Effect of transglutaminase on quality characteristics of two-structure cooked-smoked sausages

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Keywords:

Sausage
Transglutaminase
Protein
Blood plasma
Soy
Quality

Article history:

Received 1.08.2025
Received in revised form 22.11.2025
Accepted 31.12.2025

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DOI:
10.24263/2304-974X-2025-14-4-8

Abstract

Introduction. This study aimed to determine the effect of substrate interactions in composite protein mixtures and the enzyme transglutaminase on the functional-technological, structural-mechanical, and sensory properties of formed two-structure cooked-smoked sausages, as well as on their nutritional and biological value.

Materials and methods. The substrate protein mixtures differed in the proportions of animal and plant proteins and in the presence of transglutaminase. The sausage mince consisted of semi-fat pork, chicken fillet, chicken mince, and side fat. Experimental samples were produced by replacing 2.0% of semi-fat pork in the formulation with substrate protein mixtures containing transglutaminase.

Results and discussion. A binary protein mixture of porcine blood plasma and soy protein isolate, when treated with microbial transglutaminase (MTGase), effectively forms a monolithic structure in model minced meat systems for cooked-smoked sausages. This effect is attributed to the enzyme's ability to catalyze stable covalent cross-links between proteins, thereby improving textural characteristics and water-holding capacity.

To enhance the functional performance of these ingredients, their behavior in binary composite mixtures was evaluated. A mixture of porcine blood plasma protein and soy protein isolate at a 1:1 ratio in the presence of 0.9% microbial TGase exhibited the most favorable functional–technological properties, including high water-binding (78.85%) and water-holding (52.90%) capacities, along with a 5.21% increase in sausage yield. This formulation also promoted the formation of a uniform monolithic structure, improved elasticity and thermal stability of cooked-smoked sausages, and enhanced their sensory properties.

Sausage samples produced with the specified mixture composition exhibited a dense and uniform structure, along with a harmonious taste and aroma. In contrast, other formulations showed certain drawbacks: samples with the highest levels of protein and microbial transglutaminase were excessively dense, whereas those with lower protein and enzyme contents lacked sufficient elasticity.

The measured water activity of the cooked-smoked sausages ($\leq$ 0.92) indicates their suitability for long-term storage. Additionally, a reduction in the imbalance of amino acid composition, expressed by a 3.65% decrease in the difference coefficient of amino acid score (DCAS), contributed to a 4.16% increase in the biological value of the products.

Conclusion. A composite mixture of porcine blood plasma and soy protein isolate in combination with microbial transglutaminase is recommended for producing formed two-structure cooked-smoked sausages, as it improves structure, sensory quality, yield, and biological value.

Introduction

One of the methods for improving technology to form a stable structure of restructured meat products is the use of the enzyme transglutaminase (TGase) (Ivanov et al., 2021; Mirzaei, 2011). In the production of meat products, the safety of enzyme use lies in their protein nature, and consequently, their denaturation during thermal processing (Feiner, 2010). The temperature range of TGase activity is from 0 to 65 °C, with optimal chemical activity achieved approximately at 55 °C (Tarte, 2015). Denaturation of TGase begins at temperatures of 65 °C and above, and is completely halted at 70–75 °C (Whitehurst and van Oort, 2013).

In the meat processing industry, two main enzymatic preparations containing transglutaminase (TGase) are used: enzymes of bacterial origin (Feiner, 2010) and blood-based systems in which blood is fractionated according to clotting factors and subsequently recombined. In these blood-based systems, a component exhibiting transglutaminase activity is present (Prakasan et al., 2015). However, microbial transglutaminase obtained through industrial cultivation of microorganisms of the genus *Streptoverticillium* is increasingly applied in the food industry, particularly in the fish, meat, dairy, and confectionery sectors (Duarte et al., 2020; Kieliszek and Misiewicz, 2014).

The technological function of TGase is based on the structuring of protein molecules disrupted by mechanical or biochemical processes, promoting the formation of covalent bonds between amino groups and thereby creating a “cross-linking” effect in multi-component meat systems. TGase interacts differently with individual proteins depending on conditions (Ruiz-Carrascal and Regenstein, 2002; Shevchenko et al., 2020). The extent of this reaction is primarily determined by the availability of glutamine and lysine residues, as well as by reaction conditions such as pH and temperature, which must fall within the enzyme’s optimal activity range. Consequently, TGase-containing enzymatic preparations are formulated to provide the enzyme and protein substrate in the appropriate ratio (Castro-Briones et al., 2009).

Because of enzymatic action, high-molecular compounds containing intra- and intermolecular glutamyl-lysine bonds are formed. Covalent bonds formed under the action of TGase between free amino groups and gamma-carboxyl groups of glutamine are resistant to proteolysis. Bonds are formed both within the protein molecule and between its separate molecules. This allows the formation of a homogeneous dense structure in restructured meat products.

TGase also contributes to the deamination of amino acids and the biosynthesis of new ones, which improves the functional-technological properties of meat systems (Ramírez-Suárez and Xiong, 2003). The protein structure formed in this way is stable over a wide temperature range and resistant to further mechanical impacts.

However, for two-structure minced systems of cooked-smoked sausages, the specifics of combining proteins and transglutaminase enzyme in mixtures with specific substrate interaction with microbial transglutaminase are still insufficiently studied. It is important to investigate how individual ingredients (animal and plant proteins, microbial transglutaminase) and mixtures developed based on them affect the functional-technological and structural-mechanical properties of minced systems and provide synergy between recipe components. The use of optimized protein mixtures with specific substrate interaction with microbial transglutaminase will promote the formation of monolithic structure, elasticity, and thermal stability of cooked-smoked sausages.

The development of new functional protein mixtures suitable for enzymatic cross-linking by microbial transglutaminase can improve the sensory, nutritional, and biological properties of sausages, which is especially important under conditions of protein deficiency.

The aim of this study is to investigate the effect of microbial transglutaminase on composite protein mixtures in terms of their functional-technological, structural-mechanical, and sensory properties in formed two-structure cooked-smoked sausages, as well as the resulting nutritional and biological value.

Materials and methods

Materials

Substrate protein mixtures. Composition of substrate protein mixtures: porcine blood plasma protein "AProPork™" (Essentia) – a highly functional animal protein produced by spray-drying the soluble fraction of porcine blood, made from thermostable functional proteins; soy isolate (Archer Daniels Midland Company (ADM)); microbial transglutaminase (MTGase Jiangsu Zipin Biotech / BindPro®) – microbial form of calcium-independent enzyme produced by *Streptoverticillium mobamense*, with activity 50 units/g of powder.

Recipe composition of studied cooked-smoked sausage samples. One of the structural components of cooked-smoked sausages is strips of whole muscle beef, and the other is a minced system of semi-fat pork.

Model minced sausage systems were prepared based on semi-fat pork (40%), chicken fillet (20%), chicken mince (20%), and side fat (20%). During the preparation of the experimental cooked-smoked sausage samples, the developed substrate protein mixtures with various compositions of functional ingredients (Table 1) were added at 2.0%, replacing an equivalent amount of semi-fat pork.

Table 1

Recipe composition of substrate protein mixtures

Components	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Porcine blood plasma, %	2.0	–	1.6	1.4	1.0
Soy isolate, %	–	2.0	0.4	0.6	1.0
Transglutaminase (MTGase), %	0.70	0.75	0.80	0.85	0.90

Preparation of sausage samples. Model minced systems of cooked-smoked sausages consisted of strips of whole muscle beef tenderloin and minced meat from semi-fat pork, placed longitudinally along the sausage baton, parallel to the whole muscle part. The mince was prepared in a mixer for 8 minutes, formed into a protein sausage casing with a diameter of 55 mm, and thermally processed in a thermo-chamber according to the thermal processing program for cooked-smoked sausages in natural protein casing.

During the preparation of the mince for the experimental cooked-smoked sausage samples, analytically selected mixtures of enzyme and protein or protein mixtures were added in an amount of 2.0% to replace semi-fat pork. Before thermal processing, the samples were subjected to settling for 12 hours at a temperature of 4–6 °C under conditions typical for cooked-smoked sausages and for enzyme activation (at this temperature, the enzyme activity is 50 units/g) (Figure 1).

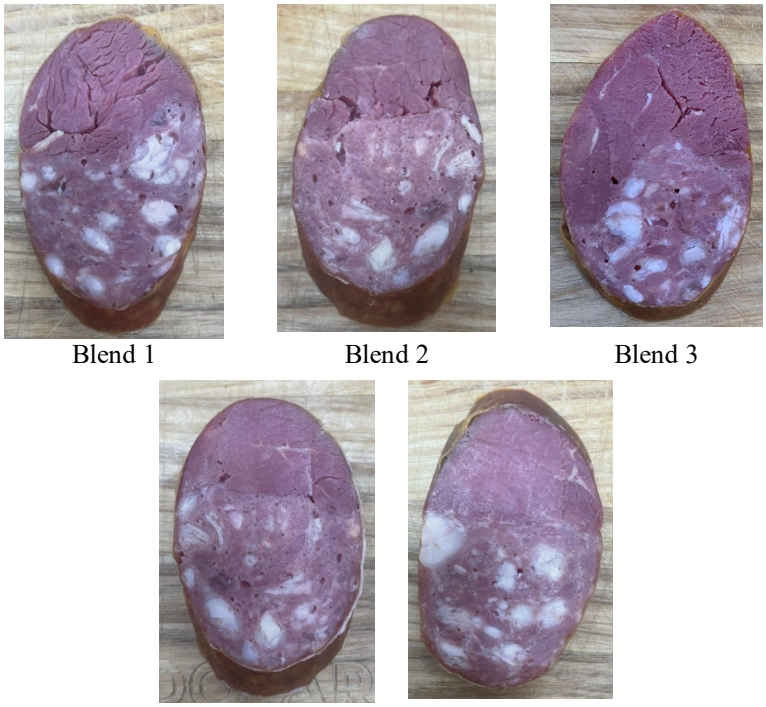

Figure 1. Sausage samples with different compositions of substrate protein mixtures

Thermal processing of experimental sausage samples was carried out until the temperature in the center of the sausage batons reached 70–72 °C. The finished sausage products were then smoked and cooled to a temperature of 12 °C.

Methods for studying the properties of substrate protein mixtures

Determination of pH of the mixture. The pH value was measured using a laboratory pH meter in an aqueous extract prepared in a ratio of mixture: water = 1:10. For this, 5 g of the mixture was placed in a 250 mL conical flask, 50 mL of distilled water was added, and it was extracted for 30 minutes with periodic stirring. After extraction, the extract was filtered through filter paper and the pH of the filtrate was measured with a laboratory pH meter (Puolanne and Kivikari, 2000).

Determination of moisture content. Moisture content was determined according to ISO 1442:1997, applicable for meat and meat products. This method involves drying a meat or meat product sample at a specific temperature to a constant mass. The weight loss of the sample during drying is considered the amount of moisture.

Determination of water-binding capacity (X_1). The water-binding capacity of the test objects was determined by the Grau-Hamm press method modified by V.I. Volovinska and B.Ya. Kelman. The method is based on extracting water from a 300 mg sample by pressing with a weight of 1 kg for 10 minutes. The size of the spot remaining on the filter paper after sorption of the released moisture is outlined with a pencil. The size of the wet spot (outer) is calculated as the difference between the total area of the spot and the area formed by the meat

(product). The water-binding capacity (percentage of bound water relative to total water) was calculated by the formula:

$$X_1 = \frac{(A - 8.4B)100}{A}$$

X_1 is a content of bound water, %, relative to total water;

A is total moisture content in the sample, mg;

B is area of the wet spot, cm².

Determination of water-holding capacity. A sausage sample weighing 0.3–0.5 g was sliced into thin pieces (2–3 mm) from the center of the product to minimize the influence of outer layers. The sample was placed between two sheets of filter paper to absorb moisture released under pressure. A weight or press was applied, typically at 5 kg/cm² for 10 minutes. After pressing, the filter paper was weighed, and the difference in weight before and after pressing was used to calculate the amount of moisture released from the sample (Baur and Ensminger, 1977).

Determination of emulsion stability (X_2). The stability of the emulsion from coarsely ground raw material was determined by heating at 80 °C for 30×60 s and cooling in water for 15×60 s. Then, four calibrated centrifuge tubes with a capacity of 50 ml were filled with the emulsion and centrifuged at a rotational speed of 500 s^{-1} for 5×60 s. The volume of the emulsified layer was then measured. Emulsion stability was calculated by the formula:

$$X_2 = \frac{V_1}{V_2}100$$

V_1 is a volume of emulsified oil, ml;

V_2 is total volume of the emulsion, ml.

Determination of penetration stress. Penetration stress of sausage products was determined using a digital Brookfield DV1 viscometer by the depth of indenter penetration into the sample at 20 °C. Three measurements were taken on the open surface of the sample at a distance of at least 10 mm from the edge of the product and at maximum distance from other measurement points to avoid deformation interference. The penetration value was then converted to penetration stress.

Determination of sensory properties of sausages. Sensory evaluation was conducted using five criteria: appearance, consistency, color of the cut, odor, and taste. Each parameter was rated on a five-point scale, where 5 = excellent and 1 = unsatisfactory. A panel of experts assessed the products according to these established parameters to determine compliance with quality standards (Fudali et al., 2021).

Determination of water activity. Water activity (a_w) of model minced systems and sausages was measured using a Rotronic Hygro Palm–23 analyzer.

Determination of protein content. Protein content was determined by the Kjeldahl method (ISO 1871:2009).

Determination of biological value. Protein biological value (BV) was determined using the corrected amino acid score, considering the limiting amino acid and "apparent" protein digestibility – PDCAAS, as proposed by FAO/WHO in 1991 according to the formula (Schaafsma, 2000).

Statistical analysis. All experiments were conducted in triplicate or more, and the results were expressed as mean±standard deviation. Statistical evaluation was performed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics (Somers, NY, USA)

Results and discussion

Compositions of substrate protein mixtures

At the first stage, the physicochemical and structural–mechanical properties of gels were studied. These gels contained varying amounts of structuring components, including porcine blood plasma proteins, soy protein isolate, and mixtures of porcine blood plasma proteins and soy protein isolate with microbial transglutaminase (MTGase). Gel-forming properties were evaluated by determining the critical gelation concentration, yield stress, and syneresis after storing the gels at 8±2 °C for 12 hours. The results are summarized in Table 1.

Table 1

Physicochemical and structural–mechanical properties of gels from substrate protein mixtures

Sample	Critical gelation concentration, %	Yield stress, kPa	Moisture separation, % (syneresis)
Blend 1 – Mixture 1 (MTGase 0.70% + blood plasma 2.0%)	0.70±0.02[a]	109.00±0.21[a]	2.90±0.08[a]
Blend 2 – Mixture 2 (MTGase 0.75% + soy protein isolate 2.0%)	0.80±0.01[b]	111.40±0.14[b]	2.80±0.09[a]
Blend 3 – Mixture 3 (MTGase 0.80% + blood plasma 1.6%, soy protein isolate 0.4%)	0.68±0.01[a]	105.50±0.13[c]	2.40±0.09[b]
Blend 4 – Mixture 4 (MTGase 0.85% + blood plasma 1.4%, soy protein isolate 0.6%)	0.85±0.01[c]	110.40±0.14[d]	2.20±0.03[c]
Blend 5 – Mixture 5 (MTGase 0.9% + blood plasma 1.0% + soy protein isolate 1.0%)	0.90±0.01[d]	113.40±0.14[e]	2.00±0.01[d]

Note: Values are the means±standard deviation. Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

It was found that increasing the content of structuring components led to a higher critical gelation concentration, which in turn resulted in an increase in yield stress (Table 1). The yield stress of the different gels ranged from 105.5 to 113.4 kPa, values that are within the acceptable range for sausages in casing. Sample 5 exhibits gel structures resistant to syneresis. An optimal ratio of animal to plant proteins (1:1) ensures uniform formation of a three-dimensional matrix, increasing gel strength and elasticity. This is explained by the synergistic action of plasma proteins and soy protein isolate. Blood plasma contains albumins and globulins, which are good donors of lysine residues, while soy protein isolate contains glutamine residues, which are good acceptors. In the presence of MTGase, ε-(γ-glutamyl)-lysine cross-links form between them, densifying the structure. Thus, the combination of blood plasma and soy protein isolate in the presence of MTGase promotes gelation through protein-protein interactions, such as cross-linking of globulins (Xiong, 2017). The minimal syneresis value (2.0%) also indicates high water-holding capacity of the system, correlating

with higher resistance to deformation. Therefore, the high yield stress of Sample 5 is explained by the most efficient formation of covalent cross-links between blood plasma proteins and soy protein isolate under MTGase action, resulting in a dense, elastic gel with reduced syneresis (Kuraishi et al., 1997; Toldrá and Reig, 2021).

Functional-technological properties of model minced systems and cooked-smoked sausages

At the next stage, studies were conducted to investigate the effect of MTGase on the functional-technological properties of model minced systems during settling. The mince included, in varying amounts as structuring components, the enzyme TGase, proteins (blood plasma, soy protein isolate), and their mixtures (Toldrá and Reig, 2021). The results of these studies are presented in Table 2.

Table 2

Functional-technological properties of model minced systems and cooked-smoked sausages depending on the composition of substrate protein mixtures

Parameter	Substrate protein mixtures				
	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Settling time (fermentation) 10 h					
Water-binding capacity, %	77.08±3.15^{a}	77.52±3.08^{a}	77.84±3.09^{a}	78.27±3.18^{a}	78.72±3.11^{a}
Water-holding capacity, %	51.14±2.23^{a}	52.12±2.15^{a}	52.37±2.24^{a}	52.41±2.51^{a}	52.84±2.15^{a}
Mass loss during thermal processing, %	22.18±0.98^{a}	21.62±0.91^{a}	21.52±0.87^{a}	21.43±0.81^{a}	20.93±0.85^{a}
Emulsion pH	6.2±0.14^{a}	6.2±0.21^{a}	6.2±0.18^{a}	6.2±0.19^{a}	6.3±0.20^{a}
Settling time (fermentation) 12 h					
Water-binding capacity, %	77.28±3.15^{a}	78.12±3.12^{a}	78.48±3.14^{a}	78.52±3.17^{a}	78.85±3.17^{a}
Water-holding capacity, %	51.16±2.06^{a}	52.19±2.15^{a}	52.51±2.24^{a}	52.48±2.51^{a}	52.90±2.51^{a}
Mass loss during thermal processing, %	21.78±0.81^{a}	20.86±0.89^{a}	20.58±0.84^{ab}	20.46±0.79^{ab}	19.96±0.82^{b}
Emulsion pH	6.2±0.14^{a}	6.2±0.20^{a}	6.2±0.17^{a}	6.2±0.18^{a}	6.3±0.17^{a}

Note: Values are the means±standard deviation. Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

According to experimental data, confirmed by statistical analysis, the functional-technological properties of model minced systems and cooked-smoked sausages remain unchanged. This can be explained by the simultaneous increase in substrate addition (mixture of blood plasma and soy protein isolate) along with increased enzyme content. Therefore, quality indicators remain stable, while the nutritional value of the product increases.

Fermentation time has a statistically significant positive effect on functional-technological properties: it increases water-binding and water-holding capacities and reduces mass loss during thermal processing. The composition of protein mixtures shows a tendency to improve the same parameters: increasing water-binding and water-holding capacities and reducing mass loss during thermal processing. The positive effect of prolonged fermentation

is observed for all types of protein mixtures, which is an important technological conclusion. Completion of the enzymatic "cross-linking" process and settling is indicated by stabilization of pH and reduction of moisture loss, not exceeding 1%, resulting from gel network densification (Table 2).

Study of structural-mechanical properties of model sausage samples

In the production of two-structure cooked-smoked sausages, an important aspect is obtaining a monolithic product with a dense structure. A disadvantage that may occur using classical technology for cooked-smoked sausages is structural separation and detachment of minced elements after thermal processing, as well as an atypical appearance of the cut, negatively affecting both sensory properties and slicing of the finished product.

To assess the degree of influence of substrate protein mixtures on structural-mechanical characteristics of model cooked-smoked sausage samples, water activity and yield were measured in samples with 2.0% of meat raw material replaced by protein mixtures of different composition. Considering the functional properties of selected protein mixtures with different structural conformations, hydration was established at a ratio of 1:4.

The dynamics of changes in shear stress, cutting force, yield, and water activity of model cooked-smoked sausage samples depending on the composition of substrate protein mixtures are presented in Table 3.

Table 3

Structural-mechanical parameters, yield, and water activity of model cooked-smoked sausage

Parameter	Substrate protein mixtures				
	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Shear stress, kPa	198.87±8.04^{b}	205.82±8.08^{a}	212.39±8.25^{a}	214.03±8.43^{a}	214.77±8.40^{a}
Cutting force, kPa	206.23±6.31^{b}	209.18±6.0^{ab}	211.16±6.23^{a}	215.12±6.40^{a}	216.02±6.34^{a}
Yield, %	77.22±2.30^{b}	82.14±2.11^{a}	82.22±2.18^{a}	82.35±2.24^{a}	82.43±2.26^{a}
Water activity, a_w	0.914±0.002^{a}	0.913±0.001^{a}	0.912±0.003^{ab}	0.910±0.001^{b}	0.909±0.001^{b}

Note: Values are the means±standard deviation. Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

Analysis of the results (Table 3) indicates that shear stress in the model sausage samples varied depending on the composition of the substrate protein mixtures. An increase in shear stress of 1.88% can be attributed to protein cross-linking, which leads to changes in their physicochemical and structural–mechanical properties. It was established that the formation of covalent bonds induced by MTGase alters the rheological properties of the sausages. Although the overall statistical analysis did not reveal significant differences among all substrate protein mixture groups, pairwise comparisons demonstrated the high efficiency of the modified mixtures. A statistically significant difference was observed between the control mixture Blend 1 (77.22±2.30%) and the most effective Blend 5 (82.43±2.26%). Replacing Blend 1 with Blends 2–5 resulted in an average yield increase of 5.2%, which is a critically important technological parameter.

Analysis of structural-mechanical parameters (shear stress and cutting force) revealed a clear trend of structure reinforcement from Blend 1 to Blend 5. Shear stress increased from

198.87 kPa (Blend 1) to 214.77 kPa (Blend 5), likely due to optimization of the gel-forming properties of the protein matrix through covalent bond formation between primary amines of Gln and Lys residues in protein molecules (Santhi et al., 2017).

Simultaneously, water activity gradually decreased from 0.914 (Blend 1) to 0.909 (Blend 5). This parameter correlates with increased yield and confirms that mixtures Blend 2–5 bind free moisture more effectively. Lower water activity is favorable for improving microbiological stability and extending shelf life of the final product.

Thus, the highest strength properties (shear stress and cutting force) (Table 3) were observed for Blend 5, which contained 0.9% MTGase, 1.0% blood plasma, and 1.0% soy protein isolate in an optimal ratio. Based on the obtained data, Blend 5 is the most technologically efficient mixture, providing the highest product yield, maximum shear stress (indicating the formation of the densest structure), and lowest water activity, creating favorable conditions for longer product shelf life.

Therefore, combining substrate protein mixtures with MTGase is reasonable to improve the structural-mechanical properties of sausages, as covalent bonds formed by transglutaminase are highly resistant to proteolysis and thermal processing (Pfleiderer et al., 2005; Toldrá and Reig, 2021).

Moreover, blood plasma proteins are a better substrate for MTGase due to their easily accessible, flexible, and open chain structure. In comparison, globular whey proteins are less reactive because disulfide bonds stabilize the globular conformation, limiting binding site accessibility. Proteins with a high glutamine content, such as soy proteins, are also good substrates for transglutaminase. Under the action of MTGase, cross-linking between glutamine and lysine residues in meat proteins leads to the formation of ε-(γ-glutamyl)lysine (ε-(γ-Glu)Lys) complexes (Han and Bertram, 2017).

Thus, the effect of MTGase in forming additional bonds between plant and animal proteins contributed to the formation of a denser and more monolithic structure in model cooked-smoked sausage samples (Table 3). These new protein bonds effectively retain moisture in the sausage matrix and increase resistance to deformation forces. MTGase facilitates interactions between meat proteins and hydrophilic groups of the substrate protein mixture, resulting in reduced moisture loss during thermal processing and a 5.21% increase in sausage yield (Lee et al., 2017).

Sausage strength with meat replacement by substrate protein mixtures increased by 1.95%. Therefore, the addition of substrate protein mixtures positively affects overall structural strength and increases yield of cooked-smoked sausages.

The study of structural-mechanical properties showed that using substrate protein mixtures significantly improves strength characteristics of thermally processed sausage samples. This is because the viscosity of the minced component of model sausages increases due to the high protein content in both substrate protein mixtures and meat raw material. Increased adhesion forces between protein molecule surfaces promote the formation of a reinforced protein structure (Mohanan et al., 2020).

Scientific studies indicate that MTGase addition increases apparent viscosity of the mince, regardless of sodium alginate addition or salt concentration. This may result from protein solubilization with covalent bonds formed between Gln and Lys (Chen et al., 2023; Moreno et al., 2008). Microbial transglutaminase catalyzes the formation of isopeptide bonds, ε-(γ-glutamyl)lysine cross-links between glutamine (Gln) residues in peptide chains and ε-amino groups of lysine (Lys) residues in plasma protein and soy protein isolate peptides, enhancing gel textural properties (Li et al., 2018; Wang et al., 2019). Therefore, inclusion of substrate protein mixtures in cooked-smoked sausages significantly improves

texture (elasticity and strength), mechanical resistance, and water-holding capacity of model samples.

Thus, shear stress and cutting force measurements on the Brookfield DV1 viscometer demonstrated that the most optimal ingredient composition is Blend 5. Its use in cooked-smoked sausages contributes to formation of the best structural properties, confirming the high functionality of its composition as a substrate protein mixture for dual-structure cooked-smoked sausages.

In conclusion, analyzing the structural-mechanical characteristics of model sausage samples shows that meat proteins in combination with substrate protein mixtures containing high amounts of Gln and Lys for cross-linking under MTGase influence positively affect overall structural strength of cooked-smoked sausages. This allows prediction of the interaction of structuring components and regulation of quality indicators in meat products (Azaza et al., 2009; Ramos-Diaz et al., 2022; Wei, 2019).

The results of water activity (a_w) studies in cooked-smoked sausages (Fig. 2) showed that model sausage samples with substrate protein mixtures can be classified as group C ($a_w \leq 0.92$) in terms of product stability. Meaning of $a_w \leq 0.92$ indicates that the product is resistant to long-term storage (Ruiz-Carrascal and Regenstein, 2002) because the amount of water biologically available for microorganisms is reduced (Shevchenko et al., 2020).

Amino acid composition of model cooked-smoked sausage samples

To assess the influence of substrate protein mixtures on the biological value of model cooked-smoked sausages, their amino acid composition was compared with that of an "ideal" protein, the mass fractions of which and overall balance fully satisfy the requirements of the human adult body. The results of amino acid composition analysis of the model cooked-smoked sausage samples are presented in Table 4.

Analysis of the amino acid composition of control and experimental sausage samples shows that the biological value index increases by 4.16% when applying the substrate protein mixture Blend 5. The analysis reveals a significant excess of the amino acid SCORE for several amino acids: valine (119.58%), isoleucine (146.42%), and tryptophan (104.5%) (Duarte et al., 2019).

In the control sample, the limiting amino acids were phenylalanine and tyrosine, whereas their content in the experimental samples was slightly higher (74.00 mg/100 g in the control vs. 77.97 mg/100 g in the experimental samples).

A reduction in amino acid imbalance, as indicated by a 3.65% decrease in the DCAS index, suggests more rational protein utilization for anabolic purposes and contributes to a 4.16% increase in biological value compared to the control. These results confirm that the biological value (BV) of dual-structure cooked-smoked sausages improves with the addition of swine blood plasma proteins and soy protein isolate, which provide a better balance of aromatic amino acids in the final product.

Sensory properties of model cooked-smoked sausages

The overall sensory evaluation of the model sausage samples was high. The highest scores were observed for taste, color, and consistency, as illustrated by the quality profiles of the samples (Fig. 2).

Table 4

Amino acid composition of model cooked-smoked sausages (mg/100 g)

Amino acid	Cooked–smoked sausage (control)		Cooked–smoked sausage with 2% lean pork replacement by Blend 5 (experimental)		Reference standard (mg/100 g)
	content	% SCORE	content	% SCORE	
Protein, mg	18050	–	19038	–	1000
Essential amino acids:	417		40.88	113.56	351
Valine	49.11	114.21	51.42	119.58	43
Isoleucine	46.07	139.61	48.32	146.42	33
Leucine	82.16	124.48	82.71	125.32	66
Lysine	86.29	156.89	77.89	141.62	55
Methionine + Cysteine	24.96	99.84	25.31	101.24	25
Threonine	43.55	124.43	44.69	127.69	35
Tryptophan	9.99	99.90	10.45	104.50	10
Phenylalanine + Tyrosine	44.40	74.00	46.78	77.97	60
Histidine	29.97	124.88	30.75	128.13	24
BV	80.34		84.50		100.00

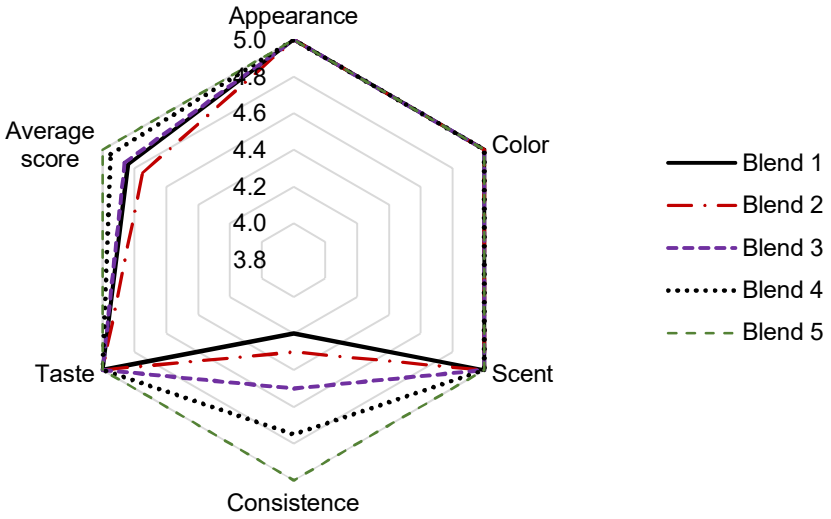

Figure 2. Quality profiles of model cooked-smoked sausage samples depending on the composition of substrate protein mixtures

Modification of proteins with MTGase allows changes in solubility, hydration, and thermal stability, thereby improving structural properties of the mince base, particularly at the junction between structural elements: whole-muscle and minced parts of dual-structure

cooked-smoked sausages. The best sensory properties were observed in Sample 5, which contained 0.9% MTGase, 1.0% blood plasma, and 1.0% soy protein isolate in an optimal ratio within the mince base.

Efficiency of substrate protein mixtures in model cooked-smoked sausages

The use of substrate protein mixtures, particularly Blend 5, effectively stabilizes meat protein systems, reduces technological losses, and prevents structural separation, positively influencing the structural-mechanical properties of sausages. The synergy between plant and animal proteins and microbial transglutaminase enhances elasticity and structural strength and increases the yield of the final product. The developed substrate protein mixture also demonstrates high functional-technological properties, confirmed by positive sensory evaluation of dual-structure cooked-smoked sausages.

The results showed an increase in water-binding and water-retention capacity of mince and thermally processed sausage samples during 12 h settling by 1.57% and 1.74%, respectively, and a reduction in mass loss during thermal processing by 1.82%. Practical application of the substrate protein mixture in the optimal component ratio of Blend 5 in cooked-smoked sausage production allows an increase in shear stress by 1.88% and cutting force by 1.95%. These values align with previous studies (Toldrá and Reig, 2021), which also reported improved rheological and textural characteristics with protein mixtures and/or transglutaminase application.

The yield of final sausages increased by 5.21%. Due to the stability of the water–fat emulsion and preservation of texture, the finished products meet high quality standards, maintaining uniform color, harmonious taste, and aroma.

The use of Blend 5 substrate protein mixture in the proposed composition can be recommended for industrial production of dual-structure cooked-smoked sausages. It improves sensory stability: products have elastic consistency, harmonious taste and aroma, enhancing market competitiveness.

The results confirm the feasibility of using substrate protein mixtures in sausage production. Their application positively affects structural strength and stability, increases mechanical characteristics, promotes monolithic structure, elasticity, and thermal stability of cooked-smoked sausages, and enhances shelf-life stability by reducing water activity to $a_w \leq 0.92$. Reduction of imbalance according to DCAS by 3.65% contributes to an increase in biological value by 4.16%.

As substrate protein mixtures are a promising tool for producing high-quality meat products with improved structural-mechanical properties, the main directions for their implementation in dual-structure cooked-smoked sausages include: controlled dosing (excess causes excessive hardness) and chemical composition modeling to optimize ingredient ratios in mince systems and restructured meat products to positively influence both sensory properties and biological value.

Conclusions

The use of composite protein mixtures, including blood plasma, soy protein isolate, and microbial transglutaminase, improves the structure, elasticity, thermal stability, and sensory properties of dual-structure cooked-smoked sausages. Partial replacement of semi-fat pork with these mixtures enhances gel formation and monolithic structure. Reduced water activity and improved amino acid balance contribute to increased storage stability and biological

value. Overall, the optimized combination of protein components and MTGase provides an effective approach for producing high quality, functional sausages.

References

Azaza M.S., Wassim K., Mensi F., Abdelmouleh A., Brini B., Kraiem M.M. (2009), Evaluation of faba beans (*Vicia faba* L. var. *minuta*) as a replacement for soybean meal in practical diets of juvenile *Nile tilapia*, *Oreochromis niloticus*, *Aquaculture*, 287, pp. 174–179, https://doi.org/10.1016/j.aquaculture.2008.10.007

Baur F.J., Ensminger L.G. (1977), The Association of Official Analytical Chemists (AOAC), *Journal of the American Oil Chemists'Society*, 54, pp. 171–172, https://doi.org/10.1007/bf02670789

Beriain M.J., Gómez I., Sánchez M., Kizkitza I., Sarriés M.V., Francisco C. I. (2020), The reformulation of a beef patty enriched with n-3 fatty acids and vitamin D3 influences consumers' response under different information scenarios, *Foods*, 9(4), pp. 506–512, https://doi.org/10.3390/foods9040506

Castro-Briones M., Calderon G.N., Velazquez G., Rubio M.S., Vázquez M., Ramírez J.A. (2009), Mechanical and functional properties of beef products obtained using microbial transglutaminase with treatments of pre-heating followed by cold binding, *Meat Science,* 83(2), pp. 229–238, https://doi.org/10.1016/j.meatsci.2009.05.002

Duarte L., Matte C.R., Bizarro C.V., Ayub M.A.Z. (2020), Transglutaminases: Part II – Industrial applications in food, biotechnology, textiles and leather products, *World Journal of Microbiology and Biotechnology*, 36(1), 11, https://doi.org/10.1007/s11274-019-2792-9

Feiner H. (2010), *Meat Products: Scientific Bases, Technologies, Practical Recommendations*, Transl. N.V. Mahdy, Profession Publishing.

Fudali A., Chełmecka I., Salejda A.M., Krasnowska G. (2021), Microbiological safety and sensory quality of homogenized sausages manufactured with commercial functional additives, *Applied Sciences*, 11(11), 11662, https://doi.org/10.3390/app112411662

Han M., Betram H.C. (2017), Designing healthier comminuted meat products: Effect of dietary fibers on water distribution and texture of fat-reduced meat model system, *Meat Science*, 133, pp. 157–165, https://doi.org/10.1016/j.meatsci.2017.07.001

ISO 1871:2009. (2009), Food and feed products — General guidelines for the determination of nitrogen by the Kjeldahl method.

Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021),Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10(1), pp. 7-36, https://doi.org/10.24263/2304-974X-2021-10-1-3

Kieliszek M., Misiewicz A. (2014), Microbial transglutaminase and its application in the food industry, A review, *Folia Microbiologica*, 59(3), pp. 241-250, https://doi.org/10.1007/s12223-013-0287-x

Kuraishi C., Sakamoto J., Yamazaki K. (1997), Production of restructured meat using microbial transglutaminase without salt or phosphate, *Meat Science*, 47(3–4), pp. 237–243.

Lee H.C., Jang H.S., Kang I., Chin K.B. (2017), Effect of red bean protein isolate and salt levels on pork myofibrillar protein gel mediated by microbial transglutaminase, *LWT - Food Science and Technology*, 76(Part A), pp. 95–100, https://doi.org/10.1016/j.lwt.2016.10.039

Li T., Li C., Quan D.N., Bentley W.E., Wang L.X. (2018), Site-specific immobilization of endoglycosidases for streamlined chemoenzymatic glycan remodeling of antibodies,

Carbohydrate Research, 458–459, pp. 77–84, https://doi.org/10.1016/j.carres.2018.02.007

Mirzaei M. (2011), Microbial transglutaminase application in food industry, *International Conference on Food Engineering and Biotechnology*, IPCBEE: http://ipcbee.com/vol9/51-B20017.pdf

Mohanan A., Nickerson M.T., Ghosh S. (2020), Utilization of pulse protein-xanthan gum complexes for foam stabilization, *Food Chemistry*, 316, 126282, https://doi.org/10.1016/j.foodchem.2020.126282

Pfleiderer C., Mainusch M., Weber J., Hils M., Fuchsbauer H.L. (2005), Inhibition of bacterial transglutaminase by its heat-treated pro-enzyme, *Microbiological Research*, 160(3), pp. 265–271, https://doi.org/10.1016/j.micres.2005.01.001

Prakasan V., Chawla S.P., Sharma A. (2015), Effect of transglutaminase treatment on functional properties of paneer, *International Journal of Current Microbiology and Applied Sciences*, 4(5), pp. 227–238.

Puolanne E., Kivikari R. (2000), Determination of buffering capacity of postrigor meat, *Meat Science*, 56, pp. 7–13, https://doi.org/10.1016/S0309-1740(00)00007-3

Ramos-Díaz J.M., Kantanen K., Edelmann J.M., Jouppila K., Sontag-Strohm T., Piironen V. (2022), Functionality of oat fiber concentrate and faba bean protein concentrate in plant-based substitutes for minced meat, *Current Research in Food Science*, 5, pp. 858–867, https://doi.org/10.1016/j.crfs.2022.04.010

Ramírez-Suárez J.C., Xiong Y.L. (2003), Rheological properties of mixed muscle/non-muscle protein emulsions treated with transglutaminase, *Journal of Food Science and Technology*, 38(7), pp. 777–785, https://doi.org/10.1046/j.1365-2621.2003.00731.x

Ruiz-Carrascal J., Regenstein J. (2002), Emulsion stability and water uptake ability of chicken breast muscle proteins affected by microbial transglutaminase, *Journal of Food Science*, 67, pp. 734–739, https://doi.org/10.1111/j.1365-2621.2002.tb10668.x

Santhi D., Kalaikannan A., Malairaj P. (2017), Application of microbial transglutaminase in meat foods: A review, *Critical Reviews in Food Science and Nutrition*, 57(10), pp. 2071–2076, https://doi.org/10.1080/10408398.2014.945990

Schaafsma G. (2000), The protein digestibility-corrected amino acid score, *Journal of Nutrition*, 130(7), pp. 1865S–1867S, https://doi.org/10.1093/jn/130.7.1865S

Shevchenko I.I., Polishchuk G.E., Filonenko M.I., Osmak T.G. (2020), Study of the structuring properties of transglutaminase in protein-containing systems, *Scientific Proceedings of the National Institute of Chemistry and Technology*, 26(2), pp. 212–219, https://doi.org/10.24263/2225-2924-2020-26-2-22

Shevchenko I.I., Polishchuk G.E., Filonenko M.I., Topchiy, O.A. (2021), Study of the features of regulating the structure of ham products using transglutaminase, *Scientific Works of the National University of Chemistry and Technology*, 27(6), pp. 140–149, https://doi.org/10.24263/2225-2924-2021-27-6-16

Tarte R. (2015), *Ingredients in Meat Products. Properties, Functionality and Applications*, Springer New York, NY, https://doi.org/10.1007/978-0-387-71327-4

Toldrá F., Reig M. (2021), Advances in the use of transglutaminase in meat processing, *Food Chemistry*, 347, 128940, https://doi.org/10.1016/j.foodchem.2021.128940

Wang J.H., Tang M.Z., Yu X.T., Xu C.M., Yang H.M., Tang J.B. (2019), Site-specific covalent immobilization of engineered enterokinase through transglutaminase-catalyzed bioconjugation, *Colloids and Surfaces,* 177, pp. 506–511, https://doi.org/10.1016/j.colsurfb.2019.02.01

Wei X. (2019), *Effects of short-term germination and autoclaving on selected compounds in faba bean and applications in low-fat pork bologna*, MSc thesis, University of Saskatchewan.

Whitehurst R.J., van Oort M. (2013), *Enzymes in the Food Industry*, Profession Publishing.

Xiong Y.L. (2017), The storage and preservation of meat: I–Thermal technologies, In: F. Toldra (Ed.), *Lawrie's Meat Science*, 8th ed., Woodhead Publishing, pp. 205–230, https://doi.org/10.1016/B978-0-08-100694-8.00007-8

Cite:

UFJ Style

Shevchenko I., Haschuk O., Moskaliuk O., Kharchenko Ye. (2025), Effect of transglutaminase on quality characteristics of two-structure cooked-smoked sausage, *Ukrainian Food Journal*, 14(4), pp. 698–712, https://doi.org/10.24263/2304-974X-2025-14-4-8

APA Style

Shevchenko, I., Haschuk, O., Moskaliuk, O., & Kharchenko, Ye. (2025). Effect of transglutaminase on quality characteristics of two-structure cooked-smoked sausage. *Ukrainian Food Journal*, *14*(4), 698–712. https://doi.org/10.24263/2304-974X-2025-14-4-8