



Independent and combined effects of ultrasound and transglutaminase on the gel properties and in vitro digestion characteristics of bay scallop (*Argopecten irradians*) adductor muscle

Jiaqi Feng, Jie Wang, Tong Zhang, Yaqiong Liu^{*}, Ran Suo, Qianyun Ma

College of Food Science and Technology, Hebei Agricultural University, Baoding, 071000, Hebei, China

ARTICLE INFO

Handling Editor: Professor Aiqian Ye

Keywords:

Bay scallop
Ultrasound treatment
Transglutaminase
Gel properties
In vitro digestion

ABSTRACT

The effects of transglutaminase (TGase) addition (0.4–1.2 g/100g), ultrasound (120–720 W, 20 min), and their combination on the gel properties and in vitro digestion characteristics of bay scallop adductor muscle were studied. The gel strength of the gel sample with TGase content of 0.8 g/100g (TG-0.8) was 58.2% higher than that of the control sample (CON). The gel sample treated with ultrasound at 480 W (UT-480) had the highest gel strength. The strength of the gel prepared by combination of 0.8 g/100g TGase and 360 W ultrasound (UT-TG) was 82.3% higher than that of CON. The whiteness and water holding capacity of the gel increased regardless of the addition of TGase or ultrasound treatment. SDS-PAGE patterns showed that the myosin heavy chain of the treated samples became thinner, and the changes of actin and tropomyosin were not significant. The scanning electron microscopy results of gel samples prepared by ultrasound combined with TGase showed a denser structure, which was related to the lowest total sulfhydryl content and TCA-soluble peptide content. The results of dynamic rheology show that the UT-TG sample had the highest G' value, followed by TG-0.8. The in vitro digestion characteristics of the selected gel samples were also discussed. The degree of protein hydrolysis and the content of free amino acids in TG-0.8 samples were the lowest, which improved after ultrasound treatment. Overall, the combination of appropriate ultrasound treatment and TGase addition provides an effective means for improving gel properties and digestibility of scallop surimi product.

1. Introduction

Surimi products such as fish balls, kamaboko and chikuwa have been recognized for their unique gelling properties and high nutritional value (Monto et al., 2021). Nowadays, with the change of consumer habits, the texture and taste of various seafood minced meat, such as crab sticks and shellfish analogues, are favored because of their sensory characteristics (Wakako YOSHIDA et al., 2003). Among them, shellfish analogues are free-shellfish meat food that contains surimi, exogenous additives and shellfish seasoning (Liu et al., 2020), its nutritional value is relatively low and cannot meet the needs of consumers. The bay scallop is very famous for the delicious taste and abundant nutrition of adductor muscle (Yi et al., 2013). However, some broken adductor muscles and small-size adductor muscles have low commercial value and can be used to produce shellfish surimi to increase its added value. But the gel properties of bay scallop adductor muscle (BSM) are poor and could not form good gel texture. At present, many reports have reported that

various exogenous additives such as transglutaminase (TGase) and hydrocolloids and/or physical treatments such as microwave, high hydrostatic pressure and ultrasound application are reported to improve the gel strength of aquatic product gels (Fang et al., 2019; Gao et al., 2021a; Liang et al., 2020b; Zhang et al., 2019; Zhu et al., 2014). However, the preparation of BSM gel by combining food additives with physical methods has not been reported.

TGase is a commonly used protein cross-linking that can improve the physical and chemical properties and network structure of the protein gel (Gaspar and de Goes-Favoni, 2015). The enzyme is able to catalyze the transfer of γ -carboxamide group of glutamine residue in proteins to the ϵ -amino group of lysine residues. This reaction results in the formation of ϵ -(γ -glutamyl) lysine isopeptide and inter-protein covalent bonding, thus improving the texture of cross-linked protein gel-like products. Zhu et al. (2014) found that TGase can improve the gel properties of Alaskan pollock surimi after high-pressure treatment. However, with an excessive addition of TGase, surimi gel shows distinct

^{*} Corresponding author.

E-mail address: shplyq@hebau.edu.cn (Y. Liu).

<https://doi.org/10.1016/j.crfs.2022.07.009>

Received 9 March 2022; Received in revised form 23 May 2022; Accepted 17 July 2022

Available online 31 July 2022

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properties that transform them from a viscoelastic to a crisp body (Singh et al., 2020), and higher cross-linking results in decreased digestibility and hence reduced the bioavailability of proteins or peptides for absorption into the body (Su and Cavaco-Paulo, 2021). Fang et al. (2019) reported that TGase induction of protein cross-linking reduced the in vitro digestive properties of silver carp. However, studies about the application of TGase to BSM gel are scarce.

In recent years, ultrasound treatment (UT) has been widely studied in the food processing field due to its non-pollution, safety and easy operation. It is an effective method to improve the food function of proteins by changing the internal structure and intermolecular force of protein molecules (Liu et al., 2017). Study have shown that ultrasound can improve the solubility of myosin (Chandrapala et al., 2011). In addition, the cavitation phenomenon generated by ultrasound could be beneficial to the structural changes of proteins and expose reactive residues to the surface. These changes facilitated protein-protein interactions and were important for the formation of elastic gels (Jambrak et al., 2014; Liu et al., 2007; Singh & Benjakul, 2017). Gao et al. (2021b) reported that ultrasound pretreatment made silver carp surimi formed a more stable gel. Valdez-Hurtado et al. (2019) found that ultrasound also greatly improved the gel properties of squid. In addition, it has been reported that ultrasound can increase the contact point and contact probability between enzyme and protein, and further promote the role of enzyme (Chen et al., 2022). Hu et al. (2015) successfully increased the degree of enzymatic cross-linking TGase cross-linked SPI with high intensity ultrasound (20 kHz, 400 W). Qin et al. (2016) found that ultrasound pretreatment significantly improved the gel properties of SPI/WG gel induced by MTGase. Ahmadi et al. (2017) reported that moderate ultrasound treatment (20 kHz, 500W) is beneficial to change the structure of whey protein and make it more vulnerable to TGase, thus improving its functional properties. Moreover, it is still unknown whether ultrasound, TGase and their combined effect will improve the gel properties of scallop protein, how their mechanism of action, and what impact they will have on the in vitro digestion characteristics of scallop gel, which still need to be further studied.

The aim of this study was to investigate the independent and combined effects of TGase and UT on the gel properties and in vitro digestibility of BSM gel. The effect on the gel properties was determined by measuring the gel strength, whiteness, water holding capacity and scanning electron microscopy. The protein changes were analyzed by SDS-PAGE, total sulfhydryl content and TCA-soluble peptides. The changes of in vitro digestibility of the gel were evaluated by measuring the degree of protein hydrolysis and the content of free amino acids.

2. Materials and methods

2.1. Materials

About 10 kg of fresh BSM was purchased from a local market (Baoding, Hebei, China). It was transported back to the laboratory of Hebei Agricultural University in half an hour and stored in a refrigerator at -20°C . TGase was purchased from Yuantai Biology Co., Ltd. (Shandong, China). All reagents used were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The instruments used for ultrasonic processing was KQ-1018 ultrasonic processor (Dongguan Keqiao Ultrasonic equipment Co., Ltd., Dongguan, China).

2.2. Preparation of BSM gel

After the BSM was thawed at 4°C , 100 g was placed into a food grinder (Jiuyang Co., Ltd, Jinan, China) and stirred for 2 min. Then, 1% salt was added and stirred for 1 min. TGase (0, 0.4, 0.6, 0.8, 1.0, 1.2 g/100g) was added into the meat paste based on the wet weight of muscle, the moisture content was adjusted to 80 g/100g with ice water. The mixture continued to be chopped for 2 min. The paste was squeezed into

polyamide casings (Dalian Zongbaiwei Food ingredients Co., Ltd., Dalian, China), and both sides were sealed tightly. The samples were heated in a water bath at 40°C for 20 min, followed by 90°C for 30 min. Finally, the gel samples were immersed in ice water for 15 min and then stored at 4°C until the following analyses.

In order to study the independent and combined effects of UT and TGase, BSM paste was treated with ultrasound at different power settings (120, 240, 360, 480, 600, 720 W) in the absence or presence of TGase at 0.8 g/100g paste. BSM gel was prepared as given previously and analyzed.

2.3. Gel strength and textural profile analysis (TPA)

The gel samples were placed at 25°C for 30 min and then cut into cylinders ($2.0\text{ cm} \times 2.0\text{ cm}$). The strength of gels was analyzed using a texture analyzer (TMS-Pro, Food Technology Corporation, USA) with a spherical plunger (diameter 5 mm, P/5 S). The test speed was 1 mm/s, the puncture distance was 10 mm, and the trigger force was 10 N. The measured result was gel strength. Every measurement was repeated six times.

The gel samples were prepared similar to the gel strength test. TPA was measured with a texture analyzer with P/50 cylindrical probe (50 mm diameter). The test speed was 1 mm/s, the compression deformation was 50%, and the trigger force was 0.3 N. The hardness, springiness, chewiness, and cohesiveness of samples were obtained.

2.4. Water holding capacity (WHC)

Gel samples were cut into thin slices with a thickness of 5 mm, weighed accurately (W_1), and then wrapped with three layers of filter paper. After centrifugation at 9690 g and 4°C for 15 min, the gel samples were quickly weighed again (W_2). The WHC was calculated using the following formula (Valdez-Hurtado et al., 2019):

$$\text{WHC (\%)} = W_2/W_1 \times 100$$

2.5. Measurement of whiteness

The L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values of gel samples were determined with a colorimeter (CR-400, Konica Minolta Ltd., Osaka, Japan). The whiteness was calculated using the following formula (Fang et al., 2019):

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

2.6. Dynamic rheology

Dynamic rheological properties were determined on an AR2000ex dynamic rheometer (TA Co. Ltd., USA). According to the method of Singh et al. (2020), measurements were carried out immediately when BSM paste added without and with TGase was subjected to UT. A 25 mm parallel steel plate and cone geometry with a 1 mm gap was used. Then, gel samples were covered with silicone oil to avoid evaporation. Samples were heated at a rate of $2^{\circ}\text{C}/\text{min}$ from 4°C to 90°C . The oscillation strain was 0.5 Pa, and the oscillation frequency was 1 Hz. The elastic modulus (G') and δ value were recorded.

2.7. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

Gel samples (2 g) were dissolved in 5% SDS solution (18 mL) and homogenized for 3 min at 9690 g. The mixture was heated in a water bath at 85°C for 45 min and centrifuged at 9690 g for 10 min at 4°C (Gao et al., 2021a). The protein concentration of the supernatant was

determined by [Lowry et al. \(1951\)](#) method and adjusted to 2 mg mL^{-1} . Then, the protein solution (2 mg mL^{-1}) was mixed with the loading buffer at 4:1 (protein: buffer). The mixture was heated at 95°C for 5 min before loading. The concentration of separating and stacking gel was 10% and 5%, respectively. Each well was loaded with a $8 \mu\text{L}$ sample. The gel was run at a constant voltage of 120 V for 90 min. Afterward, the gel was dyed at 37°C for 20 min in staining solution (Coomassie Brilliant Blue G-250) until the appearance of clear protein bands.

2.8. Scanning electron microscopy (SEM)

The microstructures of gel samples were observed by SEM. According to the method of [Hu et al. \(2015\)](#) with some modifications. Gel samples were cut into small slices (3 mm thick) and immersed in 2.5% glutaraldehyde at 4°C for 12 h for fixation. Afterward, the gel samples were rinsed with phosphate buffer (0.2 M, pH 7.0) for four times and then dehydrated in gradient ethanol of 30%, 50%, 60%, 70%, 80%, 90% and 100% (once for 10 min). The dehydrated samples were sputter-coated with gold on a bronze stub, and the structure was observed using a scanning electron microscope (PrismaE, Thermo Fisher Scientific, Waltham, MA).

2.9. Total sulfhydryl (SH) content and TCA-soluble peptides (TSP)

The total SH content in BSM gel was determined by total sulfhydryl reagent kit (Solarbio, product number: BC1375). About 0.1 g of gel samples was added to the 1 ml extract to prepare the homogenate. The supernatant was determined by 8000 g centrifugation 10 min at room temperature according to the kit method. The standard solution is diluted into standard solution with different concentration gradients with distilled water. The contrast tube, measuring tube, standard tube and blank tube are prepared according to the kit instructions. After mixed homogeneously and balanced for 10 min at room temperature, absorbance values were measured at 412 nm by a full-wavelength microplate reader (MULTISKAN GO, Thermo Fisher Scientific, Madison, USA) and recorded as A_C , A_M , A_S , A_B respectively. The standard solution concentration is x-axis and $\Delta A_S = A_S - A_B$ is y-axis. The standard curve is drawn and the standard equation $y = kx + b$ is obtained. Then the $\Delta A_M = A_M - A_C$ is substituted into the formula to get x ($\mu\text{ mol/ml}$). The total SH content is calculated according to the formula:

$$\text{Total SH content } (\mu\text{mol/g}) = x/W$$

Where W(g) is the sample mass.

TSP were determined according to the method of [Gao et al. \(2021a\)](#) with modifications. About 3 g of gel samples was mixed with 27 mL of 5% (w/v) TCA, and the mixture was homogenized for 1 min. The homogenate was kept at 4°C for 60 min before centrifuging at 9690 g for 10 min. The content of peptides in the supernatant was determined according to [Lowry et al. \(1951\)](#) method.

2.10. In vitro digestion of BSM gel

In vitro digestion was conducted using the slightly modified method of [Singh et al. \(2020\)](#). The gel samples were homogenized with distilled water (1:10 w/v), and the sample liquid was adjusted to pH 2 with 1 M HCl. Then, 1 g of pepsin was added to the sample liquid and digested on a constant temperature shaker at 37°C for 1.5 h. Thereafter, the sample liquid was adjusted to pH 7.5 with 0.5 M NaOH, and 1 g of trypsin was added to the sample liquid and digested on a constant temperature shaker at 37°C for 2 h. Samples were boiled for 5 min to terminate the reaction, followed by cooling using iced water. All samples were centrifuged at 9690 g for 10 min, and the supernatant was obtained to determine the content of free amino acids and the degree of protein hydrolysis.

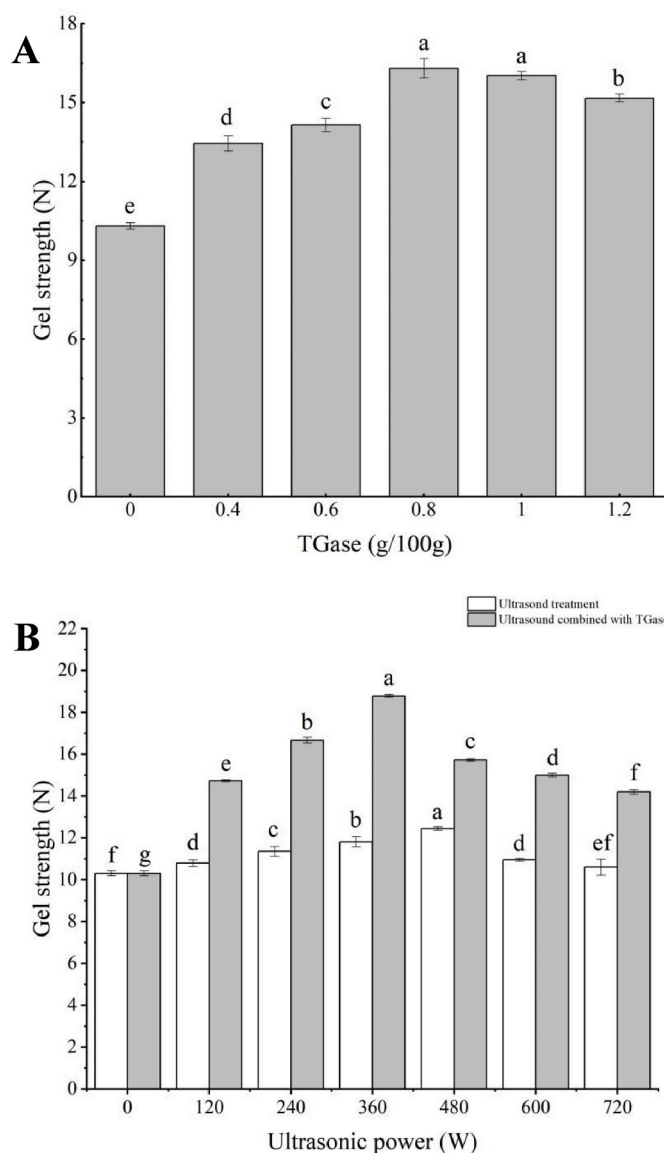


Fig. 1. Effect of TGase and UT on gel strength of Bay scallop adductor muscle gel. A: TGase alone at different concentrations; B: UT with different power and the combination of TGase at selected condition (0.8 g/100g) and UT at different concentrations. Different lowercase letters on the bars indicate significant difference ($p < 0.05$).

2.10.1. Determination of free amino acids

The digestibility of supernatant derived from the digestion solution was determined by measuring the amount of free amino acids. The determination of free amino acid content refers to the method of [Li et al. \(2019\)](#). About 0.4 mL of ninhydrin (2.0%) was added to dilute the sample (1.0 mL) with 0.4 mL of phosphate buffer (pH 8.0) and incubated at 100°C for 15 min. Then, samples were cooled to room temperature and diluted to 10 mL. The absorbance was recorded at 570 nm with a UV-2600 spectrophotometer (Unico, Shanghai) after samples were cooled to room temperature for 15 min. A blank was measured without protein solution.

2.10.2. Determination of the degree of protein hydrolysis

The degree of protein hydrolysis was slightly modified by referring to the method of [Noman et al. \(2018\)](#). To prepare neutral formaldehyde solution, 3 mL of 0.5% phenolphthalein indicator was added to 50 mL of formaldehyde solution and titrated to fine powder with 0.1 M standard sodium hydroxide solution. 2 mL of the supernatant of digested samples

Table 1
Effect of TGase and Ultrasound treatment on TPA of Bay scallop adductor muscle gel.

Groups	Component	Content of TGase	Ultrasonic power	Hardness/N	Springiness/mm	Gumminess/N	Chewiness/mJ
1	BSM	-	-	49.57±0.76 ^d	7.47±0.11 ^c	14.87±0.25 ^e	129.83±3.89 ^d
2	BSM + TGase	0.4g/100g	-	88.00±4.32 ^{bc}	7.86±0.16 ^{bc}	35.60±0.57 ^d	201.91±14.77 ^{bc}
3	BSM + TGase	0.6g/100g	-	95.93±1.92 ^b	8.17±0.10 ^a	41.45±1.62 ^b	225.05±14.33 ^b
4	BSM + TGase	0.8g/100g	-	106.43±1.48 ^a	8.26±0.45 ^a	47.63±0.45 ^a	268.01±12.32 ^a
5	BSM + TGase	1.0g/100g	-	90.37±3.91 ^{bc}	7.55±0.09 ^c	38.83±1.40 ^c	215.84±7.32 ^c
6	BSM + TGase	1.2g/100g	-	90.80±3.15 ^{bc}	7.67±0.15 ^{bc}	36.27±1.54 ^{cd}	263.00±3.46 ^{bc}
7	BSM	-	-	49.57±0.76 ^d	7.47±0.11 ^c	14.87±0.25 ^e	129.83±3.89 ^d
8	BSM + UT	-	120W	51.13±1.00 ^c	7.86±0.17 ^d	16.43±0.70 ^d	138.17±1.54 ^d
9	BSM + UT	-	240W	54.20±0.95 ^b	8.13±0.15 ^c	18.47±0.51 ^{bc}	147.56±2.07 ^c
10	BSM + UT	-	360W	55.03±1.50 ^b	8.80±0.13 ^b	19.47±0.81 ^b	154.20±4.04 ^b
11	BSM + UT	-	480W	56.60±0.46 ^a	9.10±0.13 ^a	20.77±1.15 ^a	162.94±3.55 ^a
12	BSM + UT	-	600W	50.20±0.36 ^{cd}	7.22±0.15 ^c	17.47±0.32 ^c	118.49±1.67 ^f
13	BSM + UT	-	720W	49.07±0.35 ^d	6.77±0.17 ^f	16.32±0.49 ^d	110.23±0.99 ^g
14	BSM	-	-	49.57±0.76 ^d	7.47±0.11 ^c	14.87±0.25 ^e	129.83±3.89 ^d
15	BSM + UT + TGase	0.8g/100g	120W	70.77±0.86 ^e	8.34±0.19 ^d	27.87±0.45 ^d	220.53±6.58 ^c
16	BSM + UT + TGase	0.8g/100g	240W	75.07±0.47 ^d	8.92±0.11 ^b	30.10±0.36 ^c	217.48±2.74 ^b
17	BSM + UT + TGase	0.8g/100g	360W	85.63±1.28 ^a	9.59±0.35 ^a	33.20±1.70 ^a	232.88±5.77 ^a
18	BSM + UT + TGase	0.8g/100g	480W	80.60±0.96 ^b	8.74±0.11 ^{bc}	32.17±0.31 ^{ab}	220.37±2.59 ^b
19	BSM + UT + TGase	0.8g/100g	600W	77.03±1.01 ^c	8.15±0.13 ^d	30.97±0.74 ^{bc}	208.18±3.47 ^c
20	BSM + UT + TGase	0.8g/100g	720W	74.57±1.19 ^d	7.65±0.18 ^e	28.30±0.34 ^d	205.61±1.78 ^c

was added with 5 mL of deionized water, 200 µL of 0.5% phenolphthalein indicator, and 2 mL of neutral formaldehyde on a magnetic agitator. Then, it was titrated to micro-pink with 0.01 M standard NaOH solution, and the volume of consumed NaOH solution was recorded. Deionized water was used as blank group, with three parallels in each group. The degree of protein hydrolysis was expressed by the ratio of the amino acid nitrogen content N_1 in the digested supernatant to the total nitrogen content N_2 in the samples. The formula is as follows:

$$\text{Degree of protein hydrolysis (\%)} = N_1/N_2 \times 100$$

2.11. Statistical analysis

Each experiment was repeated three times, and the experimental results were expressed as mean ± standard deviation. SPSS 26.0 (SPSS Inc., Chicago, USA) software was used to analyze the significance of the data. The significant difference was determined by Duncan's multiple range tests at 5% significant level.

3. Results and discussion

3.1. Effect of TGase, UT or combination on the gel properties of BSM gel

3.1.1. Gel strength

The gel strength of BSM gel incorporated with different concentrations of TGase is displayed in Fig. 1A. The BSM gel without any treatment (TGase or UT) named "CON" had the lowest gel strength compared with all treated samples. With the increase of TGase concentration, the gel strength increased at first and then decreased, the gel strength of BSM gel was significantly higher than that of the CON sample ($p < 0.05$). When 0.8 g/100gTGase (named "TG-0.8" sample) was added, the gel strength was the highest (16.31 N). This tendency was similarly observed in gels prepared from Zhikong scallop with TGase (Mi et al., 2021). TGase was found to catalyze the formation of non-disulfide bonds between protein molecules and promote protein cross-linking to increase its gel strength (Liang et al., 2020a,b), but excessive amounts of TGase cause gel strength to decrease because an excess of cross-links can inhibit the development of a uniform protein network (Gaspar and de Goes-Favoni, 2015).

The gel strength of BSM gel after UT is shown in Fig. 1B. When only UT was applied, the gel strength was higher than that of the CON sample ($p < 0.05$). The highest gel strength was obtained at ultrasonic power of 480 W (named "UT-480" sample). Subsequently, with the increase of

ultrasonic power, the gel strength tended to decrease. UT mainly affects the gel network formed between protein molecules by promoting protein folding and unfolding, within a certain ultrasonic power range, the formation of ultrasound induced disulfide bonds may contribute to the formation of protein-protein aggregation, resulting in a gel network with better gel strength (Chen et al., 2022). However, the decrease of gel strength indicates that excessive ultrasound power may cause damage to the gel network, which is attributed to the protein damage caused by high intensity ultrasound shock wave and strong mechanical vibration, resulting in the formation of weak gel network. thereby reducing the gel strength (C. Zhang et al., 2021).

When UT was combined with TGase, the gel strength was further improved. When the ultrasonic power was 360 W and 0.8 g/100g of TGase was added (named "UT-TG" sample), the maximum gel strength was reached (18.79 N), which was higher than that of TG-0.8 and UT-480. UT leads to the expansion of protein structure, which weakens the non-covalent bond between protein molecules and loosens the spatial structure of proteins. This loose structure combines with TGase to form a new interaction, forming a stronger gel network (Qin et al., 2017). This is consistent with the gel strength obtained by ultrasonic pretreatment of whey proteins cross-linked by TGase (T. Zhang et al., 2021). The results showed that the enhancing effect of combining UT with TGase surpassed the sum of their individual effects, indicating that UT and TGase have synergistic effect on gel strength of BSM.

3.1.2. TPA

TPA can better imitate the actions applied to the gel by the tongue and teeth (Jimenez-Munoz et al., 2019). The textural profiles of BSM gel added with TGase or UT at various levels or in combination are shown in Table 1. When only TGase was added, the hardness and springiness of BSM gel were only increased significantly ($p < 0.05$), and they all tended to increase at first and then decrease. When 0.8 g/100g of TGase was added, the hardness and springiness reached the maximum value of 106.43 N and 8.26 mm, respectively. There is a similar trend for gumminess and chewiness. The chewiness and gumminess of all samples containing TGase were 129.83–268.01 mJ and 14.87–47.63 N, respectively. This result is similar to the effect of TGase on the gel of Zhikong scallop (Mi et al., 2021). In the process of heating, the advanced structure of the protein stretches, and cross-linking occurs between the protein molecules. Proper addition of TGase can form a more stable and dense three-dimensional reticular structure between proteins, thus increasing hardness and springiness. However, the excessive addition of TGase may lead to excessive cross-linking of proteins. This makes the gel brittle and less springy, reducing its texture properties (Singh et al.,

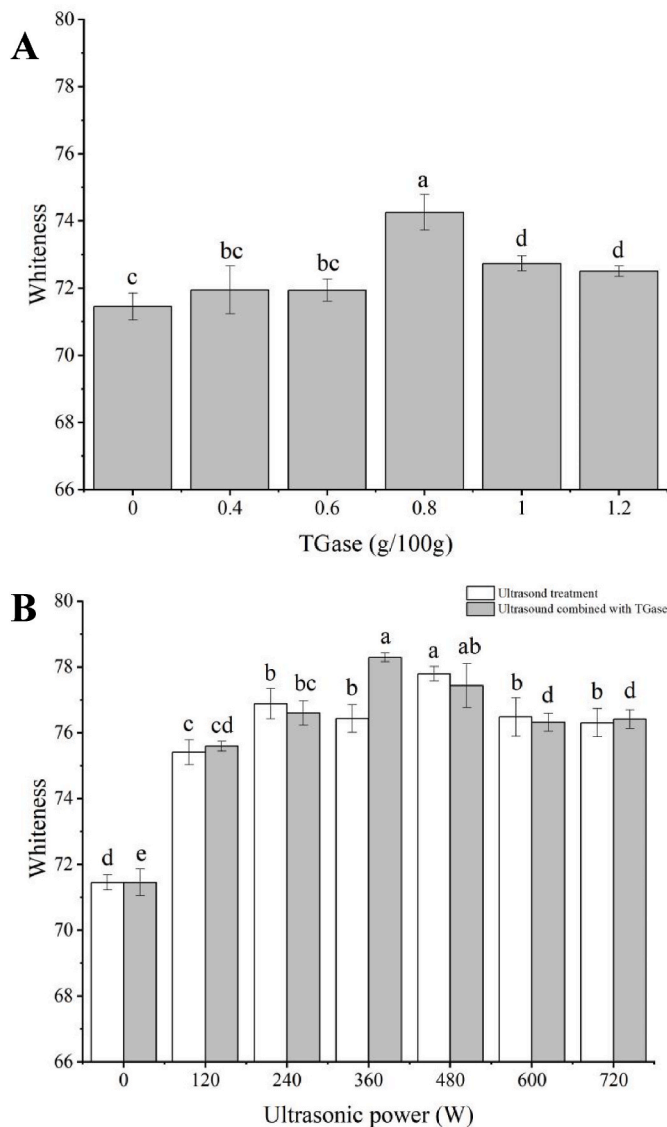


Fig. 2. Effect of TGase and UT on whiteness of Bay scallop adductor muscle gel. A: TGase alone at different concentrations; B: UT with different power and the combination of TGase at selected condition (0.8 g/100g) and UT at different concentrations. Different lowercase letters on the bars indicate significant difference ($p < 0.05$).

2020).

The texture properties of the UT gel samples and UT gel samples combined with TGase were similar to those of gel samples with TGase alone. Compared with CON samples, their hardness, gumminess, springiness and chewiness were significantly improved ($p < 0.05$). The maximum values of texture properties of the two treatments were obtained in UT-480 and UT-TG samples, respectively. The results might originate from the mechanical effects (shear force and shock waves) of ultrasound, which could improve protein solubility and promote the unfolding of protein conformation (Liu et al., 2017), as well as the interaction degree between them (Liang et al., 2020a,b). Moreover, the dispersion of small protein molecules allows TGase to promote intermolecular cross-linking. Among all samples, the highest springiness values were obtained for UT-TG sample ($p < 0.05$), followed by TG-0.8 sample. Therefore, the combination of UT and TGase can help to form elastic gel.

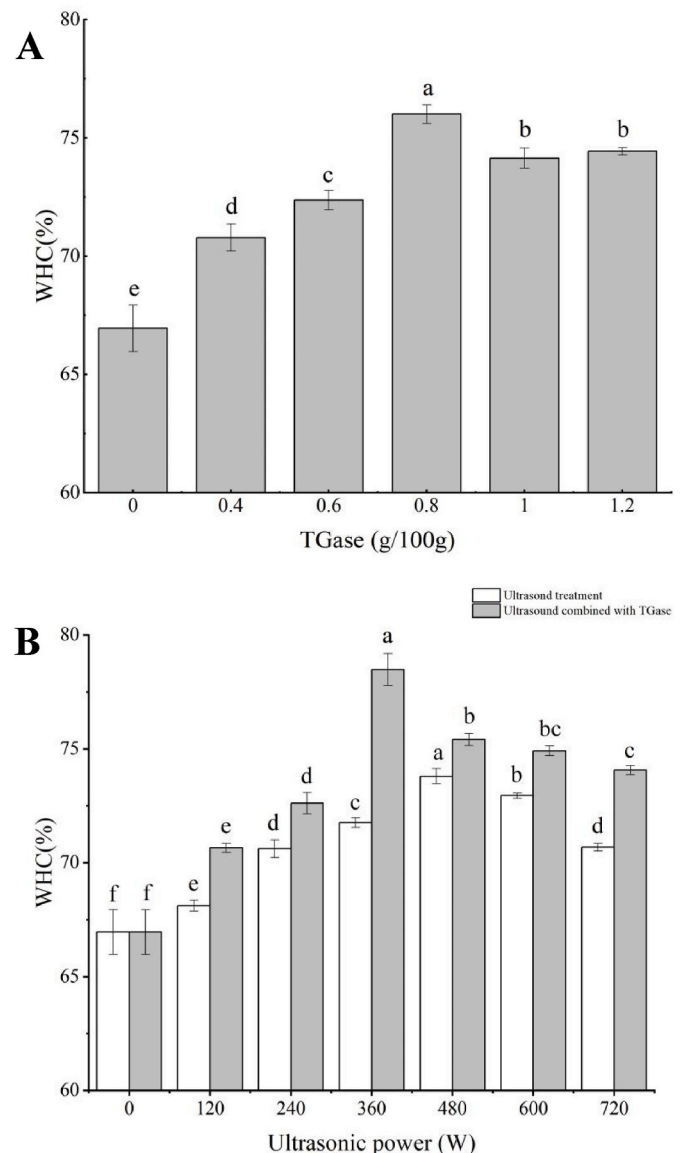


Fig. 3. Effect of TGase and UT on WHC of Bay scallop adductor muscle gel. A: TGase alone at different concentrations; B: UT with different power and the combination of TGase at selected condition (0.8 g/100g) and UT at different concentrations. Different lowercase letters on the bars indicate significant difference ($p < 0.05$).

3.1.3. Whiteness

Whiteness is an important index to measure the quality of aquatic gel products and strongly affects consumers' acceptance of aquatic products (Mi et al., 2021). The whiteness of BSM gel in the presence of different levels of TGase is shown in Fig. 2A. With the addition of TGase, the whiteness of the gel increased at first and then decreased, and tended to be stable when the TG content reached 1.0 g/100g ($p < 0.05$). The whiteness of gels containing TGase was higher than that of the CON sample ($p < 0.05$). The color of gels from aquatic muscle proteins is largely dependent on the contents and types of exogenous additives (Luo et al., 2020). TGase has been reported to promote the cross-linking between protein molecules and increase the brightness and whiteness of protein gel (Chen et al., 2020). However, Chanarat & Benjakul (2013) reported that the addition of TGase would decrease the whiteness of India mackerel fish protein isolate gels, resulting from the higher light absorption of denser gel network induced by TGase. This may also be the reason for the decrease of the whiteness of the gel when the content of

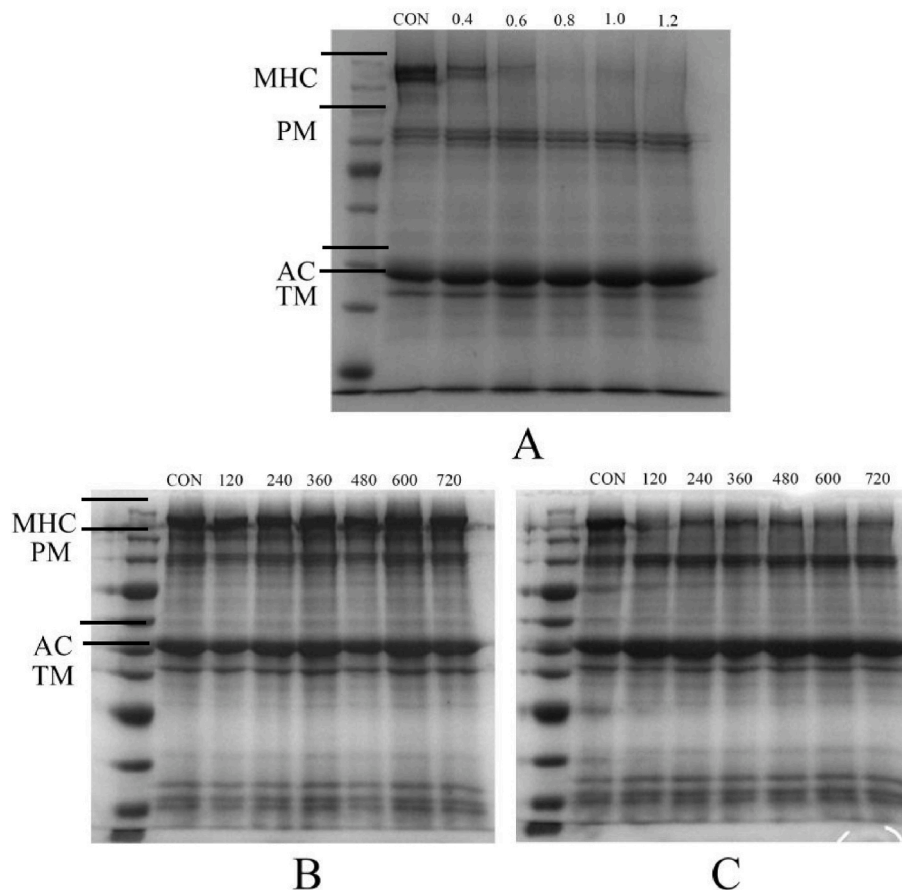


Fig. 4. Effect of TGase and UT on SDS-PAGE patterns of Bay scallop adductor muscle gel. A: TGase alone at different concentrations; B: UT alone with different power; C: the combination of TGase at selected condition (0.8 g/100g) and UT at different power. Numbers 0.4–1.2 indicate the amount of TGase and numbers 120–720 indicate the power of UT. MHC: myosin heavy chain, PM: paramyosin, AC: actin and TM: tropomyosin.

TGase was 1.0 g/100g.

The whiteness of the UT sample is shown in Fig. 2B. With the increase of ultrasonic power, the whiteness of the UT gel sample has the same trend as that of the gel sample with TGase. The whiteness of protein gel is related to protein degradation. Protein degradation and non-enzymatic reaction will lead to the decrease of gel whiteness (Liang et al., 2020a,b). It is speculated that the whiteness of UT gel samples is higher than that of CON samples because proper ultrasound can inhibit protein degradation, which is conducive to improving the whiteness of gel to a certain extent. Among all the treated samples, the whiteness of UT-TG sample is the highest at 78.3. Therefore, TGase and UT have a synergistic effect on the improvement of whiteness of BSM gel.

3.1.4. WHC

The WHC refers to the ability of proteins to bind water, and its changes affect the color, flavor, tenderness and taste of the heat-induced gel (Huang et al., 2020). The WHC of gel samples added with different amounts of TGase is displayed in Fig. 3A. The CON sample had the lowest WHC (66.96%) compared with all the treated samples ($p < 0.05$). When the gel samples contained TGase, the WHC increased gradually with the increase of TGase content. Then, it decreased and stabilized when the TGase content reached 1.0 g/100g. The results obtained in this study are consistent with the effect of MTGase on the WHC of hairtail muscle protein gel found by Hu et al. (2015). The gel containing TGase had fine three-dimensional gel network compared with the CON sample. The compact and homogeneous microstructure might retain water in the gels, leading to the enhancement of gel strength and WHC.

The WHC of BSM gel after UT is presented in Fig. 3B. In UT gel samples, the WHC tends to increase at first and then decrease with the

increase of ultrasonic power. The UT of gel samples containing TG shows the same trend. The WHC of UT-TG sample is the highest (78.49%), which was in line with the highest gel strength (Fig. 1). The cavitation and shear stress of UT may have resulted in more exposure of reaction sites, including hydrophilic residues, thus improving the ability to combine with and hold water. The results are consistent with the ultrasonic treatment of the mixture of soybean protein isolate and wheat gluten cross-linked by TGase (Chen et al., 2013). The presence of water in the BSM gel provided a juicy texture and partially contributed to the elasticity of gels.

3.1.5. SDS-PAGE patterns

The protein patterns of BSM gels in the presence of different amounts of TGase or subjected to UT with different power alone or in combination are depicted in Fig. 4. Myosin heavy chain (MHC), paramyosin (PM), actin (AC) and tropomyosin (TM) showed higher band intensity in the SDS-PAGE patterns, suggesting that they are the four major proteins in BSM gels. MHC is known to be susceptible for setting phenomenon, in which non-disulfide covalent bond is formed (Singh et al., 2020). The MHC band was retained in the CON sample and samples added with TGase (0.4–0.6 g/100g), indicating the poor setting phenomenon in BSM gel. When the content of TGase was 0.8 g/100g, the MHC band disappeared completely. A similar protein pattern was observed in the effect of MTGase on surimi protein reported by Hu et al. (2018). MHC is the preferable substrate for TGase, and the disappearance of MHC bands indicates that the formation of ϵ -(γ -glutamyl)-lysine bond induced by TGase leads to cross-linking between MHC, forming a stronger gel network (Chanarat & Benjakul, 2013; Chanarat et al., 2012), which is consistent with the result of higher WHC (Fig. 3A).

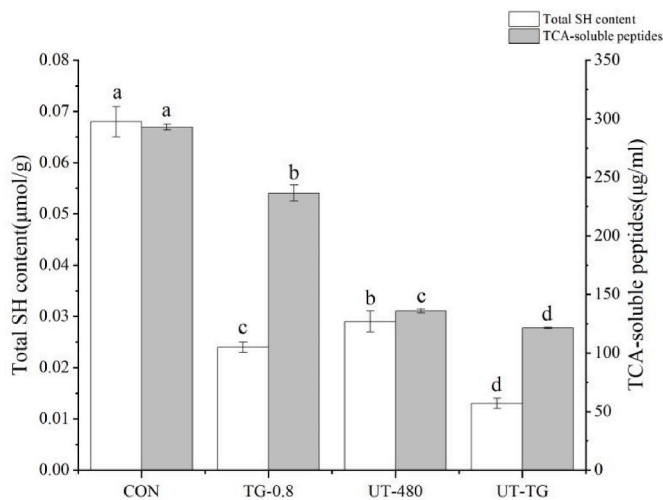


Fig. 5. Effect of TGase and UT on Total SH content and TCA-soluble peptides of Bay scallop adductor muscle gel. CON: BSM gel without TGase or UT; TG-0.8: BSM gel added with 0.8 g/100g TGase; UT-480: BSM gel with ultrasonic power of 480W; UT-TG: BSM gel containing 0.8 g/100g TGase was treated by 360 W ultrasound. Different lowercase letters on the bars indicate significant difference ($p < 0.05$).

The protein pattern of the gel sample prepared by the combination of UT and TGase was similar to that of the gel sample with TGase alone. However, the protein pattern of the samples treated by ultrasound alone showed that with the increase of ultrasonic power level, the MHC band first narrowed and then thickened. When the ultrasonic power is lower than 600W, the higher the ultrasonic power, the narrower the MHC band, and new bands appear under the MHC band. This phenomenon may be due to the degradation of MHC into smaller molecules, and the degree of degradation increases with the increase of ultrasonic power. The result was in agreement with the report of Liu et al. (2017). Nevertheless, when ultrasonic power exceeds 600 W, the MHC band was slightly thickened, possibly due to the re-enhancement of protein-protein interactions resulting from excessive ultrasound treatment, leading to increased MHC concentration (Chen et al., 2022). In all samples, AC and TM bands were relatively stable, indicating that they had little effect on the formation of gel network.

The PM rods are similar to myosin and present exclusively in invertebrates (Yang et al., 2020). In the samples treated only by ultrasound, the PM band did not change significantly. Thus, it was speculated that UT has no effect on PM. In the samples containing TGase, the PM bands were thicker than those of the CON samples. It was speculated that the presence of PM was beneficial to the formation of the gel. However, the result remains to be confirmed.

The samples with the highest gel strength (TG-0.8, UT-480 and TG-UT) were selected and compared with CON (without any treatment) for the determination of total SH content and TSP. The dynamic rheology and in vitro digestion characteristics were studied.

3.2. Characteristics of the selected BSM gels

3.2.1. Total SH content and TSP

Fig. 5 shows the total SH content and TSP of the selected gel sample (CON, TG-0.8, UT-480 and TG-UT). SH can form weak secondary bonds, which maintain the tertiary structure of proteins and play an extremely important role in protein stability. The CON sample had the highest total SH content, whereas the UT-TG sample had the lowest. The low SH content indicated the formation of more disulfide bonds. Due to cavitation effects, UT can disrupt inter- or intramolecular interactions among protein molecules and expose some target regions (Qin et al., 2016), so that TGase can better promote protein cross-linking. The total

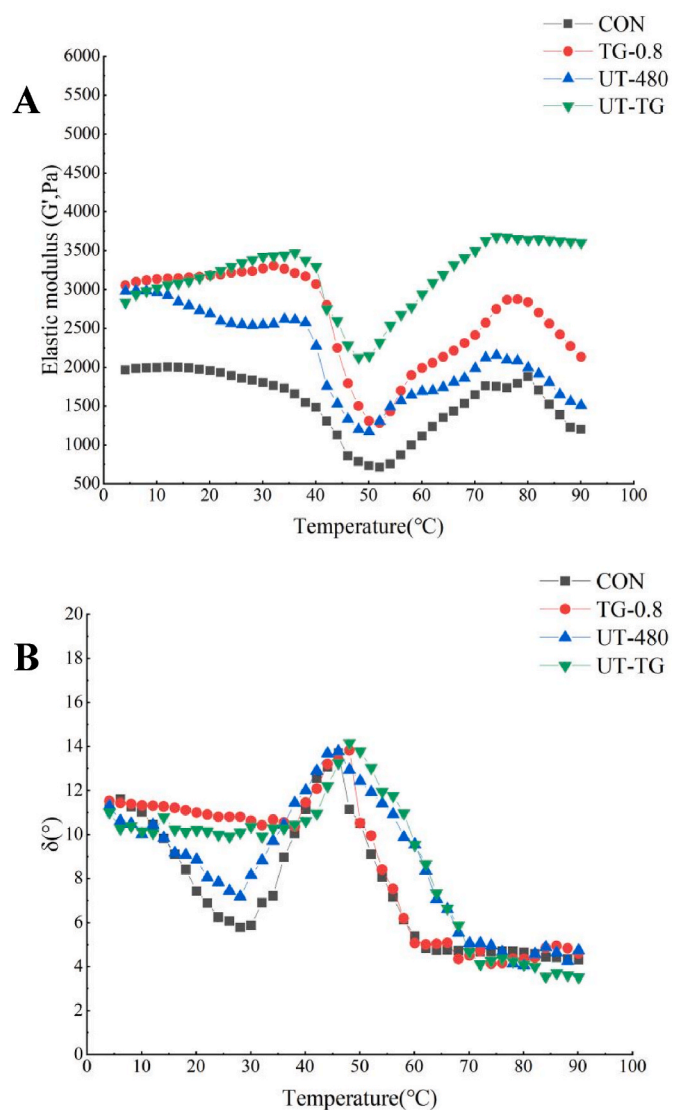


Fig. 6. Effect of TGase and UT on the dynamic rheological properties of selected Bay scallop adductor muscle gel. CON: BSM gel without TGase or UT; TG-0.8: BSM gel added with 0.8 g/100g TGase; UT-480: BSM gel with ultrasonic power of 480W; UT-TG: BSM gel containing 0.8 g/100g TGase was treated by 360 W ultrasound.

SH content of samples with treated with TGase alone and UT is higher than that of UT-TG samples, which indicates that the combination of UT and TGase could better cross-link the proteins of BSM gel.

The determination of TSP has been used by many researchers to examine the degree of hydrolysis of muscle proteins. A high level of TSP indicates a higher degradation of muscle proteins (Cao et al., 2020). The highest TSP was obtained for the CON, followed by the TG-0.8 and UT-480 samples. The UT-TG samples had the lowest TSP ($p < 0.05$). The CON sample had the highest TSP, indicating that the proteins were obviously degraded. However, the lower TSP of samples containing TGase than CON sample may be caused by protein cross-linking. The TSP of samples treated with ultrasound (with or without TG) was lower, indicating that ultrasound significantly inhibited protein degradation. Protein degradation is related to the action of endogenous proteinase (Saengsuk et al., 2021). It was supposed that UT could inactivate the endogenous proteinase. The UT-TG sample had the lowest TSP, suggesting that the combination of TGase and UT can more effectively reduce protein degradation.

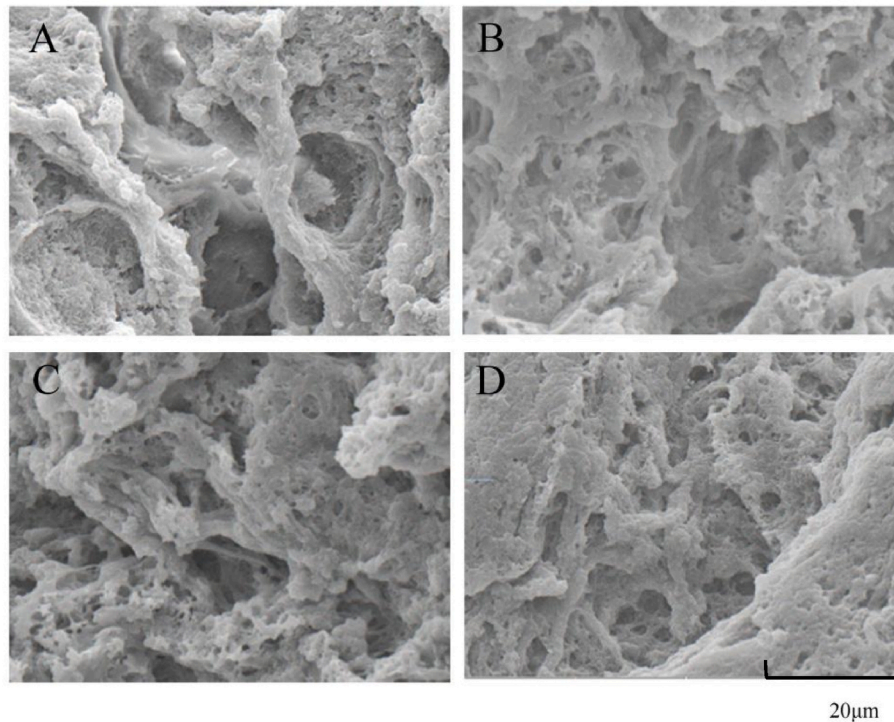


Fig. 7. Effect of TGase and UT on SEM of selected Bay scallop adductor muscle gel. Scanning electron micrographs (2500 ×). CON (A): BSM gel without TGase or UT; TG-0.8 (B): BSM gel added with 0.8 g/100g TGase; UT-480 (C): BSM gel with ultrasonic power of 480W; UT-TG (D): BSM gel containing 0.8 g/100g TGase was treated by 360 W ultrasound.

3.2.2. Dynamic rheological properties

The elastic modulus (G') and δ value of CON, TG-0.8, UT-480 and TG-UT gel samples during the conversion from sol to gel as affected by varying temperatures are described in Fig. 6. The energy stored during the formation of viscoelastic materials is defined as G' . TG-0.8 had the highest G' value at the beginning of heating. The G' value of the treated samples tended to increase at about 35 °C, which was related to the generation of protein network via hydrogen bonding between proteins (Singh et al., 2020). Then, the G' value decreased rapidly at about 40 °C and reached the lowest value at about 50 °C. The δ value showed the opposite trend. The optimum temperature for endogenous proteolytic enzyme activity was in the range of 50 °C–60 °C (Klomklao et al., 2008). This finding shows that the enhancement of protease activity led to the gel deterioration of the gel sample. In addition, heating weakens the force of weak bonds such as hydrogen bond, depolymerizes the assembly, and separates the AC–myosin complex, which enhances protein mobility, resulting in the reduction of G' and the increase of δ value.

When the paste was continued to be heated, there was a continuous increase in the G' value, and the maximum value for all samples was reached at 75 °C. The δ value began to decrease and tended to be stable at about 70 °C. The UT-TG sample showed the highest G' value followed by TG-0.8 and UT-480 samples. The CON sample had the lowest G' value. The result was in accordance with the gel strength (Fig. 1). The increase in G' value was more likely due to the increment in the cross-linking of dissociated proteins. Moreover, in the case of high temperature, the denaturation of MHC and AC caused the formation of thermo-irreversible network for the treated samples. At about 80 °C, the G' value of TG-0.8 and UT-480 samples sharply decreased, which may be related to the formation of rigid gel. Therefore, TGase combined with UT enhanced protein cross-linking in the BSM gel, which yielded a gel with high strength and elasticity.

3.2.3. SEM

The microstructures of the selected sample (CON, TG-0.8, UT-480 and TG-UT) are shown in Fig. 7. The CON sample had a coarser network

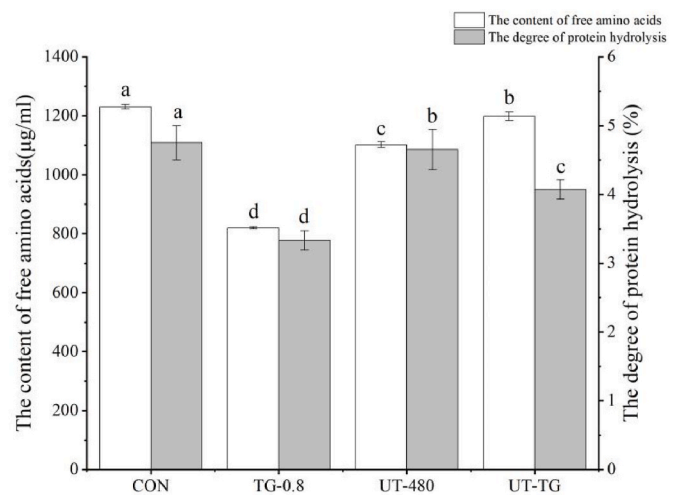


Fig. 8. Effect of TGase and UT on the free amino acid content and the degree of protein hydrolysis of Bay scallop adductor muscle gel. CON: BSM gel without TGase or UT; TG-0.8: BSM gel added with 0.8 g/100g TGase; UT-480: BSM gel with ultrasonic power of 480W; UT-TG: BSM gel containing 0.8 g/100g TGase was treated by 360 W ultrasound. Different lowercase letters on the bars indicate significant difference ($p < 0.05$).

occupied by larger cavities or voids (Fig. 7A). The gel network of TG-0.8 (Fig. 7B) and UT-TG (Fig. 7D) samples is tighter than that of CON samples and has smaller pores. Although the gel network of the UT-480 (Fig. 7C) sample is not as compact as that of the gel with TGase, it has higher connectivity and more ordered structure than the CON sample. TGase promotes the reaction between glutamine and lysine in myofibrillar proteins and induces the formation of ϵ -(γ -glutamyl)-lysine cross-linkages, which produce a firmer and more stable gel structure (Liang et al., 2020a,b), whereas UT can facilitate protein dispersion, which is

beneficial to forming a uniform network (Liu et al., 2007). The densely interconnected strands were associated with high strength in gels added with TGase or TGase in combination with UT (Fig. 1). Thus, the addition of appropriate levels of TGase in combination with UT into the BSM gel more likely induced higher connectivity and rigidity of protein strands within gel networks.

3.3. *In vitro* digestion characteristics of the selected BSM gels

Fig. 8 illustrates the free amino acid content and the degree of protein hydrolysis of the selected sample (CON, TG-0.8, UT-480 and TG-UT). The content of free amino acids after digestion by BSM gel can indirectly reflect protein digestion. The CON sample had the highest free amino acid content, and the TG-0.8 sample had the lowest ($p < 0.05$). The myosin is rich in glutamine and lysine residues, which is beneficial to the formation of isopeptide bonds catalyzed by TGase. The formation of isopeptide bonds alters the cross-linking degree of MHC and network structure (Li et al., 2019), and the increase of protein cross-linking decreases the cleavage site of proteins, which leads to protease resistance (Roy et al., 2021). Therefore, the digestibility of proteins in BSM gel with TGase is low, which affects the digestion and absorption of proteins in the human body. However, the free amino acid content of TG-0.8 samples treated by ultrasound was significantly higher than that of TG-0.8 samples. This may be because the cavitation effect and mechanical oscillation effect of ultrasonic treatment exposed the hydrophobic groups that are prone to be degraded by digestive enzymes (Jiang et al., 2021), which increases the digestibility of proteins and increases the content of free amino acids.

The degree of hydrolysis is the characterization of protein digestion efficiency. The degree of protein hydrolysis of the samples from high to low is as follows: CON, UT-480, UT-TG and TG-0.8 ($p < 0.05$). The protein hydrolysis degree of the TG-0.8 sample was 29.9% lower than that of the CON sample, whereas that of the UT-TG sample was 20.4% lower than that of the CON sample. By contrast, the protein hydrolysis degree of the UT-TG sample was higher than that of the TG-0.8 sample. The results show that the addition of TGase is not conducive to the hydrolysis of protein, and the degree of protein cleavage into peptides is low, thus affecting the absorption of proteins in the human body. However, the degree of protein hydrolysis of UT-assisted TGase samples was improved. It may be that UT destroys the internal hydrophobic interaction of protein molecules, and proteins form small aggregates through cavitation (Jiang et al., 2021), which is easier to be hydrolyzed by protease than the dense network structure formed by TGase. Overall, TGase reduced the bioavailability of gel samples due to the resistance of cross-linked proteins to digestive enzymes, and its digestibility could be restored when combined with UT.

4. Conclusion

This study found that the gel properties of BSM gels prepared by UT and TGase alone or in combination were improved. UT and TGase could improve the gel strength, WHC, whiteness and elastic modulus of BSM gel. The enhancing effect was more pronounced when UT was combined with TGase at optimum levels. These results were attributed to the fact that UT promoted the interaction between proteins, decreased the total SH content, and increased the formation of disulfide bonds, which led to the formation of a denser and more uniform gel network in the TGase-induced BSM gel. In addition, the protein oligomer formed by ultrasonic cavitation made the dense network formed by TGase more easily hydrolyzed by protease, thus improving the digestibility of BSM gel *in vitro*. Therefore, ultrasound-assisted TGase may be a relatively effective method to improve the quality of gel products. This study provides a theoretical basis for the processing and utilization of high-end scallop gel products.

Funding

This work was supported by The National Key R&D Program of China (Grant no. 2018YFD0901004).

CRedit authorship contribution statement

Jiaqi Feng: Data curation, Writing – original draft. **Jie Wang:** Investigation. **Tong Zhang:** Formal analysis. **Yaqiong Liu:** Conceptualization, Methodology. **Ran Suo:** Visualization. **Qianyun Ma:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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