International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 5
ISSN: 2088-5334

Enhancing the Sausage Quality of Indonesian Local Lamb Meat with Microbial Transglutaminase Enzyme: Physicochemical, Textural, and Microstructure Properties

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Abstract— **Indonesia Batur local lamb meat has emerged as a promising meat source for the production of emulsion-type sausages. However, the manufacturing process of this sausage typically requires high-fat content to achieve the desired quality characteristics. To address this issue, this study investigates utilizing microbial transglutaminase (MTGase) enzyme to improve local lamb meat sausage's physicochemical, textural, and microstructure features. This research aimed to develop emulsion sausages using local lamb meat by incorporating the MTGase enzyme. The experimental design encompassed various treatments, including a control group, the addition of 10% tapioca, and incremental amounts of MTGase (ranging from 0.2% to 1.0%). The sausages were evaluated comprehensively: pH value, color, tenderness, texture, and microstructure. The statistical analysis, employing ANOVA, demonstrated a significant improvement in pH, firmness, toughness, cohesiveness, and gumminess with the addition of MTGase, while also influencing the color of the sausages (P<0.05) that can be attributed to the MTGase enzyme's capacity to bind myofibrillar proteins through cross-linking reactions, enhancing texture and tenderness. Nevertheless, it was noticed that the presence of MTGase led to a* and b* values reduction due to the denaturation of globin and carotenoid pigments; however, these values remained within an acceptable range. Notably, the incorporation of 0.8% and 1.0% MTGase resulted in forming an ordered and homogeneous network microstructure, exhibiting fewer voids within the sausages. Overall, the findings of this study demonstrate the successful enhancement of the quality of sausages, thereby significantly increasing the acceptability of the final product.**

Keywords— **Emulsion-type sausage; local lamb; MTGase enzyme; tenderness; texture.**

Manuscript received 27 May 2023; revised 28 Jul. 2023; accepted 12 Aug. 2023. Date of publication 31 Oct. 2023.

I. INTRODUCTION

Meat products with high protein content, vitamins, fats, Fe, Zn, and other minerals are essential sources of macro and micronutrients for humans [1]. In response to the high demand for meat products in Indonesia, diversification of meat products by augmenting the potential of local meat could be an alternative of choice for the meat industry. One of the local livestock that has an original distribution in the territory of Indonesia is the Indonesian Batur Lambs. Batur lamb, an indigenous Indonesian livestock variety that originated in Banjarnegara Regency in 1974, is a result of hybridization between merino and slender-tailed sheep. It surpasses the weight of domestic sheep by twofold, ranging from 60 to 80 kg, and can reach a maximum weight of 140 kg [2].

Local lambs have been cultivated for generations in various regions of Indonesia. Local lamb meat is widely known and consumed by people in Indonesia in several traditional processed products such as satay, curry, *tongseng*, *rica-rica,* and many others. Producing lamb meat into sausages has great potential in the Indonesian market because, until now, it is still rarely available. In addition, the manufacture of sausages is considered an alternative in meat processing to eliminate the strong odors and tastes of local lamb meat [3]. Kasaiyan et al. [4] produced emulsion-type lamb meat sausage with olive oil and the addition of chickpeas as pulse flour and found improved hardness and chewiness compared to the control sausage. De Carvalho et al. [5] used turmeric extract to perform antioxidant activity to prevent fat oxidation that caused malodors of lamb meat sausage.

Lamb meat has been considered red meat with a relatively high-fat content. Substituting vegetable oils for fat in lamb meat has been documented as an alternate approach to enhance the nutritional advantages of lamb meat sausages without compromising their quality [6]. The fermentation of the meat sausage was also reported as an alternative technique to enhance the shelf-life and nutrition content [7]. The high fat content of lamb meat is appropriate for the emulsion-type sausage, where fat and meat form an emulsion to provide a good texture and flavor.

The fat content in conventional emulsion-style sausages varies up to 30% of animal-derived fat, which impacts the emulsions stability, cooking losses, and water-holding capacity, contributing to the rheological and structural characteristics that determine the texture and physicochemical attributes of the prepared meat sausage [8]. The presence of fat significantly influences the quality and sensory flavor of meat products. Nevertheless, elevated fat and/or cholesterol levels pose a significant issue in processed meat items [3]. Consumer awareness of the health risks caused by high animal fat intake is increasing, thus growing the demand for reduced-fat meat products [9]. Hence, it is crucial to investigate novel starters that can enhance the nutritional value of meat products while preserving their distinctive attributes and acceptance from consumers [10].

One alternative that can be done to reduce fat content is to utilize the microbial transglutaminase (MTGase) enzyme. The addition of transglutaminase enzyme can reduce fat content in the manufacture of ice cream [11], white cheese [12], and yogurt [13]. MTGase is an enzyme that facilitates the creation of iso-peptide bonds in proteins and finds extensive application in multiple manufacturing procedures. These include the production of cheese, dairy products, meat processing, bakery products, and edible films. MTGase holds significant promise in enhancing meat products' firmness, viscosity, elasticity, and water-holding capability (WHC) [14].

Several studies have focused on applying MTGase in protein-containing food products such as milk and meat [14]. MTGase emerges as a microorganism-derived binding agent facilitating the interaction between glutamine and lysine side chains [15]. The use of MTGase in meat restructuring allows the changes in the texture profile of meat products [16]. Izmail et al. [17] utilized MTGase as an additive for the fish sausages and found improved gel strength and WHC. Thephuttee and Theprugsa [18] also revealed that adding MTGase has increased the stability of meat emulsion, thereby improving the hardness and chewiness of chicken sausages. The strong binding ability of myofibrillar protein with MTGase in beef sausages was identified by Ahhmed et al. [19] as positively assisting the protein functionalities to improve the textural and gelation properties of beef sausages.

Furthermore, the effect of MTGase addition to the lamb meat sausages is limitedly studied. The addition of MTGase to the local lamb meat sausages production is expected to improve the product quality, physicochemical properties, texture, and connectivity [20], [21]. The primary objective of this study is to assess the impact of incorporating the MTGase enzyme on the physicochemical properties, including pH value, color, tenderness, texture, and microstructure, during the production of lamb sausages.

II. Materials and Methods

A. Materials and Ingredients

Meat from local lamb "Domba Batur" (age ranges 5 to 6 months) was acquired from Batur Lamb breeders in Purwokerto, Indonesia. The lamb meat and fat used were a combination of thigh meat, intermuscular fat, and deep fat. The MTGase used was purchased from Shaanxi Fonde Biotech Co., Ltd., China—collagen-based sausage Casing 10 cm. The dough ingredients consist of tapioca (Budi Starch & Sweetener Ltd., Indonesia), fine salt (UnichemiCandi, Sidoarjo, Indonesia), dextrose (Tan Putra Tama Co. Ltd., Indonesia), garlic powder (Gunacipta Multirasa Ltd., Indonesia), pepper powder (Prima Cipta Mandiri Ltd., Indonesia), paprika powder (Hoka Jaya Internasional Co. Ltd., Indonesia), and chili powder (Asean Niaga, Jakarta).

B. Treatments and Experimental Design

The formulation for the production of lamb meat sausages consisted of the following ingredients: 10% by weight of ground lamb meat, 0.5% by weight of garlic powder, 0.5% by weight of dextrose, 2% by weight of fine salt, 0.5% by weight of pepper powder, 0.5% by weight of chili powder, and 0.5% by weight of paprika powder. There were 5 levels of MTGase concentration applied to the mixture: 0.2, 0.4, 0.6, 0.8, and 1.0 % (w/w). The experiment was designed by a completely randomized design (CRD) consisting of 7 treatments and 4 replications. The treatment set in this study consisted of sausages preparation with control, 10% tapioca, 0.2% MTGase, 0.4% MTGase, 0.6% MTGase, 0.8% MTGase, and 1.0% MTGase.

C. Production of emulsion-type sausage

The procedure for making emulsion-type sausages with the addition of MTGase was prepared by adopting a modified method in previous studies [22], [23]. The meat was ground first using a grinder (WIRASTAR Meat Grinder MGD-12A, Indonesia). Then, the ground lamb meat was mixed with other ingredients (according to the treatment) and stirred until homogeneous and well emulsified. Then, the meat batter was put into the collagen sausage casing. MTGase activation was conducted at a temperature of 60 °C for 15 minutes. The subsequent gelling process was extended for 30 minutes at 80 °C, followed by cooling the pre-cooked sausages to ambient temperature. The sausages were then cooked for 15 minutes at 80 °C.

D. pH value measurement of produced sausages

A 10 g sample of sausage was suspended in 90 ml of distilled water and transferred into a measuring flask. The pH value was determined using a pH meter (Hanna HI98107, Romania). Standard pH buffer solutions with pH values of 4.01 and 7.01 were utilized beforehand to calibrate the pH meter. Duplicate measurements of the pH value were conducted for all samples.

E. Color measurement

Samples for color measurement were prepared by slicing the meat in cross-sections. Color values of the sausage sample were determined using a colorimeter (3NH Super Color Reader CR7, China) with a scale of L* (brightness), a* (reddish), and b* (yellowish) according to the International

Commission of Illumination (CIE) System. Measurements were carried out in duplicate on all samples.

F. Texture Profile Analysis

The texture characteristics of the sausage samples were assessed using the Food Texture Analyzer TA.XTplus (Stable Microsystems, UK) connected to Exponent Lite v.6.1.17.0 software. A P1/KS test probe, which is a stainless-steel cylinder with a diameter of 10 mm, was employed. The TPA (Texture Profile Analysis) value was computed by plotting force and time data on a graph. Various texture parameters, including hardness (g), adhesiveness (g), springiness, cohesiveness, gumminess, and chewiness, were recorded using specific test conditions [24].

G. Sausage tenderness analysis

The tenderness of the sausage samples was measured using a Warner Bratzler "V" shape knife equipped Food Texture Analyzer TA.XTplus (Stable Microsystems, UK). The probe used for tenderness measurement was a Blade Set (HDP/BS) [25]. The tenderness of the sausage samples can then be obtained from the results of the Warner Bratzler shear forces [26].

H. Microstructure observation

For microstructure observation, the lamb meat sausage samples were placed in a freezer (Thermo Fisher Scientific TSX Series, USA) at -20 °C for 24 h. The sausage samples were then carefully sliced with the size of 0.8 x 0.8 cm and 10 µm of thickness. The sliced samples were placed on the sample holder with a carbon double-tip and then gold-sputtered. The samples were then observed under Scanning Electron Microscopy (SEM JEOL Series 6510 LA, Japan) at a magnification of 50 – 1000 times.

I. Statistical analysis

The collected sample data was subjected to statistical analysis using Analysis of Variance (ANOVA) with Duncan's New Multiple Range Test (α = 0.05). The SAS Program (SAS® Institute Inc., Cary, NC, USA) Version 9.4 performed the analysis. The recorded values are presented as the mean ± standard error.

III. Results and Discussions

A. Effects of MTGase on the pH and Color of Lamb Meat Sausage

The experimental results of the pH value measurement for prepared local lamb sausages are summarized in Table 1. Adding MTGase can significantly increase the pH value (P<0.05). The results of this study indicate that at least the addition of MTGase in the manufacture of sausages can increase the pH by 0.4%, and the addition of 0.4% to 1% has a pH value that is not significantly different. The pH value is measured as one of the physicochemical characteristics of sausage products because pH is a very important property that can affect color, taste, WHC, protein properties, and the shelf life of lamb meat sausages [27]. The pH value reflects the activity of microorganisms and the degree of biogenic lipid and amino oxidation [28]. In sausage products, the pH value is important in evaluating the quality of fermented sausages during cooking [29]. Similar results were also reported by previous studies, presenting a pH value of 6.13 in pork sausage [30], a range of 6.21 – 6.34 in bologna sausage [31], and 6.12 – 6.24 in lamb sausage [31]. The expected pH value in meat studies is in the range of 5.1 – 6.1 [32]. The pH value is related to the growth rate of microorganisms that can deteriorate the lamb sausage product.

TABLE I

pH VALUE AND COLOR PROFILE OF LOCAL LAMB MEAT SAUSAGES WITH MTGASE ADDITION (MEAN ± STANDARD DEVIATION, N = 4)

Treatment	pH value*	Color*		
		L*	a*	b*
Control	6.34 ± 0.02^b	21.98 ± 0.86^d	30.94 ± 4.14^a	18.57 ± 3.77^a
+ 10% tapioca	6.23 ± 0.01^b	18.17 ± 0.04^e	33.88 ± 1.38^a	4.57 ± 0.59^b
+ 0.2% MTGase	6.34 ± 0.13^b	29.33 ± 1.16^b	18.97 ± 3.42^b	6.06 ± 0.93^b
+ 0.4% MTGase	6.63 ± 0.17^a	29.73 ± 0.53^b	16.57 ± 2.55^b	6.17 ± 0.51^b
+ 0.6% MTGase	6.76 ± 0.12^a	30.21 ± 0.21^a	17.19 ± 0.47^b	6.43 ± 0.19^b
+ 0.8% MTGase	6.78 ± 0.18^a	28.43 ± 0.45^{bc}	20.89 ± 1.04^b	4.19 ± 0.39^b
+ 1.0% MTGase	6.82 ± 0.17^a	27.44 ± 1.32^c	22.7 ± 6.42^b	3.81 ± 1.44^b

* The distinct letters in the same column indicate statistically significant variances (P<0.05).

In product development, color is an important parameter because it is an apparent parameter that influences consumers in purchasing products [33]. Discoloration of sausage products can reduce the level of consumer preference. The incorporation of the oil greatly influences the instrumental color of the sausage. Table 1 also displays the color profile of local lamb sausages, which include the L* (brightness), a* (reddish), and b* (yellowish) scales. The addition of MTGase to the production of local lamb sausages had a significant effect (P<0.05) on all three measured scales. Adding 0.2 – 0.6% MTGase to the production of lamb sausages increased the L* value. The highest L* value was found in the sausage sample with the addition of 0.6% MTGase. The further addition of 0.8-1.0% MTGase decreased the L* value. Greater L* values indicate enhanced light reflection owing to the decreased size of the emulsified oil droplets [34]. The control treatment and adding 10% tapioca showed the highest a* value. Adding MTGase can reduce the * value produced in lamb sausages. A decrease in a* value was also reported in bologna sausage formulated with echium oil [30]. The noticeable reduction in the a* value can be ascribed to the existence and denaturation of globin pigments in the lamb meat sausages during the heating process in the presence of MTGase. In contrast to the current findings, the a* value of fish mince sausage made from Fresh Indian major carp meat, utilizing MTGase, exhibited a substantial increase against the control variable (p < 0.05), potentially owing to the presence of pigments in the minced sausage [35]. The control sausage showed the highest b* value. Adding MTGase and tapioca lowered the b* value of lamb sausage products. The potential cause for this could be the degradation of carotenoid pigment in lamb meat that occurs during the cooking process. Additionally, a minor change in the b* (yellowness) values was observed when lizard fish mince was subjected to the influence of MTGase. [36]. In comparison, other studies did not find a significant change of b* value of sausage products with MTGase addition [37].

B. Effects of MTGase Addition on The Lamb Meat Sausages Tenderness

This study analyzed the tender values of local lamb sausages in terms of firmness and toughness tests (Table 2). The incorporation of MTGase resulted in a substantial enhancement in the firmness and toughness measurements of prapared sausages (P<0.05). However, the addition of 0.6% - 1.0% MTGase had a slightly decreased firmness value. Choi et al. [38] reported comparable findings, showing that chicken sausages treated with 1.0% combined Laminaria japonica and 1.0% MTGase exhibited the highest tenderness and juiciness scores (P < 0.05).

TABLE II
TENDERNESS OF LOCAL LAMB MEAT SAUSAGES WITH MTGASE ADDITION (MEAN ± STANDARD DEVIATION, N = 4)

Treatment	Firmness* (g)	Toughness* ($g.s^{-1}$)
Control	780.5 ± 59.9[b]	6,825.3 ± 203.1[b]
+ 10% tapioca	592.7 ± 68.8[b]	4,405.4 ± 205.5[b]
+ 0.2% MTGase	1,217.7 ± 104.9[a]	10,205.1 ± 565.6[a]
+ 0.4% MTGase	1,466.3 ± 36.5[a]	11,938.3 ± 830.9[a]
+ 0.6% MTGase	1,407.4 ± 128.2[a]	11,263.9 ± 1,056.1[a]
+ 0.8% MTGase	1,321.8 ± 339.9[a]	11,613.3 ± 3,161.8[a]
+ 1.0% MTGase	1,319.5 ± 200.9[a]	12,260.7 ± 1,979.4[a]

*The distinct letters in the same column indicate statistically significant variances (P<0.05).

The increase of firmness and toughness by adding MTGase could be attributed to the linking of meat protein by transglutaminase during the comminution of the raw batters [39]. Canto et al. [40] also found that sodium salt in meat products alongside MTGase significantly influences meat tenderness. According to reports, the application of MTGase treatment resulted in enhanced chewiness, hardness, tenderness, and juiciness of restructured beef and pork meat products [41]. The cross-linking of meat poly-peptide facilitated by MTGase can alter the emulsifying and rheological properties and hydration capability, ultimately leading to increased gel strength, viscosity [42], and improved tenderness attributes.

C. Effect of MTGase Texture Profile Analysis

Examination of the TPA, as presented in Table 3, shows the measurement data of the texture for the emulsion-type lamb meat sausages, including hardness, springiness, cohesiveness, gumminess, and chewiness. The incorporation of MTGase had a significant (P<0.05) effect on cohesiveness and gumminess. However, there is no significant difference for other parameters. The addition of MTGase improves the cohesiveness characteristics of the resulting sausages. Adding 1%, MTGase produced lamb meat sausage products with the highest cohesiveness. As for the gumminess parameter, adding 0.8%, MTGase produced the highest gumminess score of sausage products. These textural properties of lamb meat sausages could be correlated with the gel matrix formation of meat protein during the cooking process that can maintain the components contained therein. So, the treatment in the manufacture of lamb sausages with the addition of MTGase is expected to result in the components of the gel emulsion surviving in the meat protein gel [43]. Protein oxidation can cause changes in gelation, viscosity, solubility, emulsification, texture, juiciness, and an increase in sausage hardness [44].

TABLE III
TEXTURE PROFILE ANALYSIS OF LOCAL LAMB MEAT SAUSAGES WITH MTGASE ADDITION (MEAN ± STANDARD DEVIATION, N = 4)

Treatment	Texture profiles*				
	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)
Control	1,912. 4 ± 112.8	88.9 ± 2.5	0.57 ± 0.01[c]	1,086.9 ± 116.3[ab]	965.6 ± 98.2
+ 10% tapioca	1,717.8 ± 164.6	89.5 ± 1.6	0.65 ± 0.05[b]	1,113.9 ± 84.1[ab]	997.9 ± 85
+ 0.2% MTGase	1,381 ± 710.3	83 ± 7.1	0.66 ± 0.03[ab]	923.4 ± 468.2[b]	787.5 ± 458.7
+ 0.4% MTGase	1,939.3 ± 278.9	85.9 ± 4.2	0.68 ± 0.04[ab]	1,312.6 ± 236.9[ab]	1,132.6 ± 249.4
+ 0.6% MTGase	1,794.3 ± 82.8	82.5 ± 2.26	0.68 ± 0.01[ab]	1,209.3 ± 51.5[ab]	998.6 ± 69.1
+ 0.8% MTGase	2,214.7 ± 259.3	85.9 ± 3.3	0.69 ± 0.01[ab]	1,532.2 ± 187.3[a]	1,312.9 ± 109.4
+ 1.0% MTGase	1,904.2 ± 853.9	87.3 ± 1.3	0.73 ± 0.05[a]	1,353.3 ± 549.7[ab]	1,183.8 ± 488.1

*Mean values (± standard deviation) with distinct letters in the same column indicate statistically significant variances (P<0.05)

D. Microstructure Properties of Lamb Meat Sausages Using SEM

Figure 1 shows sequential SEM results from control sausages, 10% tapioca addition, the addition of 0.2 MTGase; 0.4; 0.6; 0.8, and 1.0%. In Figures 1A (control) and 1B (addition of 10% tapioca), it is noticed that the sponge is much less compared to the other samples with MTGase addition. These findings suggest that the protein matrix in Figures 1A and 1B demonstrates an increased level of organization, characterized by a more interconnected network and condensed structure, with minimal occurrence of a porous structure and a more even distribution. These results imply that both the regular sausage and the sausage supplemented with 10% tapioca exhibit a beneficial interaction with water and/or fat. These characteristics contribute to increased stability of the meat emulsion in both samples. The reason behind this phenomenon can be attributed to the presence of tapioca flour, which is abundant in amylopectin and fiber. These constituents exhibit a robust ability to bind with water and fat, which aids in elucidating the observed outcomes [45]. Figure 1C-1E presents a rather rough structure with many cavities, and the structure looks like a sponge. The formation of this cavity could be attributed to the expansion of various constituents, particularly fat, water, or air [46]. The microstructure of the reduced fat shows a larger cavity.

Figures 1F and 1G (0.8% and 1.0% MTGase addition) illustrate improved microstructure and homogeneous network of lamb meat sausages. The incorporation of MTGase in protein form leads to the formation of intermolecular ε-(γ - glutamyl) lysine cross-links, resulting in the development of an organized gel network characterized by protein aggregation through hydrophobic interactions, disulfide bonds, and/or other interactions during the heating process [36]. Hence, the present investigation demonstrates that incorporating MTGase at specific concentrations during the preparation of lamb meat sausages can lead to a decline in the structural quality of the resultant sausages.

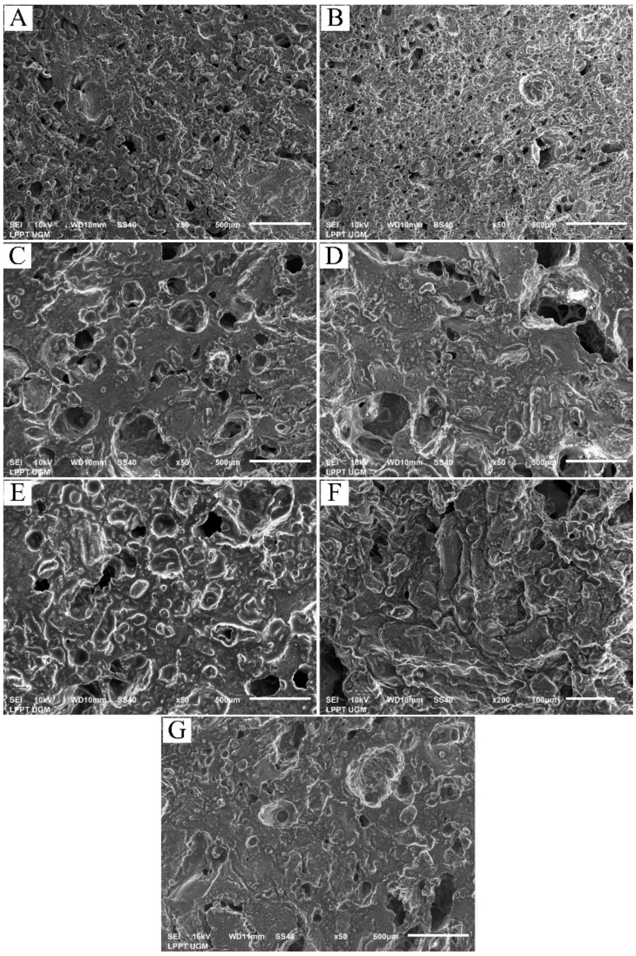

Fig. 1 SEM images of local lamb meat sausages with different concentrations of MTGase addition: (A) Control; (B) 10% tapioca; (C) 0.2% MTGase; (D) 0.4% MTGase; (E) 0.6% MTGase; (F) 0.8% MTGase; (G) 1.0% MTGase

IV. CONCLUSION

Incorporating MTGase in the processing of local lamb meat sausages has enhanced their physicochemical properties, resulting in improved quality characteristics. The experimental findings unequivocally demonstrate the significant impact of MTGase on the overall quality of the sausage products. One considerable advantage of incorporating MTGase is its ability to positively affect the pH value, thus contributing to the extended shelf life of the sausages. Moreover, the presence of MTGase in emulsion-type lamb meat sausages has notably enhanced their textural attributes and tenderness by facilitating cross-linking reactions between primary amines, inducing the formation of high-molecular-weight polymers. Importantly, the color of the lamb meat sausages, following MTGase addition, falls within the range of consumer acceptance, ensuring favorable market reception.

Furthermore, this study has unveiled intriguing observations regarding the microstructure of the sausages. A porous microstructure was observed at certain MTGase concentrations (0.2% - 0.6%), whereas higher concentrations (0.8% - 1.0%) resulted in an ordered and homogeneous network structure. Consequently, MTGase-incorporated lamb meat sausages present an enticing option as value-added products with excellent market acceptability.

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