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Insight into the mechanism of physicochemical influence by three polysaccharides on myofibrillar protein gelation

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ABSTRACT

In this study, the effect and mechanism of myofibrillar protein (MP) gelation influenced by the hydration characteristic of three polysaccharides were studied through puncture test, paraffin section, SEM and Raman spectroscopy. The gel strength and water holding capability reflect that MP gelation only significantly improves until modified starch (MS) addition beyond 1.0%. The MS granule improves MP gel property through simply physical swelling effect. At gelatinization temperature, MS absorbs the moisture nearby to compress the MP three-dimensional networks, but the swelling effect is limited. The insoluble dietary fiber (IDF) improves MP gelation property through moisture stability. The IDF addition could lessen the appearance of moisture channel in MP gel networks and promote the interaction of hydrophobic groups. The MP gelation with 2.0% IDF addition has the highest gel strength (279 g) and water holding capability (91.87%). The konjac glucomannan (KG) (> 1.0%) could degrade gel property of MP gelation through interpenetrate structure, because the KG hydrogel hinders the aggregation of the MP gel networks. In conclusion, the IDF, which has strong water-holding capability at room temperature and distribute individually, is the best polysaccharides-based fat replacement in low-fat restructured products.

1. Introduction

Restructured meat products such as frankfurters and bologna have been favored by customers. This is not only because these products have satisfactory sensory properties including texture, flavor and juiciness but also they supply necessary dietary protein, energy, vitamins, and minerals. Restructured meat products, however, contain over 30% animal fat that includes a high content of cholesterol and saturated fat. Excess intake of animal fat results in an increasing incidence of various diseases such as obesity, hypertension, and cardiovascular diseases (Colmenero, 1996; Siri-Tarino, Sun, Hu, & Krauss, 2010). Hence, the development of healthier restructured meat products with low animal fat has become a key target for the meat industry. However, animal fat plays an important role in restructured meat products, including improving the product quality by reducing cooking loss and providing an appropriate flavor substance (Jimenez-Colmenero, 1996). The most efficient and economic approaches for reducing the fat content in the restructured meat products while retaining their overall acceptability are the use of fat replacements. Currently, the polysaccharide-based fat replacements are widely used in low-fat products formulation to improve the cooking yield, enhance the water-holding ability, reduce formulation costs, modify texture and improve the freezing stability of restructured meat products (Debusca, Tahergorabi, Beamer, Matak, & Jaczynski, 2014; Ramírez, Uresti, & Vázquez, 2011; Talukder, 2015).

However, numerous studies only focus on utilization of novelty polysaccharides in restructured meat product. Besides, the polysaccharides from various plants parts and plants species gave rise to a variety of physical and chemical characteristics. Hence, the various polysaccharides result in the different effects on low-fat quality of restructured meat products; for example, some starch and gum such as

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modified starch and carrageenan improved the textural properties of the restructured meat products (Ayadi, Kechaou, Makni, & Attia, 2009; Sun, Xiong, & Zhao, 2014); while some gum and cellulose derivatives such as xanthan gum and carboxymethyl cellulose have a negative effect on the restructured meat products (Gibis, Schuh, & Weiss, 2015; Montero, Hurtado, & Pérez-Mateos, 2000). Hence, the restructured meat industries are hard to choose a specific and efficient polysaccharides-based fat replacement to use in low-fat restructured sausage and it is necessary to elucidate the effect and mechanism of the myofibrillar protein gel properties influenced by various polysaccharides.

Based on the hydration characteristic, the polysaccharides used in the low-fat products mainly divide into three categories: starch, which absorbed the moisture and swelled to form the semitransparent granule at the gelatinization temperature (Kong, Ogawa, & Iso, 1999); Gums, which could gelatinize through hydrogen bonds at room temperature and fluidize with the breaking of hydrogen bonds at high temperature (Ramírez et al., 2011); Cellulose derivative, which is the insoluble dietary fiber and has inherent physical structure at various temperatures. In this study, we choose cross-linked acetylated cassava starch, konjac glucomannan and sugarcane insoluble dietary fiber to represent the three categories of polysaccharides, respectively. In the restructured meat products, the cross-linked acetylated cassava starch, konjac glucomannan and insoluble dietary fiber is most typical and efficient fat replacement to improve the quality of low-fat sausage. Therefore, the objective of the present study was to elucidate the effect and mechanism of the gel properties of myofibrillar protein influenced by the hydration characteristic of three polysaccharides (modified starch, konjac glucomannan and insoluble dietary fiber), and it could offer instructive guide for the low-fat meat industry interested in healthier formulations.

2. Materials and methods

2.1. Materials

The modified starch (MS) is cross-linked acetylated cassava starch and purchased from Hangzhou Starpro Starch Co. Ltd (Hangzhou, Zhejiang, China). Based on the test result report from the company, the cross-linked acetylated cassava starch was obtained by adding 0.15 mol of acetylated group per mole of glucose residue and the degrees of cross-linking (DC) were determined to be 0.001 through the spectrophotometric method. In the MS the contents of moisture and ash were determined to be 9.3% and 0.5%, respectively. The konjac glucomannan (KG) were purchased from Shanghai Beilian Biotechnology Co., Ltd (Shanghai, China) and the content of glucomannan is 85%. In the KG the contents of moisture and ash were determined to be 7.7% and 0.7%, respectively. The sugarcane insoluble dietary fiber (IDF) was purchased from Lanzhou Waterrice Biotechnology Co., Ltd (Lanzhou, Gansu, China) and the IDF was modified with alkaline hydrogen peroxide treatment. The composition of IDF was cellulose 59.6%, hemicellulose 26.13%, and lignin 9.2%. Fresh pork ham meat (24 h postmortem, 72.18% moisture, 20.17% protein, 6.75% fat; AOAC 2000) was purchased from Muxuyuan market. The excess fat and connective tissue was cut off, and the meat was stored at −20 °C until required for the MP extraction. All chemicals used were of analytical grade.

2.2. Preparation of polysaccharide-MP composite system

The myofibrillar protein extraction from pork ham was carried out using the method described by Zhuang and Zhou (2016). The thawed meat ground (10 s, 2000 rpm/min) with blender (GM 200, Retsch, Germany) and the process repeated three times. The ground muscle was mixed with four volumes insolation buffer (10 mM Na_2HPO_4/NaH_2PO_4, 0.1 mM NaCl, 2 mM $MgCl_2$, 1 mM EGTA, pH = 7.0, 4 °C) and homogenized (T25, IKA, Inc. Germany) three times for 30 s at 8000 × *g*. The homogenate was filtered through a 20-mesh sieve (0.9 mm) and centrifuged (Model 225, Beckman Coulter, Inc., California, USA) at 5000 × *g* for 10 min. The step above repeated three times and then the precipitant further was homogenized in four volumes of salt solution (0.1 M NaCl), centrifuged (5000 × *g* for 10 min), and washed three times. The final precipitant was collected as myofibrillar protein. The entire preparation process was carried out in a walk-in cooler (4 °C). The biuret method was used to determine the protein concentration of pure MP with bovine serum albumin as the standard. The three polysaccharides added at 4 concentrations: 0.5, 1, 1.5 and 2 g/100 g of MP and the MP concentration of the all final blend systems (0.6 M NaCl, pH = 7.0) were 40 mg/mL (Zhuang et al., 2018). To ensure the uniform distribution of polysaccharides, the blend system was homogenized (1 min, 4 °C) with a power whisk (AHM-P125A, Appliance company Co., LTD, Shanghai, China). Portions of the polysaccharides-MP solutions in plastic tubes (50 mL) were heated at 80 °C for 20 min in water bath (TW20, Julabo Co., Ltd., German) after the centrifugation treatment (800 × *g*, 3 min) to remove internal bubbles (Zhuang & Zhou, 2016). Then, all the samples stored at 4 °C before test.

2.3. Gel strength

The gelling properties of the blending gels were measured according to the method of Liu et al. (2013) with some modifications. The polysaccharides-MP gels were cut (height of 2 cm) and analyzed with a texture analyzer (TA-XT Plus, Stable Micro System Co., U.K.) at room temperature. The gel was subjected to a puncture test with a 0.5-cm-diameter plate probe and the test parameters were as follows: pretest speed of 2.0 mm/s, test speed of 1.0 mm/s, post-test speed of 2.0 mm/s and trigger force of 5 g. The maximum sustained force was used as the gel strength value. The gel samples were placed into cylinders with filter paper, which were suspended inside centrifuge tubes and then centrifuged at 10,000 g (Model 225, Fisher Scientific, Pittsburgh, Pa., U.S.A.) for 10 min at 4 °C. The WHC are reported as percentage (w/w) of water retained after centrifugation. Each sample was analyzed six times.

2.4. Low-field NMR

The samples (approximately 2 g) were filled into cylindrical quartz tubes and heated at 80 °C for 20 min. The measurements of the transverse relaxation time (T_2) were performed on a Niumag Benchtop Pulsed NMR analyzer (Niumag PQ001; Niumag Electric Copporation, Shanghai, China) operating at 22.6 MHz. The T_2 was measured using a CarrePurcelleMeiboomeGill (CPMG) with 16 scans, 12,000 echoes, 6.5 s between scans, and 250 ms between pulses of 90 and 180. The lengths of the pulses were 23 ms for the 180 pulse. The NMR data was primarily analyzed by continuous distribution inverse and discrete exponential fitting. Each sample was analyzed six times.

2.5. Light microscopy of gel structures

Sections of the samples (8 μm thick) were cut using a microtome (CM1900, Leica, German) and then fixed and stained with hematoxylin-eosin following the procedure outlined by Wu, Xiong, and Chen (2011). Slides were observed and photographed using a light microscope (Axio Imager, Zeiss, Oberkochen, Germany) mounted with a digital camera.

2.6. Scanning electron microscopy (SEM) of gel structures

Composite MP gels were examined with a Hitachi S-3000 N scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 20 kV, according to the method described by Han, Xu, and Zhou (2014).

2.7. Raman spectroscopy of protein gels

Raman experiments were performed using a modified version of the procedure by a Jobin Yvon 141 Labram HR800 spectrometer (Horiba Jobi Yvon S.A.S., Longjumeau, France). The spectra were obtained in the range of 600–3050 cm^{-1}. Each spectrum of the samples was obtained under the following conditions: three scans, exposure time of 30 s, resolution of 2 cm^{-1}, sampling speed of 120 cm^{-1}/min, and data collection every 1 cm^{-1}. The spectra were smoothed, baseline corrected, and normalized against the phenylalanine band at 1003 cm^{-1} using Labspec version 5.0 (Horiba/Jobin Yvon, Longjumeau, France).

2.8. Statistical analysis

The entire experiments were independently replicated four times, and a completely randomized design was used. Statistical analysis of the results was performed using the Statistical Analysis System (SAS 9.0, SAS Institute Inc., Cary, NC). The data was evaluated using a one-way ANOVA. Significant differences between the means were identified using Duncan's multiple range tests.

3. Results and discussion

3.1. The hydration characteristic of three polysaccharides in aqueous solution during the thermal process

The Fig. 1 showed the hydration characteristics of three polysaccharides in aqueous solution heated at three temperature points (20 °C, 60 °C and 80 °C) for 20 min. The modified starch (MS) granules do not have hydration with moisture at 20 °C and just deposit in aqueous solution. While the MS granules closed to the moisture phase start to swell and become semitransparent after heating at 60 °C for 20 min. The MS granules completely swell fully and stay at the bottom of the glass bottle after heating at 80 °C for 20 min. The phenomenon is the MS gelatinization, which is a process of breaking down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water, resulted in the irreversibly dissolution of starch granule in aqueous solution. Compare to the MS at 20 °C, the MS at 80 °C hydrate with moisture and increased in volume several times. The insoluble dietary fiber (IDF) can absorb more than 10 times its volume of water and deposited in an aqueous solution. Besides, the IDF had the inherent physical structure and the hydration characteristic of IDF does not change at any temperatures. Compared to the gelatinized MS, the swelling volume of IDF in aqueous solution is much larger, even at 80 °C. The konjac gum (KG) is water-soluble polysaccharide and its aqueous solution is transparent. Due to the interaction between the KG and the water molecules, the KG aqueous solution was viscous and the viscosity sharply increased with the KG concentration. After placing the solutions into a glass bottle, the aqueous solutions with 1.5% and 2% KG do not flow at 20 °C, suggesting that gel networks have formed and the gel immobilizes the moisture migration. The hydrogen bond breaks down with increasing temperature, resulting in the destruction of the weak gel network (Gómez, Míguez, Yáñez, & Alonso, 2017; Zhou et al., 2014). After heating at 80 °C for 20 min, the KG aqueous solutions with various concentrations will flow after the inversion of the glass bottle. Therefore, the phenomena described above reflect the fact that three polysaccharides in aqueous solutions at various temperatures have various specific physical conditions that may have a significant effect on the quality of myofibrillar protein gelation.

3.2. Gel strength and water holding capability

The gel strength and water holding capacity (WHC) are generally used to objectively evaluate the quality and the yield of meat and meat products. Gel strength and WHC indicate the aggregation and water immobilization capability of MP gel networks, respectively. Therefore, Fig. 2 illustrates the effect of the three polysaccharides on gel strength and WHC of MP gelation. The gel strength and WHC are both significantly affected by the concentration and type of polysaccharides ($P < 0.05$). The MS and IDF addition significantly improved the gel strength and WHC of the composite MP gel, with strongest improvement obtained by IDF. The concentration of the added IDF in the range of 0 − 2% significantly improves the gel strength and WHC of the

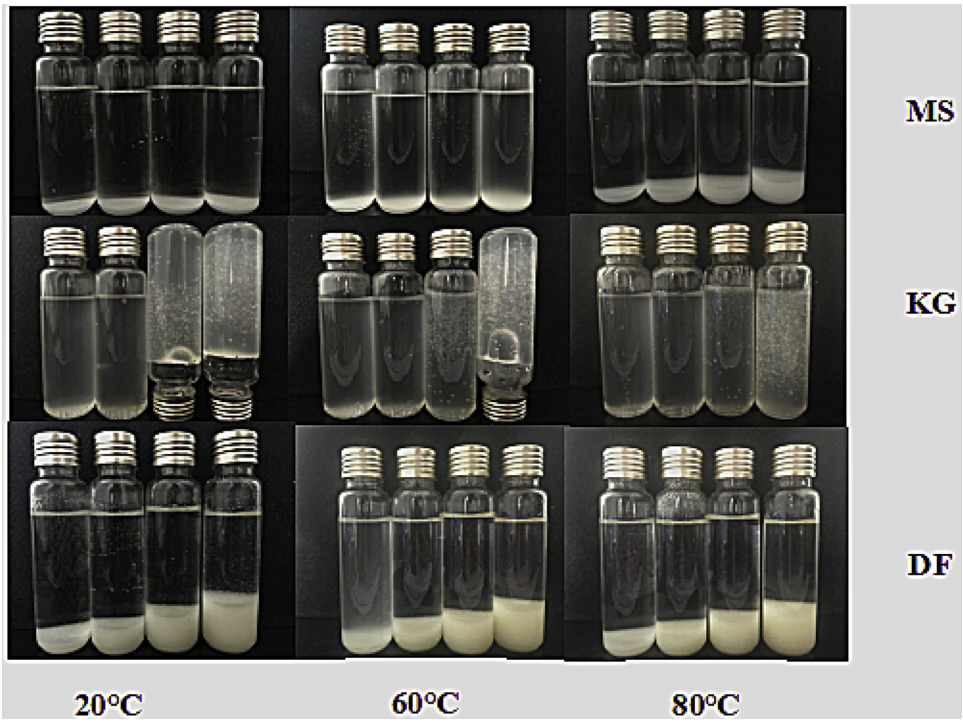

Fig. 1. The physicochemical changes of three polysaccharides in aqueous solution heated from 20 °C to 80 °C.

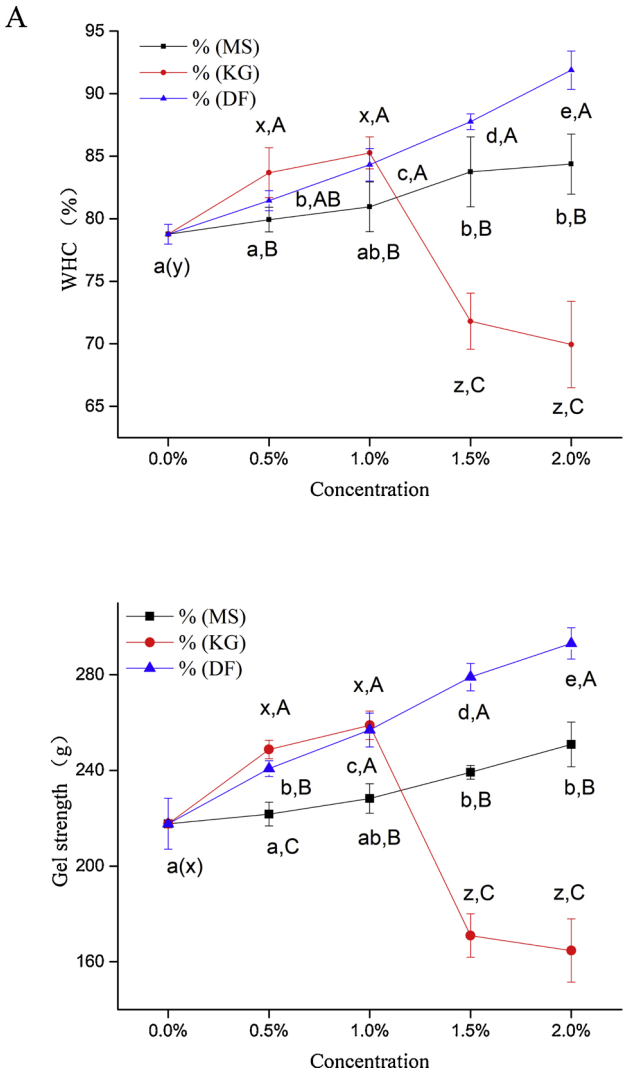

Fig. 2. Gel strength (A) and Water Holding Capacity (B) of MP composite gels with three polysaccharides. Different letters (a–e) indicate significant differences ($P < 0.05$) between treatments with various concentration of same polysaccharide. Different letters (A–C) indicate significant differences ($P < 0.05$) between treatments with same concentration of different polysaccharides.

composite gel, increasing the gel strength and WHC from 221.75 to 279.00 g and from 78.76 to 91.87%, respectively. By contrast, MS addition does not lead to a distinctive improvement in the gel strength and WHC until the concentration exceeds 1.0%. The gel strength and WHC of the composite gel with 2% MS increased significantly to 250.87 and 84.37, respectively ($P > 0.05$). Additionally, the improvements in gel strength and WHC due to the addition of IDF are better than those for the addition of MS at the same concentration. KG has a positive impact on the composite gel in the range of 0.5–1% of the additive concentration, and had the opposite effect beyond this range. This indicates that KG has a limitation for the additive amount to increase the gel strength and WHC. Under the same additional concentration (0.5% and 1%), KG treatment shows the largest improvement of gel textural properties among the three polysaccharides. When the concentration exceeds 1%, the gel strength and WHC of KG treatment are even worse than that of the control.

Some researchers reported that MS, IDF, and KG are entrapped as filler ingredients and influence the formation of the continuous gel matrix, modifying the distribution of the aqueous phase and/or influencing the texture of the final product. The effects of the various cassava starches on gel properties of myofibrillar protein were studied, and it was found that all cassava starches significantly improve the WHC of the composite gel but that only the hydroxypropylated cassava starch and cassava starch significantly improve the gel strength (Sun et al., 2014). Debusca et al. (2014) studied the effect of wheat dietary fiber on the physicochemical properties of surimi gels and found that the addition of WIDF (2%–8%) significantly increased the hardness of the composite gel and that the WHC has the maximum value of 90.62 at the highest examined added content (8%). Furthermore, carrageenan was also studied and was demonstrated to significantly improve turkey meat sausages textural properties at 1.5% (Ayadi et al., 2009).

3.3. Paraffin section

In the paraffin section (400×), the distributions of myofibrillar protein (MP) and three polysaccharides in composite gels were observed by light microscopy (Fig. 3). Fig. 3A shows that the pure MP gel is composed of dense and homogeneous structure with numerous moisture cavities. With the addition of MS, the MS granules absorb the free moisture and swell during the thermal process, forming many large similar circular cavities and embedding into the MP three-dimensional gel networks. The number of the MS cavities increased remarkably with the concentration of the MS additive. Meanwhile, IDF retains its inherent physical structure and acts as an active hydrating polymer, binding a large amount of moisture on its surface. Therefore, similar to the MS, there are many IDF cavities embedded in the gel networks. However, the volumes of the IDF cavities are much larger than those of the MS cavities. KG dissolved in the aqueous solution, and formed a weak hydrogel at high concentration. When a small amount of KG was added, the backbone of the composite gel network is MP and the KG, as the filler, trapped in the protein network (Fig. 3K 1.0%). However, when the KG concentration reached 2%, MP and KG form a protein network and a polysaccharide network, respectively. Additionally, the backbone of the composite gel network is not the MP but the "interpenetrated" structure. Montero et al. (2000) compared the structural behavior of the MP-hydrocolloids composite gels. All of the MP-hydrocolloids composite gels exhibited a two-phase separation system and hydrocolloids were trapped in the MP gel networks. In addition, the hydrocolloids formed a substructure: thickening hydrocolloids (locust bean gum and carboxymethyl cellulose) as a mesh of filaments, and the gelling hydrocolloids (carrageenan) as a continuous structure.

The spatial distribution and morphology of the protein-polysaccharide composite gel network are thermodynamically incompatible, giving rise to phase separation and the formation of a two-phase system. The three polysaccharides with different physicochemical properties resulted in the various spatial distribution and morphology as described above: the polysaccharide phase is embedded in the protein phase; the two separated phases interpenetrate each other. In the former spatial distribution the MP gel network was the backbone structure of the composite gel, and in the latter spatial distribution the backbone structure of the composite gel is the interpenetrated structure.

3.4. SEM

SEM micrographs show the irregular and porous three dimensional network microstructure resulted from the MP denaturation and aggregation during the thermal process. The SEM micrographs clearly reveal that the addition of three polysaccharides results in the significant variations of the MP microstructures. The microstructure of pure MP is crisscrossed by moisture channels (shown in Fig. 4) resulting from the water exudation of protein denaturation during the thermal process (Zhuang et al., 2018). The existence or formation of water channels could block the aggregation and lead to the fragmentation of the MP gel network. However, the water channels in the MP gel network decrease significantly with IDF addition. The water channels

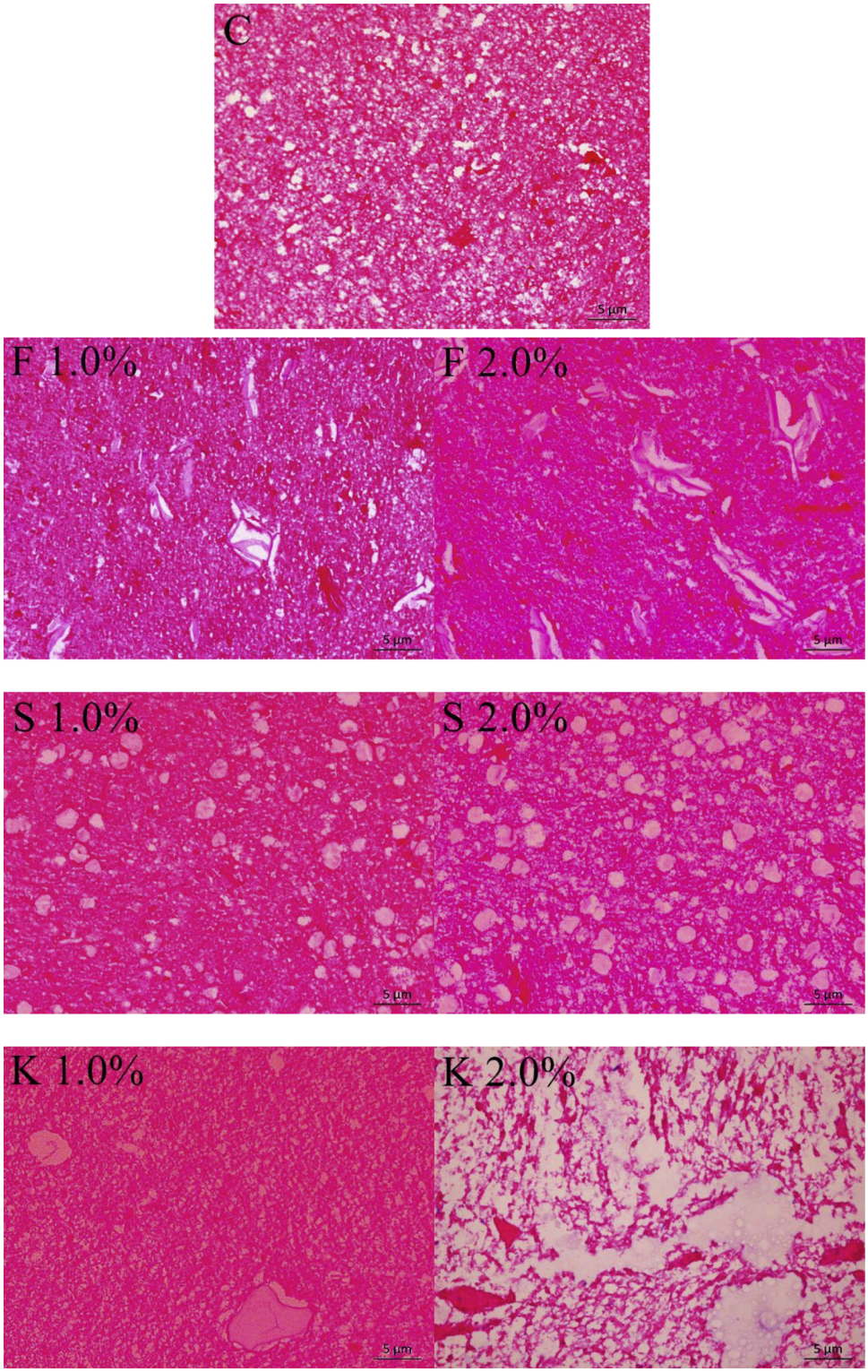

Fig. 3. Micrograph images (400×) of formalin-fixed composite gels with three polysaccharides. C: pure MP; F1.0: MP with 1.0% DF; F2.0: MP with 2.0% DF; S1.0%: MP with 1.0% MS; S2.0%: MP with 2.0% MS; K1.0%: MP with 1.0% KG; K2.0%: MP with 2.0% KG.

finally disappear and the gel network forms a compact and homogeneous structure until the IDF concentration reached 2%. IDF shows a strong water-holding capability and removes some of the moisture from the MP prior to the heating process. The "concentrated" MP promotes the interaction of the hydrophobic groups and decreases the frequency of occurrence of the moisture channels, leading to a compact and homogeneous structure. So in the SEM we could find that the IDF addition could have the significant improvement on the compactness of MP three-dimensional networks.

The microstructure of the MP gel networks with 1% MS addition is similar to that of the control. When the MS concentration reaches 2%, the water channels in the MP gel networks show a small reduction. As described in Section 3.1 we found that the MS granule is insoluble and deposits underwater. The MS granules start to swell and hydrate with

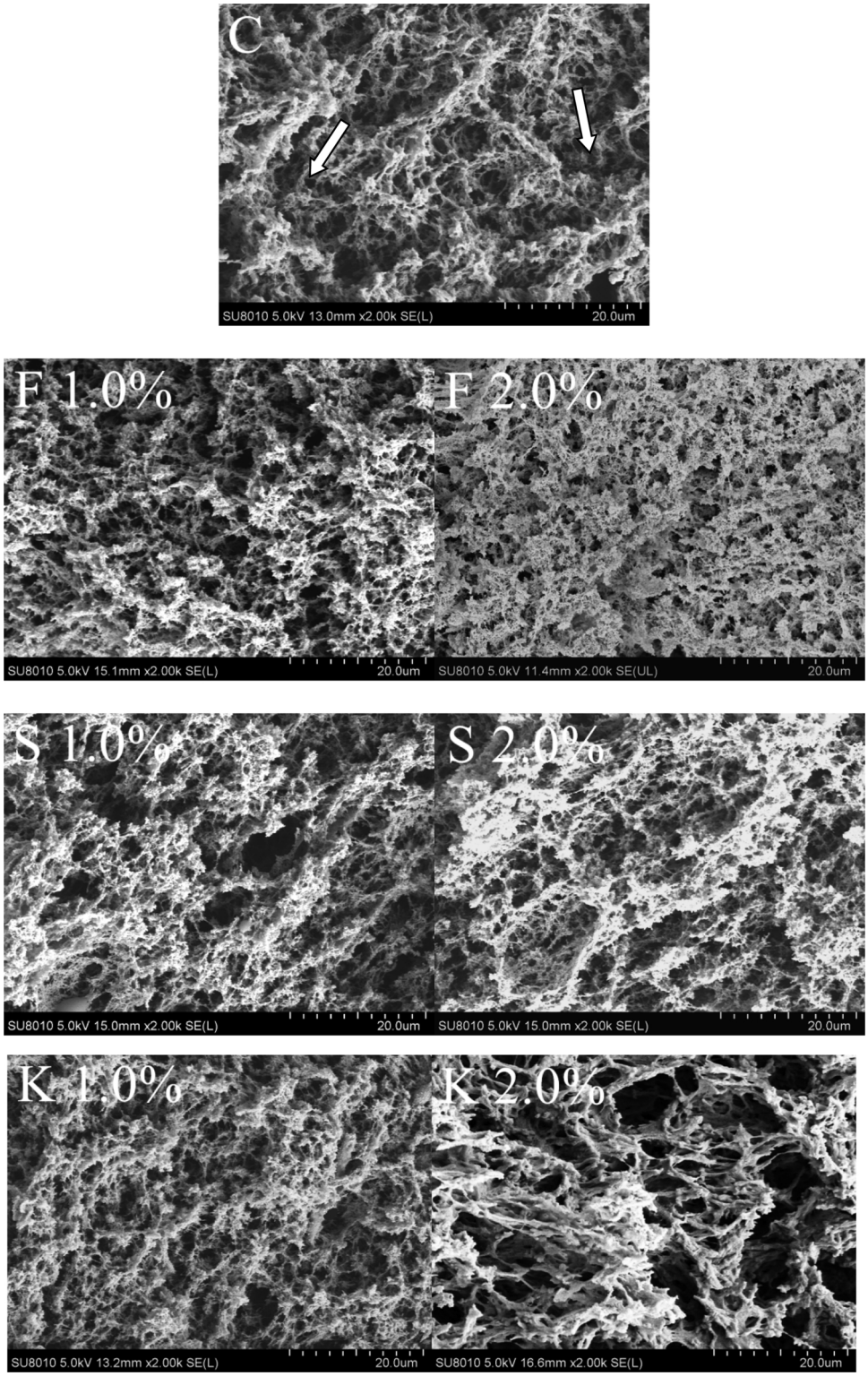

Fig. 4. SEM (2000×) of MP gel networks with three polysaccharides. C: pure MP; F1.0: MP with 1.0% DF; F2.0: MP with 2.0% DF; S1.0%: MP with 1.0% MS; S2.0%: MP with 2.0% MS; K1.0%: MP with 1.0% KG; K2.0%: MP with 2.0% KG.

moisture in the gelatinization temperature. However, at this temperature the MP three-dimensional networks and the moisture channels have been initially formed. The moisture around the MS granules is limited and restricted the swelling of the MS granule. The swelling MS granule could compress of the MP structure; however, the pressure effect is limited. So in the SEM the MS addition could not have the significant improvement on the compactness of MP three-dimensional networks.

For KG addition of less than 1%, the MP is the framework of the composite gel and the improvement of KG on the MP network was even better than that of the IDF. By contrast, the MP gel networks become loose and assume cluster shape with as KG addition was increased to

2%. KG (2%) formed a weak hydrogel through hydrogen bonding and interpenetrated with MP prior to the thermal treatment. With the temperature increase the protein denatures and the hydrophobic groups unfold. However, the unfolded hydrophobic group could not make bonds due to the space hindrance of the KG hydrogel, leading to poor gel quality (Section 3.1).

In the paraffin section (400×), the pure MP gel has a dense and homogeneous structure, but the textural property is worse than that of the composite gel with large polysaccharide cavities. However, when the MP three-dimensional networks amplify 2000 times, it was found that IDF and KG (< 1.0%) could stable moisture, lesson the moisture channels, promote the aggregation of MP gel and finally form the compactness and homogeneous gel networks. The swelling MS granule could compress of the MP structure; however, the pressure effect is limited. So in the SEM the MS addition could not have the significant improvement on the compactness of MP three-dimensional networks. Besides, KG (2%) formed a weak hydrogel through hydrogen bonding, hinder the aggregation of hydrophobic group and final form the loose three-dimensional networks.

3.5. Low-field NMR

In emulsion sausage, the moisture content almost reached 55% and is bounded in the MP gel network in different ways. In the MP gel, there were three main distinct water categories: protein-associated water; immobilized water (trapped within the three-dimensional networks); free water (Han et al., 2014). The mobility and structural properties of the three distinct moisture molecules in MP gel matrix could be measured by low-field NMR (Kuntz & Kauzmann, 1974). Additionally, the presence of a water species with a shorter relaxation time indicates that it associates with the MP molecules more firmly (Yasui, Ishioroshi, Nakano, & Samejima, 1979). Hence, the relaxation time of immobilized water (trapped within the three-dimensional networks) could directly reflect the aggregation degree of the MP gel networks. The Table 1 shows the distribution of the T_2 relaxation times and its corresponding proportion of the composite MP gelation heat-induced at 80 °C for 20 min. For all treatments, three distinct water populations centered at approximately 1–10 ms (T_{2b}, protein-associated water), 200–300 ms ($T_{21,}$ immobilized water), 1000–2000 ms (T_{22}, free water) were observed. The relaxation time T_{2b} and its corresponding proportion are not significantly influenced, because this part of moisture can be associated with protein molecules through a covalent bond and depend on the MP itself. However, the relaxatioan time T_{21} and its corresponding proportion were significantly affected by the types and concentration of polysaccharides. The relaxation time T_{21} decreases and its corresponding proportion significantly increases with the IDF addition concentrations changing from 0.5% to 2%, from 286.11 to 253.13 ms and 82.26 to 91.51%, respectively. By contrast, MS addition shows no distinctive improvement until the concentration exceeds 1.0% ($P > 0.05$). The relaxation time T_{21} of the treated sample (2% MS) decreased to 275.93 ms and its corresponding proportion significantly increased to 88.00%. KG has a positive impact on the relaxation time T_{21} and its corresponding proportion of the composite gel in the additional range of 0.5–1%, and the opposite result is obtained for KG addition beyond 1%.

The changes in the relaxation time T_{21} and its corresponding proportion directly reflect the water immobilization capability of the heat-induced gel network. The MS granules absorb the free moisture in the gelatinization temperature and trapped in the MP three-dimensional gel networks, so the proportion of free water in MP gelation with MS addition significantly reduced. Besides, the swelling MS granules also could press against MP gel networks through swelling at gelatinization temperature. The pressure effect could promote the moisture immobilization capability of the MP gel network, which results in the reduction of T21 relaxation time. However, the T21 relaxation time of MP gelation significantly reduced until the MS addition reached 1.5%.

Table 1
the relaxation times and corresponding peak areas of composite gels with three polysaccharides.

	MS					KG				
	0.0	0.5	1.0	1.5	2.0	0.0	0.5	1.0	1.5	2.0
T2b (ms)	4.89 ± 2.45	6.51 ± 3.12	6.72 ± 2.27	5.58 ± 2.07	4.45 ± 2.45	4.89 ± 2.45	6.51 ± 3.12	3.75 ± 0.68	4.05 ± 2.08	6.73 ± 2.28
PT2b (%)	0.86 ± 0.08	1.00 ± 0.13	1.09 ± 0.11	1.13 ± 0.18	1.22 ± 0.12	0.86 ± 0.08b	1.30 ± 0.13ab	1.12 ± 0.42	1.16 ± 0.38	1.09 ± 0.11ab
T21 (ms)	294.73 ± 7.69a	292.95 ± 6.05a	287.19 ± 2.66ab	280.35 ± 3.36bc	275.93 ± 2.93c	294.73 ± 7.69b	279.75 ± 5.27c	271.19 ± 5.5c	253.13 ± 3.21d	275.00 ± 5.11c
PT21 (%)	80.32 ± 1.83a	82.14 ± 1.05ab	84.94 ± 2.74bc	86.59 ± 2.32cd	88.00 ± 1.25d	80.32 ± 1.83b	85.22 ± 1.58a	86.10 ± 0.99c	88.80 ± 1.42b	87.60 ± 1.33a
T22 (ms)	1481.65 ± 120.33	1596.25 ± 134.66	1598.94 ± 135.30	1553.30 ± 146.74	1652.63 ± 150.23	1481.65 ± 120.33a	1381.39 ± 167.36	1451.13 ± 168.21	1612.07 ± 165.16	1492.23 ± 153.57
PT2b (%)	18.82 ± 1.82a	16.86 ± 0.94ab	13.97 ± 2.64bc	12.28 ± 2.48cd	10.77 ± 1.27d	18.82 ± 1.82b	13.48 ± 1.06c	12.78 ± 0.92c	10.053 ± 1.21d	7.34 ± 1.39e

	KG		IDF			
	1.5	2.0	0.0	0.5	1.0	2.0
T2b (ms)	5.58 ± 2.28	4.45 ± 2.45	4.89 ± 2.45	2.26 ± 1.68	3.75 ± 0.68	2.82 ± 0.87
PT2b (%)	1.39 ± 0.31a	1.36 ± 0.31a	0.86 ± 0.08	0.96 ± 0.05	1.12 ± 0.42	1.17 ± 0.21
T21 (ms)	319.75 ± 5.85a	326.51 ± 8.01a	294.73 ± 7.69a	286.11 ± 1.61b	271.19 ± 5.5c	234.32 ± 8.87e
PT21 (%)	72.44 ± 3.12c	68.51 ± 4.10c	80.32 ± 1.83e	83.26 ± 1.25d	86.10 ± 0.99c	91.51 ± 0.87a
T22 (ms)	1287.72 ± 135.44	1256.12 ± 146.42	1481.65 ± 120.33	1512.66 ± 147.61	1451.13 ± 168.21	1492.23 ± 153.57
PT2b (%)	26.31 ± 4.12a	30.19 ± 4.11a	18.82 ± 1.82a	15.76 ± 1.23b	12.78 ± 0.92c	7.34 ± 1.39e

0.0: control; MS: modified starch; KG: Konjac gum; IDF: dietary fiber.
Different letters (a–d) in a row indicate significant differences ($P < 0.05$) between treatments with various concentrations of same polysaccharide.

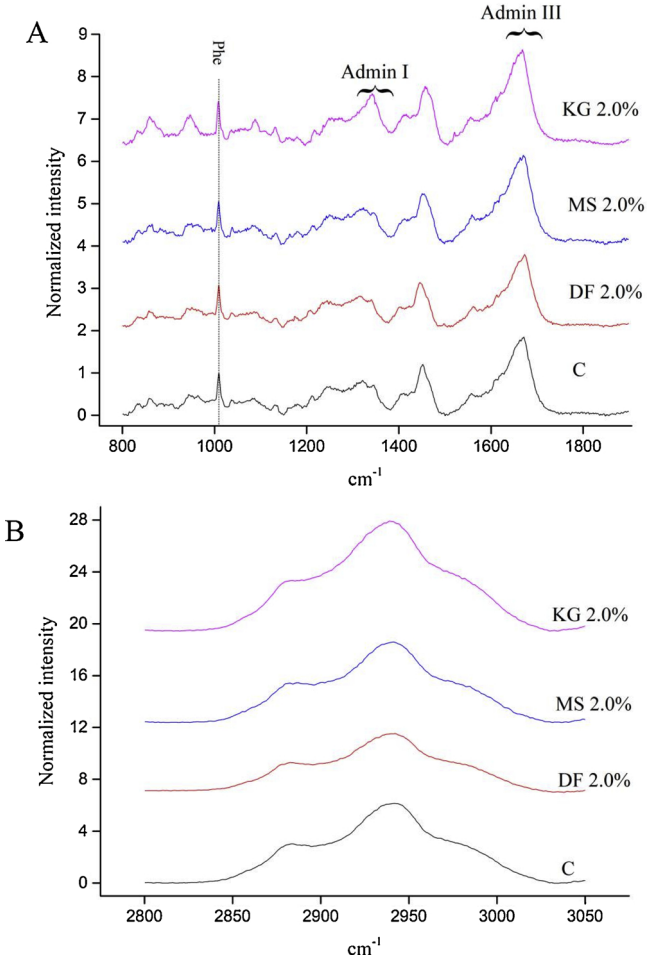

Fig. 5. Raman spectrum (900–1900 and 2800–3050 cm^{-1}) of the composite MP gels with three polysaccharides at 2.0%.

Compared to the MS, the MP gelation with IDF addition has shorter T21 relaxation time and less proportion of free water at same additional concentration. Combination to the microstructure, it suggested that IDF hydration can take moisture and trapped in gel network to prevent exudation. And IDF eliminated the formation of the interconnect moisture channels, and promoted to form a compact gel network, leading to lower T2 relaxation time. With KG addition beyond 1%, the KG formed a weak hydrogel and interpenetrated with MP, which hinder the unfolding of protein and aggregation of hydrophobic groups. Hence, the loose MP gel networks with KG addition (> 1.0%) have highest T2 relaxation time and proportion of free water. The low-field NMR could reflect the changes in the MP gel network with the addition of the three polysaccharides showing results similar to those obtained by SEM.

3.6. Molecular conformation

We further study the change in the MP molecular conformation influenced by the polysaccharides addition through Raman spectroscopy. Raman spectra of the composite MP gel with three polysaccharides at the concentration of 2% are shown (Fig. 5) in the regions of 700–2000 cm^{-1} and 2800–3050 cm^{-1}. The frequency and intensity changes of the characteristic peaks are discussed in detail below, and indicate the changes of the secondary protein structure and the MP local environments.

3.6.1. Changes in the secondary structures

The secondary structure of the protein (α-helix, β-sheet, β-turn and random coil) is indicated by the characteristic peaks in the Raman spectra of the amide I (mainly C═O stretching and less C−N stretching, Cα−C−N bending, and N−H in-plane bending), amide II and amide III bands (complex bands resulting from several coordinate displacements) (Herrero, 2008). However, the changes in the MP secondary structure rarely lead to the modification of the amide II frequency and intensity, and therefore, the present study mainly examines the secondary structure modification using the amide I and amide III bands (Alix, Pedanou, & Berjot, 1988). The amide I band is located in the 1650–1657 cm^{-1} range in the Raman spectra. Most studies on the MP molecular conformation found in the literature reported the presence of a correlation between the frequencies of the amide I and amide III bands and the change of protein secondary structure. Thus, generally the α-helix, β-sheet, and random coil structures correspond to the 1658–1650, 1680–1665, and 1665–1660 cm^{-1} ranges of the amide I band, respectively (Bouraoui, Nakai, & Li-Chan, 1997; Li-Chan, 1996). In the Fig. 5A, it is observed that the polysaccharides addition results in the modification of the amide I band in the Raman spectra. The characteristic peak of the control (amide I) is at 1669 cm^{-1} and located in the β-sheet range at 1680–1665 cm^{-1}, indicating that the β-sheet dominates the secondary structure in the heat-induced gelation. This result corresponds to the phenomena that the thermal process inducing a decrease in the α-helical content and an increase in the β-sheet content during the MP denudation (Chen & Han, 2011; Xu, Han, Fei, & Zhou, 2011). The MS did not influence the change in the characteristic peak in amide I at various concentrations, reflecting the fact that MS addition does not affect MP denaturation. The similar results were found by Fan, Hu, and Huang (2017), who studied the effect of the modified starch on the gel characteristics of fish myofibrillar protein. IDF addition results in the significant redshift, from 1670.77 to 1674.90 cm^{-1} of the characteristic peak in amide I, indicating the decrease in the α-helix content and the increase in the β-sheet proportion in heat-induced composite gelation. Similar results were found, who studied the Raman spectra of surimi gelation with added wheat dietary fiber (WIDF). Compared with the pure gel, the characteristic peak (amide I) of the surimi gel with WIDF shifted toward higher frequencies. Quantitative analysis of the secondary structure showed a significant increase in the β-sheet proportion accompanied by the decrease in the α-helix proportion. The relationship between the textural property and secondary structure of Raman spectra was studied through the principal component analysis and the textural property was found to have a positive correlation with the β-sheet fraction and a negative correlation with the α-helix fraction (Xu et al., 2011). In the present study the improvement in the textural property also accompanied the raise of the β-sheet fraction and the decrease of the α-helix fraction. KG addition induces a redshift of the characteristic peak in amide I at 1% and then blueshift at 2%. The SEM images show that thick myofilaments are present in clusters and rarely contact each other at 2%. Therefore, the KG hydrogel could slow down the denaturation of the MP, with less of α- helix unfolded and less hydrophobic groups exposed, forming a loose gel network.

The amide III mode involves C−N stretching and N−H in plane bending vibrations of the peptide bond and as well as contributions from Cα−C stretching and C−O in-plane bending. The band of α-helix and β-sheet structure bands in amide III mainly focus on the region of 1300–1260 cm^{-1} and 1250–1240 cm^{-1} regions of the spectrum, respectively (Pelton & McLean, 2000; Schweitzer-Stenner, 2006). The characteristic peaks in amide III of the composite gel show various changes with the polysaccharides addition (Fig. 5B). The MS addition did not result in the shift of the characteristic peaks in amide III. However, the characteristic peaks in amide III significantly shift toward lower frequency with the increase of the IDF. KG induces the shift of the characteristic peak in amide III toward lower frequency at the concentration of 1%, and then toward higher frequency at the concentration of 2%. The trend for the changes of the secondary structure with polysaccharides addition in amide III is consistent with the change in the amide I band.

Table 2
Normalized intensities of the 2945 cm^{-1} band (CH2 and CH3 bending and C\H stretching vibrations of aliphatic resides) and the frequency of characteristic peak in Admin I and Admin III of the composite MP gel with three polysaccharides.

	MS					KG			
	0	0.5	1.0	1.5	2.0	0	0.5	1.0	
Admin I	1670.77 ± 0.94	1669.77 ± 0.90	1670.24 ± 0.90	1670.46 ± 0.90	1671.24 ± 0.62	1670.77 ± 0.94b	1671.14 ± 0.77c	1672.32 ± 0.47c	
Admin III	1251.37 ± 0.94	1251.97 ± 1.87	1250.71 ± 1.29	1250.95 ± 1.71	1251.70 ± 2.08	1251.37 ± 0.94a	1246.46 ± 1.51b	1246.74 ± 1.28b	
I2945/1003	6.27 ± 0.21	6.22 ± 0.37	6.22 ± 0.37	6.28 ± 0.58	6.13 ± 0.61	6.27 ± 0.21c	5.83 ± 0.31c	5.79 ± 0.29b	
	KG		IDF						
	1.5	2.0	0	0.5	1.0	1.5	2.0		
Admin I	1668.46 ± 1.01a	1667.87 ± 1.13a	1670.77 ± 0.94c	1671.37 ± 0.47bc	1672.32 ± 0.47b	1674.20 ± 0.91a	1674.90 ± 0a		
Admin III	1252.26 ± 1.82a	1252.87 ± 1.12a	1251.37 ± 0.94a	1249.27 ± 1.42ab	1246.98 ± 2.07bc	1244.97 ± 1.51c	1244.72 ± 1.01c		
I2945/1003	6.83 ± 0.28a	6.94 ± 0.21a	6.27 ± 0.21a	5.96 ± 0.29ab	5.73 ± 0.19bc	5.39 ± 0.23c	4.86 ± 0.32d		

0.0: control; MS: modified starch; KG: Konjac gum; IDF: dietary fiber.
Different letters (a–d) in a row indicate significant differences ($P < 0.05$) between treatments with various concentrations of same polysaccharide.

3.6.2. Changes of C\H stretching and CH_2 and CH_3 bending vibrations

The Raman spectrum bands at 2950 cm^{-1} are assigned to C\H stretching and CH_2 and CH_3 bending vibrations of aliphatic amino acids, peptides, and proteins, respectively (Herrero, 2008). The intensity of this band is positively correlated with the exposure of the aliphatic residues in the MP. The hydrophobic interactions (aliphatic residues) are the dominant factor in MP aggregation and the intensity decrease of this band may indicate that the hydrophobic groups (aliphatic residues) are well-aggregated. Table 2 shows the intensity changes of the C\H stretching band in the composite gel with three polysaccharides. The intensity of the Raman spectra bands at 2950 cm^{-1} do not change with MS addition. However, the IDF significantly decreased the band intensity from 6.27 (0%) to 4.86 (2%). Additionally, KG induces the intensity decrease at the concentration of 1%, and followed by an increase at the concentration of 2%. Based on the results of the bands of amides I and III and the band at 2950 cm^{-1}, it was found that the secondary structure unfolds, leading to the exposure of hydrophobic groups during thermal processing. Subsequent hydrophobic interactions induce the formation of three-dimensional gel networks. The more secondary structure (amide I, III) unfolds, the more hydrophobic groups aggregated, leading to compact and homogeneous gel networks.

3.7. Schematic model

The gel strength and WHC indicate that the MP gel quality significantly improved with the MS, KG (within 1.0%) and IDF addition, and the improvement by IDF at 2% is the greatest. However, the spatial distributions of MP and polysaccharides in paraffin section (400×) indicate that the polysaccharides do not associate directly with MP and just physically trapped in the gel network. Compare to the control (pure MP) with homogeneous and compact microstructure, the composite gel networks is filled with amount of large cavities of polysaccharides, which might degrade to be a loose and inhomogeneous microstructure. However, the gel strength and WHC of the control are worse than the treatments with polysaccharides addition. Besides, low-field NMR also indicated that the MP-polysaccharide gel had shorter relaxation time T_{21} and higher its corresponding proportion. So to explain the phenomenon above, the microstructure MP gel networks and protein molecular conformation are further studied.

Based on the results of microstructure MP gel networks and protein molecular conformation, the schematic model of the MP gelation property improved by IDF and MS was showed in Fig. 6. From the MP solution to heat-induced gelation, the swelling MP would denature and then exude moisture. The moisture exudation happened in the interior gelation would form water channels or water cavities (arrow in Fig. 4C), which would hinder the further aggregation of MP hydrophobic groups and degrade the integrity of gel network, further resulted in deterioration of MP gel textual property. The IDF has strong water-holding capability and migrates the part of moisture from the MP before heating process. The “concentrated” MP promotes the interaction of hydrophobic groups and lessens the appearance of water channel, leading to a compact and homogeneous structure. The result of SEM and low-field NMR directly and indirectly proved the conclusion above. In addition, the Raman spectroscopic analysis indicated that the IDF addition significantly affect the changes of secondary structure and hydrophobic amino acid residues, like aliphatic residues. The unfolding of secondary structure (the decrease of α-helix) leading to the exposure of hydrophobic groups during thermal processing. Subsequent hydrophobic interactions induce the formation of the three-dimensional gel networks. Finally the more compact and homogeneous gel networks is formed.

However, the addition of MS addition did not significantly affect the MP gel microstructure until reached 2%. The MS granule would absorb water and swell until the gelatinization temperature. However, in the gelatinization temperature the MP three dimensional networks and the

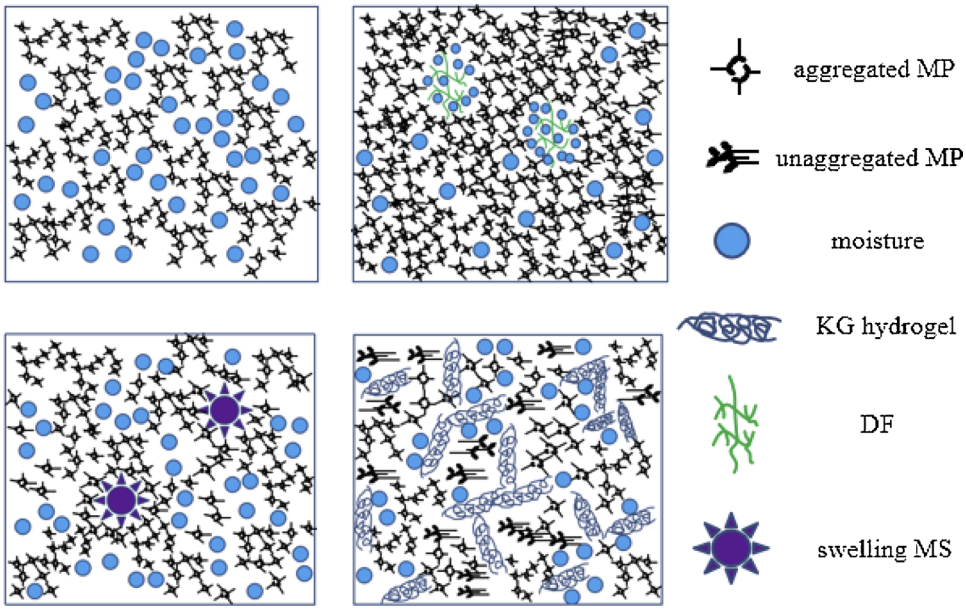

Fig. 6. Schematic model of three polysaccharides with various physicochemical properties on the MP gel functionality.

moisture channels have initially formed. The moisture around the MS granule is limited, so the MS addition could improve the textual property of MP through physical swelling effect. In the microstructure MS addition do not have the significant improvement on the compactness of MP three-dimensional networks, and in molecular conformation MS addition do not change the secondary structure and hydrophobic amino acid residues. But the MS granules could swell pressure against MP gelation through gelatinization. Hence, the MS granule addition improves the textural property of MP gel through simply physical swelling pressure and the improvement is limited. The result of textual properties and low-field NMR show that the gel network only could significantly improve by MS until at 1.5%. Besides, the Raman spectra analysis indicated that the MS addition do not affect the changes of secondary structure and hydrophobic amino acid residues. The mechanism of MP gelation improved by KG is similar to IDF in the additional concentration ranged at 0.5% and 1.0%.

With the concentration beyond 1.0%, the KG would form a weak hydrogel through hydrogen bond and then interpenetrated with MP before thermal treatment. Due to the fragmentation of MP distribution, the unfolded hydrophobic group could not further have connection with each other, formed an incomplete and loose gel networks, leading to poor textual property of composite gel (Section 3.1). The result of Raman spectra analysis also could prove.

In conclusion, the hydration characteristic of three polysaccharides could significantly influence on the moisture stability in MP system, which play an important role in the textural quality of the MP gelation. And the polysaccharide, which has strong water-holding capability at room temperature and could distribute individually, is the best additive to improve the textural property of MP gelation.

4. Conclusion

The results reveal the effect and mechanism of the MP gelation influenced by three polysaccharides. Paraffin section showed that the polysaccharides and MP are thermodynamically incompatible system and finally forms a two-phase separation gel after the thermal process. So three polysaccharides did not direct contact with the MP. The MS granule addition improves MP gel property through physical swelling. The SEM and Raman spectroscopy shows that the swelling effect of MS do not have significantly influence on the microstructure and molecular conformation of MP gel networks, which play the dominant role in the viscoelasticity of product. Hence, the improvement of MP gelation with MS addition has limitation and the textural property only significantly improved until MS addition beyond 1.0%. The IDF improves MP gelation property through moisture stability. IDF has a strong water-holding capability and migrates the part of moisture from the MP before heating process. The SEM and Raman spectroscopy shows that the moisture stability of IDF could lessen the crossed water channels in the MP three-dimensional gel network and promotes the unfolding of protein and the interaction of the hydrophobic groups, leading to a compact and homogeneous structure. The WHC and gel strength of MP gelation significantly improved with the increase of IIDF concentration. The mechanism of MP gelation improvement by KG is similar to that of IDF in the additional concentration range from 0.5% to 1.0%. With the concentration beyond 1.0%, KG formed the weak hydrogel and interpenetrated with MP solution prior to thermal treatment. In the thermal process, the KG hydrogel separates the MP distribution and further hinder the interaction of the unfolded hydrophobic groups, leading to the degraded textual property. So the hydration characteristic of three polysaccharides have significantly influence on the MP gel property, and the polysaccharides with good moisture stability in common temperature and individual distribution is the ideal fat replacement.

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