Effects of Transglutaminase on the Gel Properties of Indonesian Catfish Surimi Using Response Surface Methodology

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Received: 20 January 2025
Accepted: 10 August 2025
Published: 30 August 2025
Academic Editor: Lukita Purnamayati, S.TP, M.Sc

 Accreditation Number:148/M/KPT/2020. ISSN: 2089-5690, e-ISSN: 2406-9272. https://doi.org/ 10.15578/squalen.1027

Abstract

Surimi is a myofibrillar fish protein, extracted mainly from marine fish, which is commonly used to produce fish balls, crab imitation products, and various other seafood substitutes. However, finding an alternative fish from freshwater, such as freshwater catfish, is important, although its drawbacks include low gel strength and water-holding capacity. To address this problem, transglutaminase addition can maintain the surimi quality. This study aimed to improve catfish surimi characteristics by optimizing surimi production with various transglutaminase concentrations and incubation times. It used Response Surface Methodology (RSM) with Central Composite Design (CCD) to evaluate the effect of Enzyme Concentration and Incubation Time. The result showed that the catfish surimi gel strength, chewiness, whiteness, and water-holding capacity were respectively 927.513 g.cm, 3,747.18 g, 79.95 %, and 91.37 %. It was obtained by the optimum condition of surimi production with the addition of 0.85 % w/w transglutaminase and an incubation time of 36 minutes. The overall study provides insight for surimi producers to maintain surimi characteristics from freshwater fish, such as catfish.

Keywords: fish protein, crosslinking, physicochemical, optimization

Introduction

The Surimi industry holds significant potential for development and expansion in Indonesia. With an estimated 23 Surimi factories running as of 2020 (Sihono *et al*., 2021), Indonesian surimi manufacturers are mostly found in northern Java. Indramayu, Tegal, Pekalongan, Kendal, Rembang, Tuban, Lamongan, Sidoarjo, Pasuruan, and Probolinggo house these factories. The main obstacle in the Indonesian surimi sector is the declining raw material supply, which results from overfishing and the application of government control regulation No. PERMAN-KP/2015. This control forbids trawl fishing, which also significantly reduces the availability of raw materials (Sihono *et al*., 2021). This shortage calls for the development of new technologies or the search for substitute raw materials for surimi manufacture, such as freshwater fish (Phetsang *et al*., 2021).

Many freshwater fish species have been successfully grown with significant production levels. Particularly competitively priced and the highest production rate among farmed fish is catfish. Technical benefits of catfish include ease of processing, absence of scales, and the possibility of being transported alive. It also generates a rather high amount of surimi in comparison to other farmed fish. According to the Ministry of Marine Affairs and Fisheries, compared to wild-caught fish, farmed fish offer better freshness, simpler handling, and freedom from seasonal fluctuations as the raw material for surimi. On the other hand, the low gel strength of farmed fish makes surimi manufacture difficult since it is less than that of white-fleshed marine fish.

Three elements define surimi quality: water-holding capacity, whiteness, and gel strength. Catfish's low gel-forming capacity (Phetsang *et al*., 2021) makes the traditional three-stage washing process

inappropriate for them. Changes to the manufacturing process are therefore needed to generate surimi quality. Studies by Sihono (Sihono *et al*., 2021) show that transglutaminase improves surimi's texture, water retention, and protein binding characteristics, raising its general quality. Surimi-based products and minced meat's elasticity and gel formation are produced in part by transglutaminase, a transferase enzyme. It helps food proteins' polymerization and cross-linking of ã-glutamyl-lysine bonds between lysine residues and glutamine. Transglutaminase can be generated on a lab scale using submerged fermentation using bacteria, including *Streptomyces mobaraensis*. Between 5 and 8 is the ideal pH for the enzyme; its ideal temperature for enzymatic activity is 50 to 55 °C (Nugroho *et al*., 2019). Optimizing the concentration of transglutaminase, the quantity of time, and the temperature will help to improve the mechanical qualities of surimi. Transglutaminase enhances the strength of surimi gels by inducing the glutamyl-lysin (GL) covalent bond in fish protein (Huang *et al*., 2023), that improves their capacity to retain water (Water-holding Capacity), and their binding power of proteins (Sihono *et al*., 2021) without changing the taste of surimi (Miwa, 2020). Compared to animal-origin transglutaminase, microbial transglutaminase (MTGase) has a much lower price in comparison to its activity from animal origins (Kieliszek & Misiewicz, 2014). Moreover, MTGase keeps the pH from increasing during storage, preserving the quality of the product (Tokay *et al*., 2021). This inhibition of pH is crucial in extending the shelf life of such foods by preventing spoilage either through microbial activity or protein degradation. This kind of stabilization effect is advantageous for surimi producers because it manages to retain the desired texture and flavor of the product during extended storage.

The Indonesian surimi industry presents significant opportunities for further growth and development, providing innovative solutions and alternative raw materials. Although transglutaminase has been applied to species such as silver carp (Yang *et al*., 2024), threadfin bream (Fang *et al*., 2021), and milkfish (Yuliana *et al*., 2021a), its use in Indonesian catfish remains relatively unexplored. In this study, catfish is introduced as a sustainable environmental alternative source of raw materials for Indonesia's surimi production, discussing optimization of microbial transglutaminase concentration and incubation time as means of improving its gel characteristics. The use of catfish as an alternative to marine fish and the application of transglutaminase will become a promising approach for improving the status of the surimi industry. The addition of 0.3 % transglutaminase was effective in enhancing the gelation properties of silver carp surimi (Liu *et al*., 2025). The study of (Fang *et al*., 2021) showed that 0.5 % of transglutaminase, combined with emulsified lard, resulted in higher hardness and chewiness of threadfin beam surimi. The milkfish surimi gel strength and whiteness have been measured by comparing various concentrations of transglutaminase (0.1, 0.3, and 0.5 %), and 0.5 % transglutaminase was the most effective in increasing gel strength and whiteness. In the study of (Yang e*t al*., 2020), the addition of transglutaminase at different temperatures and incubation times showed that the highest gel strength resulted from 35 °C and 2 hours of incubation. Therefore, it needs optimization and characterization of surimi gel at different transglutaminase concentrations and incubation times, which could be an excellent approach to further improvement in the qualities of surimi made from Indonesian catfish. This study aimed to optimize surimi production using different levels of transglutaminase and incubation times. Thus, this study represents an applied approach that might be of interest to the food industry, especially to those regions where catfish is abundant and inexpensive, offering a very good alternative in surimi production against both sustainability and quality.

Materials and Methods

Materials

Freshwater catfish (*Clarias batrachus*) were purchased from local fish farming with an average of total length of 40 - 50 cm and an average weight of 700 - 800 g. Grade AA surimi was purchased from Blue Sea Industry. The transglutaminase used in this study was purchased from Agung Mulia Chemindo. Sucrose and Sodium Chloride were analytical-grade chemicals from Merck.

Preparation of Surimi Gels

The production of surimi gels begins with separating the flesh from the bones. The fish flesh is then minced, washed, and filtered using cold water at a temperature of 10°C, with a ratio of fish flesh to washing water is 1:3 (w/v). Afterward, the flesh is filtered and pressed. The fish flesh is mixed with 4% sucrose, an important cryoprotectant, then packed and stored at a temperature of -18°C. The frozen surimi was thawed to a temperature of 5°C, then 2.5% Sodium Chloride was added. Sodium chloride is a common additive that contributes to the gelation of surimi (Cao *et al*., 2022). Subsequently, transglutaminase was added in varying concentrations based on the experimental design. The mixture was then homogenized at 5°C for 5 minutes until a surimi paste was formed. The surimi samples

were then heated in a water bath at the optimum temperature for Transglutaminase activity (50°C) at different incubation times based on the experimental design. Enzyme inactivation was performed by heating the samples in a water bath at 90°C for 20 minutes. Finally, the samples were cooled to room temperature and stored at 4°C for further analysis. The schematic diagram of the surimi process is presented in Figure 1.

Figure 1. Schematic Diagram of Surimi Gel Processing.

Experimental Design Using Response Surface Methodology

The impact of two independent variables of enzyme concentration (A) and incubation time (B) on gel strength (Y1), chewiness (Y2), whiteness (Y3), and water-holding capacity (Y4) was optimized by RSM. This study used the central composite design (CCD) and analyzed by Design Expert 12.0 to evaluate the effect of Enzyme Concentration (A) and Incubation Time (B) on four response parameters: Gel Strength, Chewiness, Whiteness, and Water-holding Capacity (WHC). The experimental design involved 13 runs without blocks, with the main model being quadratic for a given response.

Analysis of Gel Properties

Analysis of Gel Strength

The gel strength of surimi was measured according to (Zeng *et al.*, 2025) with slight modification, using a TA-XT Plus (Stable Micro Systems, Surrey, UK) at the Laboratory of Agroindustry, Agro and Biomedical Industry Technology Development Laboratory (LAPTIAB), the National Research and Innovation Agency, Indonesia. Surimi gel samples were cut into cylindrical shapes with dimensions of 25 mm in height, 30 mm in width, and 35 mm in length, then equilibrated at 25°C for 30 minutes. The gels were positioned on a heavy-duty platform (HDP 90) and vertically compressed using a P/0.25S ¼ spherical stainless probe at a constant speed of 1 mm/s. After pressing to 15 mm, the probe returned to its original height. The gels were compressed to 10% of their original height, with the trigger force of 10 g. Experiments were repeated three times for each sample. Gel strength is the peak force in grams multiplied by the distance of rupture measured in centimeters. The value produced has units of gram-centimeter. The calculation formula of gel strength was:

$$\text{Gel strength (g.cm)} = \text{Breaking force (g)} \times \text{Breaking distance (cm)}$$

Analysis of Whiteness

The color was determined according to the method of (Tan *et al.*, 2024). Prior to sample determination, the colorimeter undergoes whiteboard calibration and blackboard zeroing. The color coordinates (L*, a*, and b* values) were measured using a benchtop spectrophotometer (Agera HunterLab Associates Laboratory Inc., Reston, VA, USA) at Agro and Biomedical Industry Technology Development

Laboratory (LAPTIAB), National Research and Innovation Agency, Indonesia. It can be obtained (L*) brightness; (a*) indicates red-greenness, b* indicates yellow-blue, and whiteness values. The whiteness calculation formula was as follows:

$$W = \sqrt{100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]}$$

Analysis of Chewiness

The chewiness of Catfish surimi was measured using the TPA (Texture Profile Analysis) method of (Liu *et al*., 2025) with slight modification using the TA-XT Plus (Stable Micro Systems, Surrey, UK) equipped with P/36R probe at Agro and Biomedical Industry Technology Development Laboratory (LAPTIAB), National Research and Innovation Agency, Indonesia. The surimi sample is prepared with a width of 30 mm, a length of 30 mm, and a height of 25 mm, at room temperature (25°C) and placed on a heavy-duty platform (HDP 90), then applying 40 % strain and trigger force of 10 g at a test speed of 1.0 mm/s. Chewiness is defined as the product of gumminess x springiness (which equals hardness x cohesiveness x springiness) and is therefore influenced by the change of any one of these parameters.

Analysis of Water-holding Capacity

The water-holding capacity (WHC) is determined by the initial moisture content of the surimi and the residual water present in the gel. Surimi gel WHC was measured by the hardness tester method (An *et al*., 2018) using a TA-XT Plus (Stable Micro Systems, Surrey, UK) at Agro and Biomedical Industry Technology Development Laboratory (LAPTIAB), National Research and Innovation Agency, Indonesia. Weighing as W1, the Surimi gel was cut into 5 mm slices. The slice was next wrapped in filter paper. The slice was weighed again as W2 after being compressed for one minute with a 5 g force from the hardness tester. The water-holding capacity calculation formula was as follows:

$$\text{WHC} = 100\,\% - (\text{W1} - \text{W2}) \times 100\%/\text{W1}$$

Analysis of Molecular Weight

The molecular weight of surimi was analyzed by SDS-PAGE (Copeland, 1994). The surimi sample of 100 mg was homogenized with 1 ml of analytical grade phosphate buffer pH 7 from Merck. The analysis was conducted at the Laboratory of Biocatalyst, LAPTIAB, National Research and Innovation Agency, Indonesia.

Analysis of Microstructure Using Scanning Electron Microscopy (SEM)

The surface morphology of surimi gels from Indonesian catfish was examined using scanning electron microscopies (SEM), where gel samples were coated with platinum (Pt) after being freeze-dried with an accelerated voltage of 20 kV (Kaberova *et al*., 2020). The gels were observed using JSM-6510LA JEOL, Tokyo, Japan at the Laboratory of Polymer, National Research and Innovation Agency, Indonesia.

Results and Discussion

The yield of catfish surimi to whole fish in this study is 23.49 %, while the yield of fish fillet to whole fish is 48.06 %. The differences in surimi yield may be affected by species, fish size, and the method of removing the flesh (Priyadarshini *et al*., 2018). The Enzyme Concentration factor was tested by Response Surface Methodology (RSM) with Central Composite Design (CCD) in the range of 0 - 1% (code: -1 to +1). The Incubation Time was tested in the range of 15-60 minutes, and the data are shown in Table 1. The 3D surface plot of the experimental design (Figure 2) showed an interaction between two dependent variables, the concentration of transglutaminase (%) and incubation time (minutes), toward the responses of gel strength, chewiness, whiteness, and water-holding capacity of the surimi product. The data showed that the response parameters had different sensitivities to changes in the experimental factors, reflecting the complex interactions among the variables.

The overall study showed that Gel Strength and Whiteness were responsive to changes in experimental parameters, while Chewiness showed a more complex relationship, and Water-holding Capacity was not significantly affected. The resulting model can be used as a guide to optimizing surimi formulation, with a focus on optimizing Enzyme Concentration and Incubation Time to achieve strong gel characteristics and desired visual appearance (You *et al*., 2024) Upon acquisition of the empirical data, a numerical optimization procedure was employed using the point prediction capability of statistical software to ascertain the specific combination of factor levels that would yield the most desirable response according to predefined criteria. Model prediction using Response Surface Methodology design resulted in an optimum condition for each response, with Enzyme Concentration at 0.85 % and Incubation Time at 36 minutes. The effect of transglutaminase concentration and incubation time gives different results on surimi gel properties in some studies. The optimum of enzyme concentration and incubation time were 0.7 % at 4 hours (Van Muoi *et al*., 2019), 0.6 % at 40 minutes (Wang *et al*., 2025), or

0.4 % at 1 hour (Liang *et al*., 2020). Further studies with an expanded range of factors and additional parameters may improve the understanding of this system (You *et al*., 2024).

Table 1. Response of the Experimental Design Using *Response Surface Methodology*

Treatments	Independent Variables		Responses			
	Enzyme Conc (%)	Incubation Time (min)	Gel Strength (g.cm)	Chewiness (g)	Whiteness (%)	Water-holding Capacity (%)
1	0.50	60.00	878.72 ± 191.26	3,004.45 ± 94.89	79.86 ± 0.55	90.92 ± 0.43
2	0.15	21.59	362.16 ± 69.92	3,402.87 ± 124.97	79.62 ± 0.25	92.29 ± 0.81
3	0.50	37.50	664.52 ± 25.35	4,376.95 ± 184.04	80.12 ± 0.33	92.79 ± 0.50
4	0.50	15.00	397.42 ± 15.01	4,055.94 ± 547.06	79.05 ± 0.32	90.76 ± 0.85
5	0.50	37.50	918.39 ± 191.27	4,644.23 ± 362.60	80.00 ± 0.38	90.69 ± 3.47
6	0.00	37.50	265.72 ± 32.25	2,849.19 ± 24.40	80.17 ± 0.37	89.14 ± 0.55
7	0.15	53.41	533.58 ± 143.52	2,176.23 ± 192.82	80.04 ± 0.08	90.83 ± 0.48
8	0.50	37.50	632.69 ± 100.68	4,170.83 ± 263.34	80.09 ± 0.37	92.08 ± 0.37
9	0.50	37.50	691.05 ± 116.23	3,267.50 ± 173.12	79.88 ± 0.22	93.29 ± 0.79
10	1.00	37.50	919.70 ± 85.53	3,186.80 ± 141.92	79.80 ± 0.35	92.11 ± 0.85
11	0.50	37.50	762.12 ± 126.22	3,117.82 ± 196.82	80.06 ± 0.27	90.42 ± 1.28
12	0.85	21.59	717.25 ± 65.05	4,094.97 ± 363.09	79.79 ± 0.52	90.53 ± 0.55
13	0.85	53.41	999.26 ± 63.48	3,564.55 ± 211.55	79.74 ± 0.36	91.93 ± 0.55

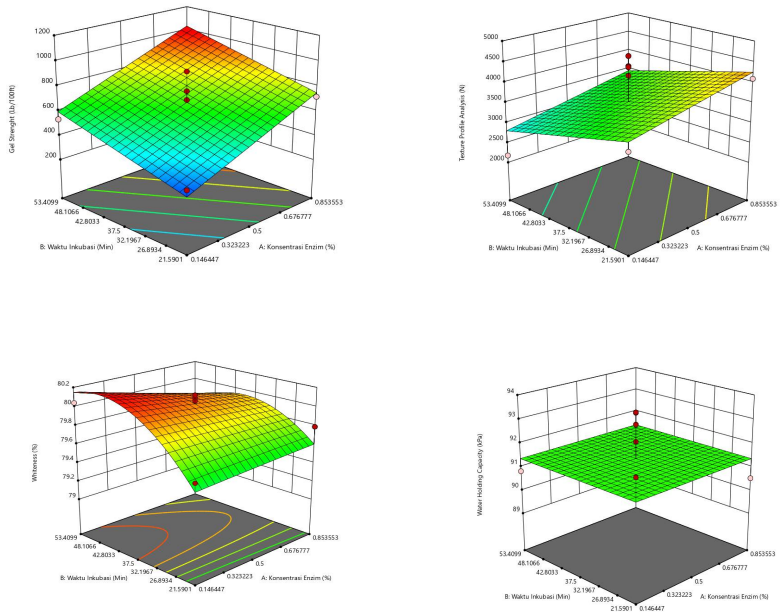

Figure 2. 3D Surface Plot of Experimental Design.

The four predicted responses include Gel Strength (927.513 g.cm), Chewiness (3747.18 g), Whiteness (79.946 %), and Water-holding Capacity (91.368 %). All these values were generated from a model that has been tested for reliability based on statistical parameters such as Adjusted R^2, Predicted R^2, and lack-of-fit. Confirmation results showed that the model predictions for Gel Strength and Whiteness were highly accurate, with actual values falling within the predicted range. However, Chewiness and Water-holding Capacity showed larger deviations, indicating the need for further model development. Optimization of experimental parameters, especially Enzyme Concentration and Incubation Time, remains a key focus in improving practically relevant responses.

Optimization of enzyme concentration and incubation time on surimi gel strength

The Gel Strength response is based on Table 1. was analyzed using a significant linear model ($p<0.0001$), with an R^2 of 0.8469, Adjusted R^2 of 0.8162, and Predicted R^2 of 0.7759. This model showed that Enzyme Concentration ($p<0.0001$) and Incubation Time ($p=0.0023$) were both significant in influencing this response. The highest coefficient was Enzyme Concentration, which contributed more than Incubation Time. The prediction model for Gel Strength was formulated as:

$$\text{Gel Strenght} = 672.51 + 218.22\,A + 141.77\,B$$

Enzyme concentration significantly influences gel strength, where higher enzyme concentration corresponds to higher gel strength values substantially as enzymes accelerate the formation of protein bonds in the surimi matrix (Yuliana *et al*., 2021). Incubation time also plays an important role, with longer incubation duration increasing gel strength to a certain extent before it stabilizes or decreases due to protein degradation (Yang *et al*., 2020). Protein degradation may cause softening of surimi gel (Okita *et al*., 2021). The interaction between enzyme concentration and incubation time suggests that an optimal combination of these two factors is required to produce maximum gel strength in surimi (Huang *et al*., 2023). The prediction for Gel Strength is 927.513 g.cm with a 95% confidence interval of [826.372 - 1028.65 g.cm]. These results indicate that the linear model was able to capture the variation in response well, evidenced by the Adjusted R^2 of 0.8162 and Predicted R^2 of 0.7759. In experimental confirmation, the actual value was 879.49 g.cm, which is within the model's predicted range. This indicates the high accuracy of the prediction. The effects of Enzyme Concentration (A) and Incubation Time (B) were both significant in the model ($p<0.05$). The strongest gel (999.26 g.cm) was observed at an enzyme concentration of 0.85% and an incubation time of 53.41 minutes, suggesting this combination as optimal for achieving maximal gel strength in surimi. In contrast, lower enzyme concentrations (e.g., 0.00% and 0.15%) produced significantly weaker gels, as indicated by the lowest gel strength of 265.72 g.cm at 0.00% enzyme concentration and 37.50 minutes of incubation. This result aligns with established biochemical mechanisms, where the transglutaminase enzyme catalyzes the formation of covalent protein cross-links, resulting in a stronger and more robust gel network, and consistent with previous research highlighting the critical role of protein solubilization and gelation kinetics (Grossmann & Mcclements, 2022), influenced by enzyme activity and processing conditions, in determining the textural properties of surimi products.

Optimization of enzyme concentration and incubation time on surimi chewiness

In the Texture Profile Analysis response, The multiplication of hardness × cohesiveness × springiness gives chewiness, assessing the easiness of chewing the surimi in the human mouth (Yang et al., 2024). This result suggested a quadratic model although the overall model significance was low ($p=0.1688$), with an Adjusted R^2 of 0.3621 and a Predicted R^2 of 0.1049. This response had no significant factors, although the effect of Incubation Time ($p=0.0920$) was close to significant. The resulting predictive model was:

$$\text{Chewiness} = 3532.03 + 320.23A - 405.01B$$

The model for this response shows that Enzyme Concentration has a positive effect on surimi chewiness while incubation time has a negative impact. However, this model requires further optimization, especially due to the low Predicted R^2 (based on Table 1). Enzyme concentration contributes positively to its chewiness, as transglutaminase plays its role by catalyzing protein cross-linking formation, which caused a stronger gel network structures (Yang et al., 2024). With increasing enzyme concentration tends to increase the textural strength of surimi through the formation of denser protein bonds (Setiadi et al., 2018). However, the incubation time has a negative effect on chewiness, where longer incubation time leads to a decrease in texture due to protein degradation or weakening of the gel structure (Han & Li, 2024). The combination of optimal enzyme concentration with controlled incubation time is essential to produce an ideal surimi texture, given the complex relationship between these two factors on texture strength and elasticity (Yuliana *et al*., 2021). The experimental results differ slightly from the model prediction, but these deviations were

not extreme. The prediction for Chewiness is 3747.183 g with a 95 % confidence interval of 3118.98 - 4375.39 g. In the experimental confirmation, the actual value is 4574.84 g, which is outside the prediction interval, showing a significant difference between the experimental results and the model prediction. This can be explained by the low Predicted R^2 (0.1049), although the adjusted R^2 is at a higher level (0.3621). The Incubation Time factor (B) has a significant negative impact (p=0.0920), suggesting the model may require refinement or additional data collection to improve the prediction accuracy. This anomaly suggests that while the primary gel structure was strengthened, other factors not controlled in this study likely had a more dominant influence on the complex textural properties.

Optimization of enzyme concentration and incubation time on surimi whiteness

Whiteness response data in Table 6 showed that the quadratic model produced the best results with an R^2 of 0.8637, Adjusted R^2 of 0.7663, and Predicted R^2 of 0.2217. Incubation Time (p=0.0071) and the square of Incubation Time (B2, p=0.0016) were significant factors affecting Whiteness. The prediction model was formulated as:

$$\text{Whiteness} = 80.03 - 0.0817A + 0.1894B$$

The dominance of the quadratic effect of Incubation Time suggests that the optimization of this parameter is important to achieve the maximum Whiteness value. Enzyme concentration had a minimum influence on the whiteness of surimi, indicating that changes in enzyme concentration did not significantly affect the final color appearance (Yuliana *et al*., 2021). Incubation time exerts a more dominant influence, where optimum incubation duration increases Whiteness, but a longer incubation time may cause discoloration due to protein degradation reactions (Buamard & Benjakul, 2019). The quadratic effect of incubation time indicates that precise duration adjustment is essential to maximize whiteness, making this variable more significant than enzyme concentration in determining the brightness of surimi (Somjid *et al*., 2021). The surimi color was formed by some factors such as fish species, heat treatment and time, rinsing condition, additives, and freshness. The initial improvement in whiteness could be due to the formation of a uniform protein network that enhances light scattering. However, prolonged incubation and enzyme activity might trigger other minor biochemical reactions or structural rearrangements that slightly diminish this effect, leading to the observed curved relationship. In this study, the concentration of 0.8 % transglutaminase, combined with moderate incubation time, has a positive effect and gives the best whiteness of 80.2 %. However, the use 0.1 u/g transglutaminase incubated at 50 °C for 1 hour showed the highest whiteness index of 40 – 40.5 % of tilapia surimi gels (Huang *et al*., 2023). The surface plot is higher at lower incubation times and flattens as it increases, especially for higher transglutaminase concentration. This plot suggests a moderate enzyme concentration and incubation time to maximize whiteness in surimi.

Optimization of enzyme concentration and incubation time on surimi water-holding capacity

Water-holding Capacity (WHC) showed the ability of the gel to retain moisture (Xiong *et al*., 2023). Higher water-holding capacity may givi higher juiciness of the food which give lower cooking loss (Yang *et al*., 2024). The result showed insensitivity of WHC to both experimental factors with an R^2 of 0.0000, Adjusted R^2 of 0.0000, and Predicted $R^2<0$. None of the parameters were significant, and the average-based model (WHC=91.37) was sufficient to describe this response. With these results, it can be concluded that neither variations in Enzyme Concentration nor Incubation Time significantly affected the water-holding capacity of the surimi under the experimental conditions tested. The water-holding capacity values range from around 89.14 to 93.29 %. However, the water-holding capacity is mostly unaffected by both variables. Cross-linking by microbial transglutaminase enhances the textural gel qualities of silver carp surimi by increasing the degree of cross-linking within the gels, leading to a gradual reduction in surface hydrophobicity and the formation of high-molecular-weight polymers (Fang *et al*., 2019). The water molecules were entrapped in a surimi gel network which transformed the partially free water to bound water, resulting in a higher WHC (Zhao *et al*., 2023). The prediction for Water-holding Capacity was 91.368 % with a 95 % confidence interval of 90.6738 - 92.0616 %. The actual value from the experiment was 98.44 %, which was well outside the predicted range, signaling that the model failed to describe an adequate relationship for Water-holding Capacity. The negative Predicted R^2 (-0.1736) and low Adjusted R^2 indicate that the variation in Water-holding Capacity cannot be explained by the model, making the mean value the best prediction.

The factors of Enzyme Concentration and Incubation Time were not significant in influencing Water-holding Capacity (Van Muoi *et al*., 2019). It strongly implies that the potential positive effect of enzymatic cross-linking was completely negated by other, more powerful factors. Some research shows that enzyme concentration does not show a significant effect on the Water-holding Capacity (WHC) of surimi,

indicating that changes in enzyme levels and incubation time (Yan *et al*., 2024) do not directly affect the ability of surimi to hold water (Nakamura *et al*., 2021). These results indicated that the WHC of surimi is more influenced by other factors beyond enzyme concentration and incubation time, such as protein composition or additional processing methods (Liu *et al*., 2021). According to Buamard et al (Buamard *et al*., 2024), transglutaminase in combination with coconut husk extract may enhance WHC and breaking force. Additionally, combining transglutaminase with a heating treatment at 40°C further improves the gel properties of vacuum-freeze-dried catfish surimi through the formation of myosin heavy chain (MHC) cross-links (Guo *et al*., 2019). The dissociation of myofibrillar proteins can be effectively achieved by monovalent salts such as Sodium Chloride, which primarily convert á-helix structures to â-sheets and establish a uniform and dense gel network (Zhao *et al*., 2023). According to the data analysis based on the parameters above, the optimum conditions were generated by the transglutaminase concentration of 0.85 % at 36 minutes of incubation time.

Molecular Weight of Surimi using SDS-PAGE

By raising the degree of cross-linking inside the gels, cross-linking by microbial transglutaminase improves the textural gel properties of catfish surimi by gradually lowering surface hydrophobicity and producing high-molecular-weight polymers. Myofibrillar proteins solubilize and denature in salt and heat, revealing their highly reactive surfaces. These sites form intermolecular bonds between protein molecules. Four essential bonds for protein network formation are hydrogen bonds, ionic linkages, covalent bonds, and hydrophobic interactions (Yingchutrakul *et al*., 2022). By means of myosin heavy chain (MHC) cross-links, combining transglutaminase with a heating treatment at 40°C enhances the gel qualities of vacuum-freeze-dried catfish surimi even more. Figure 3 shows the SDS-PAGE of surimi gel with transglutaminase, without transglutaminase, and compared to commercial surimi products. A myofibrillar protein of surimi gel primarily contains MHC and actin (Petcharat & Benjakul, 2018).

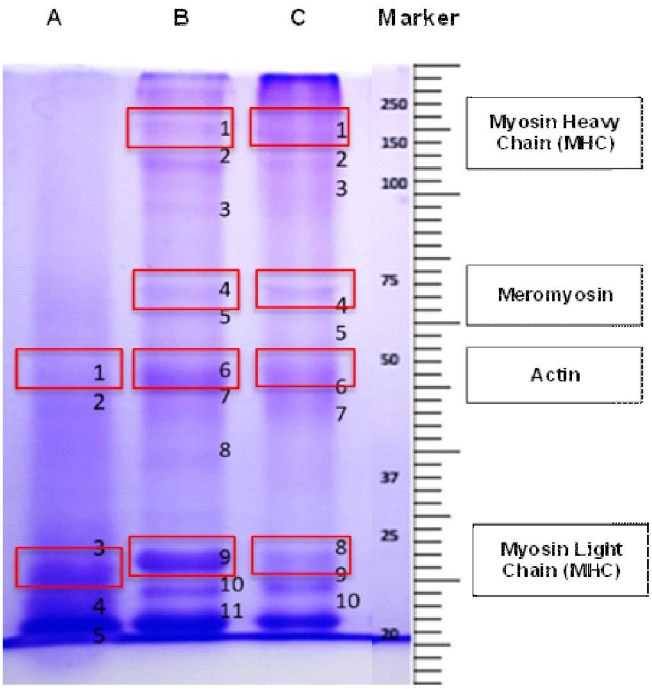

Figure 3. Molecular Weight of Surimi Gel (A: Commercial Surimi, B: Catfish Surimi with 0.85 % w/w of Transglutaminase, C: Catfish Surimi without Transglutaminase).

Surimi has bands close to 200 kDa (myosin heavy chains) (Yang *et al*., 2024), 70 kDa (light meromyosin), between 20 and 25 kDa (myosin light chains), and between 43 and 45 kDa (actin) (de Oliveira *et al*., 2021). The myosin heavy chain (MHC) bands were detected around 172 kDa for catfish surimi with transglutaminase and 180 kDa without transglutaminase. The meromyosin bands were detected around 73 kDa for catfish surimi with or without transglutaminase. Meanwhile, the MHC and meromyosin bands were not detected on commercial surimi. The actin bands were detected around 48 kDa, 45 kDa, and 46 kDa for commercial surimi and catfish surimi with and without transglutaminase, respectively.

The myosin light chain (MLC) bands were detected around 24 kDa for commercial surimi and around 25 kDa for catfish surimi with and without transglutaminase. Actin bands were present in the catfish sample, which indicates that transglutaminase did not interfere with the actin. The study of Petcharat & Benjakul (Petcharat & Benjakul, 2018) also stated that actin was not a preferred substrate for transglutaminase. However, all samples showed actin and MLC bands, which indicates that they were more stable and not easy to form protein cross-linking(Yang *et al*., 2024).

Microstructure of Surimi using Scanning Electron Microscopy (SEM)

Myofibrillar proteins break down into actomyosin, myosin, and other fragments when extracted, then aggregate into a denser, more polished protein network. Promoting protein aggregation and the building of a stable three-dimensional gel structure depends critically on salt (Zhao *et al*., 2023). Figure 4 shows the microstructures of surimi gels derived from Indonesian catfish improved with transglutaminase.

The functional properties of a gel depend critically on the three-dimensional network structure (Leng *et al*., 2022). The cryoprotectants take part in preventing the denaturation and aggregation of the myofibrillar proteins (Walayat *et al*., 2022). The coarse and heterogeneous microstructure will be formed if the protein aggregation is faster than the unfolding speed (Chen *et al*., 2021). However, transglutaminase addition produced a denser and more consistent surimi gel network (Figure 4.2) than the control gel without Transglutaminase (Figure 4.3). In the presence of transglutaminase, myofibrillar proteins may experience enhanced cross-linking, resulting in a denser and more compact gel network (Zhang & Chang, 2023). The irregular structure with larger pores was observed in surimi that did not contain transglutaminase. The study Leng *et al.* (2022) indicates that polysaccharides can create a dense surface structure in surimi gel. Additionally, an increase in polysaccharide concentration leads to the formation of more aggregates. The network with larger pores exhibited insufficient strength and resistance to the applied force (Zhou *et al*., 2019).

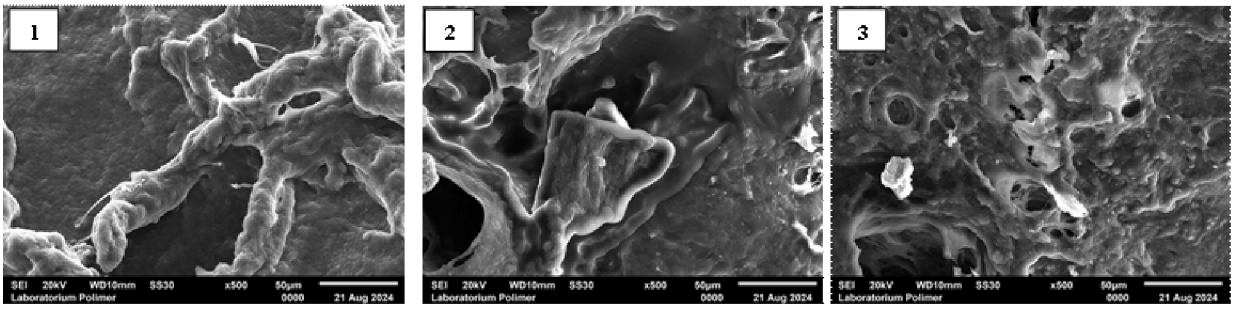

Figure 4. Morphological Structure of Surimi Gel (1: Commercial Surimi, 2: Catfish Surimi with 0.85 % w/w Transglutaminase, 3: Catfish Surimi without Transglutaminase).

Conclusion

This study aims to enhance catfish (freshwater fish) surimi properties by optimizing surimi production with various transglutaminase levels under different incubation times. The food sector may find this method interesting, particularly in areas where catfish is plentiful and reasonably priced, providing a good substitute in surimi manufacture. Separating the flesh from the bones, mincing, washing, filtering, pressing, mixing with sucrose, and refrigeration at -18°C generate surimi gels. To make the surimi gels, the frozen surimi is thawed, added with Sodium Chloride and Transglutaminase, and then homogenized into surimi paste. Response Surface Methodology was applied in this study to optimize the effects of enzyme concentration and incubation time on gel strength, chewiness, whiteness, and water-holding capacity. The study sought to ascertain how incubation time and enzyme concentration affected gel characteristics, including gel strength, chewiness, whiteness, and water-holding capacity. SDS-PAGE was used for molecular weight analysis, while Scanning Electron Microscopy (SEM) was used to investigate surface morphologies of surimi gels from Indonesian catfish. Results revealed a two-dependent variable interaction between enzyme concentration and incubation time toward the responses of gel strength, chewiness, whiteness, and water-holding capacity of surimi gel. The unusual findings may be attributable to the distinct physicochemical properties of catfish muscle proteins. To better understand what determines surimi quality, subsequent research should investigate other potentially important factors. Future studies should also analyze the individual components of Texture Profile Analysis (TPA), such as hardness, cohesiveness, springiness,

and chewiness as separate responses. This approach could reveal more nuanced effects, as the enzyme might alter one parameter significantly while not affecting another, a detail that was missed in the current analysis. However, this study provides actionable insights for surimi processors, suggesting that adjusting enzyme concentration and incubation time can improve product quality by enhancing the gel strength of freshwater fish to substitute for marine fish.

Acknowledgments

The authors would like to express their sincere gratitude to the Agro and Biomedical Industry Technology Development Laboratory (LAPTIAB), National Research and Innovation Agency, Indonesia, for their invaluable contributions in providing the facilities for this research. This study was supported by Research Grant No. 6/III.11/HK/2024 of the Research Organization of Agriculture and Food, National Research and Innovation Agency.

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