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Gel properties of surimi gel from clam *Meretrix meretrix* as affected by microbial transglutaminase

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To exploit high-value-added clam (*Meretrix meretrix*) products, the effects of pretreatment methods and microbial transglutaminase (MTGase) on the gelation process of clam surimi were studied. After comparing three pretreatment methods, mechanical chopping combined with rinsing was identified as a suitable method for pretreating the clam material. Based on this finding, MTGase was shown to improve the gel properties of clam surimi. When MTGase was added up to 2.0 U g surimi^{-1}, the hardness, gumminess, chewiness, and breaking force of the samples were enhanced by 38.5%, 28.9%, 31.2%, and 28.6%, respectively. At the microscopic level, the pores in the gel network gradually became more homogeneous and smaller in size. The results of SDS-polyacrylamide gel electrophoresis, intermolecular interactions, and protein cross-linking degree suggested that MTGase promoted the aggregation of the clam protein molecules. In summary, pretreatment *via* mechanical chopping with rinsing and the addition of MTGase are conducive to the preparation of high-quality surimi gel from clam meat.

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Sustainability spotlight

As is known, the clam (*Meretrix meretrix*) is one of the common bivalves in China, North Korea, and Japan. Clam meat has high nutritional value, is rich in polysaccharides and protein, and is known for its delicious taste. However, the development of clam meat products is very limited, and the product forms are only focused on fresh, cured, frozen and dried meat products, with few reports on the deep processing of clam meat. Therefore, the exploration of high added-value clam products, such as clam surimi, has great development prospects and economic value. This study may provide more information and guidance for the sustainable utilization of clam resources and give new ideas for the development of bivalve food materials.

1. Introduction

Surimi products are favored by the public due to their distinctive taste, convenience, and versatility. These products are primarily prepared through the gelation of surimi, following rinsing, salt-chopping, and other processing steps.[1] During the salt-chopping process, salt-soluble proteins are solubilized and then undergo heat-induced denaturation to form a compact gel network structure.[2] Currently, fish[3,4] and shrimp[5,6] are the primary raw materials used for surimi production. Shellfish, such as clams, scallops, mussels, and oysters, represent the second-largest category of aquaculture products in China,[7] showing significant potential for application in surimi product processing.[8] Mi *et al.* (2021)[9] investigated the gel properties of the adductor muscle in Zhikong scallop (*Chlamys farreri*). Stangierski *et al.* (2021)[10] studied the physicochemical characteristics of surimi gel from freshwater mussels (*Sinanodonta woodiana*). However, there have been relatively few studies on improving the gelation characteristics of shellfish surimi, and the underlying mechanism remains unclear.

The clam (*Meretrix meretrix*) belongs to the phylum Mollusca and is a prevalent bivalve species native to China, North Korea, and Japan.[11] Clam meat is nutritious, rich in proteins and polysaccharides, and renowned for its delicious taste.[11] Most current research on clams focuses on their health benefits. For example, some oligopeptides in clams have been shown to have immunomodulatory effects,[12] anti-inflammatory potential,[13] and protective effects against high-fat liver disease.[14] Furthermore, clam polysaccharides have been found to exhibit immune-enhancing effects.[15] However, the direct processing of clam meat has not been reported. Therefore, the exploration of high-value-added clam products, such as clam surimi, has considerable development prospects and economic value. Notably, the high protein content in clams is beneficial for the preparation of clam surimi gel. Nevertheless, the presence of

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edible visceral mass in clams has been shown to adversely affect the properties of heat-induced surimi gels. Endogenous proteases and water-soluble proteins in the mass can disrupt the formation of the surimi gel network.[16] Conventional rinsing is commonly employed to alleviate this disruption of the gel network.[6] Therefore, identifying suitable raw material pretreatment methods is crucial for the production of high-quality clam surimi gels. Furthermore, given that the gelation properties of shellfish surimi differ significantly from those of fish and shrimp surimi,[17] developing exogenous additives for clam surimi to enhance gel formation is essential for its commercial production.

Microbial transglutaminase (MTGase) is widely used in food processing as a protein cross-linking agent to enhance the gel properties of surimi products.[8] Isopeptide bonds can be generated by MTGase through the formation of intramolecular and intermolecular cross-links.[18] Previous studies have reported its effectiveness in improving the gelation of fish[2,19] and shrimp[20] surimi. Similarly, MTGase has also been observed to have positive effects in shellfish surimi products. For example, Feng *et al.* (2022)[21] found that the textural properties of adductor muscle gels from bay scallop (*Argopecten irradians*) could be improved by MTGase supplementation. Mi *et al.* (2021)[9] investigated the gel-enhancing effect of MTGase combined with soybean protein isolate in adductor muscle gels of Zhikong scallop. Nevertheless, while previous studies have primarily focused on the effect of MTGase in enhancing the macroscopic properties of shellfish surimi gels, a systematic investigation into the dose–response relationship and the fundamental mechanisms of MTGase in shellfish surimi gelation is still lacking. Moreover, given that the protein structure, amino acid composition, and functional characteristics vary among species,[22] the effect of MTGase is likely a species-specific effect. Therefore, elucidating the dose–response relationship of MTGase and its underlying mechanism in clam surimi gelation is essential for developing high-quality clam surimi products.

To produce high-quality clam surimi products, this study developed a pretreatment method (combining mechanical chopping and rinsing) for clam raw materials to reduce the adverse effects of visceral mass. Also, a negative control (mechanical chopping without rinsing) and a positive control (manual visceral removal followed by rinsing) were established to evaluate the beneficial effect of the combined pretreatment method. Furthermore, the dose–response relationship of MTGase on the gel properties of clam surimi was characterized, and the underlying mechanism was explored. This research could contribute valuable insights for enhancing the utilization of clam resources and the application of MTGase in clam surimi products.

2. Materials and methods

2.1. Materials and chemicals

Frozen clam meat was obtained from the Zhumi Food Business Department and stored at a temperature of −45 °C. Food-grade MTGase derived from *Streptomyces mobaraensis* (enzyme activity: 125 U g^{-1}) was sourced from Zhengzhou Taichung Food Additives Co., comprising 1% enzyme and 99% maltodextrin with the recommended operating temperature range between 40 °C and 60 °C. The sodium chloride used was food grade, and the other chemicals were analysis grade.

2.2. Pretreatment of clam meat

Frozen clam meat was thawed, and then washed with deionized water to eliminate surface impurities. To enhance the extraction of water-soluble components from clams, three different pretreatments were applied. A chopper (MQ 5025 plus, Germany) was utilized for the preparation of all samples. In the control group, the clam meat was chopped without rinsing at 1000 rpm for 10 s. In the mechanical group, the clam meat was chopped under the same conditions, and then rinsed 4 times with deionized water. Also, for the manual group, the clam was manually removed the visceral mass, followed by the same chopping and rinsing process. After removal of the excess surface moisture, all minced clam meat was stored at 4 °C until further processing.

2.3. Production of heat-induced clam surimi gel

The clam surimi gel was prepared as reported by Ueki *et al.* (2019)[16] with some modifications. Firstly, the water content of the pretreated clam meat was adjusted to maintain approximately 80%. Subsequently, the clam surimi was obtained by adding 1% NaCl (w/w) and mincing at 3000 rpm for 2 min. The mixed surimi was then transferred to a 50 mL centrifuge tube. The clam surimi gel was formed by heating in a water bath. Briefly, the surimi was initially heated at 45 °C for 45 min. Then, the sample was transferred rapidly to a 90 °C water bath for a further 15 min of heating. All samples were refrigerated at 4 °C overnight prior to the subsequent analysis.

Moreover, the effects of MTGase on the gel properties of clam surimi and its mechanism of action were explored. Minced clam meat obtained by mechanical pretreatment was used as the raw material. Different concentrations of MTGase (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 U g surimi^{-1}) were mixed with the surimi and continued to mince for an additional 1.5 min before transfer. The other preparation processes were carried out as described above.

2.4. Mechanical properties

The breaking force and texture profile analysis (TPA) of the clam surimi gel were tested using a TA-XT Plus texture analyzer (Stable Micro System, Surrey, UK) according to the method reported by Chen *et al.* (2024).[20] For TPA, a P/50 probe was used for this test with pre-test, test, and post-test speeds of 2.0 mm s^{-1}, 1.0 mm s^{-1}, and 1.0 mm s^{-1}, respectively. Also, the fracture gel evaluation was carried out using a P/0.5 probe. The test conditions were as follows: pre-test speed of 2.5 mm s^{-1}, test speed of 1.0 mm s^{-1}, and post-test speed of 1.0 mm s^{-1}. For all the tests, 40% deformation was applied using a 5 g trigger force. Prior to testing, all the surimi gels were precut into cylindrical shapes with a diameter of 20 mm and height of 10 mm.

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2.5. Whiteness

The whiteness of the samples with the addition of different MTGase concentrations was measured using an UltraScan Pro (Hunter Lab, USA) according to the method described by Chen *et al.* (2024).[20] To evaluate the whiteness, eqn (1) was used:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (1)$$

2.6. Cooking loss

The cooking loss of the clam surimi gel was assessed as described by Mi *et al.* (2021).[9] At first, the clam surimi with different MTGase concentrations was placed in a centrifuge tube and its weight was noted as Q_1. The samples were then prepared as previously detailed. Upon cooling the sample to 4 °C, the remaining liquid weight was recorded as Q_2. The cooking loss could be calculated using eqn (2), as follows:

$$\text{Cooking loss (\%)} = (Q_2/Q_1) \times 100\% \quad (2)$$

where Q_1 is the weight of the initial materials and Q_2 is the weight of the precipitated liquid.

2.7. Expressible water content (EWC)

To measure the water holding capacity of the clam surimi gel, EWC was conducted by the method reported by Dong *et al.* (2020).[19] 1 g sample was measured and denoted as W_1. Subsequently, the samples were enveloped in filter paper and centrifuged at 5000 rpm for 20 min to eliminate the expressible water. Afterwards, the gel was removed from the centrifuge tube, and the weight was recorded as W_2. Eqn (3) was used to measure the EWC, as follows:

$$\text{EWC (\%)} = [(W_1 - W_2)/W_1] \times 100\% \quad (3)$$

where W_1 is the weight of the sample before centrifugation and W_2 is the weight of the sample after centrifugation.

2.8. Water distribution

The water distribution of the clam surimi gel was measured using a low-field nuclear magnetic resonance (LF-NMR) tester (MesoMR23-060V-1, Niumag Analytical Instrument Co., Ltd, China) as described by Chen *et al.* (2022).[6] The heat-induced gels were cut into the same cylinders, and the test was carried out at 22 MHz with the echo number of 2000 and the magnetic field strength of 0.6 T.

2.9. Rheological properties

Rheological analysis can be used to assess the viscoelasticity of a gel. It has been reported that the optimum temperature range for MTGase activity is between 40 °C and 70 °C.[23] Therefore, in this study, the suwari gel formed after the initial stage of heating (45 °C/45 min) was subjected to rheological analysis according to the methods reported by Chen *et al.* (2024)[20] with some modifications. The mixed clam surimi, containing different MTGase concentrations (0.0–3.0 U g surimi^{-1}), was placed on the bottom plate of a Discovery HR-1 rheometer (TA Instruments Menu Co., Ltd, USA) and heated at 45 °C for 45 min using a 20 mm parallel plate. Subsequently, the plate temperature was reduced to 25 °C at a rate of 4 °C min^{-1}. At 25 °C, the strain sweep test with 0.01–100% strain was performed at a frequency of 1 Hz, and a strain value of 0.3% was determined from the linear viscoelastic region (LVR), serving as a constant parameter for the following frequency sweep model. The storage modulus (G') and loss modulus (G'') were recorded in the range of 0.1 to 10 Hz at 25 °C.

2.10. Protein cross-linking degree

The degree of protein cross-linking in the clam surimi gels was determined by the methods reported by Jia *et al.* (2016).[24] Briefly, the clam surimi gel was mixed with borate buffer (containing 0.07 M SDS) and homogenized at 7200 rpm for 1.5 min. The homogenate was heated at 75 °C for 15 min, and then at 60 °C for 2 h. Subsequently, borate buffer was added to adjust the protein concentration in the supernatant to 1 mg mL^{-1}. The TNBS test, as outlined by Chen *et al.* (2024),[20] was employed to measure the concentration of myosin free amino groups. The cross-linking degree of the sample was calculated using eqn (4), as follows:

$$\text{Cross-linking degree (\%)} = [1 - (a/a')] \times 100\% \quad (4)$$

where a' is the amount of free amino groups in the uncrosslinked raw surimi and a is the amount of free amino groups in the cross-linked surimi gel.

2.11. Intermolecular interaction

To investigate the intermolecular interactions that stabilized the clam surimi gel, the solvent destruction method was conducted in accordance with the method reported by Zhang *et al.* (2024).[25] Briefly, five solutions were prepared, and the protein contents in solution I (0.6 mol L^{-1} NaCl), II (1.5 mol L^{-1} urea and 0.6 mol L^{-1} NaCl), III (8 mol L^{-1} urea and 0.6 mol L^{-1} NaCl), IV (8 mol L^{-1} urea, 0.6 mol L^{-1} NaCl, and 0.5 mol L^{-1} β-mercaptoethanol), and V (1 mol L^{-1} NaOH) represent the level of ionic bonding, hydrogen bonding, hydrophobic interactions, disulfide bonding, and insoluble protein, respectively. Initially, 15 mL solution I was mixed with 3 g sample and homogenized for 3 min at 6000 rpm. Subsequently, to make sure the protein fully dissolved, the mixture was subjected to shaking at 140 rpm for 1 h. The supernatant was collected after centrifuging for 20 min at 10 000×*g*, and the precipitate was mixed with 15 mL solution II. The supernatant was collected after homogenization, shaking and centrifugation were performed under similar conditions. Then, the precipitate was further mixed in 15 mL solution III, IV, and V.

2.12. Determination of Fourier transform infrared (FTIR) spectroscopy

The FTIR test was performed according to the method reported by Deng *et al.* (2023)[26] with some modifications. At first, the clam surimi gel samples were freeze-dried, and then mixed with

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KBr in the ratio of 1 : 100. After grinding to powder form using a mortar and pestle, the FTIR spectra of the samples were recorded using an FTIR spectrometer (PerkinElmer, USA) in the wavenumber range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}.

2.13. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein profiles of the clam surimi gels were obtained using SDS-PAGE following the methods reported by Stangierski *et al.* (2021).[10] Briefly, the samples were lyophilized, and then dissolved in 2× uploading buffer at a concentration of 1.5 mg mL^{-1}. High molecular weight markers ranging from 5 to 245 kDa were used, and Coomassie Brilliant Blue R-250 was utilized for gel staining.

2.14. Microstructure

Cryo-SEM images were taken using the methods reported by Yan *et al.* (2022).[27] Firstly, the clam surimi gels were immersed in a liquid nitrogen slush. To remove the free water, the frozen sample was transferred to a preparation chamber (PP3010T cryo-SEM preparation system, Quorum Technologies, UK) and subjected to 40 min of vacuum treatment at −60 °C. Pt was then sprayed onto the surface of the sample. The images were taken using an SU8000 scanning electron microscope (Hitachi Co., Ltd, Tokyo, Japan) at an acceleration voltage of 10.0 kV. The pore size of the sample microstructure was analyzed using ImageJ2 software (National Institutes of Health, USA).

2.15. Data analysis

The mechanical properties of the samples were measured at least six times, while other experiments were carried out in triplicate. Graphs were created using Origin Pro 2019 (Origin Lab Corporation, MA, USA). The SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA) was used to conduct analysis of variance (ANOVA), followed by post-hoc testing including Duncan's multiple range test and least significant difference (LSD) test, to ensure the statistical validity. $p < 0.05$ was deemed statistically significant, and the results were presented as the mean ± standard deviation.

3. Results and discussion

3.1. Effects of different pretreatment regimens on the breaking force and whiteness of the clam surimi gels

To eliminate the negative effects caused by the clam visceral mass, the pretreatment method for the raw clam materials was optimized to streamline the operational process for subsequent experiments. The whiteness and breaking force were chosen to assess the effect of three pretreatment methods. As shown in Fig. 1, the gel achieved from mechanical chopping without rinsing clam meat exhibited a greener color, with the whiteness level approximately 8.5% lower than that of the mechanically pretreated gel and 10.8% lower than that of the manually pretreated gel. Moreover, the breaking forces of both the mechanical and manual pretreated gels greatly surpassed that of the control group, with the mechanical pretreatment gel showing an improvement of 61.2% compared to the control group. Notably, there was no significant difference in breaking force between the mechanically pretreated gel and the manually pretreated gel.

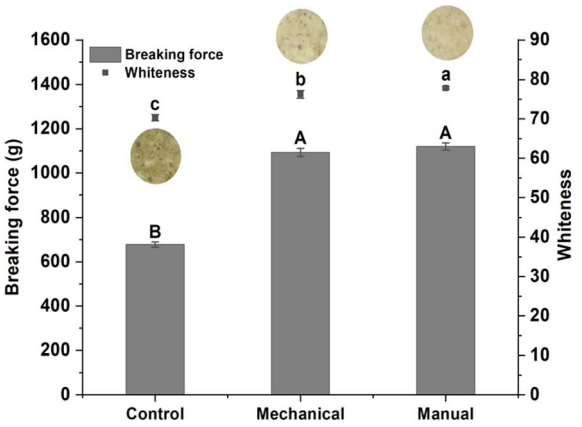

Fig. 1 Effects of different pretreatments on the breaking force and whiteness of the clam surimi gels. The bars represent the standard deviations from triplicate determinations. The different lowercase letters and uppercase letters in the same group indicate significant differences between different pretreatments ($p < 0.05$).

The visceral mass might account for the differences in the appearance and whiteness of the clam surimi gel. Clams feed mainly on algae and plankton, which results in their visceral mass having a dark green color, affecting the color of the samples. Therefore, the control group without rinsing might retain more pigment, decreasing the whiteness. The difference in breaking force could be because some of the myofibrillar proteins were destroyed by endogenous proteases.[28] In general, rinsing, a common procedure in surimi products processing, has been shown to improve the gelation of various aquatic raw materials, such as shrimp,[6] fish,[29] and bivalve.[10] Owing to the rinsing process, the breaking forces of the treated groups were much greater than that of the control group (Fig. 1). Notably, there was no significant difference between the mechanical pretreatment sample and the manual pretreatment sample (Fig. 1). Thus, to simplify the process, mechanical pretreatment was chosen for further study. The following experiments were carried out to determine the effect of MTGase on the gelation of clam surimi.

3.2. Visible appearance and cooking loss of clam surimi gels with different MTGase

The visible appearance and cooking loss can reflect the quality of surimi products. A denser visible structure is more popular with consumers, while cooking loss quantifies the loss of moisture and nutrients during heating. As given in Fig. 2A, the clam surimi gel exhibited multiple irregular voids in the control group, with relatively lower cooking loss (Fig. 2B). No significant difference ($p > 0.05$) was found in the cooking loss among the samples with MTGase added at levels below 2.0 U g $surimi^{-1}$, but a significant increase ($p < 0.05$) occurred when more than 2.0 U g $surimi^{-1}$ MTGase was incorporated (Fig. 2B).

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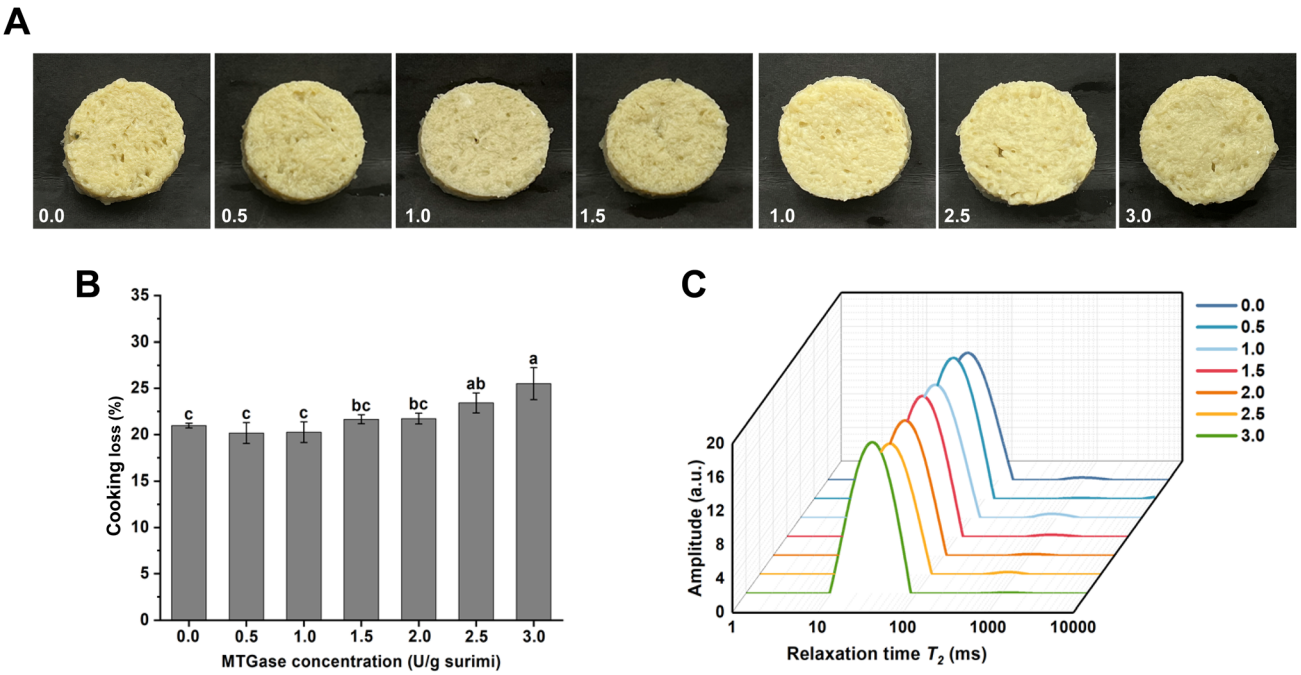

Fig. 2 Effects of different MTGase concentrations on the appearance (A), cooking loss (B), and T_2 relaxation times (C) of clam surimi gels. The bars represent the standard deviations from triplicate determinations, and the different lower-case letters in the same group indicate significant differences between the different MTGase concentrations ($p < 0.05$) in (B). MTGase, microbial transglutaminase. The numbers 0.0–3.0 in (A) and (C) refer to the concentration of MTGase addition (U g surimi^{-1}).

MTGase has been found to promote myofibrillar protein interactions,[18] resulting in a denser cross-sectional structure in the surimi gel. This denser structure indicates a more compact internal gel network. However, the increased compactness of the gel network might lead to higher cooking loss. This differed from the results outlined by Chen *et al.* (2024),[20] who reported that MTGase reduced the cooking loss in shrimp surimi gels. One possible reason for this is that the denser network structure provides less space for water retention, thereby leading to a higher cooking loss.[19] A similar phenomenon was observed by Jia *et al.* (2016),[24] where the cooking loss of black carp actomyosin gels showed a rise with increasing MTGase concentrations.

3.3. Texture properties and breaking force of clam surimi gels with different MTGase

TPA analysis can be used to obtain a range of data by simulating the chewing behavior of food in the mouth, and the breaking force can be measured by compressing the gel.[20] As shown in Table 1, when the concentration of MTGase was increased from 0.0 to 2.0 U g surimi^{-1}, the hardness, gumminess, and chewiness of the samples improved significantly ($p < 0.05$), increasing from 1931.9 ± 42.3 g, 1605.7 ± 23.0, and 1464.6 ± 37.5 to 2467.4 ± 77.7 g, 2068.3 ± 66.5, and 1921.8 ± 115.2, respectively. Furthermore, there was a consistent increase in the breaking force of the clam surimi gels with an increase in MTGase concentration. The breaking force is a key component of gel strength, reflecting the maximum force required to rupture the gel.[30] Also, a higher gel strength generally corresponds to greater breaking force. Specifically, the addition of 2.0 U g surimi^{-1} MTGase led to a 28.6% improvement in the breaking force of the sample (Table 1). However, further elevating the MTGase concentration from 2.0 to 3.0 U g surimi^{-1} did not result in a significant ($p > 0.05$) modification of the texture properties or breaking force.

Within a suitable concentration range, the improved mechanical properties of clam surimi gels might be attributed to the fact that an appropriate concentration of MTGase could generate cross-links between myofibrillar proteins, which corresponded with the more compact visible appearance of the sample with added MTGase (Fig. 2A). However, the excessive cross-links induced by high MTGase concentrations might disrupt the formation of an ordered gel matrix, making it brittle[31] and preventing a significant sustained increase in breaking force and textural properties (Table 1). In addition, the relatively low content of myofibrillar protein in raw clam meat also hindered a sustained increase in breaking force. Therefore, the mechanical properties of the clam surimi samples were strengthened by the addition of MTGase. It is worth noting that the optimal concentration of MTGase for enhancing the mechanical properties of clam surimi gels (2 U g surimi^{-1}) differs from that for snakehead fish (0.28 U g surimi^{-1})[32] and shrimp *Trachypenaeus curvirostris* (30 U g surimi^{-1}).[33] This difference might be attributed to the variations in the protein composition of different species and the specific properties (*e.g.*, source and activity) of the MTGase used.

3.4. Water status of clam surimi gels with different MTGase

The water status of surimi gel plays a critical role in influencing its gel properties.[34] In this study, both the EWC and relaxation times (T_2) were used to analyze the water status of the samples.

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Table 1 Effects of different MTGase concentrations on the breaking force and textural properties of the clam surimi gels[a]

MTGase concentration (U g surimi^{-1})	Hardness (g)	Gumminess	Chewiness	Breaking force (g)
0.0	1931.9 ± 42.3^{d}	1605.7 ± 23.0^{d}	1464.6 ± 37.5^{e}	1093.6 ± 18.3^{e}
0.5	2153.8 ± 121.8^{c}	1791.0 ± 87.7^{c}	1615.3 ± 134.8^{d}	1160.8 ± 44.4^{d}
1.0	2217.2 ± 65.6^{c}	1863.9 ± 62.3^{c}	1712.9 ± 33.5^{cd}	1236.0 ± 29.5^{c}
1.5	2359.4 ± 33.3^{b}	1987.8 ± 26.9^{b}	1778.4 ± 117.0^{bc}	1306.8 ± 21.2^{b}
2.0	2467.4 ± 77.7^{ab}	2068.3 ± 66.5^{ab}	1921.8 ± 115.2^{ab}	1406.0 ± 23.8^{a}
2.5	2494.5 ± 26.3^{a}	2103.3 ± 37.8^{a}	1966.1 ± 113.8^{a}	1388.2 ± 47.7^{a}
3.0	2534.4 ± 39.4^{a}	2157.2 ± 30.0^{a}	2005.0 ± 24.5^{a}	1421.4 ± 47.6^{a}

[a] Data are reported as mean ± SD from triplicate determinations. Different letters in the same column indicate significant difference.

A lower EWC corresponds to superior water holding capacity.[35] As presented in Table 2, the group without the incorporation of MTGase exhibited the highest EWC of 25.3%. When the amount of MTGase added was increased from 0.5 to 2.0 U g surimi^{-1}, the EWC gradually decreased to a minimum value of 17.6%, and no significant difference ($p > 0.05$) was observed beyond 2.0 U g surimi^{-1}. Similarly, the T_2 patterns showed the same trend. LF-NMR can be applied to assess the mobility of water molecules in a static magnetic field by measuring their spin–spin relaxation times (T_2).[36] Fig. 2C shows two distinct peaks in the T_2 spectra of the clam surimi gels, mainly at 10–100 ms (T_{22}) and approximately 1000 ms (T_{23}), representing immobile water and free water, respectively. Among the samples, T_{22} predominated, accounting for over 99% of the total. T_{22} is attributed to water tightly held within the spaces between muscle fibers, demonstrating its strong entrapment by the structural protein network.[37] As the enzyme concentration increased (Fig. 2C), the peak apex of T_{22} gradually shifted to the left. For instance, as displayed in Table 2, the T_{22} of the samples was accelerated from 42.94 ms to 31.08 ms when the MTGase concentration reached 3.0 U g surimi^{-1}, accompanied by a proportional decrease in EWC. This suggested that the samples exhibited better water holding properties.

The above-mentioned improvement might be attributed to the promotion of protein cross-linking by MTGase, resulting in more uniform and narrower voids in the gel network, thereby retaining more water.[20] Also, this could correspond to the more uniform cross-section of the samples with the addition of MTGase (Fig. 2A). The progressive shortening of T_{22} indicated an enhanced water binding capacity, resulting in the reduced EWC of the sample with the addition of MTGase (Table 2). Previous research has demonstrated that MTGase induced conformational changes in myofibrillar proteins, enhancing their interactions with water molecules, and consequently restricting their mobility.[38] Luo *et al.* (2020)[34] also reported that a decrease in T_{22} occurred with the increasing MTGase addition in silver carp surimi gels. In contrast to our findings, An *et al.* (2021)[18] observed an increase in the proportion of free water in MTGase-treated silver carp surimi gels. This discrepancy may arise from the intrinsic differences in the water-binding properties of proteins from different species. These results indicated that MTGase resulted in the tighter binding of water molecules within the clam surimi gel.

Table 2 Effects of different MTGase concentrations on the T_{22} relaxation time and expressible water content of the clam surimi gels[a]

MTGase concentration (U g surimi^{-1})	EWC (%)	Relaxation time T_{22} (ms)
0.0	25.4 ± 0.8^{a}	42.9 ± 1.2^{a}
0.5	23.9 ± 1.1^{ab}	42.3 ± 1.7^{a}
1.0	22.4 ± 0.8^{b}	39.2 ± 1.9^{b}
1.5	20.3 ± 0.8^{c}	39.2 ± 0.5^{b}
2.0	17.6 ± 0.5^{d}	35.2 ± 2.4^{c}
2.5	16.9 ± 1.4^{d}	33.3 ± 0.4^{cd}
3.0	16.0 ± 0.7^{d}	31.1 ± 0.4^{d}

[a] Data are reported as mean ± SD from triplicate determinations. Different letters in the same column indicate significant difference.

3.5. Rheological properties of clam suwari gels with different MTGase concentrations

The rheological properties of surimi gels reflect the changes in their viscoelastic behavior under external forces, as defined by the storage modulus (G') and loss modulus (G''). G' represents the elastic properties of the surimi gels, while G'' shows their viscous properties.[39] The optimum temperature range for MTGase activity has been reported to be between 40 °C and 70 °C.[23] Therefore, to explore the effect of MTGase on clam surimi, the suwari gel formed at 45 °C (the first step heating) was chosen for this experiment. The impacts of different MTGase levels on the G' and G'' of the clam suwari gels are depicted in Fig. 3A. When the MTGase concentration increased, both G' and G'' increased, and then decreased. The highest G' was obtained at an enzyme concentration of 2.0 U g surimi^{-1} compared to the control group (0.0 U g surimi^{-1}), elevating from 9915.09 Pa to 14 321.40 Pa at 10 Hz. There was no significant difference between the samples with 2.0 and 2.5 U g surimi^{-1} MTGase. However, the addition of 3.0 U g surimi^{-1} MTGase resulted in a decline in G'.

The higher values of G' might be attributed to the increased cross-linking between the myofibrillar proteins. At 45 °C, the actin-myosin complex starts to be separated by endogenous proteolysis, resulting in increased protein mobility.[6] Additionally, 45 °C was in the optimum temperature range for MTGase activity,

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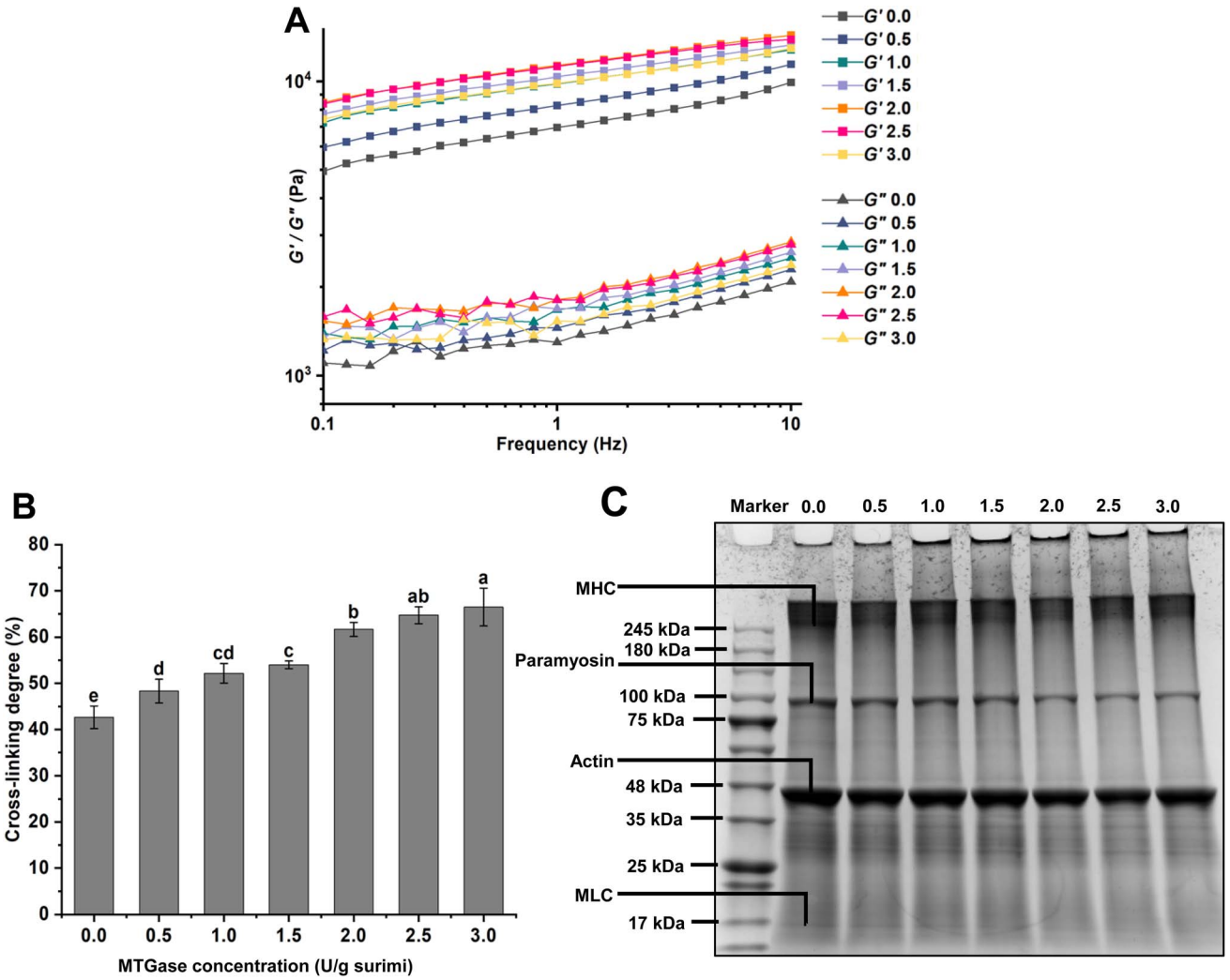

Fig. 3 Rheological properties (A) of the clam suwari gels, protein cross-linking degree (B) and SDS-PAGE patterns (C) of the clam heat-induced gel treated with MTGase at different concentrations. The bars represent the standard deviations from triplicate determinations, and the different lower-case letters in the same group indicate significant differences between the different MTGase concentrations ($p < 0.05$) in (B). MTGase, microbial transglutaminase. The numbers 0.0–3.0 in (A) and (C) refer to the concentration of MTGase addition (U g surimi^{-1}).

facilitating acyl transfer reactions that promote greater cross-linking.[23] As mentioned earlier, enhanced protein cross-linking would provide better stiffness support for samples, consequently elevating both G' and G''. Seighalani *et al.* (2017)[2] also showed that the G' of red tilapia surimi gel at 40 °C was elevated by the addition of MTGase. This result corresponded with the outcome of the mechanical properties (Table 1). As MTGase concentration increased, a more continuous gel network was formed, leading to enhanced mechanical and rheological properties of the clam surimi gels. However, at MTGase concentrations exceeding 2.5 U g surimi^{-1}, the decrease in G' and G'' suggested that continuous MTGase addition could promote excessive isopeptide bonding formation, potentially compromising the gel network by weakening other intermolecular interactions.[18] Consequently, the gel might become brittle. Chen *et al.* (2022)[3] also reported that the MTGase-induced surimi network mainly provided hardness and crispness.

3.6. Protein cross-linking degree of the clam surimi gels with different MTGase concentrations

The level of protein cross-linking serves as an important indicator for revealing the effect of MTGase on surimi gels. MTGase can help proteins unfold and expose their amino acid groups, which is a prerequisite for MTGase-catalyzed protein cross-linking (An *et al.*, 2021).[18] The effects of MTGase on the protein cross-linking degree in the clam surimi gels are shown in Fig. 3B. As the concentration of MTGase increased, the degree of protein cross-linking exhibited a gradual increase from 42.7% to 66.5%, but the rate of increase decelerated once the MTGase amount exceeded 2.0 U g surimi^{-1}.

This result demonstrated that MTGase indeed increased the covalent cross-linking of clam myofibrillar proteins, which is consistent with the findings of MTGase in Zhikong scallop[9] and black carp actomyosin.[24] However, their limited glutamine and lysine content constrained the rate of protein cross-linking

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progression. The rheological properties (Fig. 3A) and textural properties (Table 1) of the samples corroborated these outcomes. As protein cross-linking intensified, the gel network gradually became dense, leading to increased G' values and mechanical properties. Despite this, when the MTGase concentration exceeded 2.0 U g surimi^{-1}, the G' and textural properties could not be enhanced consistently.

3.7. Intermolecular interactions of clam surimi gels with different MTGase concentrations

The above-mentioned experimental results suggested that MTGase contributed to enhancing the gel properties of the clam surimi gels, potentially leading to alterations in the intermolecular interactions maintaining the gel network. As presented in Table 3, covalent bonding played an important role in maintaining the gel network. Disulfide bonds were dominant and affected by MTGase, which increased from 48.0% to 58.7%, and then decreased to 49.5%. Concomitantly, the proportion of insoluble protein increased gradually from 4.6% to 20.3%. In addition, hydrophobic interactions were the main non-covalent interactions maintaining the clam surimi gel (Table 3), which decreased with an increase in MTGase concentration from 44.4% to 26.1%. Notably, as the enzyme level increased from 0.0 to 2.0 U g surimi^{-1}, the amount of hydrogen bonds gradually declined (Table 3).

Disulfide bonds are engaged in the formation of gel networks during heating. The addition of MTGase results in the exposure of additional sulfhydryl groups, leading to an increase in disulfide bonds. Nevertheless, the disulfide bonds could be reduced by the addition of excess MTGase.[40] The increase in insoluble proteins was mainly attributed to MTGase-induced crosslinking. This phenomenon is in line with the findings of Zhong *et al.* (2023),[41] where the proportion of disulfide bonds declined in liver carp surimi gels upon the addition of MTGase, accompanied by an increase in the insoluble protein content. Therefore, based on the data in Table 3, it is reasonable to speculate that during the low-temperature gelation of clam surimi (45 °C for 45 min), MTGase at a concentration of 2 U g surimi^{-1} might mainly promote the aggregation of clam protein molecules by stronger covalent interactions.

In addition, non-covalent bonds also play an irreplaceable role in gel formation. The slight decrease in hydrophobic interactions might be attributed to the higher presence of amino cross-links and disulfide bond formation (Table 3), which reduced the binding sites of clam myofibrillar proteins to water molecules. Consequently, the reduced interaction with water molecules might lead to an increase in cooking loss (Fig. 2B), while the denser gel network limited the movement of water molecules, resulting in an increase in water holding capacity (Fig. 2C and Table 2). However, in silver carp surimi gels, the excessive cross-linking induced by MTGase increased the proportion of hydrophobic interactions.[18] This effect may be attributed to the differential response of various protein types to MTGase.

3.8. Protein patterns of clam surimi gels with different MTGase concentrations

The protein patterns illustrate the impact of MTGase on protein aggregation. As displayed in Fig. 3C, the bands representing myosin heavy chains (MHC, approximately 250 kDa) became progressively weaker, while the bands with molecular weights above 250 kDa, as well as uptake port residues, correspondingly became stronger. Moreover, the bands corresponding to paramyosin (approximately 90 kDa) and myosin light chain (MLC, approximately 17 kDa) also exhibited a decrease in intensity. It has been reported that during gelation, transglutaminase-catalyzed cross-linking reactions mainly target the myosin heavy chains of myofibrillar proteins.[42] In addition, the weakened bands corresponding to paramyosin and MLC suggested the formation of isopeptide bonds facilitated by MTGase. This finding aligns with the report by An *et al.* (2021),[18] who studied the influence of MTGase on silver carp surimi gels. Additionally, the protein aggregation behavior in clam surimi gels exhibited a consistent trend without a distinct turning point as the MTGase concentration increased, which aligned with the observed pattern in the protein cross-linking degree (Fig. 3B).

3.9. FTIR spectra of clam surimi gels with different MTGase concentrations

FTIR is an effective analytical method for identifying functional groups and changes in the conformation of biomacromolecules.[27] As shown in Fig. 4A, the band initially red shifted from 3364 cm^{-1} to the maximum of 3410 cm^{-1} as the MTGase concentration increased from 0.0 to 2.0 U g surimi^{-1}.

Table 3 Effects of different MTGase concentrations on the relative proportions of chemical forces of the clam surimi gels[a]

MTGase concentration (U g surimi^{-1})	Relative proportions of chemical forces (%)				
	Ionic bonds	Hydrogen bonds	Hydrophobic interactions	Disulfide bonds	Insoluble proteins
0.0	1.9 ± 0.1^{d}	1.1 ± 0.2^{a}	44.4 ± 1.3^{a}	48.0 ± 1.1^{d}	4.6 ± 0.3^{g}
0.5	2.2 ± 0.1^{abc}	1.1 ± 0.1^{a}	34.8 ± 0.2^{b}	55.8 ± 0.1^{b}	6.2 ± 0.3^{f}
1.0	2.2 ± 0.1^{bc}	1.0 ± 0.0^{ab}	30.8 ± 1.4^{c}	57.8 ± 1.5^{ab}	8.2 ± 0.2^{e}
1.5	2.1 ± 0.1^{cd}	0.9 ± 0.0^{b}	27.0 ± 0.5^{d}	58.7 ± 0.9^{a}	11.2 ± 0.4^{d}
2.0	2.3 ± 0.1^{ab}	0.9 ± 0.1^{b}	26.1 ± 0.5^{d}	55.7 ± 0.4^{b}	15.1 ± 0.2^{c}
2.5	2.4 ± 0.3^{a}	1.0 ± 0.1^{ab}	26.5 ± 1.6^{d}	51.5 ± 2.4^{c}	18.7 ± 0.6^{b}
3.0	2.4 ± 0.0^{ab}	1.0 ± 0.0^{a}	26.8 ± 1.1^{d}	49.5 ± 1.0^{cd}	20.3 ± 0.4^{a}

[a] Data are reported as mean ± SD from triplicate determinations. Different letters in the same column indicate significant difference.

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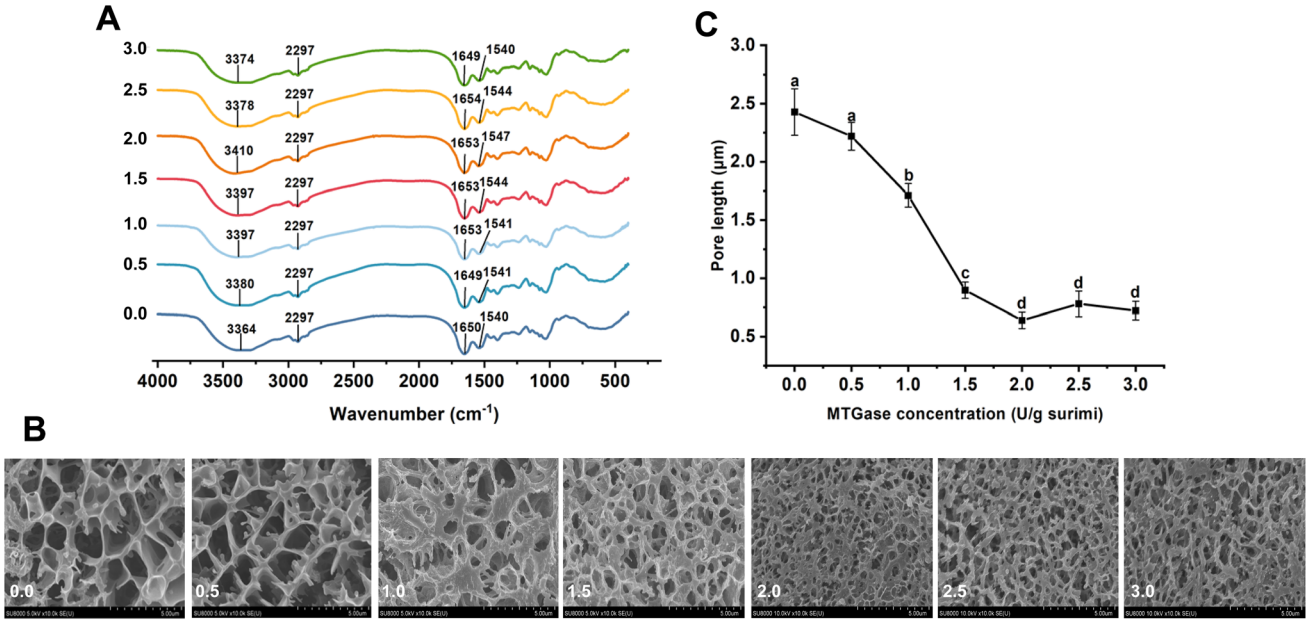

Fig. 4 FTIR spectra (A), cryo-SEM images (B), and pore length (C) of the clam surimi gel induced by MTGase at different concentrations. The bars represent the standard deviations from triplicate determinations, and the different lower-case letters in the same group indicate significant differences between the different MTGase concentrations ($p < 0.05$) in (C). MTGase, microbial transglutaminase. The numbers 0.0–3.0 in (A) and (B) refer to the concentration of MTGase addition (U g surimi^{-1}).

Subsequently, when the MTGase concentration was further increased to 3.0 U g surimi^{-1}, the band exhibited a blue shift to 3374 cm^{-1}. In addition, the characteristic peak of the clam surimi gel at 1600–1700 cm^{-1} (amide I band) and 1530–1550 cm^{-1} (amide II band) shifted upon the addition of MTGase.

The band at 3000–3600 cm^{-1} corresponds to the O–H stretching vibrations of hydroxyl groups.[26] The red shift indicates a decrease in O–H binding through hydrogen bonding, leading to the shift to a higher frequency.[26] In addition, the amide I and II bands represent the two most important characteristic absorption peaks, which directly reflect the conformation of the protein backbone and the state of hydrogen bonds, respectively.[27] Marcelino & Gierasch (2008)[43] reported that the shift in protein from α-helix to β-turn results in a decrease in hydrogen bonds. The observed changes might indicate that MTGase facilitated a structural transition in the clam proteins from α-helix to β-turn, which is consistent with the alteration in functional properties. An *et al.* (2021)[18] also observed that after the incorporation of MTGase, the secondary structure of silver carp (*Hypophthalmichthys molitrix*) protein was altered from α-helix to β-sheet and β-turn.

3.10. Microstructure of clam surimi gels with different MTGase concentrations

The gel network can be directly characterized by its microstructure. Fig. 4B and C show the microstructure of the clam surimi gels with and without MTGase, respectively, as well as the pore size of their gel network. As the addition of MTGase gradually increased to 2.0 U g surimi^{-1}, the pores in the gel network became smoother and more ordered, with thicker pore walls observed compared to the control group. Compared to the control group, the pore length of the sample with MTGase at 2 U g surimi^{-1} significantly decreased from 2.43 to 0.64 μm. This result is in agreement with the findings of Jia *et al.* (2016),[24] who reported that the network structure of the black carp actomyosin gel was improved with the addition of MTGase. Notably, when MTGase concentration exceeded 2.0 U g surimi^{-1}, minimal changes were observed in the microstructure (Fig. 4B), and no significant difference in pore size was detected (Fig. 4C). This observation correlated with the non-significant change ($p < 0.05$) in breaking force (Table 1) and slower rate of protein cross-linking (Fig. 3B).

The above-mentioned results confirm our conjecture that as the enzyme concentration increases, more protein cross-linking (Fig. 3B) leads to a denser gel network (Fig. 4B), which provides more rigid support for the clam surimi gel, subsequently improving its mechanical properties (Table 1). Beyond that, the gel network is associated with the rheological properties obtained *via* frequency sweep in the linear viscoelastic region.[44] G' can be considered as the elastically active chains in the network.[45] As the MTGase concentration increased, the more compact network (Fig. 4B) induced by MTGase corresponded to a higher G' value (Fig. 3A), indicating that more protein chains were connected. This is also evidenced by the results of the protein pattern (Fig. 3C) that the aggregation of the clam proteins was enhanced by MTGase, thus increasing the content of disulfide bonds (Table 3). However, when the MTGase concentration exceeded 2.0 U g surimi^{-1}, the ratio of disulfide bonds and hydrophobic interactions was reduced. This disruption resulted in an excessively rigid structure, causing the gel to become brittle and reducing the storage modulus (G') of the samples (Fig. 3A). Additionally, the smaller pore (Fig. 4C)

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leads to increased cooking loss (Fig. 2B). However, its compact structure traps moisture within the network, enhancing the water holding capacity (Table 2 and Fig. 2C).

4. Conclusion

In the present study, mechanical chopping combined with rinsing was found to be an appropriate method to mitigate the detrimental effects caused by clam visceral mass. In addition, MTGase at 2 U g surimi^{-1} was identified as the optimal concentration for enhancing the gelation of clam surimi, as evidenced by the improvements in mechanical properties and water holding capacity. During gelation, MTGase promoted myofibrillar protein aggregation in the clam surimi, which thereby facilitated the formation of more disulfide bonds. In summary, this study provides a scientific foundation for the development of MTGase-induced clam surimi products. Further study can focus on quantitative prediction models to characterize the effect of MTGase with other substances, thereby meeting the specific requirements of various clam surimi products.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data would be available on request.

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