Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(4): 3033 – 3056 (2025)
www.ejabf.journals.ekb.eg

Aloe Vera Extract as a Gelling Agent in Improving the Texture Quality of Mackerel Sausage

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ARTICLE INFO
Article History:
Received: April 11, 2025
Accepted: June 25, 2025
Online: Aug. 25, 2025

Keywords:
Transglutaminase,
Aloe vera,
Mackerel sausage,
Texture,
Microstructure

ABSTRACT

Processed fish products generally exhibit lower elasticity than red meat-based products due to the softer structure of fish protein fibers. To address this, synthetic gelling agents are often used to enhance texture, though their long-term use raises potential health concerns. Thus, safe and effective natural alternatives are needed. This study evaluated the effectiveness of transglutaminase extracted from *Aloe vera* in improving the texture quality of mackerel sausages. Results showed that 4% *Aloe vera* crude extract transglutaminase increased sausage texture quality by 8% compared to the control. The optimum incubation time for cross-linking reactions was 60 minutes, resulting in a 10% improvement over the control. However, the thermal stability of protein gels treated with *Aloe vera* transglutaminase decreased after 80 minutes of heating at both 80 and 100°C. Microstructural analysis revealed that sausages treated with 4 and 5% *Aloe vera* transglutaminase displayed a more compact protein matrix than the control. These findings suggest that *Aloe vera* transglutaminase is a promising natural alternative to synthetic gelling agents for enhancing the texture quality of fish sausages in the food industry.

INTRODUCTION

Mackerel is one of the key fish species targeted by fishermen in Indo-Pacific waters. It can weigh up to 45kg and reach a length of 3.4m, with seasonal peaks of abundance. As a large pelagic species with high catch volumes, mackerel is an important economic resource due to its year-round availability. The high production level necessitates diversification of processed products to increase added value and to generate income for coastal communities. Common mackerel-based products include sausages, fish cakes,

grilled or fried fish, stews, fillets, frozen fish, and other preparations **(Fiore *et al.*, 2011; Silovs, 2018; Ninan & Ravishankar, 2021; Silva *et al.*, 2020)**.

Nutritionally, mackerel is rich in niacin and omega-3 fatty acids, making it valuable in maintaining dietary health **(Venugopal, 2005; Pagarkar *et al.*, 2012; Strobel *et al.*, 2012; Majewski & Lebiedzińska, 2014; Morrissey & DeWitt, 2014)**. Processed mackerel products thus provide both food security and high nutritional value. Techniques such as rapid freezing, innovative packaging, and cooling systems can help improve quality and consumer acceptance **(Venugopal, 2005; Morrissey & DeWitt, 2014)**. Additional processing methods, including salting, partial cooking, and the use of natural antioxidants, are also applied to enhance sensory and nutritional properties **(Fiore *et al.*, 2011; Barros *et al.*, 2023)**.

The demand for high-quality, safe, and convenient fish products is increasing. Value-added processing enhances product appeal while aligning with consumer preferences for ready-to-cook foods. Sustainable and eco-friendly processing practices can further increase marketability and consumer confidence **(Morrissey & DeWitt, 2014; Sone *et al.*, 2019)**. Modern consumers favor fish products that combine nutrition, convenience, and sensory quality, which can be achieved through innovative processing and packaging **(Pagarkar *et al.*, 2012; Silva *et al.*, 2020)**.

One key challenge in fish processing is texture. Compared with red meat, fish proteins—mainly myosin and actomyosin—differ biochemically and physiochemically, resulting in a softer structure that requires specialized processing methods **(Ramírez *et al.*, 2011)**. Fish also contain lower fat levels, and their smoother, less dense muscle fibers are more susceptible to structural changes during processing **(Nilsen *et al.*, 2009; Ramírez *et al.*, 2011)**. This often affects elasticity, an important sensory parameter influencing consumer acceptance.

Various processing techniques, such as extrusion and the use of hydrocolloids, are applied to improve fish product texture. Hydrocolloids modify protein gelation, enhance structural integrity, and reduce cooking loss **(Ramírez *et al.*, 2011; Gao *et al.*, 2015; Lankatillake *et al.*, 2023; Zhong *et al.*, 2023)**. However, improper handling can lead to texture degradation, making process optimization essential **(Backi, 2018)**.

Mackerel sausage, a high-protein, low-fat product rich in omega-3 fatty acids, is one example of a value-added product. With a digestibility rate of over 85%, it is considered suitable for health-conscious consumers **(Cardoso *et al.*, 2008; Kim *et al.*, 2014; Pourashouri *et al.*, 2020)**. Production involves grinding, mixing with binders and spices, packaging, and cooking **(Zhong *et al.*, 2023)**. To achieve optimal gel strength and texture, additives are often required **(Kim *et al.*, 2013; Yi *et al.*, 2020)**. While such additives improve elasticity and chewiness, they may negatively affect consumer acceptance due to undesirable changes in hardness, color, or aroma.

Elasticity remains a critical sensory attribute for consumer acceptance of processed fish products **(Kim *et al.*, 2013; Huang *et al.*, 2021)**. Methods to improve elasticity include

adding non-starch hydrocolloids or modifying gelation to produce softer textures for specific consumer groups, such as the elderly **(Kasapis, 2009; Fei *et al.*, 2024)**. Chemical additives such as phosphates are commonly used, but natural alternatives are increasingly sought. Hydrocolloids and gelling agents derived from natural sources (e.g., guar gum, carrageenan, seaweed extracts, microbial polysaccharides, *Aloe vera*) have been explored for improving meat and fish products **(Imeson, 2009; Gao *et al.*, 2015; Habib *et al.*, 2015; Tahmouzi *et al.*, 2023; Yuan *et al.*, 2025)**.

Aloe vera has demonstrated potential as a gelling and stabilizing agent in meat systems. It improves texture, reduces lipid oxidation, suppresses microbial growth in mutton nuggets **(Rajkumar *et al.*, 2015)**, and functions as a fat substitute in emulsions, creating stable matrices **(Kumar *et al.*, 2016; Angioletti *et al.*, 2024)**. In addition, *Aloe vera* increases water-holding capacity, a key factor in sausage freshness and texture **(Zhao *et al.*, 2014; Hasan *et al.*, 2021)**. Sensory analysis has shown that *Aloe vera* gel can positively influence taste, texture, and overall consumer acceptance **(Rajkumar *et al.*, 2015)**.

Unlike previous studies that primarily used *Aloe vera* gel, the present study explores the potential of transglutaminase extracted from *Aloe vera*. Transglutaminase is an enzyme capable of cross-linking proteins to form stronger gel structures **(Sidauruk *et al.*, 2017)**. Prior research has shown that commercial transglutaminase enhances gel strength in fish ball products **(Nugroho *et al.*, 2019)**, and recent work confirmed transglutaminase activity in *Aloe vera* extracts **(Indarto *et al.*, 2025)**. Based on these findings, *Aloe vera* transglutaminase shows strong potential as a natural alternative for improving the texture of protein-based foods such as mackerel sausages. Its use could replace synthetic gelling agents, reduce production costs if locally sourced, and provide a competitive advantage by meeting consumer demand for natural, health-conscious, and sustainable products.

Accordingly, this study aimed to evaluate the application of transglutaminase extracted from *Aloe vera* as a natural substitute for chemical gelling agents to improve the texture and overall quality of mackerel sausages.

MATERIALS AND METHODS

1. Material

The materials used in this study were mackerel, beef fat, *Aloe vera*, commercial transglutaminase, sausage casings, sucrose, NaCl, 0.2 M phosphate buffer, 0.2 M acetic acid, Whatman paper, 50% maltodextrin. The equipment used is a TA.XT. Plus texture analyzer (Stable Micro Systems, United Kingdom), *Scanning Electron Microscope* (SEM) (FEI Inspect S50, United States), knife, large pot, food processor, tablespoon, casing, teaspoon, scales, blender, gas stove, cutting board, woolen thread, sieve, scissors, gloves, analytical scales, 3 kg scales, and measuring cups.

2. Research design

The research design used was a Completely Randomized Design with *Aloe vera* Transglutaminase concentrations of 1, 2, 3, 4, 5, and a control. This study was conducted with three replicates. The research experimental design is presented in Table (1).

Table 1. Design of research treatment

Treatment	*Aloe vera* concentration
1	Control (0%)
2	1%
3	2%
4	3%
5	4%
6	5%
7	Commercial Transglutaminase (2%, WBS500)

3. Making mackerel sausage

The sausage-making process uses mackerel as the primary raw material (65%), beef fat (30%), NaCl (3%), and sucrose (2%). The mackerel is separated into meat and bones using a food processor. *Aloe vera* transglutaminase was added to the raw sausage dough at 1, 2, 3, 4, 5, and a control. It was then incubated at the optimal temperature of *Aloe vera* transglutaminase (50°C) for 30 minutes. The mixture was then placed in a sausage casing to be stored in a refrigerator for 24 hours and was then steamed for 30 minutes. The resulting mackerel sausage was stored in a refrigerator at 4°C **(Hidayah & Sofyaningsih, 2022; Usman *et al.*, 2024)**.

4. Extraction of aloe vera transglutaminase

Aloe vera leaf transglutaminase extract was extracted using a water solvent containing NaCl salt, 0.2 M phosphate buffer, and 0.2 M acetic acid. Extraction was carried out with a weight ratio of material to solvent of 1:3. It was then ground and filtered with Whatman paper. The resulting filtrate was centrifuged at 10.000 x g for 20 minutes at 4°C. The supernatant obtained was purified by the precipitation method at 40% saturation, and maltodextrin was added at 50% of the precipitate weight **(Juniantito & Prasetyo, 2006)**.

5. Observation parameters

5.1 Organoleptic testing

Hedonic quality testing was performed using an organoleptic test on 20 semi-trained panelists. Panelists were asked to rate mackerel sausage's texture, color, and flavor. The rating was based on the panelists' level of liking, which consisted of strongly dislike (1), dislike (2), like (3), very like (4), and strongly like (5).

5.2 Determination of the best concentration of mackerel sausage

The determination of the effect of concentration was carried out using ANOVA analysis. The test to determine the best treatment uses the De Garmo technique. De Garmo is a method for determining the best product based on mathematical equations **(DeGarmo *et al.*, 1984)**. The determining parameter for the best concentration is based on the level of fish sausage elasticity and organoleptic tests. The treatment with the highest yield value declares the treatment with the best formulation **(DeGarmo *et al.*, 1984)**.

5.3 Analysis of the rate of the Aloe vera transglutaminase catalyst in mackerel sausages

The transglutaminase catalyst rate analysis was carried out to measure the optimal reaction time of *Aloe vera* transglutaminase on fish sausage protein. The catalyst rate of transglutaminase was measured using TA.XT. Plus texture analyzer (Stable Micro Systems, United Kingdom) in which hardness parameter is used as an indicator of protein cross-linking by transglutaminase. The measurement was proposed based on research by **Ahhmed *et al.* (2007)**, **Kawahara *et al.* (2007)** and **Sakamut *et al.* (2025)** that stated adding transglutaminase enhanced cross-linking of myofibrillar proteins and hardness. Transglutaminase reacted with mackerel fish sausage dough for different durations, namely 30, 45, 60, and 75 minutes, and the dough was incubated at the optimum temperature of *Aloe vera* transglutaminase.

5.4 Thermal stability of mackerel sausage texture

This research stage aimed to measure the stability of gel protein in mackerel sausages and the effect of *Aloe vera* transglutaminase application. The stability of gel protein was evaluated based on the hardness parameter **(Rigdon *et al.*, 2020)** using a 35-mm cylinder probe TA.XT. Plus, texture analyzer (Stable Micro Systems, United Kingdom). The texture of fish sausages before steaming is expressed as 100%. Steaming was carried out at temperatures of 80 and 100°C for 0, 20, 40, 60, 80, and 100 minutes.

5.5 Analysis and visualization of the cross-linking profile of proteins in gel formation

The analysis of the formation of cross-linked protein catalyzed by *Aloe vera* transglutaminase in the gel formation process of fish sausage products was studied by

performing visualization to observe the microstructure with the help of a scanning electron microscope (SEM) (FEI Inspect S50, United States). The procedure of sample preparation to observation followed the guidelines of **An *et al.* (2021)**. Microstructure analysis of the best treatment using SEM was used to analyze the compactness of the structure in mackerel fish sausage products that have undergone protein cross-linking modification using *Aloe vera* transglutaminase.

RESULTS AND DISCUSSION

1. The effect of transglutaminase on gel strength and analysis of the interaction of transglutaminase with product ingredients

Aloe vera is a plant that contains natural transglutaminase, potentially improving the texture of protein-based foods. One of the texture parameters in food ingredients is the hardness value. Table (2) shows the results of measuring the hardness value in fish sausages treated with the addition of transglutaminase *Aloe vera*.

Table 2. Effect of *Aloe vera* transglutaminase and commercial transglutaminase comparator on the hardness of mackerel sausages

Treatment	Hardness Fish Sausages (%)
Control	100.00 [a]
1% *Aloe vera* extract (transglutaminase)	101.76 [a]
2% *Aloe vera* extract (transglutaminase)	101.49 [a]
3% *Aloe vera* extract (transglutaminase)	104.38 [b]
4% *Aloe vera* extract (transglutaminase)	107.64 [c]
5% *Aloe vera* extract (transglutaminase)	108.77 [cd]
Commercial transglutaminase (2%, WBS500)	110.54 [d]

Note: *The averages in the same column and accompanied by different letters indicate a difference in Aloe vera transglutaminase's effect at P≤0.05.*

Table (2) shows that the hardness value of mackerel sausage ranges from 100-110.54%. The lowest hardness value was obtained in the control treatment and the highest in the commercial transglutaminase treatment. The treatment of adding *Aloe vera* transglutaminase concentration has a significant effect on the hardness value of mackerel sausage ($P< 0.05$). Table (2) shows that increasing the concentration of *Aloe vera* transglutaminase can increase the hardness value of mackerel sausage compared to the control, but it is still lower than commercial transglutaminase. Fish sausage products with extract concentrations of 3, 4, and 5% produced hardness that did not differ significantly ($P\leq0.05$) (104.38, 107.64, and 108.77%, respectively). Commercial transglutaminase produces the highest increase in hardness of up to 110.54%, compared to the control, where

the control hardness is expressed as 100%. The increase in hardness in sausages with the addition of *Aloe vera* transglutaminase is relatively small, possibly due to the looser protein matrix in the sausage or the inhibitory effect of the fat content in the product **(Ahhmed *et al.*, 2007; Kawahara *et al.*, 2007; Lim & Chin, 2018b)**. The higher fat content in sausages can inhibit the formation of protein cross-links, thus limiting the effect of transglutaminase **(Lim & Chin, 2018a; Prayitno *et al.*, 2024)**.

2. Organoleptic analysis

Organoleptic analysis can be used to analyze a product's physical quality characteristics. The organoleptic analysis of mackerel sausages was conducted using 20 panelists to score the taste, aroma, and texture parameters. The organoleptic analysis uses a scale of 1-5. Table (3) shows the results of measuring the organoleptic value of mackerel sausages with the addition of *Aloe vera* transglutaminase.

Table 3. Effect of *Aloe vera* transglutaminase and commercial transglutaminase comparator on the hardness of mackerel sausages

Treatment	Taste	Aroma	Texture
Control	4.3	4.0	3.5
Transglutaminase 1%	4.2	3.8	3.5
Transglutaminase 2%	3.9	4.1	3.7
Transglutaminase 3%	4.1	4.0	3.7
Transglutaminase 4%	4.4	4.0	4.3
Transglutaminase 5%	4.2	4.1	4.0
Commercial transglutaminase (2%, WBS500)	4.2	3.5	4.3

Table (3) shows that the organoleptic value of mackerel sausage with the addition of transglutaminase *Aloe vera* does not cause many changes in organoleptic parameters. The taste value obtained in mackerel sausage ranges from 3.9 to 4.4. The lowest flavor value was obtained in mackerel sausages with the addition of 2% *Aloe vera* transglutaminase. In comparison, the highest flavor value was obtained in mackerel sausages with the addition of 4% transglutaminase. This value is still higher than the control without adding transglutaminase and the commercial transglutaminase control. The treatment of adding *Aloe vera* transglutaminase had no significant effect on the taste value of mackerel sausages ($P > 0.05$). The transglutaminase enzyme plays an important role in the process of protein binding and amino acid formation, thus affecting the resulting flavor. Previous research results show that fish sausages with transglutaminase have a higher level of acceptance and a better flavor profile than sausages without the enzyme **(Valencia *et al.*, 2015; Chareonthaikij *et al.*, 2018; Prayitno *et al.*, 2024)**. For example, sausages with 0.3 and

0.6% transglutaminase show higher average scores for texture and flavor, resulting in a high acceptance rate (79.86 to 84.03%) **(Prayitno *et al.*, 2024)**.

Aroma is the complex combination of volatile compounds that contribute to the smell of food products, including sausages **(Hemmerling & Spiller, 2016)**. Aroma is important in the overall sensory experience and acceptance of sausage products. Table (3) shows that the aroma value of mackerel sausage ranges from 3.8 to 4.1. The lowest aroma value is found in the treatment with 1% transglutaminase addition, and the highest is aligned with the addition of 2 and 5% transglutaminase. The aroma value of mackerel sausage with the addition of *Aloe vera* transglutaminase is generally higher than that of commercial transglutaminase but not much different from sausage without the addition of transglutaminase. Adding transglutaminase has no significant effect on the aroma value of mackerel sausage ($P > 0.05$).

Transglutaminase significantly improves the texture of fish products, which can indirectly affect aroma perception. Improved texture can result in better overall sensory acceptance, including aroma. The enzyme's ability to increase water-holding capacity and reduce cooking loss can help retain volatile compounds that contribute to aroma **(Vácha *et al.*, 2006; Valencia *et al.*, 2015; Hemmerling & Spiller, 2016; Cavenaghi-Altemio *et al.*, 2018; Chareonthaikij *et al.*, 2018)**. The texture value of fish sausage products refers to a combination of sensory and instrumental attributes that describe the physical properties of the sausage, which significantly affect consumer perception and satisfaction **(Rahman *et al.*, 2007)**. Table (3) shows that the texture value of mackerel sausage with the addition of *Aloe vera* transglutaminase with ranges fluctuating from 3.5 to 4.3. The lowest texture value is found in the 1% *Aloe vera* transglutaminase treatment, and the highest is obtained with the addition of 4% *Aloe vera* transglutaminase. The higher the addition of transglutaminase concentration, the higher the texture value, but it decreases at a concentration of 5%. In general, the texture value in *Aloe vera* transglutaminase treatment is almost the same as that using the commercial transglutaminase, and they are better than the control treatment without transglutaminase. The treatment of adding *Aloe vera* transglutaminase has a significant effect on the texture value of mackerel sausages ($P < 0.05$).

Transglutaminase increases the hardness and compactness of fish sausages. Research on the application of transglutaminase shows that it can increase the hardness and compactness of products, making them firmer and more cohesive **(Vácha *et al.*, 2006; Rahman *et al.*, 2007; Alves *et al.*, 2021)**. This enzyme also increases the elasticity and strength of the gel, which is very important for the desired texture of the sausage. It is achieved by cross-linking proteins, which strengthens the gel matrix **(Vácha *et al.*, 2006; Rahman *et al.*, 2007)**.

3. The best treatment

The best treatment is determined based on hardness, taste, aroma, and texture parameters using De Garmo's Effectiveness Index method. The parameters are weighted based on the assessment of five experts. Table (4) shows the results of calculating De Garmo's Effectiveness Index on mackerel sausages by adding transglutaminase *Aloe vera.*

Table (4) illustrates that in determining the Effectiveness Index using De Garmo's method, the highest productivity obtained was 0.88, which is the 4% *Aloe vera* transglutaminase treatment, so the 4% *Aloe vera* transglutaminase treatment was chosen as the best treatment, based on the parameters of hardness, taste, aroma, and texture. The results of the organoleptic analysis in Table (4) also show that the highest average value is obtained from the treatment of adding 4% transglutaminase compared to the treatment without transglutaminase (control) and commercial transglutaminase.

Table 4. Calculation of the Effectiveness Index of mackerel sausage quality parameters influenced by *Aloe vera* transglutaminase treatment

Treatment	Index	Hardness	Taste	Aroma	Texture	Total
Weight	-	0.38	0.25	0.13	0.25	1.0
Control	NE	0	0.8	0.8	0	
	NP	0	0.2	0.11	0	0.31
Trans.	NE	0.17	0.6	0.5	0	
1%	NP	0.1	0.15	0.007	0	0.28
Trans.	NE	0.14	0	1	0.25	
2%	NP	0.05	0	0.13	0.06	0.25
Trans.	NE	0.42	0.4	0.83	0.25	
3%	NP	0.16	0.1	0.11	0.06	0.43
Trans.	NE	0.72	1	0.83	1	
4%	NP	0.28	0.25	0.11	0.25	**0.88**
Trans.	NE	0.83	0.6	1	0.63	
5%	NP	0.316	0.15	0.13	0.16	0.75
Comerc.	NE	1	0.6	0	1	
Trans.	NP	0.38	0.15	0	0.25	0.78

Note: NE = Effectiveness value and NP = Productivity value.

Adding transglutaminase to mackerel sausages can significantly improve their sensory value, effectiveness, and productivity. Several previous studies have reported that transglutaminase improves texture, taste, and color while increasing water holding capacity and reducing cooking loss, resulting in higher yields and better consumer acceptance **(Ando *et al.*, 1989; Motoki & Seguro, 1998; Tseng *et al.*, 2000; Kuraishi *et al.*, 2001; Yokoyama *et al.*, 2004)**.

4. Analysis of the speed of the transglutaminase catalyst in mackerel sausages

Table (5) shows the reaction time of *Aloe vera* transglutaminase in forming cross-links of protein in the mackerel sausage dough. This study evaluated the effect of the *Aloe vera* transglutaminase incubation time on the gel strength (hardness) of fish-based sausages. The results showed a significant increase in hardness in fish sausage products.

Table 5. The reaction speed of *Aloe vera* transglutaminase extract in forming protein cross-links in sausages

Reaction time (minutes)	Fish sausage hardness (%)
0	100.00[a]
30	104.81[b]
45	105.55[b]
60	110.04[c]
75	109.96[c]

Note: The averages in the same column and accompanied by different letters indicate a difference in the effect of Aloe vera transglutaminase at $P \leq 0.05$.

Table (5) shows that the hardness value of mackerel sausage ranges from 100-110.04%. The lowest hardness value was obtained from the incubation process of 0 minutes, and the highest value was found in the 60-minute incubation treatment. Based on the data in Table (5), it can be concluded that the longer the incubation time, the higher the hardness value. It is caused by the enzymatic reaction between transglutaminase and protein, which forms coagulation in the protein and, at the same time, affects the hardness of the product. It is due to the ability of the enzyme to catalyze the formation of covalent bonds between proteins, resulting in a denser and stronger structure **(Kuraishi *et al.*, 2001; Herrero *et al.*, 2008)**. The length of treatment with transglutaminase enzyme incubation significantly affects the hardness value of mackerel sausage ($P < 0.05$).

Adding transglutaminase to the fish sausage formulation significantly increases the product's hardness. This effect is caused by the enzyme's ability to catalyze protein cross-linking, resulting in a denser and stronger structure. Previous research using commercial transglutaminase with a 0.4% to 1% usage rate has effectively increased hardness without compromising other quality attributes. Combining transglutaminase with other ingredients, such as carrageenan or potato starch, can further improve the texture of fish sausages **(Ando *et al.*, 1989; Motoki & Seguro, 1998; Tseng *et al.*, 2000; Kuraishi *et al.*, 2001; Yokoyama *et al.*, 2004; Herrero *et al.*, 2008)**. An interesting thing is the plateau effect of the long-incubation influence of transglutaminase, especially in mackerel sausage products, where an increase in the incubation time of up to 60 minutes will reach saturation so that it does not increase the hardness of the sausage anymore with the addition of incubation time.

5. Thermal stability of mackerel sausage texture

Thermal stability refers to the ability of food products to retain their texture when exposed to heat. In the context of fish sausages, this involves how well the sausages retain their firmness, elasticity, and overall mouthfeel during cooking and storage. A stable texture is essential for consumer acceptance and product quality. The texture of the fish sausage before steaming is expressed 100%. Steaming is performed at 80 and 100°C for 0, 20, 40, 60, 80 and 100 minutes.

Table 6. Thermal stability of mackerel sausages: the effect of temperature and heat exposure on the decrease in stability is expressed in %, and stability at time 0 is expressed as 100% stability

Steaming Time (minutes)	Steaming Temperature 80ºC	Steaming Temperature 100ºC
0	100	100
20	98	97
40	96	92
60	90	90
80	87	80
100	80	68

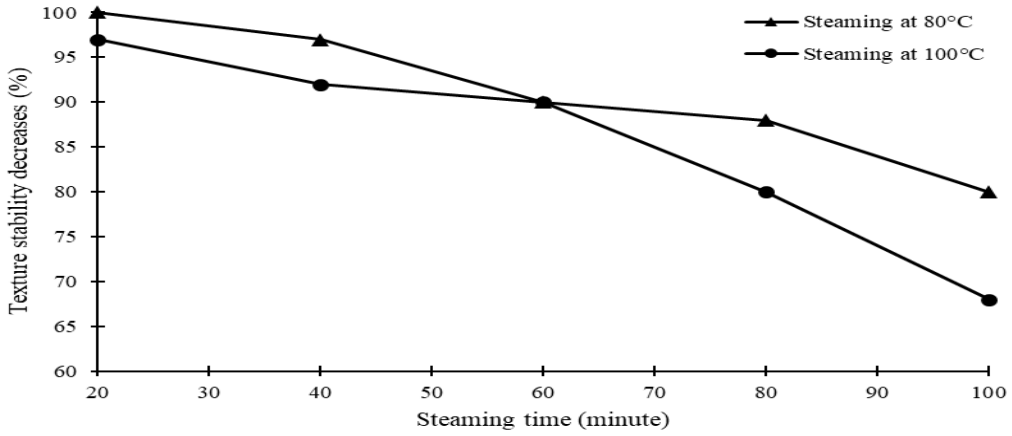


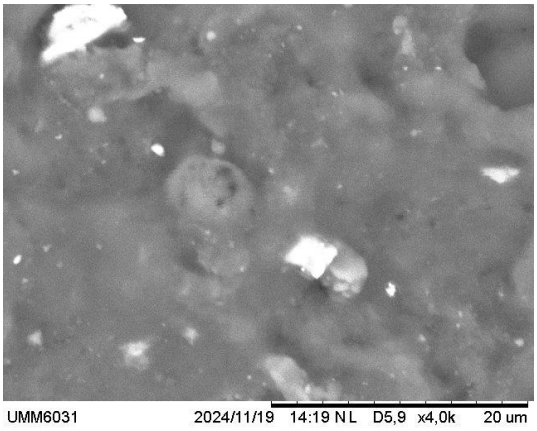
Fig. 1. The addition of transglutaminase *Aloe vera* at different steaming temperatures and durations influencing the stability of mackerel sausages

Table (6) and Fig. (1) show that the textural stability value for fish sausages ranges from 80-100% for sausages steamed at 80°C and 68-97% for sausages steamed at 100°C. Fig. (1) explains that the longer the steaming time, both at 80 and 100°C, the lower the texture stability value of the mackerel sausage. The texture stability value of mackerel at a high temperature (100°C) results in lower stability compared to a lower temperature (80°C). It is because the protein gel formed because the protein's cross-links are damaged by high-temperature treatment, disrupting the strength of the mackerel sausage gel. The time and temperature of steaming play an important role in determining the texture stability

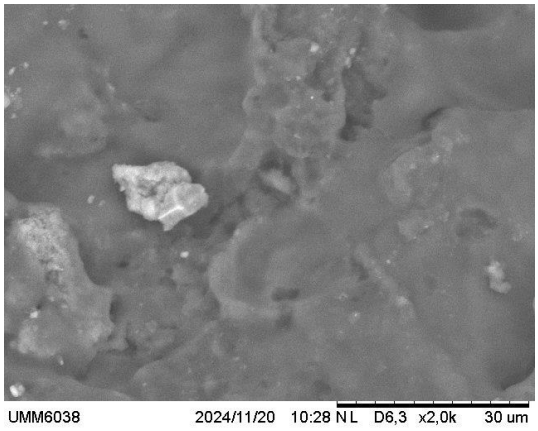
of fish sausages. Lower temperatures and shorter steaming times are generally more effective in maintaining the desired texture properties, minimizing cooking losses, and maintaining water-holding capacity **(Szczesniak, 2002; Vaudagna *et al.*, 2002; Kong *et al.*, 2007; Pietrasik *et al.*, 2007; Baldwin, 2012)**.

6. Scanning electron microscope (SEM) testing meat and fish meatballs and sausages

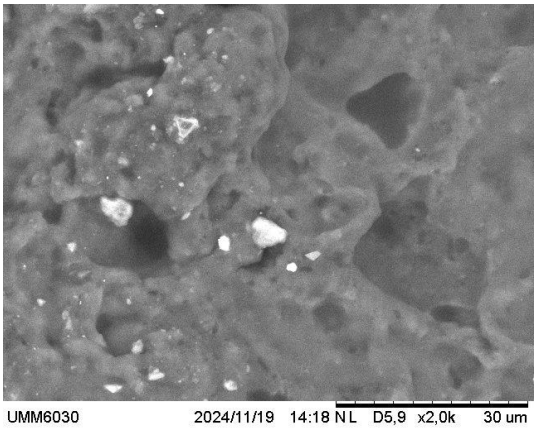
Research on the effect of transglutaminase from *Aloe vera* on protein-based products, namely mackerel sausages, is critical to improve their quality. Transglutaminase is an enzyme that can form cross-links between protein chains, improving the product's texture, stability, and binding power. The effectiveness of *Aloe vera*-based transglutaminase as an innovative additive to enhance the texture of fish sausages was tested using SEM to visualize the effect of protein cross-linking in fish sausages catalyzed by transglutaminase from Aloe vera.



A (control)



B (4% *Aloe vera* transglutaminase)



C (5% *Aloe vera* transglutaminase)

Fig. 2. Results of SEM analysis on fish sausage products with *Aloe vera* transglutaminase treatment

Fig. (2) shows the microstructure analysis results of mackerel sausage using a scanning electron microscope (SEM). Applying *Aloe vera* transglutaminase to sausage

products significantly changes the microstructure of the protein. Transglutaminase is an enzyme that can form cross-links between amino groups in proteins, resulting in a tighter and more stable protein network. In this study, treatment with 5% *Aloe vera* transglutaminase (C) produced the most compact microstructure compared to the control treatment (A) and 4% *Aloe vera* transglutaminase treatment (B). This compactness shows that an increase in enzyme concentration is directly proportional to the effectiveness of protein cross-linking. A tighter protein network increases the stability and durability of the product's texture. It is because transglutaminase effectively improves the texture of protein-based foods by forming a dense and stable protein matrix. Research shows that transglutaminase can strengthen the binding between protein molecules, which is very important in the development of sausage products that have a good texture **(Ahlawat & Khatkar, 2011; Zanuzzo *et al.*, 2012; Sadoyu *et al.*, 2021)**.

In this study, treatment with 5% *Aloe vera* Transglutaminase (C) concentration showed the most optimal results in creating a more compact mackerel sausage microstructure compared to the control treatment (A) and treatment with 4% *Aloe vera* transglutaminase (B) concentration. This concept is in line with the concept that increasing the enzyme concentration positively affects the formation of protein cross-links, increasing the compactness and stability of the final product.

Transglutaminase connects one protein's amine group to another protein's carboxyl group, creating a tighter protein network. This compactness results in a better texture and contributes to the product's durability, making it more stable against damage due to heat during cooking **(Radha & Laxmipriya, 2015; Mesbah *et al.*, 2016)**. The presence of transglutaminase activity in *Aloe vera*, which is capable of catalyzing protein bonds, has also been reported in previous researchers who postulated that *Aloe vera* has various bioactive compounds that can support cell regeneration and structure **(Ahlawat & Khatkar, 2011; Sadoyu *et al.*, 2021)**. From the results obtained, it can be concluded that the use of higher concentrations of transglutaminase in combination with *Aloe vera* extract can produce superior mackerel sausage products in terms of texture and gel stability, the effect of increasing the number of bonds between proteins.

CONCLUSION

Based on the study's results, transglutaminase extracted from *Aloe vera* improved mackerel's texture effectively. The crude extract of *Aloe vera* transglutaminase 4% improves the texture quality of fish sausages by 8% compared to the control. The optimum incubation time for the cross-linking reaction of protein in fish sausage products by *Aloe vera* transglutaminase is 60 minutes, with a 10% improvement in texture quality compared to the control. The thermal stability of the mackerel sausage protein gel with *Aloe vera* transglutaminase treatment decreased at a heating time of 80 minutes, both at 80 and 100°C. Microstructural analysis of mackerel sausages with 4 and 5% *Aloe vera* transglutaminase treatment showed a more compact structural matrix than the control.

ACKNOWLEDGMENT

The highest appreciation is expressed to the Directorate of Research, Technology and Community Service, Directorate General of Higher Education, Research and Technology, Ministry of Education Republic of Indonesia (BIMA), which fully funded this research management and publication, with contract Number 015/UN.46.4.1/PT.01.03/2024.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this article.

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