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Novel insights into induced low-salt Mandarin fish (*Siniperca chuatsi*) surimi gel with transglutaminase and microwave heating

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Abstract

The present work examined water bath (WB) heating with microwave heating (WM) under different levels of transglutaminase (TGase) additions in a low-salt Mandarin fish surimi to obtain excellent gel properties. The breaking force was gradually enhanced with increased TGase additions (0.0 - 0.8 U/g), while WM with 0.8 U/g TGase produced the maximum gel strength. In the texture analysis, WM depicted a reduced hardness and an increased springiness at the corresponding TGase additions than that of WB. In low-field nuclear magnetic resonance spectroscopy (LF-NMR) studies, TGase reduced water protons' relaxation times to different extents, while WM increased their water-binding abilities, especially at 0.8 U/g. Fourier transform infrared spectroscopy (FT-IR) revealed that the relative contents of the secondary structures did not apparently change at 0.2 - 1.0 U/g. Therefore, using WM and adding 0.8 U/g TGase improved the gel quality of low-salt Mandarin fish surimi gel, providing a new strategy for preparing healthy surimi products.

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Introduction

Freshwater fish have become China's primary raw material for high-quality surimi products owing to their growing consumer demand, and high protein and amino acid contents (Wang *et al*., 2023). Known as Mandarin fish (*Siniperca chuatsi*), this freshwater fish species is widely distributed in China (Chen *et al*., 2023). In traditional surimi processing, 2 - 3% salt is usually used to enhance myofibrillar protein solubility, and inhibit microbial growth (Fu *et al*., 2012). An elastic gel is produced when the dissolved swelling protein undergoes thermal aggregation and crosslinking to create a fine three-dimensional solid network from a continuous matrix (Xiong *et al*., 2021). However, excessive salt intake leads to kidney diseases such as hypertension and arteriosclerosis (Chen *et al*., 2023). Therefore, reasonable salt intake is crucial in the food industry to develop low-salt surimi (Jose *et al*., 2006). Low sodium, however, prevents myofibrillar proteins from fully dissolving and crosslinking, which weakens the surimi gel's gel strength and water-holding capacity (WHC), and leaves it with a poor three-dimensional network structure (Ye *et al*., 2022).

Microwave heating may be utilised to manufacture surimi products, because a rapid heating rate will let surimi travel through the gel rupture zone rapidly, and prevent the "*modori* phenomenon" (thermal breakdown of the gel) (Cao *et al*., 2020). Feng *et al*. (2017) reported that a microwave heating gel developed a denser network structure than two-step water bath heating. However, microwave heating did not improve gel quality in some cases because local hot spots led to structural defects in the gel matrix owing to non-uniform microwave heating (Fu *et al*., 2012). In addition, water bath heating followed by microwave heating can avoid non-uniform performance, save energy, and significantly improve the gel strength and WHC of surimi products (Meng *et al*., 2021).

Transglutaminase (TGase), with a special catalytic function, is widely used in surimi processing to improve the gel properties of surimi products (Lee

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and Chin, 2013). TGase catalyses the glutamic acid (Glu)-γ-carboxamide group in myosin with the ε-amino group of lysine (Lys) to generate an intramolecular or intermolecular ε-(γ-Glu)-Lys non-disulphide covalent bond (Liang *et al*., 2020a). Fish type, protein structure, substrate content, enzyme addition amount, and temperature affect the ability of TGase to cross-link myofibrillar proteins (Chanarat *et al*., 2012). TGase has successfully catalysed numerous fish proteins, such as silver carp, Alaska pollock, and mackerel (Zhu *et al*., 2014; Abdollahi *et al*., 2019; Fang *et al*., 2020). These raw fish species significantly differ in protein composition, leading to pronounced variance in product quality under the same processing conditions. According to Yuliana *et al*. (2020), adding 0.1 U/g TGase enhances the gel strength of milkfish surimi, and improves its physical quality. Zhang *et al*. (2022) demonstrated that adding 0.3 U/g TGase improved the gelation and quality of longtail southern cod surimi gel when the fish were stored in the cold.

Microwave energy can be directly applied to TGase because the enzyme has a certain polarity during the microwave treatment of surimi (Monto *et al*., 2022). According to Cao *et al.* (2018), enzyme conformation can be changed by microwave energy, which can also improve the surimi gels' mechanical and functional qualities. Microwave heating has significant potential for processing surimi products containing TGase (Liang *et al*., 2020b; Monto *et al*., 2022). Improving gel properties by TG combined with water bath heating has become a research hotspot. Nevertheless, no research has been done on how TGase and WM affect the quality of the gel in Mandarin fish. Therefore, it is necessary to study the improvement of gel properties of Mandarin fish surimi by microwave-assisted TGase.

The present work thus compared the gel properties of Mandarin fish surimi, including WHC, gel strength, colour, and texture, using microwave heating (plus water bath heating) and traditional water bath heating, with different TGase additions. FT-IR and LF-NMR spectroscopy were used to detect the protein secondary structure and water distribution of the surimi gel. The present work provided theoretical support for the microwave treatment of low-sodium freshwater fish surimi, and provided a new method for developing new energy and low-sodium freshwater fish surimi products for the aquatic food industry.

Materials and methods

Materials

Dalianwei Agricultural Development Co., Ltd. (Dongzhi County, Chizhou City, Anhui Province) and Beijing Solaibao Technology Co., Ltd. supplied frozen Mandarin fish and TGase (activity: 100 U/g), respectively. All chemicals used in the experiments were of analytical grade, and purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of surimi gels

The preparation of surimi gel was slightly modified following the method of Li *et al*. (2019) and Liang *et al*. (2020b). Frozen Mandarin fish were thawed, peeled, and deboned. Fish was cut into small pieces, and chopped in a high-speed masher for 1 min to obtain uniform surimi. The appropriate water content of the surimi was measured by a water analyser (Metter Toledo, Switzerland), and controlled at 80 ± 2%. Next, NaCl of 1.5% and different addition amounts of TGase (0, 0.2, 0.4, 0.6, 0.8, and 1.0 U/g) were blended with 100 g surimi, and further emulsified for 1 min. Surimi was then packed into a plastic casing (diameter: 34 mm), and sealed at both ends.

Surimi sausage was heated using two approaches, including water bath (WB) and water bath plus microwave (WM). WB samples were set at 40°C for 30 min, followed by heating at 90°C for 20 min. WM samples were kept at 40°C for 30 min to ensure that the samples were fully gelled, and their shape maintained, and then heated in a microwave oven (Model M1-L213B, Midea, China) for 60 s at a power level of 5 W/g. Each sample was cooled to ambient temperature, and then kept in 4°C for 12 h.

WHC

Under suitable changes, the present work carried out WHC as suggested by Yi *et al.* (2020). First, 50 mL centrifuge tube was filled with around 5 g (M1) of surimi gel slices that had been triple-wrapped in filter paper. An H1750R centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Hunan) was used to centrifuge the samples for 10 min at 4°C at 6,000 rpm. The mass of surimi gel after centrifugation was weighed again (M2), and Eq. 1 was used to compute the WHC:

$$WHC = \frac{M2}{M1} \times 100\% \quad \text{(Eq. 1)}$$

Gel strength

The present work used the Liang *et al*. (2020b) method to test gel strength using a TA.XT Plus texture analyser (Stable Micro System Ltd., Surrey, UK). Before the gel strength tests, the samples were divided into 25 mm-thick sections, and allowed to acclimate for 2 h at room temperature. Specific parameter settings = trigger type: Auto (Force); trigger force: 5.0 g; speed before test: 2.0 mm/s; speed during test: 5.0 mm/s; speed after test: 2.0 mm/s; and deformation: 20 mm. The surimi gels' breaking force (g) and deformation (mm) were measured on the samples by a compression test with a spherical probe P/5S. The gel strength was obtained using Eq. 2:

Gel strength (g × cm) =
Breaking force (g) × Deformation (cm) (Eq. 2)

Colour

Using a colorimeter (CR-400, Konica Minolta, INC., Japan), the L* (brightness), a* (red or green), and b*(yellow or blue) of the surimi gels were ascertained in accordance with Petcharat and Benjakul (2017). Before analysis, samples were divided into 20 mm-thick pieces, and allowed to acclimate for 1 h at ambient temperature. The whiteness of surimi gels was determined using Eq. 3:

Whiteness = 100 - [(100 - L*)2+a*2+b*2]$^{1/2}$ (Eq. 3)

Texture profile analysis (TPA)

The method described by Gao *et al.* (2020) was used to determine the TPA with slight adjustment. A TA.XT Plus texture analyser (Stable Micro System Ltd., Surrey, UK) was used to perform gel TPA. Before analysis, the samples were split into 15 mm-thick sections, and allowed to acclimate for 2 h at ambient temperature. They were then put into a P-50 cylinder with a flat bottom. Specific parameter settings = trigger type: Auto (Force); trigger force: 5.0 g; speed before test: 1.0 mm/s; speed during test: 5.0 mm/s; speed after test: 5.0 mm/s; and deformation: 20 mm.

LF-NMR

LF-NMR were carried out using a slightly modified protocol outlined by Wang *et al.* (2016) on a MesoMR23-060H-I Niumag Benchtop-Pulsed LF-NMR analyser (Niumag Electronic Technology Co., Ltd.; Shanghai, China). For each sample, a glass tube containing around 2 g of the material was used to hold the NMR probe. Using a Carr-Purcell-Meiboom-Gill (CPMG) sequence run at 23 MHz, the transverse relaxation time (T2) was determined. The following were the primary parameters = 8 scans (NS): 5000 collected echoes (NECH); wait time (TW): 1800 ms; and echo time (TE): 0.2 ms.

FT-IR

According to Wang *et al.* (2014), FT-IR analysis of the surimi gel was carried out using a Fourier infrared spectrometer (Thermo Fisher Scientific, Massachusetts, USA). Using an agate mortar, 1 - 2 mg of each freeze-dried sample and 200 mg of KBr were combined for this experiment. Under infrared light, mixtures were homogenised into a fine powder, and then spread out on a thin sheet. An infrared spectrometer with a scanning time of 32 and resolution of 4 cm^{-1} were used to measure the wave number absorption spectra in the region of 4000 - 400 cm^{-1}. Under the protection of nitrogen, an infrared Fourier transform analysis was carried out.

Statistical analyses

The sample was performed at least three times. All statistical analyses were performed using One-way analysis of variance (ANOVA) and independent-sample *t*-tests using SPSS Statistics (version 16.0) (SPSS Inc., Chicago, IL, USA). There were statistically significant variations between the groups based on the Duncan's multiple range test results ($p < 0.05$).

Results and discussion

Gel strength

The effects of TGase on the breaking force, deformation, and gel strength are displayed in Figure 1. The gel with a larger deformation was more elastic, and the gel with a smaller breaking force was softer (Liang *et al*., 2020b). In the WB treatment, the breaking force of the gel with the addition of 0.4 or 0.8 U/g TGase was significantly higher than that with the addition of 0.0 U/g TGase ($p < 0.05$). In contrast, the breaking force of the gel with the addition of 0.2, 0.6, or 1.0 U/g TGase was slightly higher than that of 0.0 U/g TGase ($p > 0.05$). In WM treatment, the breaking force of the gel with the addition of 0.4, 0.8,

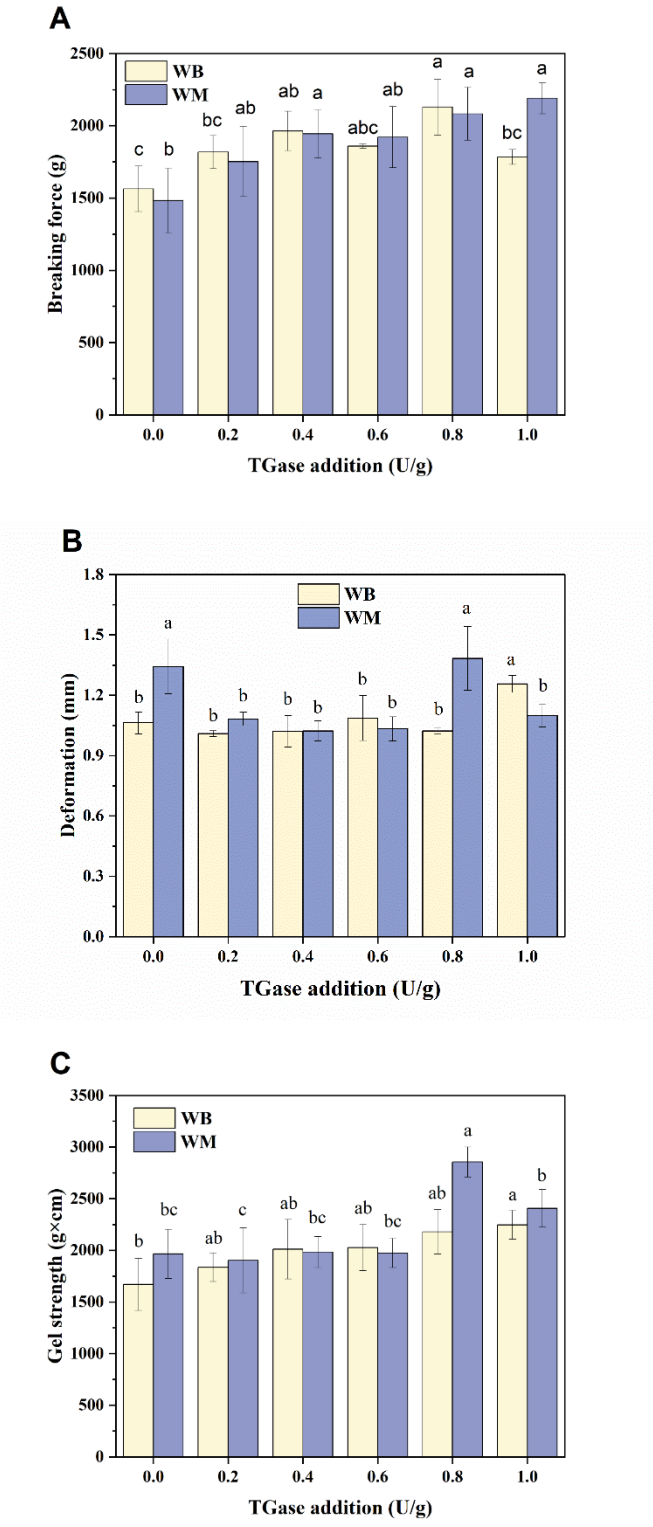


Figure 1. Effect of WB and WM treatments on breaking force **(A)**, deformation **(B)**, and gel strength **(C)** of surimi gels with different TGase additions. Columns represent mean of three replicates (n = 3) with error bars indicating standard deviations. Columns with different lowercase letters are significantly different (p < 0.05) between gels with different TGase additions under WH and WB, respectively.

or 1.0 U/g TGase was significantly higher than that with the addition of 0.0 U/g TGase (p < 0.05). In contrast, the breaking force of the gel with the addition of 0.2 or 0.6 U/g TGase was slightly higher than that of 0.0 U/g TGase (p > 0.05). The gel-breaking force demonstrated an increasing trend in the WB and WM treatments when TGase was increased from 0.0 to 0.6 U/g. In WB treatment, the deformation of TGase with the addition of 1.0 U/g was significantly higher than that of TGase with the addition of 0.0 - 0.8 U/g (p < 0.05); however, no significant differences were observed between TGase with the addition of 0.2 - 0.8 U/g (p > 0.05).

Gel strength is an important indicator for evaluating surimi products, and gel quality directly influences consumer acceptability (Foegeding *et al*., 2010). In WB and WM treatments, the gel strength gradually increased with increasing TGase addition (0.0 - 1.0 U/g), except at 0.8 U/g TGase in the WM group. Gel strength of the WM group at TGase's addition of 0.0 U/g was higher than the WB group, by the extent of 294.60 g × cm. The gel strength reached the maximum when TGase was 0.8 U/g in the WM group. The enhancement effect of the microwave treatment was more obvious than that of the WB treatment. The difference in gel strength between the WB and WM group was 677.16 g × cm.

These results might indicate that TGase catalysed the acyl transfer between lysine and glutamine residues in protein, causing the protein gel after TGase action to form ε-(g-glutamyl) lysine cross-links between the protein chains as compared to the blank group with TGase addition of 0.0 U/g, and these non-disulphide covalent bonds enhanced gel strength (Qian *et al*., 2021). Previous studies have illustrated that higher levels of TGase may lead to excessive protein cross-linking (Hu *et al*., 2015). The present work demonstrated that the protein's gel strength was incompletely formed by the low salt content (adding 1.5% low-salt); therefore, adding 0.8 U/g TGase compensated for the gel strength loss to some extent. These results were consistent with those of Canto *et al.* (2014), who studied the effect of low sodium concentration in combination with 1.0% TGase treatment on the quality of beef steaks. In addition, a study illustrated that microwaves provide a higher heating rate, and help the gel quickly pass through the gel crack (Jiao *et al*., 2019). Therefore, adding TGase could improve gel strength, and WM treatment could enhance the strengthening effect based on water bath heating.

WHC

WHC is the ability of gel system to retain water. The WHC of surimi gel with different TGase addition after WB and WM heat treatment is shown in Figure 2.

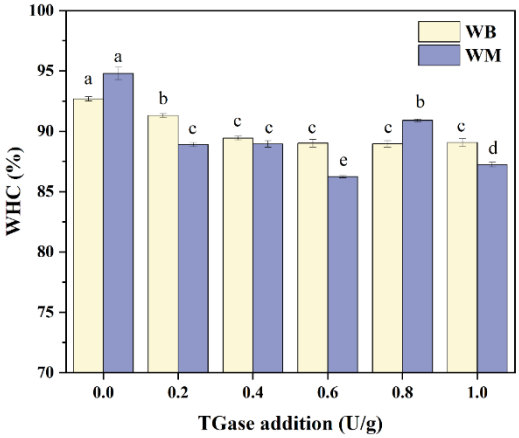


Figure 2. Effects of WB and WM treatments on WHC of surimi gels with different TGase additions. Columns represent mean of three replicates (n = 3) with error bars indicating standard deviations. Columns with different lowercase letters are significantly different (p < 0.05) between gels with different TGase additions under WH and WB, respectively.

Without TGase, WHC in WM group was higher than that in WB group. Ye *et al.* (2022) reported that microwave heating could significantly improve the WHC of tilapia surimi gel because the non-thermal effect of microwaves prevents degradation or polymerisation of the protein. In WB group, WHC value decreased with the increase of TGase addition. The reason was that TGase induced the formation of (ε-(γ-glutamyl) lysine) non-disulphide covalent bonds between peptide segments, which promoted intermolecular or intramolecular cross-linking of myofibrillar proteins, reduced the binding between protein molecules and water molecules, and reduced the WHC of the gel formed after heating (Singh *et al*., 2020). These results were consistent with the study by Du *et al*. (2019), which indicated that relative low addition of TGase could reduce WHC of gels. In WM group, with the increase of TGase addition, WHC first decreased then increased, and finally decreased. In WM group, when 0.2 - 1.0 U/g of TGase was added, the WHC values at 0.8 U/g TGase were significantly higher than other groups (p < 0.05). The WHC at the addition of TGase of 0.8 U/g was significantly higher (about 2.25%) than at 0.2 and 0.4 U/g (p < 0.05). The WHC of TGase with 0.8 U/g was higher than at 0.6 and 1.0 U/g by 4.66 and 3.67% (p < 0.05). It showed that the WHC of surimi with 0.8U/g TGase was slightly improved by microwave-assisted heating technology. Therefore, it is speculated that the WHC of Chinese Mandarin fish surimi gel can be improved by adding exogenous substances properly, and choosing appropriate heating technology, and the specific reasons need further study.

Colour

After WB and WM treatments, the colour of the surimi gel containing different levels of TGase (Figure 3) demonstrated that the values of L*, a*, and

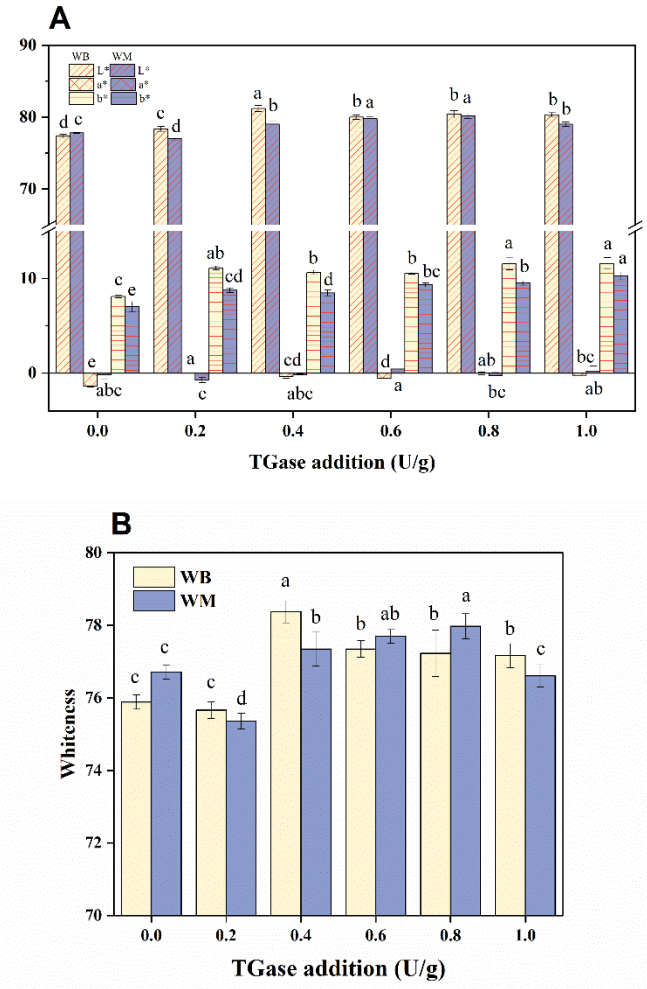


Figure 3. Effects of WB and WM treatments on colour parameters **(A)** and whiteness **(B)** of surimi gels with different TGase additions. Columns represent mean of five replicates (n = 5) with error bars indicating standard deviations. Columns with different lowercase letters are significantly different (p < 0.05) between gels with different TGase additions under WH and WB, respectively.

b* with TGase (0.2 - 1.0 U/g) were significantly higher than those without TGase ($p < 0.05$) in the WB group. In the WM group, the a* value of TGase with adding 0.6 U/g was significantly higher than at 0.0 U/g ($p < 0.05$). In addition, the b* values at the TGase addition at 0.2 - 1.0 U/g were significantly higher than at the TGase addition at 0.0 U/g ($p < 0.05$). Whiteness assists consumers in visually evaluating gel quality, while the effect of TGase on gel colour is primarily attributed to the difference in colour parameters between TGase and surimi (Petcharat and Benjakul, 2017). When the TGase was 0.4 - 0.8 U/g, the gel whiteness of the WB and WM groups was significantly higher than the blank group ($p < 0.05$); with 0.6 - 0.8 U/g TGase, the whiteness of the WM group was higher than that of the WB group. Consistent with these results, Karayannakidis *et al.* (2010) reported that adding TGase positively affected the whiteness index of heat-induced proteins in sardine muscle. Therefore, the whiteness of the gel could be improved by adding 0.4 - 0.8 U/g TGase. In addition, adding 0.6 - 0.8 U/g TGase showed a better microwave treatment effect than water bath heating.

TPA

The effects of TPA of the two heat-treated surimi gels (Table 1) illustrated that cohesiveness, chewiness, gumminess, and resilience had no significant changes in gels with TGase additions (0.2 - 1.0 U/g) in the WB and WM groups ($p > 0.05$). The gumminess and chewiness of the WM and WH groups gradually increased when TGase was added at 0.2 - 1.0 U/g. Resilience did not change significantly in the WB group ($p > 0.05$). With increased TGase addition, the hardness and springiness of WB and WM groups gradually increased, which was consistent with the report by An *et al.* (2022). In the WM group, the springiness was maximum when 0.8 U/g TGase was added. In addition, the hardness with 0.8 U/g TGase was significantly higher than that at 0.0 U/g by 4234.31 g ($p < 0.05$). The springiness of WM group was relatively higher than that of the WB group at the corresponding TGase addition; however, the opposite hardness results were observed. The texture in the WM group gels improved owing to the formation of ε-(γ-glutamyl) lysine bonds by cross-linking with TGase, and the formation of disulphide and non-disulphide covalent bonds by microwave interaction, which promoted highly flexible network production (Meng *et al*., 2021). Several studies have proven that WM with WB can yield softer and more elastic surimi gels (Fang *et al*., 2019), consistent with the changes in the gel breaking force and deformation obtained in the present work.

LF-NMR

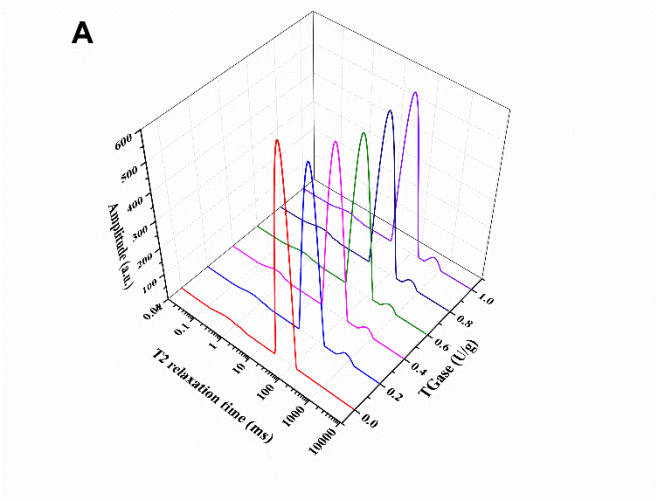
The transverse relaxation time reflects the free state of the water molecules in the surimi gel. The T2 spectrum after inversion analysis (Figures 4A and 4B) depicted that NMR attenuation signals of surimi gel fitted into three peaks: T_{21} (0 - 10 ms), T_{22} (10 - 200 ms), and T_{23} (200 - 1000 ms), representing bound water, non-mobile water, and free water, respectively. In addition, the main water component of the surimi gel was non-mobile water. A high non-mobile water content indicates that the microstructures and sub-microstructures of the gel retain more water (Ye *et al*., 2022). In WB and WM groups, the free movement degree of T_{21} slightly changed with an increase in TGase addition (0.455 - 0.561 ms), and the relaxation time of T_{22} first decreased and then increased. Moreover, the relaxation time at T_{22} in the WM group was shorter than in the WB group with the same addition of TGase. The relaxation time at T_{23} moved to a lower degree, indicating an increased network force of free water. The changes in relaxation time indicated that adding TGase stabilised the non-mobile and free water in the surimi gel. Furthermore, microwave treatment enhanced the binding force of fixed water compared with water bath heating. The peak area values of T_{21}, T_{22}, and T_{23} after the mass normalisation treatment, representing the relative water content in each phase (Figures 4F - 4H), showed that the larger the peak area, the more water in the phase. The non-mobile water decreased after adding TGase; however, the A_{22} of WM group was higher than WB group when 0.6 - 1.0 U/g TGase was added. In addition, the free water content in the WM group was lower than in the WB group when the 0.6 - 0.8 U/g TGase was added.

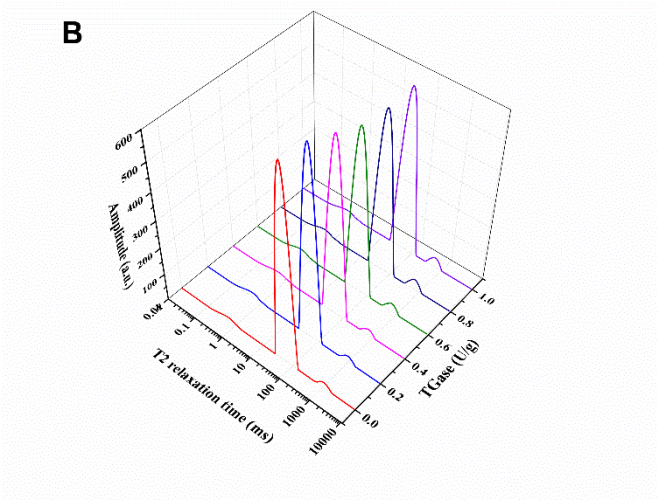
The increase in bound water content indicated that TGase crosslinking increased binding with myofibrillar proteins and bound water components. In addition, the microwave treatment increased the content of non-mobile water in the gel matrix. The amide group in the protein molecule prevented water from flowing easily because the group combined with water and a small bond energy formed a multi-molecular layer of water in the protein gel. The T_{22} in the group treated with TGase was lower than in the

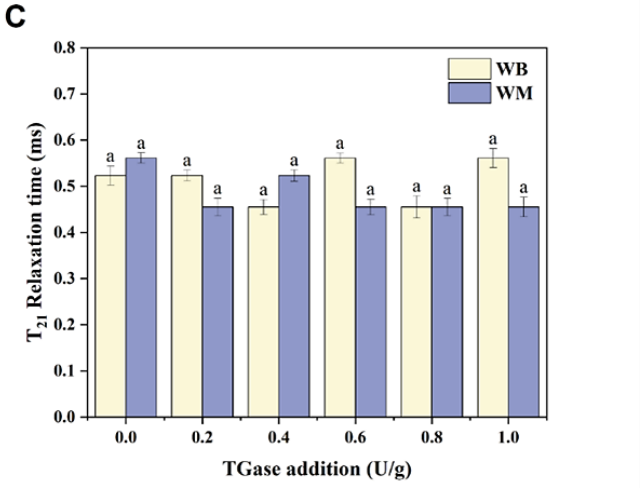
Table 1. Effect of WB and WM treatments on TPA parameters of surimi gels with different TGase additions.

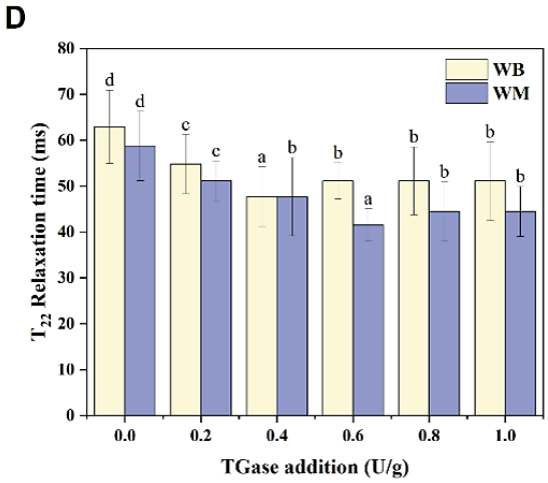
Parameter	Group	0 U/g	0.2 U/g	0.4 U/g	0.6 U/g	0.8 U/g	1.0 U/g
Hardness (g)	WB	11697.93 ± 556.04^{d}	13008.82 ± 729.83^{d}	14631.01 ± 110.68^{c}	16132.55 ± 474.06^{c}	17858.56 ± 505.47^{b}	21175.13 ± 1005.06^{a}
	WM	10916.49 ± 911.06^{e}	11490.98 ± 735.80^{de}	13239.41 ± 488.86^{cd}	14700.17 ± 202.08^{bc}	15150.80 ± 148.57^{bc}	20093.44 ± 1244.81^{a}
Springiness	WB	0.92 ± 0.02^{a}	0.93 ± 0.02^{a}	0.92 ± 0.04^{a}	0.94 ± 0.02^{a}	0.95 ± 0.03^{a}	0.95 ± 0.01^{a}
	WM	0.94 ± 0.01^{a}	0.95 ± 0.03^{a}	0.95 ± 0.00^{a}	0.96 ± 0.01^{a}	0.96 ± 0.01^{a}	0.96 ± 0.02^{a}
Cohesiveness	WB	0.48 ± 0.02^{a}	0.44 ± 0.02^{a}	0.44 ± 0.01^{a}	0.45 ± 0.07^{a}	0.44 ± 0.02^{a}	0.42 ± 0.07^{a}
	WM	0.50 ± 0.12^{a}	0.37 ± 0.01^{a}	0.43 ± 0.04^{a}	0.40 ± 0.06^{a}	0.40 ± 0.04^{a}	0.47 ± 0.04^{a}
Gumminess (g)	WB	5564.26 ± 329.31^{b}	5131.38 ± 1297.89^{b}	6437.78 ± 201.18^{ab}	7263.03 ± 1326.98^{ab}	7798.68 ± 509.37^{ab}	8888.15 ± 1755.82^{a}
	WM	5389.61 ± 1044.36^{b}	4298.50 ± 391.22^{b}	5735.04 ± 248.59^{b}	5922.80 ± 876.82^{b}	6001.08 ± 565.23^{b}	9418.83 ± 968.70^{a}
Chewiness (g)	WB	5135.65 ± 201.94^{bc}	4732.64 ± 1096.95^{c}	5920.14 ± 60.28^{bc}	6792.95 ± 1082.36^{abc}	7364.33 ± 373.61^{ab}	8477.48 ± 1688.37^{a}
	WM	5085.28 ± 949.03^{b}	4070.89 ± 301.42^{b}	5451.13 ± 236.29^{b}	5661.42 ± 850.99^{b}	5746.65 ± 571.43^{b}	9078.73 ± 899.87^{a}
Resilience	WB	0.16 ± 0.01^{a}	0.17 ± 0.03^{a}	0.15 ± 0.01^{a}	0.15 ± 0.03^{a}	0.16 ± 0.02^{a}	0.16 ± 0.01^{a}
	WM	0.18 ± 0.03^{a}	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.14 ± 0.02^{a}	0.13 ± 0.03^{a}	0.16 ± 0.02^{a}

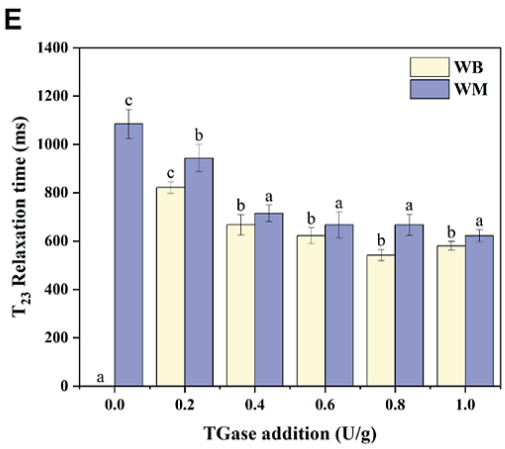
Data are mean ± standard deviation of five replicates (n = 5). Different lowercase superscripts within similar rows indicate significant difference ($p < 0.05$) between gels with different TGase additions under WB and WM, respectively.

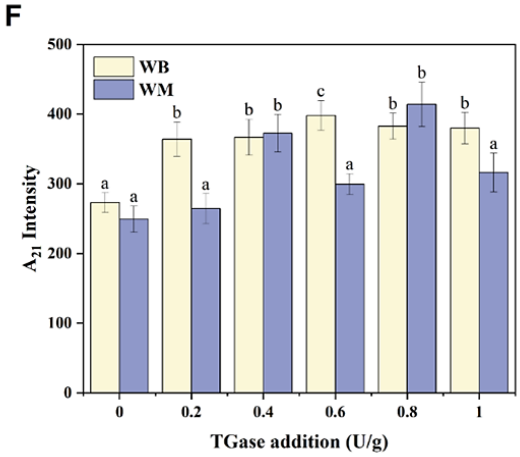

A
Amplitude (a.u.)
T2 relaxation time (ms)
TGase (U/g)


B
Amplitude (a.u.)
T2 relaxation time (ms)
TGase (U/g)


C
WB
WM
T21 Relaxation time (ms)
TGase addition (U/g)


D
WB
WM
T22 Relaxation time (ms)
TGase addition (U/g)


E
WB
WM
T23 Relaxation time (ms)
TGase addition (U/g)


F
WB
WM
A21 Intensity
TGase addition (U/g)

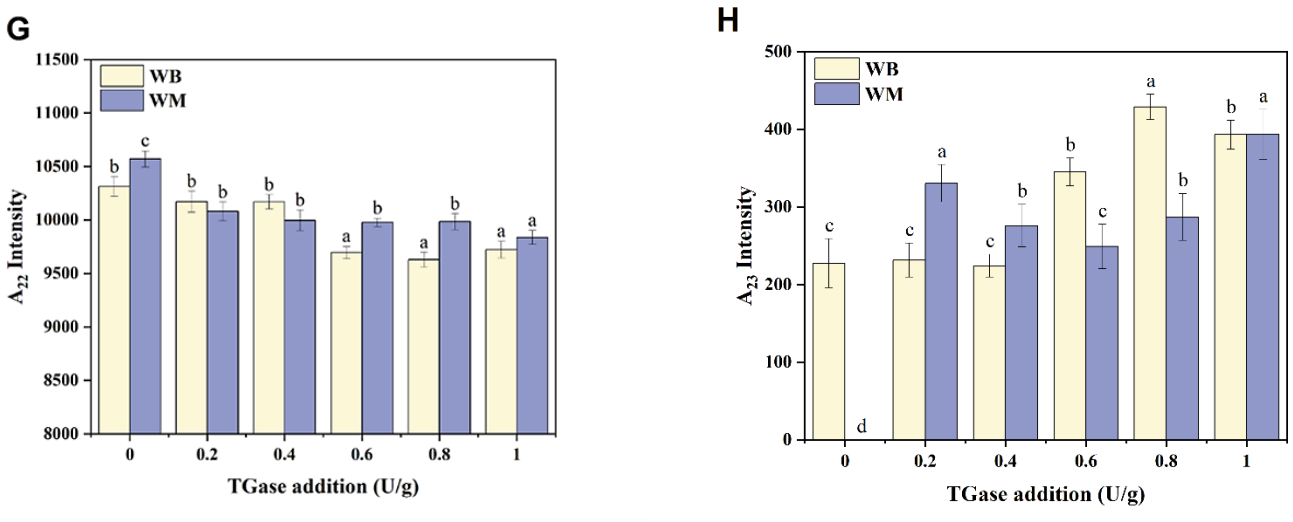


Figure 4. Relaxation spectrogram of surimi gels heat-induced by WB **(A)** and WM **(B)**, distribution of T2 relaxation time **(C - E)**, and the peak areas **(F - H)** for gels with different TGase additions. A_{21}, A_{22}, and A_{23} indicate peak areas for T_{21}, T_{22}, and T_{23}, respectively. Columns represent mean with error bars indicating standard deviations. Columns with different lowercase letters are significantly different ($p < 0.05$) between gels with different TGase additions under WH and WB, respectively.

group without TGase. Although it can be demonstrated that the close combination of the gel matrix and protein is enhanced by TGase, and strongly bound by the gel network structure, the combination of non-mobile water and protein is relatively poor, and its content is easily influenced by the external environment (An *et al*., 2021). This previous study indicated that the cross-linked structure occupied the original position of water, and the gel formed replaced the water, increasing the fluidity of water (Wang *et al*., 2020). Consistent with these findings, the present work concluded that TGase could lead to less mobile water and a decrease in WHC.

FT-IR

The secondary structure of a protein refers to the spatial conformation of the primary chain atoms in the peptide chain. The present work employed FT-IR to obtain the vibration spectrum of each sample (500 - 4000 cm^{-1}; Figure 5A). The results demonstrated that the amide I band was very sensitive to the hydrogen bonding mode, dipole-dipole interaction, and geometry of the terminal peptide backbone. It is the most important characteristic band in the muscle fibre spectrum (Liang *et al*., 2020a). Without TGase, microwave treatment increased the α-helix and random coil content, and decreased the β-turn and β-sheet content compared with water bathing

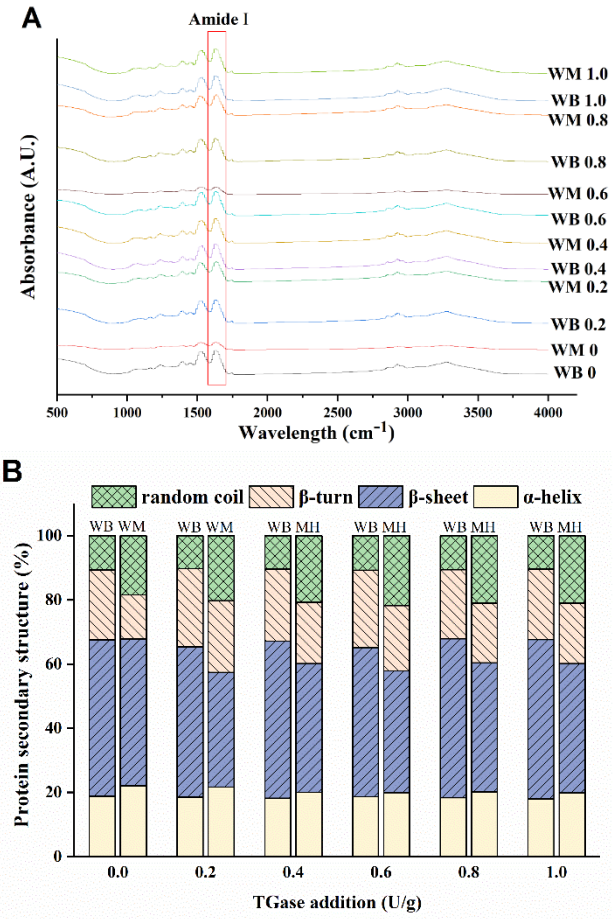


Figure 5. Effects of TGase on Fourier transform infrared spectroscopy (500 - 4000 cm^{-1}) **(A)** and protein secondary structure **(B)** of surimi gels subjected to WB and WM treatments.

treatment (Figure 5B). A study reported similar results when investigating the effect of microwave combined with water bath heating on the gel properties of a surimi-crab meat mixture (Liang *et al*., 2020a). Compared with the blank group, the α-helix and β-sheet contents of samples with TGase added in the WB and WM groups decreased, while the random coils content increased. However, the relative contents of the four protein secondary structures did not markedly change when 0.2 - 1.0 U/g TGase was added to WB and WM groups. Another study found that aggregated α-helix and β-sheet structures were higher in surimi gels containing TGase because of the higher proportion of high-valence cross-links in the sample (Herranz *et al*., 2013). However, the α-helix content in this experiment decreased, possibly because the low NaCl content affected protein conformation. Ye *et al*. (2022) reported that microwave field treatment with 1.5% NaCl decreased the relative α-helix content of myofibrillar protein (Jiang *et al*., 2016). In addition, some studies have shown that higher levels of random coils and lower levels of α-helices lead to a stable and orderly three-dimensional network of the protein skeleton that can retain moisture and improve muscle texture. This might be the reason why the gel of Mandarin fish surimi with TGase and microwave heating had better texture characteristics.

Conclusion

The present work improved the quality of low-sodium Mandarin fish surimi gel using TGase and WM. In the group with TGase, gel in WM with 0.8 U/g TGase yielded better WHC. TGase improved the surimi gel deformation, gel strength, and whiteness, and the strengthening effect was more significant after WM. TPA results depicted that WM could produce elastic and soft surimi gels. In addition, T2 relaxation time analysis showed that WM assisted the non-mobile water to bind in the gel network, and adding TGase reduced the non-mobile water, consistent with the WHC study. The FT-IR results demonstrated that TGase promoted the covalent cross-linking of proteins, and formed a more stable three-dimensional network structure in WM. The present work provided a theoretical basis for developing low-sodium freshwater fish products using TGase and microwave heating to produce high-quality and healthier consumer products.

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