Comparison of rheological behaviors and nanostructure of bighead carp scales gelatin modified by different modification methods

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Abstract In this study, microbial transglutaminase (MTGase) and pectin were compared to modify bighead carp (*Hypophthalmichthys nobilis*) scale gelatin. The functional properties of modified fish scales gelatin (FSG) were largely improved, including melting temperature and rheological behavior. While, modification methods decreased the triple-helix content and destroyed the single left-hand helix chain of modified FSG as investigated by X-ray diffraction. MTGase could induce the denser and finer gels network, but had none significant effect on nanostructural properties of fish gelatin. Pectin inserted itself into the fish gelatin gels network and caused aggregations, forming crystalline peaks and various nanostructures. In particular, compared with pectin modified FSG, MTGase produced FSG with lower storage modulus and

apparent viscosity, but higher gel points and melting points.

Keywords Bighead carp scales gelatin · Microbial transglutaminase · Pectin · Rheological behavior · Nanostructure

Introduction

Gelatin, a protein derived from acidic or alkaline heat-denatured collagen, is widely used in food products and pharmaceutical industries due to its excellent multiple functions (Karim and Bhat 2009; Sha et al. 2014). Currently, about 98.5% of the world's gelatin production is extracted from cattle hides, beef bones and pork skin (Karim and Bhat 2009; Sow and Yang 2015). However, applications of mammalian gelatin have religious restrictions as well as food safety concern because these gelatin may be contaminated by pathogenic vectors such as prions from the diseased animal (Sow and Yang 2015; Huang et al. 2017). Since then, there has gained great interest in gelatin alternatives source, such as fish processing by-products (skin, bone, scale et al.) (Sha et al. 2014). Nevertheless, compared with mammalian gelatin, fish gelatin generally possesses poorer gelling and inferior rheological properties, which limit its large-scale applications (Karim and Bhat 2009).

In order to overcome these traits, various approaches have been put forward for improving its gel and rheological properties, such as high pressure (Montero et al. 2002); genipin, glutaraldehyde and caffeic acid (Chiou et al. 2006; Hernández-Balada et al. 2009), MTGase (Mohtar et al. 2013; da Silva et al. 2015), κ -carrageenan (Pranoto et al. 2007) et al. Among all the modification methods, MTGase

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and polysaccharides exhibited excellent superiority in terms of faster crosslinking rates, lower price and non-toxicity (Karim and Bhat 2009). Pectin is an acidic water-soluble, inexpensive, nontoxic, and natural polysaccharide, which has high nutritional value, and is widely used in pharmaceutical and food industry as biodegradable and biocompatible polymer (Liang et al. 2015). Associative interactions between pork gelatin and pectin had been reported to improve rheological properties of mixtures (Liu et al. 2007). However, few reports about comparing the rheological and nanostructural properties of MTGase and pectin modified fish gelatin.

In our previous work, we extracted FSG from bighead carp (*Hypophthalmichthys nobilis*) scales and reported its physical–chemical properties and structural characteristics (Sha et al. 2014; Tu et al. 2015). Nevertheless, there is no information regarding modifying bighead carp scales gelatin. Therefore, in this study, we strived to compare the effect of MTGase and pectin on the melting temperature and rheological behavior of modified bighead carp scales gelatin. The X-ray diffraction, microstructure and nanostructure were also determined to characterize the properties of modified FSG to obtain a good modification method.

Materials and methods

Materials

Bighead carp (*H. nobilis*) scales were purchased from a local fresh fish market (Nanchang, China). High-methoxyl citrus pectin was bought from Sigma Chemical Co (P9135, Shanghai, China). Microbial transglutaminase (MTGase) was kindly donated by Yiming company (Taixing city, China) with activity of 100 U/g powder according to information provided by Yiming Company. All other chemicals used were of analytical grade. Deionized water was used for all the experiments.

Preparation of gelatin gel

FSG was extracted according to our previous reports (Sha et al. 2014). The lyophilized gelatin was dissolved with deionized water in a water bath at 50 °C with constant mild stirring for preparing 6.67% (w/v) gelatin solution. The pH of gelatin solutions was adjusted to 6.5 using 0.1 M NaOH and HCl solution.

MTGase modified FSG solution was prepared by adding the enzyme to gelatin solution at the concentrations of 0, 0.02, 0.04, and 0.06% (w/v), named control, TG-FG_(0.02%), TG-FG_(0.04%) and TG-FG_(0.06%) respectively. Mixture solution was incubated at 40 °C for 40 min. Subsequently,

the enzyme was irreversibly inactivated at 100 °C for 10 min. Pectin was added into the 6.67% (w/w) FSG solution to get the final pectin concentration at 1, 3, and 5% (w/v), named P-FG_(1%), P-FG_(3%) and P-FG_(5%) respectively. The mixture was heated in a water bath with constantly stirring at 80 °C for 1 h. At the end of the treatment, all the modified FSG were cooled down to room temperature and kept in refrigerator (4 °C).

Determination of melting temperature

The melting temperature of gelatin gels was measured as described by Hernández-Balada et al. (2009). Gelatin solution was transferred into a 10 × 100 mm (diameter × length) screw-cap test tubes, leaving some headspace in the tube. The test tube was held upside down in a 4 °C refrigeration for 16–18 h, and then transferred into a water bath with constant temperature at 10 °C for 10 min. The water was warmed gradually at a rate of 0.5 °C/min. The melting temperature was recorded as the temperature at which a bubble started to move up to the headspace. The experiment was conducted in triplicate.

Rheological measurement

Rheological measurements of gelatin samples were performed according to the method of Mohtar et al. (2014). Briefly, the experiment was performed using a shear stress-controlled rheometer (Paar–Physica MCR 302, Anton Paar, Germany) with a cylinder geometry (Couette geometry, CC27). Approximately 19 mL of gelatin solution was transferred to cylinder and kept at 40 °C for 10 min. Temperature sweeps were carried out from 40 to 5 °C and 5 to 40 °C with heating/cooling rates of 0.5 °C/min. The measurements were performed at 0.5% strain and 1 Hz frequency.

To assess the behavior of modified gelatin gels, a dynamic frequency sweep measurement was applied. Each sample was held at 5 °C for 30 min before test. The frequency sweep measurement was applied at a constant strain of 0.5% by varying the frequency from 0.01 to 10.0 Hz.

The apparent viscosity (Pa·s) of gelatin solution was determined at a various shear rates (0.01–100.00/s, 1/s) at 25 °C. All the measurements were carried out in triplicate within the identified linear viscoelastic region.

X-ray diffraction measurement

The X-ray diffraction analysis of gelatin was performed on a D8 Advance Bruker X-ray diffractometer (Bruker AXS Co., German) according to Zhang et al. (2011). Copper Ka was used at 40 kV and 35 mA. The scanning regions were collected from 5° to 50° (2θ) with a step size of 0.05° (2θ).

Microstructure analysis

The microstructures of gelatin gels were visualized using a scanning electron microscopy (SEM) according to the method of Wangtueai et al. (2010) with some modifications. Gels having a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h at 4 °C. Then they were rinsed with distilled water three times for 10 min, and were dehydrated in a serial concentration of 30, 50, 70, 80, 90, and 100% (v/v) ethanol, respectively. The dried samples were mounted on a conducting resin. The specimens were observed using an environmental SEM Quanta200F (FEI Deutschland GmbH, Germany) at an acceleration voltage of 20 kV.

Atomic force microscopy (AFM)

Characterization of gelatin nanostructure was assessed by Atomic force microscopy (Agilent 5500, Agilent technologies, USA) according to the work of Sow and Yang (2015). The original gelatin solution (6.67%, w/w) was diluted 10 times (approximately 0.667%) for the AFM imaging. About 10 µL of each diluted solution was pipetted rapidly onto a piece of freshly cleaved mica sheet, and kept in a desiccator for at least 12 h before imaging. The imaging process was conducted in tapping mode with resonance frequency around 330 kHz, constant force of 48 N/m and scan rate of 0.6 Hz. All the experiments were conducted at room temperature.

Statistical analysis

All measurements were made in triplicate to get mean values $\pm$ standard deviation. The results were analyzed using general linear model (SPSS 17.0 for Windows; SPSS Inc, Chicago, Illinois, USA) with Duncan's multiple range test at significant level of $p < 0.05$.

Results and discussion

Increased melting temperature of modified FSG

Melting temperature is one of the most important functional properties of gelatin beside gel strength, viscoelastic and gelling temperature (Pranoto et al. 2007). The melting temperatures of fish gelatin (11–28 °C) were much lower than that of mammalian gelatins (28–31 °C), and that limited its applications in many practical commercial industries (Karim and Bhat 2009). In this study, the melting temperatures of FSG are shown in Table 1. With the similar measuring methods, the melting temperature of original bighead carp scales gelatin (28.1 °C) was

Table 1 Melting temperature, swelling ratios, diffraction peaks and peaks intensity of original/modified fish scales gelatin

	Control	TG-FG			P-FG		
		TG-FG _(0.02%)	TG-FG _(0.04%)	TG-FG _(0.06%)	P-FG _(1%)	P-FG _(3%)	P-FG _(5%)
Melting temperature (°C)	28.1 $\pm$ 0.07a	28.55 $\pm$ 0.07b	29.15 $\pm$ 0.21c	30.2 $\pm$ 0.07d	31.45 $\pm$ 0.07e	33.3 $\pm$ 0.14f	34.9 $\pm$ 0.14g
First diffraction peak (°)	7.96 $\pm$ 0.01b	7.88 $\pm$ 0.02b	7.86 $\pm$ 0.01b	7.85 $\pm$ 0.01a	8.19 $\pm$ 0.03c	8.28 $\pm$ 0.03d	8.99 $\pm$ 0.02e
Intensity of first peak (count)	170 $\pm$ 0.4g	163.84 $\pm$ 0.1f	150.45 $\pm$ 0.01e	124.85 $\pm$ 0.34b	110.26 $\pm$ 0.14a	131.85 $\pm$ 0.05c	142.45 $\pm$ 0.5d
Second diffraction peak (°)	19.77 $\pm$ 0.01d	19.73 $\pm$ 0.01d	19.47 $\pm$ 0.01b	18.05 $\pm$ 0.01a	19.38 $\pm$ 0.02c	20.77 $\pm$ 0.02e	20.83 $\pm$ 0.02e
Intensity of second peak (count)	237.88 $\pm$ 0.02g	177.79 $\pm$ 0.06f	175.91 $\pm$ 0.02e	164.02 $\pm$ 0.12d	133.28 $\pm$ 0.34a	149.90 $\pm$ 0.29b	150.19 $\pm$ 0.44c

a–g: means within a column with different letters are significantly different ($p < 0.05$)

significant higher than that of red tilapia skin gelatin (22.45 °C) (Jamilah and Harvinder 2002), tiger-toothed croaker skin gelatin (20.3 °C) (Koli et al. 2011). Therefore, bighead carp scales gelatin can be used as a good source for modification to obtain gelatin with higher melting temperature and could be used instead of mammalian gelatin in selected food applications.

As Table 1 shows, all the modified FSG exhibit higher melting temperature than that of control. The melting temperatures of TG-FG increased from 28.55 to 30.2 °C as the concentration of MTGase increased from 0.02 to 0.06% (w/v). In this study, MTGase modified bighead carp scales gelatin showed higher melting temperature than that of MTGase modified tiger-toothed croaker skin gelatin (20.3–22.7 °C) (Koli et al. 2011). This significant difference might be attributed to the amino acids difference in various fish gelatin sources. MTGase could catalyze lysine and glutamine of gelatin to create cross-linked peptide bonds (Mohtar et al. 2013; da Silva et al. 2015), propitiating stronger and higher stability of the gel network.

Moreover, the melting temperature of P-FG increased from 31.45 to 34.9 °C as pectin content increased from 1 to 5% (w/v), which was much higher than that of carrageenan modified fish gelatin (28.55 °C) (Pranoto et al. 2007). Gelatin is a flexible protein, which is able to form a maximum number of binding interactions with the other polysaccharides strongly (Doublier et al. 2000). Therefore, more hydrogen bonds were produced from shielding of electrostatic interaction as increased pectin content. During the process of cold maturation, low temperature was favorable for the formation of side-by-side associations of gelatin chains and the hydrogen bonds were enhanced to strengthen the gel network (Al-Ruqaie et al. 1997).

Additionally, it was worth noting that two different measuring methods were used to illustrate the gel-sol transition, static methods (constant temperature water bath) and dynamic method (rheology). The melting temperatures of modified FSG were significantly different ($p < 0.05$). As measured by static methods, the highest melting temperature of TG-FG (30.2 °C) is lower than that of P-FG (34.9 °C) (Table 1). However, interestingly, the result is on the contrary when measured by rheometer (Table 1 and Table S1). This may be due to the different modification mechanisms of MTGase and pectin, as well as the different measurement process (such as instrument, maturation temperature and time, measuring parameters et al.). Therefore, MTGase presents more stable efficient in modifying fish gelatin than that of high-methoxyl pectin. The modified gelatin with higher melting temperature may overcome the drawback of gelatin of “melt in the mouth”. It can be used in candy and capsule industry, as well as in the condition of higher storage temperature needed.

Rheological behaviors

Temperature sweep measurements

The temperature sweep was performed at constant frequency of 1 Hz and strain of 0.5% to determine the gelation kinetics and gel properties of FSG. Storage modulus (G') and loss modulus (G'') of the gelatin samples as a function of temperature are shown in Fig. 1 and Fig. S1. The result shows that gelatin exhibited liquid behavior with very low G' and G'' at the initial temperature (40 °C) (Fig. 1a and Fig. S1 A). As the gelatin start to aggregate, a gel begins to form and G' increased markedly as temperature is decreased. The transition of solution to gel (sol-gel) of gelatin samples corresponds to a temperature in which a crosslinked material undergoes phase transition from a liquid to a solid-like state (Fonkwe et al. 2003), as depicted in Fig. S2 A. The gelation temperature (T_{gel})

Fig. 1 Storage modulus (Pa) of unmodified/modified fish scales gelatin gels cooled from 40 to 5 °C (a) and heated from 5 to 40 °C (b). Control: unmodified fish scales gelatin (FSG); TG-FG_(0.02%), TG-FG_(0.04%), TG-FG_(0.06%): FSG modified by various MTGase concentration (0.02, 0.04, 0.06% w/v); P-FG_(1%), P-FG_(3%), P-FG_(5%): FSG modified by various pectin content (1, 3, 5% w/v)

increased from 21.51 to 23.87 °C with an increase of MTGase concentration, but decreased from 21.62 to 19.43 °C with increasing pectin concentration (Table S1). MTGase and lower pectin content could improve T_{gel} , due to the formation of covalent networks (Koli et al. 2011) and coupled networks (Fonkwe et al. 2003; Hill et al. 1998) respectively. However, high concentration of pectin formed segregative interactions and the system was evolved into two coexisting phase, resulting in decreasing the possibility for gelatin to recover the original triple helix conformation, decreasing T_{gel} . Moreover, higher T_{gel} indicated individual polymer and small aggregates were more quickly became part of larger groups, and resulted in higher T_{mel} value (Table S1).

Generally, the final G' of modified FSG decreased with increasing concentration of MTGase and pectin, except P-FG_(1%) (Table S1). TG-FG_(0.06%) presented higher G' value than that of TG-FG_(0.04%) ($p < 0.05$), implying the presence of covalent networks do not prevent triple-helics formation as suggested by da Silva et al. (2015). P-FG_(1%) showed markedly higher values of G' than that of TG-FG, indicating pectin could lead to form complex gel with higher modulus. This was because low content of pectin contributed to the electrostatic interactions and hydrogen bond interactions between pectin and gelatin molecules, which contributed to gelatin triple-helix formation (Hill et al. 1998; da Silva et al. 2015).

During heating (Fig. 1b and Fig. S1B), the G' and G'' decreased, suggesting that there was only a single-phase transition (gel-sol) within the temperature range (Fig. S2B). Melting temperature (T_{mel}) was increased from 29.41 to 31.24 °C as increased MTGase. However, T_{mel} was decreased from 29.49 to 28.49 °C as increased pectin content (Table S1). Pectin may make the interaction between pectin and gelatin change to phase separation and disturb the network of the mixed system during rheological measurements (Rafieian et al. 2015), forming weaker gel network.

Frequency sweep measurements

The strength of gelatin gel network can be obtained from change of G''/G' ($\tan \delta$) upon the frequency sweep (Kaewruang et al. 2013). The $\tan \delta$ of co-gel was lower than 0.1, which is an indicator of a good gel network or more solid-like nature of the material. Compared with the $\tan \delta$ of P-FG, TG-FG had lower value (Fig. 2a), suggesting TG-FG formed a better gel network. This also revealed that P-FG possessed lower T_{mel} than TG-FG (Table S1). Meanwhile, $\tan \delta$ of TG-FG gels decreased upon frequency increased, while P-FG gels decreased at beginning and then increased as frequency increased, illustrating that TG-FG possessed stronger gels than those

Fig. 2 The values of G''/G' ($\tan \delta$) of unmodified/modified gelatin gels upon different frequency (a), apparent viscosity of unmodified/modified gelatin solution upon shear rate from 0.01 to 100 1/s (b)

of P-FG. All the $\tan \delta$ of P-FG increased with increasing frequency, demonstrating that a tendency toward weakening gel network behavior at higher frequencies (Gornall and Terentjev 2008).

Apparent viscosity

Viscosity is the second most important commercial property for gelatin, because it is critical in process control to ensure proper handling in industrial process (Wulansari et al. 1998). Viscosity can be significantly affected by variables such as shear rate, pH, temperature, pressure and time of shearing (Marcotte et al. 2001). The modified FSG showed higher apparent viscosity values than that of original gelatin (Fig. 2b). MTGase catalyzed the formation of intra- and intermolecular cross-links, producing higher molecular weights aggregation, resulting in increasing the initial apparent viscosity of TG-FG. Chen et al. (2005) also reported MTGase could increase collagen solutions

viscosity. In comparison to MTGase, pectin seemed more efficiently increased the initial apparent viscosity, as a consequence of viscosity characteristic of pectin, multi-molecular clusters were forming through electrostatic interactions and hydrogen bonding between polymers, resulting in strongly enhanced viscosity. This was in agreement with Eleya and Turgeon (2000) reported that k-carrageenan— β -lactoglobulin complex gels with high viscosity.

In general, the apparent viscosity of gelatin decreased with increasing shear rates, showing non-Newton behavior. Interestingly, in this study, TG-FG_(0.04%), TG-FG_(0.06%), P-FG_(3%) and P-FG_(5%) exhibited Newton behavior at low shear rates and non-Newton behavior at high shear rates. This phenomenon might be caused by the changes in gels dispersity, molecular shape and strength of intra-molecular chemical bonds upon various shear rates (Huang et al. 2017). Furthermore, the higher liquid viscosity of modified fish gelatin produced lower stress at lower shear rate. Increasing shear rate leads to particle clustering and consequently increasing viscous stresses (Fig. S3), resulting in shear thickening.

Compared with TG-FG, P-FG had higher viscosity, demonstrating P-FG could increase the physical stability of foods and the overall mouth-feel properties as suggested by Marcotte et al. (2001). Furthermore, we speculated that P-FG with higher viscosity needed longer time and higher temperature to make gas move up, and resulted in higher melting temperature as measured by static methods (Table 1).

X-ray diffraction measurements

According to Sha et al. (2014), fish gelatin exhibited two diffraction peaks, which were related to the triple-helix structure and the single left-hand helix chain respectively. In this study, gelatin display two characteristic diffraction peaks at 7–9° and 17–20° as shown in Fig. 3. These were similar to the reports of Zhang et al. (2011) and Liu et al. (2012), who detected two diffraction peaks at around 7–8° and 17–18.5° for walleye pollock skin gelatin, and 7.42° and 19.58° for grass carp fish scale gelatin, respectively.

As Table 1 shows, modified FSG presents characteristic diffraction peaks at around 7–9° and 17–20°, but the intensity is lower than that of control. All the intensity of diffraction peaks of TG-FG showed continuously decreasing trends, indicating MTGase could decrease triple helix content and destroy left-hand helix. MTGase catalyzed formation of covalent cross-linkage junctions along the chains of gelatin and decreased possibility of forming hydrogen bonds between glycine and proline to stabilize the triple helix of fish gelatin, and resulted in inhibiting fish

Fig. 3 X-ray diffraction patterns of unmodified/modified gelatin with and without modification

gelatin to assemble into triple-helices, as suggested by da Silva et al. (2015) and Giraudier et al. (2004).

Moreover, the intensity of two diffraction peaks for P-FG became flat and broad gradually with increasing the amount of pectin, suggesting that pectin also could decrease the triple-helix content and destroy left-hand helix of gelatin. The characteristic intense peaks of pure pectin at around 9.18°, 18.36°, 19.64° and 28.24° (2 θ) (Jiang et al. 2012). Interestingly, P-FG_(5%) possessed peaks at around 9° and 19.64° were the same as pure pectin, demonstrating it exhibited crystalline behavior. This might be due to high concentration of pectin could bury X-ray diffraction peaks of pure gelatin through the hydrogen bonding molecular interaction between pectin and gelatin (Sundaralingam, 1968). Given the above results, it reflected that chemical and physical modifications could alter the tridimensional network of gelatin through cross-linking process.

Microstructure of gelatin gels

The microstructures of fish gelatin gels are visualized by SEM (Fig. 4). The bighead carp scale gelatin gels exhibited a much looser network with fairly uniform strands and the narrow range of pore sizes, compared with the modified gelatin gels. TG-FG exhibited finer and denser strains by the formation of covalent bonds and resulted in greater intermolecular aggregation between adjacent molecules in the gels structure (Jongjareonrak et al. 2006; Wangtueai et al. 2010). Prominently, the amount of denser strain aggregates increased along with increasing MTGase

Fig. 4 The microstructures of unmodified/modified gelatin gels with and without modification (magnification 3000 $\times$) and the last indicates P-FG_(5%) (magnification 12,000 $\times$)

content, especially when it reached to 0.06% (w/v). This result was similar to the melting temperature (Table 1 and Table S1).

However, P-FG showed a more homogenous and “swollen network” than the other gelatin gels, indicating it is very without any phase separation. This suggests that the interaction between pectin and gelatin is smooth leading to structural integrity. This could clearly found that pectin inserted itself in gelatin gels network at 5% (w/v) pectin content, since gelatin and pectin could form polyanion-polycation complexes. This was similar with Fonkwe et al. (2003), who reported that the coupled complex networks could be observed in the low-acyl gellan–gelatin gels. The cross-linking network might be associated with the higher melting temperature of gelatin gel (Table 1), which was similar with Papageorgiou et al. (1994)’s report that the gellan could increase melting temperature of gelatin markedly.

Nanostructure of gelatin

AFM can be applied as a novel tool to help understand the underlying mechanism of changed properties of gelatin (Huang et al. 2017). As shown in Fig. 5a, the original gelatin shows a conglomerate structure with different diameters. The image shows the mean diameter of small aggregates typically is 593.12 ± 134.55 nm in length and 5 nm in height. It had been reported that these aggregates

appear to be clusters of molecules presumably linked by intermolecular triple helix formation (Farris et al. 2011). All the TG-FG possessed typical spherical with mean diameter was 210.06 ± 100.93 and 265.41 ± 163.73 nm for TG-FG_(0.04%) and TG-FG_(0.06%), respectively. All the TG-FG possessed typical spherical. These demonstrated that there were no significant differences for the spherical nanoparticle aggregates in the TG-FG. This was due to gelatin aggregation occurred as a multimeric association (or cluster association) process, where multimers of any size could form the cluster structures and associated further into spherical aggregates (Yang et al. 2008).

Addition of pectin led to a completely different scenario (Fig. 5b). As concentration of pectin increased, the nanostructures of P-FG were different accompanying by the increase of mean diameter of nanoparticle. The mean size was 296.32 ± 201.55 , 512.83 ± 268.81 nm for P-FG_(3%) and P-FG_(5%), respectively. Clearly, the size of aggregations tended to be larger as increased pectin content. Compared with the nanostructure of TG-FG, P-FG showed various structures, such as spherical, conglomerate, coacervates, and small massif. These indicated that pectin had significant effect on the nanostructure of fish gelatin. Pectin and gelatin formed complex nanostructure, and this was consistent with the results from the SEM analysis. This confirmed that the compatibility between the two components, which generated a relative large particle, yielding a more pronounced physical polyion complex.

Fig. 5 Nanostructure of fish gelatin and fish gelatin modified by different concentrations of MTGase (a) and pectin (b)

Conclusion

This study was aimed at comparing MTGase and pectin influence the functional and structural properties of FSG, finding out MTGase and pectin could improve melting temperature and apparent viscosity of FSG. P-FG exhibited higher storage modulus (G') and apparent viscosity than those of TG-FG. In particular, MTGase and pectin could disturb the formation of triple-helix network, as well as decreased the content of triple helix and destroyed left-hand helix. The addition of pectin to the gelatin system would result in formation of crystal peak. SEM showed that pectin and gelatin could form complex aggregations by filling pectin in the network of gelatin gels, and this phenomenon also could be obtained from the image of AFM. MTGase could enhance the strength of gels network, but had none significant effect on the nanostructure.

MTGase had significant effect on the improvement of melting temperature of fish gelatin, demonstrating that TG-FG possessed higher melting temperature could overcome melt in the mouth. P-FG showed higher viscosity, indicating P-FG could increase the physical stability of foods and the overall mouth-feel properties. These modified fish scale gelatins could replace mammalian gelatin and are suitable for a variety of applications in the food, pharmaceutical and biological industries.

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