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Insight into the mechanism of fracture properties modulated by microstructure in the myofibrillar protein and polysaccharide gel systems

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Abstract

The objective of this study was to investigate the mechanism of fracture properties modulated by microstructure in the myofibrillar protein (MP) and polysaccharides gel systems. Compare to the modified starch, the dietary fiber significantly improved the fracture stress and reduced the fracture strain at same concentration. The treatment with 2% dietary fiber had the highest value of fracture stress and the lowest value of fracture strain, which were 259 g and 1.12 respectively. From the skeleton structure, the Raman spectroscopy result showed that dietary fiber addition significantly reduced the intensity at 2945 cm^{-1}, which suggested that the aggregation of hydrophobic groups was improved. The SEM showed that the treatment with 2% dietary fiber had the highest fractal dimension value of 1.7772 and the lowest lacunary value of 0.258. From the filling structure, the paraffin section showed that the polysaccharides were just simply trapped in MP gel networks and formed numerous large volumes and no-elastic of cavities. The principal component analysis suggested that the compactness of three-dimensional gel networks determined fracture stress of composite gel. The no- no-elastic of cavities formed by modified starch and dietary fiber resulted in the reduction of fracture strain. These results would promote the development of innovative nutritional meat product formulation with satisfied textural property.

Keywords: Fracture properties, Microstructure, Protein gelation, Dietary fiber, Polysaccharides, Fat substitution
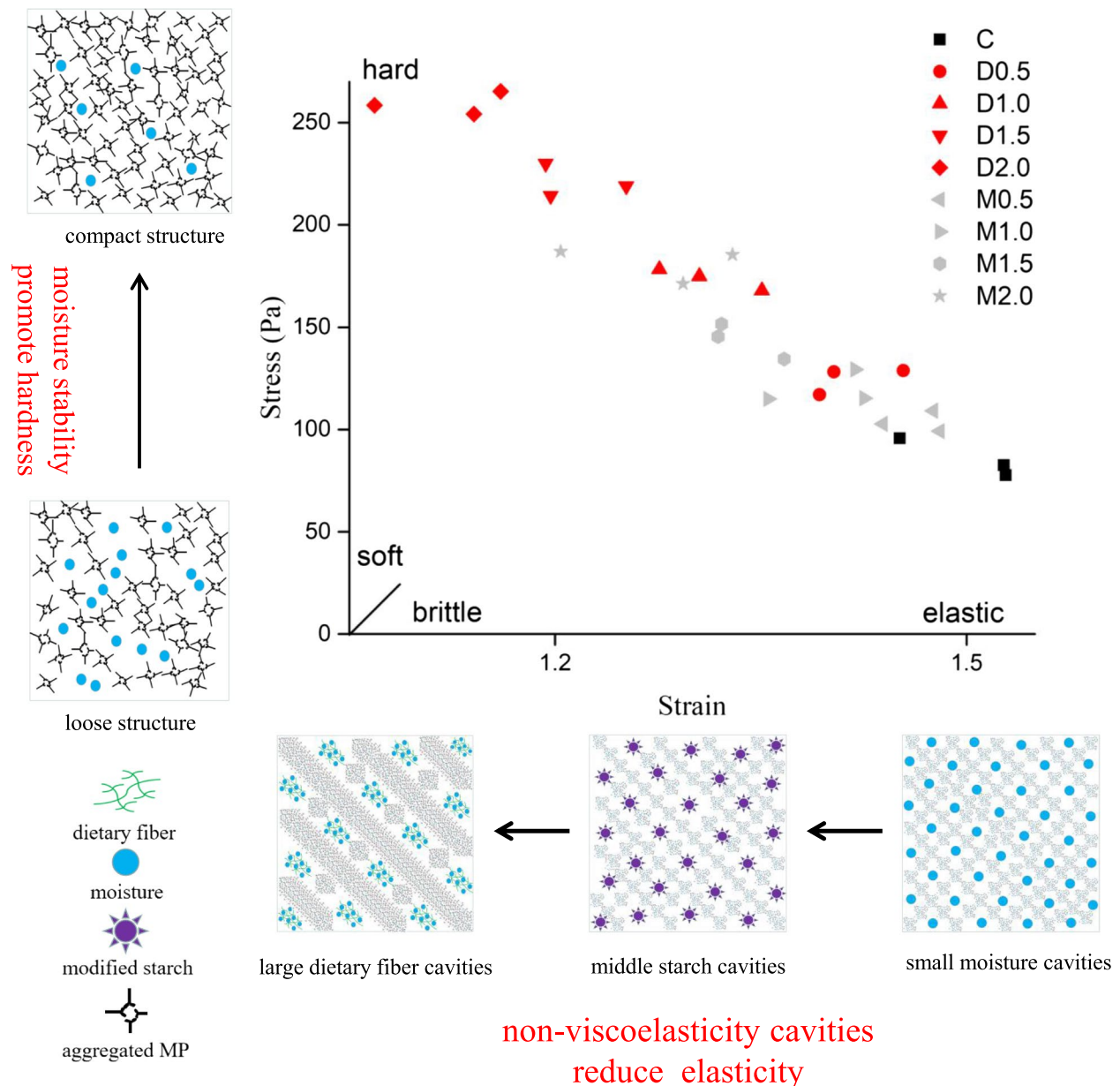
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Graphical Abstract

Introduction

In recent years, with the improvement of health consciousness, low fat and low calorific value meat products have been highly concerned and favored by the public (Carvalho et al. 2019; Ztürk-Kerimolu 2021). Processed meat product even contained more than 30% animal fat, which had high content of cholesterol and saturated fat, especially frankfurter sausage, pork ham and so on. Excessive intake of animal fat caused various chronic diseases such as atherosclerosis and elevated blood cholesterol. However, simply reducing the animal fat content could result in poor taste of processed meat product (Hu & Yu 2015; Ye 2021). The food gel system with satisfied texture property could provide the enjoyable and pleasant experience, which was one of the main factors to occupy market share. In

Luo *et al. Food Production, Processing and Nutrition*　　　(2022) 4:30

Page 3 of 14

previous study, we found that sugarcane dietary fiber addition could significantly improve the physicochemical properties of low-fat meat batter. Pintado et al. (2018) noticed that fresh sausages reformulated with polysaccharides as fat replacer could improve the total texture property of sausages. Besides, Hu et al. (2015) found that rice dietary fiber as animal fat replacement could significantly improve the hardness of meatball. Therefore, partial replacement of animal fat with polysaccharide would be the optimal solution to enhance the healthiness of meat product with satisfied overall acceptability. The modified starch (cross-linked hydroxypropylated cassava starch and cassava starch) had been always utilized to immobilize moisture, enhance emulsion stability and improve textural characteristics in low-fat meat products (Kong et al. 2016; Sun et al. 2014). Dietary fiber had some prominent healthy function as seventh nutriment and good processing characteristics, so it was ideal fat substitute to utilize in the low-fat healthy meat products (Choi et al. 2013; Mehta & Ahlawat 2015).

In theoretical perspective, the achievement of healthy meat product with satisfied textural property should understand the relationships between the molecules (protein and polysaccharides), microstructure and texture. In protein–polysaccharides gel system, the mainly structures included the skeleton structure of myofibrillar protein (MP) and the filling structure of polysaccharides. MP could aggregate and form three-dimensional gel network during the heating process, which determined the texture and water retention of final products. However, the polymerization of MP was easily affected by other factors during the heating process, including pH, ionic strength and additives. The polysaccharide was not directly cross-linked with MP, but only physically adsorbed in the gel network. The storage modulus, volume fraction, concentration and spatial distribution of polysaccharides could influence textural property of the composite gel system. Sun et al. (2014) studied the effect of different modified starches on textural characteristics of low-fat meat products, and found that the swelling volume of modified starch had positive correlation with the hardness of meat gelation. The different physicochemical characteristics of modified starch and dietary fiber significantly changed microstructure of the composite gel, and final influenced the textural property of products. Therefore, it is essential to gain insight on how textural properties can be modulated by microstructure in MP gel system when introducing polysaccharides.

Hence, the objective of this study was to illustrate the mechanism of fracture properties modulated by microstructure in the MP-polysaccharides gel systems. Large deformation measurements, microscopy and Raman spectroscopy were combined to explain the modulate relationship between fracture properties and microstructure.

Materials and methods

Materials

The phosphate ester double starch was purchased from Hangzhou Starpro Modified starch Co. Ltd. (Hangzhou, Zhejiang, China). The oat dietary fiber was purchased from Hangzhou Linran Biotechnology Co., Ltd. (Hangzhou, Zhejiang, China). The particle sizes were all 100-mesh. Fresh pork hind leg (70.92% moisture, 23.61% protein, 4.17% fat and 0.76% ash) and pork fat (92.91% fat, 1.67% protein, 4.86% moisture) were purchased from a local market (Suguo Supermarket, NanJing, China). All chemicals used are analytical grade.

Preparation of the composite protein gel system

After cutting off the excess fat and connective tissue, fresh pork ham meat (Sugo Supermarket, Jiangsu, China) was stored at -18 °C for 24 h until protein extraction. Extraction of MP was carried out as the following steps. The ground muscle was mixed with four volumes of isolation 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 6,000 r/min. The homogenates were filtered through a 20-mesh sieve (0.9 mm) and centrifuged (Model 225, Beckman Coulter, Inc., California, USA) at $3000 \times g$ for 15 min. The supernatant was decanted, and the aforementioned steps were repeated two times. The final pellet was collected as pure MP. The biuret method was used to determine the protein concentration of MP using bovine serum albumin as the standard, and then MP was diluted to 50 mg/mL (0.6 M NaCl, pH 7.0). The modified starch and dietary fiber (0.5%, 1.0%, 1.5% and 2.0%) were added to the MP solution and then homogenized with glass rod. All samples were stored in a refrigerator (4 °C) until testing.

Textural property

Large deformation

The fracture stain and stress of composite gel were tested through texture analyzer (TA-XT plus, Stable Micro Systems Ltd., Godalming, U.K.). The composite MP solutions were heated at 80 °C for 20 min, and cut into cylindrical pieces (20 mm in height, 20 mm in diameter) using a steel wire after cooling to room temperature. Samples were compressed with P50 probe to 90% of their

Luo *et al. Food Production, Processing and Nutrition*   (2022) 4:30

Page 4 of 14

initial height at speed of 1 mm/s. True stress and true strain can be expressed by Eq. (1), and Eq. (2).

$$strain = \int_{H_0}^{H} \frac{1}{H} dH = In\left(\frac{H}{H_0}\right) \qquad (1)$$

$$Stress = \frac{F}{A} \qquad (2)$$

where H_0 was the initial specimen height and H was the final height after deformation. Where F was the force measured during compression and A was the compressed area of the sample.

Small deformation
The creep-recovery tests of the composite gels were measured using a rheometer (MCR301, Anton Paar, Austria) as described by Dzadz et al. (2015). In the creep phase, the composite gels were applied with constant strain (7%), and the creep response was measured over 180 s. In the recovery phase, the recovery response was measured for 360 s after removing the stress. The creep-recovery curves of the MP composite gels were fitted to Burger's model (Huang, Zeng, Xiong, & Huang 2016). The Burger's model could be expressed by Eq. (3), and the recovery (R %) is expressed by Eq. (4):

$$J(t) = J_0 + J_1 \times \left(1 - e^{\left(-\frac{t}{\lambda}\right)}\right) + \frac{t}{n} \qquad (3)$$

$$R\% = \frac{J_0 + J_1}{J_{max}} \times 100\% \qquad (4)$$

Where $J(t)$ represents the compliance at any time t, J_0 is the instantaneous elastic compliance, J_1 is the retarded elastic compliance, λ is the retardation time, η is the zero-shear viscosity, and J_{max} is the maximum compliance.

Microstructure analysis
### *Spatial distribution of composite gel system*
The gel samples (0.8 cm × 0.8 cm × 3 cm) were treated using formalin fixation, ethanol dehydration, and paraffin embedding procedures. The samples were cut into 8 μm thick sections by a microtome (CM1900, Leica, German), then stained with haematoxylin−eosin and fixed on a slide. The microstructure of the composite gels was investigated by light microscopy (Axio Imager, Zeiss, Germany). Micrographs were threshold and transformed into binary images as the followed steps. The average size and number of polysaccharide pores (modified starch and dietary fiber) in the images were calculated using ImageJ v1.47 software

(Davila et al. 2007). Five images of each replicate were calculated.
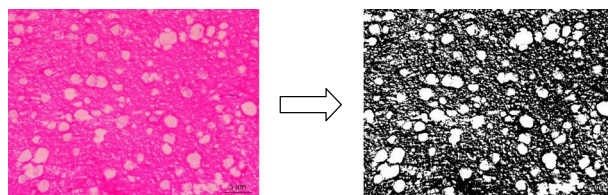
Microstructure of three-dimensional gel networks
The samples were treated as described by Han et al. (2014) and observed through a Hitachi S3000 N scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 20 kV. Micrographs were threshold and transformed into binary images as the followed steps. The fractal dimension and lacunary of the binary images were calculated using ImageJ v1.47 software and the FracLac 2.5v plugin for ImageJ (Davila et al. 2007).
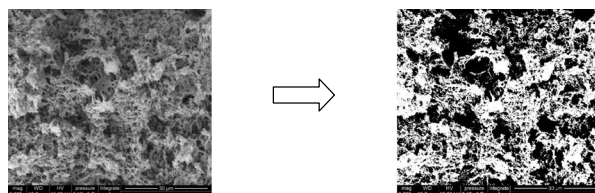
Raman spectroscopy of protein gels
The experimental parameters and process were conducted in accordance with the method of Zhuang et al. (2018) with an HR800 spectrometer (Horiba Jobi Yvon S.A.S., Longjumeau, France). The spectra were smoothed, baseline corrected, and normalized against the amplitude of the band at 1003 cm^{-1} (phenylalanine band) by Labspec version 5.0 (Horiba Jobi Yvon S.A.S., Longjumeau, France).

Statistical analysis
The entire experiment was replicated three times on different occasions with a completely randomized design. The pure MP was named the control; the treatment with 0.5%, 1.0%, 1.5% and 2.0% modified starch were respectively named M0.5, M1.0, M1.5, and M2.0; the treatments with 0.5%, 1.0%, 1.5% and 2.0% dietary fiber were respectively named D0.5, D1.0, D1.5 and D2.0. The data was analyzed using Statistical Analysis System 9.0 (SAS Institute Inc., Cary, NC) with one-way ANOVA. Significant differences between means were identified using Duncan's multiple range tests. Principal component analysis was performed with R language.

Luo *et al. Food Production, Processing and Nutrition*   (2022) 4:30

Page 5 of 14

Results and discussion

Fracture properties of composite protein gels

The composite protein gels were measured through large deformation test. The fracture strain and fracture stress were calculated by the equations above and showed in Fig. 1. The modified starch and dietary fiber addition had the same effect on fracture property of the composite gel, and the dietary fiber was more pronounced. The results suggested that fracture stress significantly ($p < 0.05$) increased with modified starch and dietary fiber concentrations and the fracture strain significantly ($p < 0.05$) reduced with modified starch and dietary fiber concentrations.

The fracture stress reflects hardness of the composite gel, and fracture stress has positive correlation with hardness (Czerner et al. 2016). The related literature had reported that modified starch addition could improve the sausage hardness, and in present study fracture stress had same phenomenon (Fan et al. 2017; Sun et al. 2014). Compare to modified starch, fracture stress of the composite gels with dietary fiber had higher ($p < 0.05$) values. The fracture stress of 1% dietary fiber treatment was even higher than that of 2% modified starch treatment. We also found that fracture stress of the composite gels presented a liner distribution as a function of modified starch and dietary fiber concentrations. Linear fitting of the data was carried out with the addition concentration as the independent variable and fracture stress as the dependent variable. The fitting liner equation of the composite gel with modified starch and dietary fiber were $y = 23.52x + 94.82$ ($R^2 = 0.94$) and $y = 83.78x + 91.56$ ($R^2 = 0.98$) respectively.

The fracture strain reflects deformation of the composite gel, and positively relates with elasticity (Stieger & van de Velde 2013). The modified starch and dietary fiber addition all reduced fracture strain, and the effect of dietary fiber addition was more obvious. The fracture stress of the composite gels also had a liner distribution as a function of modified starch and dietary fiber concentrations. The fitting liner equation of the composite gel with
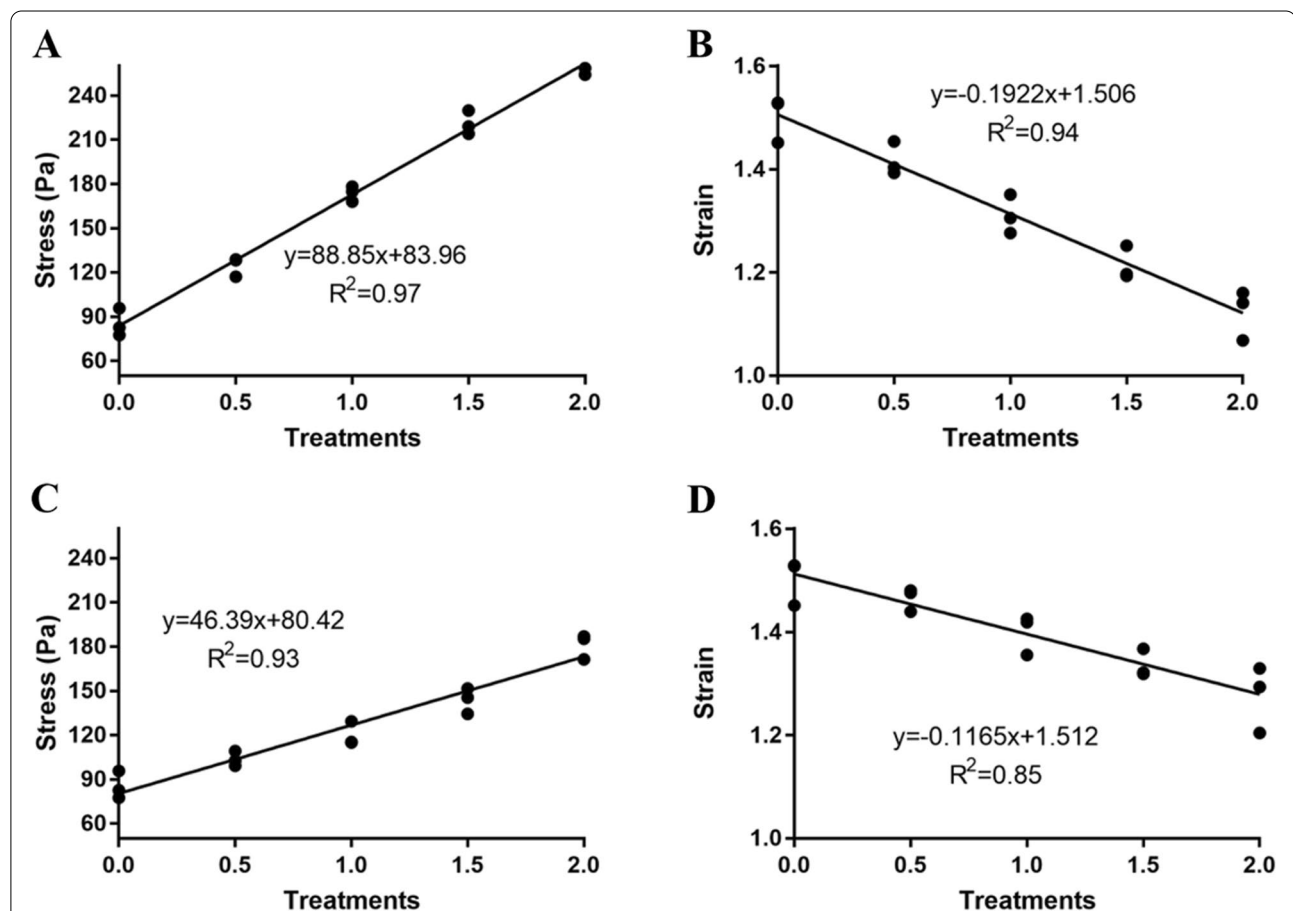

Fig. 1 The fracture stress and fracture strain of composite gel with modified starch and dietary fiber addition. The fracture stress (**A**) and fracture strain (**B**) of composite gel with dietary fiber addition. The fracture stress (**C**) and fracture strain (**D**) of composite gel with modified starch addition

Luo *et al. Food Production, Processing and Nutrition*   (2022) 4:30

Page 6 of 14

modified starch and dietary fiber were y = -0.1005x + 1.504 (R²= 0.90) and y = -0.1922x + 1.506 (R²= 0.94) respectively.

Creep-recovery property
The creep-recovery test was a nondestructive measure of the viscous and elastic parts of composite gels. The stress applied in creep-recovery test belongs to the linear viscoelastic range (Dzadz, et al. 2015; Huang et al. 2016). The creep-recovery curves of the composite gels with modified starch and dietary fiber were exhibited in Fig. 2. In the first 180 s, creep compliance of the composite gel gradually increased under constant pressure, and reached the maximum value at 180 s. After removing the constant pressure, the creep compliance decreased gradually. In the creep stage, the maximum creep compliance of the composite gel with dietary fiber was lower than the treatment with modified starch at same concentration. The value of creep compliance (J_{max}) was negatively

correlated with the compactness of the gel networks (Dzadz et al. 2015; Huang et al. 2016). The creep compliance of the composite gel gradually decreased with the concentration increase, indicating that the modified starch and dietary fiber addition could promote the aggregation of protein and the formation of a compact gel networks. In the recovery stage, the compliance values of treatments with dietary fiber also were lower than that of treatments with modified starch. Hence, the composite gel with dietary fiber addition could resist the deformation more effectively.

The Burger's model was used to fit the creep-recovery curves and R^2 values of fitting equations were all > 0.95. The specific parameters of J_0, J_1, λ and J_{max} obtained from Burger's model were showed in the Table 1. The instantaneous creep compliance (J_0), delayed creep compliance (J_1) and final compliance (J_{max}) of composite gel significantly decreased with modified starch and dietary fiber addition. Especially,
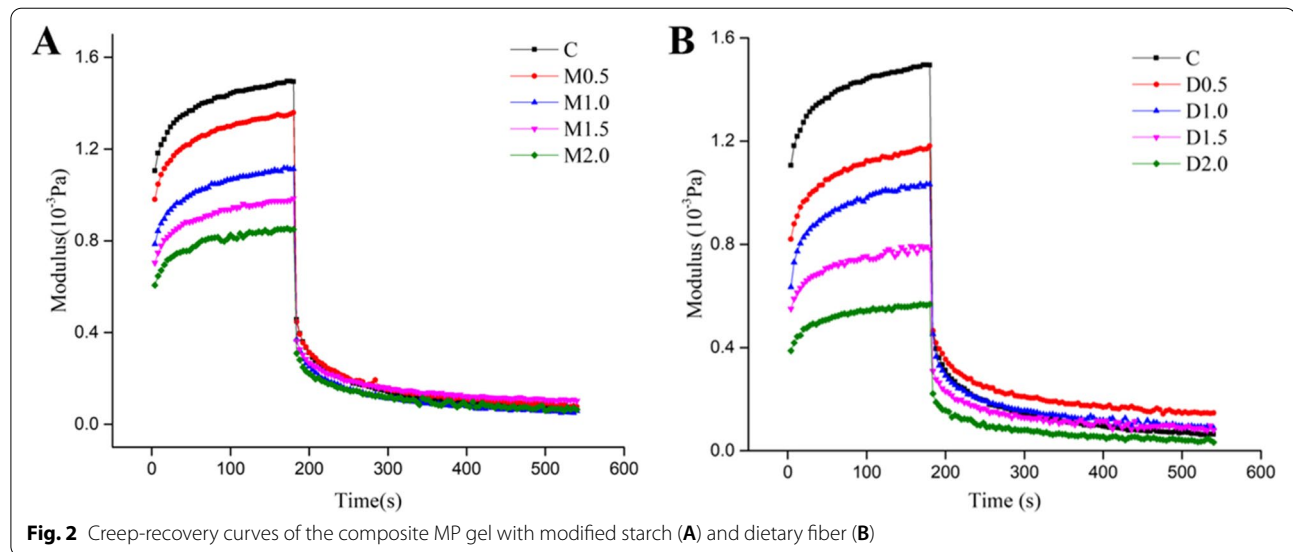

Fig. 2 Creep-recovery curves of the composite MP gel with modified starch (**A**) and dietary fiber (**B**)

Table 1 the parameter of creep-recovery of composite gels with modified starch and dietary fiber

Treatments	$J_0(\times 10^{-3}$ Pa$^{-1})$	$J_1(\times 10^{-4}$ Pa$^{-1})$	$J_{max}(\times 10^{-3}$ Pa$^{-1})$	λ (s)	R%
C	0.89 ± 0.02a	2.30 ± 0.09a	1.56 ± 0.10a	50 ± 5a	91.5 ± 1.1a
M0.5	0.65 ± 0.04b	2.17 ± 0.08ab	1.32 ± 0.03b	46 ± 2ab	90.5 ± 0.3ab
M1.0	0.61 ± 0.04b	1.95 ± 0.12c	1.13 ± 0.11c	45 ± 3ab	88.7 ± 0.4 cd
M1.5	0.50 ± 0.04 cd	1.67 ± 0.13d	0.98 ± 0.02d	42 ± 1bcd	88.2 ± 0.7d
M2.0	0.47 ± 0.04d	1.53 ± 0.11d	0.82 ± 0.09ef	41 ± 2bcd	87.4 ± 1.2de
D0.5	0.63 ± 0.02b	2.09 ± 0.10bc	1.18 ± 0.03c	43 ± 2bc	90.2 ± 0.4abc
D1.0	0.52 ± 0.03c	1.63 ± 0.14d	0.96 ± 0.08de	42 ± 2bcd	88.8 ± 1.4bcd
D1.5	0.39 ± 0.03e	1.32 ± 0.06e	0.72 ± 0.09f	39 ± 2 cd	87.6 ± 1.7de
D2.0	0.31 ± 0.01f	1.19 ± 0.05e	0.53 ± 0.08 g	37 ± 2d	86.2 ± 0.2e

Different letters (a-g) in a column indicate significant differences *(p < 0.05)*

the treatment with 2% dietary fiber addition had the lowest value of J_{max}, which was 0.53×10^{-3} Pa. The retardation time (λ) reflected the elasticity. Generally, the shorter the retardation time was, the better the elasticity of treatment would be. Compare to modified starch, λ value of treatments with dietary fiber addition significantly decreased at same concentration. R % was the percentage of the total recoverable modulus. The R% did not influenced by the dietary fiber at small addition, but significantly reduced with the addition beyond 1%. The same phenomenon was reported by Wu et al. (2015), who studied the effect of curdlan (2%, 4% and 6%) on the textural properties of restructured surimi gel. The addition of 4.0% curdlan was a critical point for the composite surimi gel, and the Jmax values of the MP-KG composite gel ranged from small to large. They suggested that a less orderly structure and lower cross-linking density were presented in the surimi gels with excess curdlan addition (Jiang et al. 2020; Wu et al. 2015).

Microstructure

The spatial distribution of composite gel system

The spatial distribution of composite gel system was exhibited in Fig. 3. In paraffin section the red part was the MP gel networks; the gray branch-like part was dietary fiber; and the white circular part was modified starch or moisture. The pure MP networks were the humongous entirety, and filled with numerous moisture cavities. The modified starch and dietary fiber addition significantly changed the uniformity of MP gel. The modified starch and dietary fiber were physically embedded in the MP gel networks; and the polysaccharides and protein had no direct interaction. We even could observe blank space between dietary fiber and MP, which was the moisture absorbed around the surface of dietary fiber. The particle size of modified starch and dietary fiber were all 100-mesh, but in paraffin section the volume of dietary fiber was much larger than modified starch. Because modified starch was insoluble at room temperature, then absorbed moisture until reaching the gelatinization temperature. However, at the gelatinization temperature the aggregation of MP gel networks was almost completed and the moisture was immobilized in it. Compare to modified starch, dietary fiber could immobilize large volume of moisture at room temperature (Zhuang et al. 2020).

To further objectively and quantitatively compare the effect of modified starch and dietary fiber on the spatial distribution of the composite gel system, the particle size and number of polysaccharides (modified starch and dietary fiber) cavities in gel networks were analyzed through image software and the related data was exhibited in Table 2. The characteristic of moisture
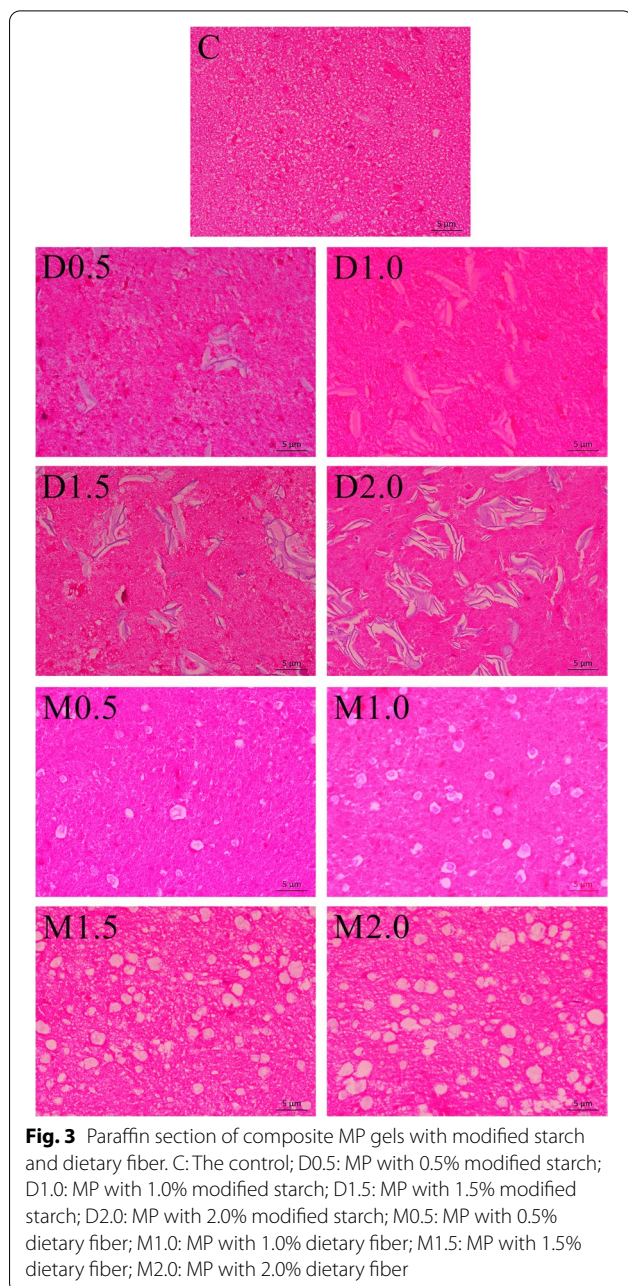

Fig. 3 Paraffin section of composite MP gels with modified starch and dietary fiber. C: The control; D0.5: MP with 0.5% modified starch; D1.0: MP with 1.0% modified starch; D1.5: MP with 1.5% modified starch; D2.0: MP with 2.0% modified starch; M0.5: MP with 0.5% dietary fiber; M1.0: MP with 1.0% dietary fiber; M1.5: MP with 1.5% dietary fiber; M2.0: MP with 2.0% dietary fiber

cavities in pure MP were in large quantity and in small size, which were 543 and 43 respectively. On the contrary, the characteristic of dietary fiber cavities in the composite gel were in small quantities and in large size, which were 61 and 2780 respectively. The various size and number of cavities (moisture, modified starch and dietary fiber) reflected that pure MP had the most humongous structure, followed by modified starch and dietary fiber. The MP gel networks were elastic structure; the gelatinized starch was viscous; and dietary

Luo *et al. Food Production, Processing and Nutrition*　　　(2022) 4:30

Page 8 of 14

Table 2 Number, average size and sum of cavities in the gel networks; lacunary and fractal dimension of the MP gel networks

Treatments	Fractal dimension	Lacunary	Number	Size (pixel* pixel)	Sum(Number × Size)
C	1.7576 ± 0.0057d	0.333 ± 0.03a	543 ± 216a	43 ± 5e	19,867 ± 3231f
M0.5	1.7491 ± 0.0082d	0.310 ± 0.015ab	65 ± 7e	473 ± 11d	31,071 ± 2842ef
M1.0	1.7603 ± 0.0051 cd	0.301 ± 0.032ab	139 ± 8d	470 ± 20d	65,504 ± 4964d
M1.5	1.7605 ± 0.0051bcd	0.291 ± 0.043ab	195 ± 7c	468 ± 20d	91,806 ± 7601c
M2.0	1.7615 ± 0.0017abc	0.288 ± 0.018ab	243 ± 8b	517 ± 17d	125,787 ± 947b
D0.5	1.7616 ± 0.0089abc	0.307 ± 0.012bc	21 ± 4 h	2082 ± 80c	43,625 ± 2729e
D1.0	1.7670 ± 0.0118abc	0.297 ± 0.009bc	36 ± 2gh	2455 ± 78bb	83,549 ± 6248c
D1.5	1.7692 ± 0.0117ab	0.283 ± 0.009bc	48 ± 3 fg	2779 ± 59a	129,852 ± 5634b
D2.0	1.7772 ± 0.0056a	0.258 ± 0.006b	61 ± 6ef	2780 ± 192a	170,738 ± 8452a

Different letters (a-f) in a column indicate significant differences *(p < 0.05)*

fiber had no viscoelasticity. The modified starch and dietary fiber addition could reduce the elasticity of the composite MP gels, especially dietary fiber. The results of fracture strain and R% could prove the assumption above.

Microstructure of three-dimensional gel networks

The SEM images of MP gel networks were shown in Fig. 4. In SEM images, the white part represented MP three-dimensional networks, and the gray part represented moisture pores. The MP denatured and aggregated during heating process, finally formed an irregular and porous three-dimension networks. In salt solution the MP would depolymerize and then adsorb lager volumes of moisture. The subsequent heating process induced the denaturation of MP, which would result in moisture exudation. The moisture exudation in the gel networks formed moisture channels or moisture cavities (Andrew J. Gravelle et al. 2017; Andrew J Gravelle et al. 2016).

The pure MP gel had the loose cross-linked three-dimension networks structure, which filled with numerous moisture channels. Compare to pure MP, the gel networks with 1.0% modified starch addition had firmer structure. The modified starch improved the compactness of the gel networks through swelling effect. The modified starch granules would absorb moisture and swell at the gelatinization temperature, which would press MP gel. The gel networks with dietary fiber addition were totally different from the treatments with modified starch, especially at 2%. When dietary fiber addition reached to 2%, the moisture channels finally disappeared and the gel networks had compact and homogeneous structure without moisture channels. Zhuang et al. (2020) studied the mechanism of MP gelation property improved by insoluble dietary fiber, and indicated that

the dietary fiber could remove the moisture from the MP through strong water holding capability. So the "concentrated" MP promoted the interaction of the hydrophobic groups and improved the compactness of gel networks.

The gel networks directly determined the hardness and viscoelastic properties of final products. To expound the differences of gel networks with various polysaccharides addition and the relationships between gel networks and textual characteristics, the gel networks further objectively and quantitatively analyzed through the image software. The fractal dimension and lacunary represented the complexity and homogeneity of MP gel networks, respectively. The value of fractal dimension had positive correlation with the complexity of the MP gel networks, while the value of lacunary had negative correlation with the homogeneity of MP gel networks. The analysis results showed that pure MP gel networks had the lowest value of fractal dimension (1.7576) and the highest value of lacunary (0.333), however the composite gel networks with 2% dietary fiber addition had the highest value of fractal dimension (1.7772) and the lowest value lacunary (0.258).

Raman spectral analysis

The MP molecular conformation influenced by modified starch and sugarcane dietary fiber addition was studied through Raman spectroscopy. The Raman spectra of different treatments were shown in Fig. 5. The frequency and intensity changes of the characteristic peaks indicated the changes of the secondary structure and the local environments of the MP.

Many literatures had reported the correlation between the frequencies of the amide I band and the types of protein structure conformation (Alix et al. 1988; Schweitzer-Stenner 2006). In the Raman spectra the amide I (1645–1685 cm^{-1}) regions contain

Luo *et al. Food Production, Processing and Nutrition*   (2022) 4:30
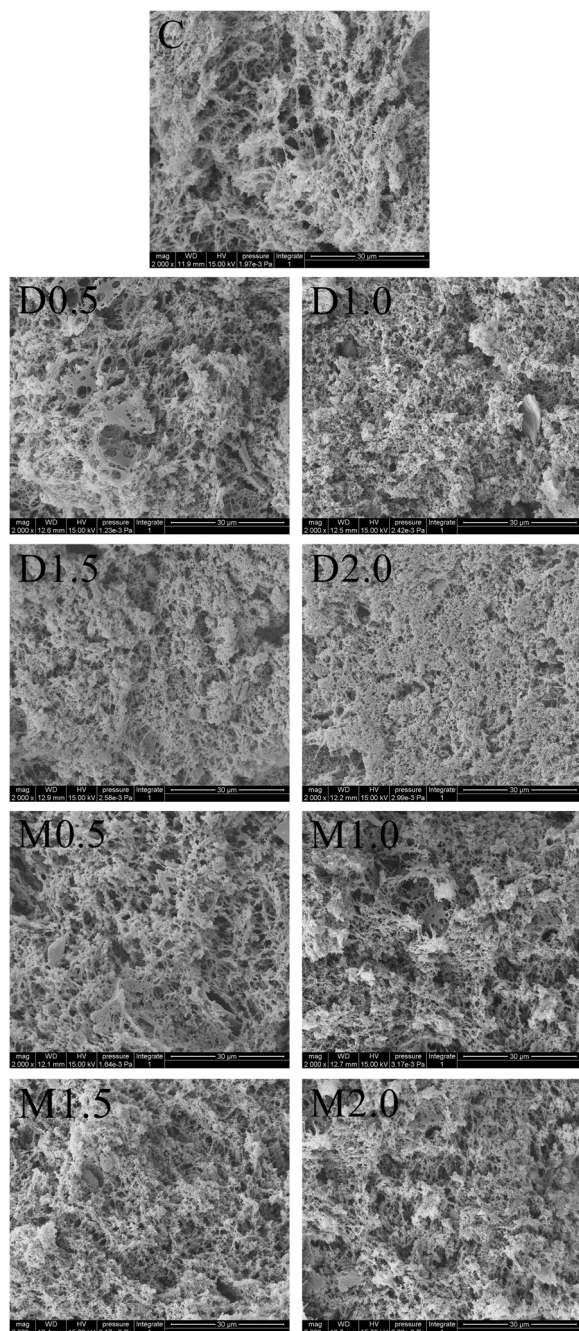
Page 9 of 14

Fig. 4 SEM of composite MP gels with modified starch and dietary fiber. C: The control; D0.5: MP with 0.5% modified starch; D1.0: MP with 1.0% modified starch; D1.5: MP with 1.5% modified starch; D2.0: MP with 2.0% modified starch; M0.5: MP with 0.5% dietary fiber; M1.0: MP with 1.0% dietary fiber; M1.5: MP with 1.5% dietary fiber; M2.0: MP with 2.0% dietary fiber

characteristic peaks of secondary structure, and the ranges of 1658–1650, 1680–1665, and 1665–1660 cm^{-1} in the amide I band were respectively presented the α-helices, β-sheets, and random coil structures. Compared to the control, the characteristic peak of the amide I band did not have significant movement with modified starch addition. It suggested that the modified starch addition did not affect the changes of MP secondary structures during the thermal process. However, the characteristic peak of the amide I band significantly shifted from 1669.2 to 1672.1 with 2% dietary fiber addition. Xu et.al utilized Raman spectroscopic to study the heat-induced MP gelation and its relationship with textural characteristic. The results suggested that the right shift in Raman spectra reflected the increase of β-sheets, β-turns and random coil proportion, which were related positively to the aggregation of MP. The intensity of the band at 2950 cm^{-1} could reflect the change of aliphatic amino acids present in peptide and proteins. Table 3 showed the intensity at 2945 cm^{-1} of various treatments. Compare to the control, the intensity at 2945 cm^{-1} did not have significant change with modified starch addition, but it significantly reduced with dietary fiber addition. Especially, the intensity at 2945 cm^{-1} of the treatment with 2% dietary fiber addition reduced from 5.35 to 4.33. During the heating process, the MP molecular structure has two transformation processes: the unfolding of protein conformation and the aggregation of hydrophobic groups. As the temperature initially increases, the double helix structure of myosin tail started to denature and unfold. And numerous hydrophobic groups inside the protein conformation could expose. Hence, in the Raman spectra the right movement of the characteristic peak in the amide I band reflected the reduction of α-helices proportion and the increased exposure of hydrophobic groups. As the temperature further increases, the exposed hydrophobic groups started to aggregate through hydrophobic interaction. Hence, in the Raman spectra the reduction of intensity at 2945 cm^{-1} reflected the firmer aggregation of hydrophobic groups, leading to form more compact three-dimensional networks.

Principal component analysis (PCA)

The principal component analysis (PCA) showed that the first principal component could contribute to 70.7% of the total variation and first two components contribute
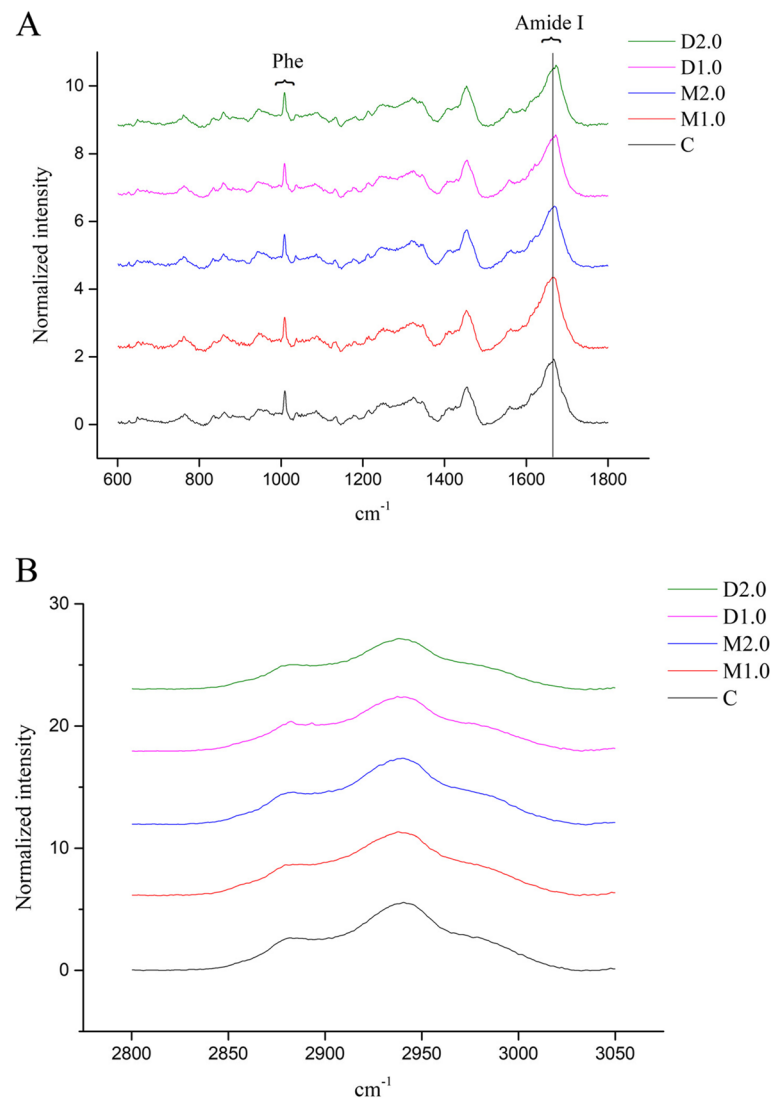
Fig. 5 Raman spectrum between 600 and 1800 cm^{-1} of composite MP gels with modified starch and dietary fiber (**A**); Raman spectrum between 2800 and 3050 cm^{-1} of composite MP gels with modified starch and dietary fiber (**B**). C: The control; D1.0: MP with 1.0% modified starch; D2.0: MP with 2.0% modified starch; M1.0: MP with 1.0% dietary fiber;; M2.0: MP with 2.0% dietary fiber

Table 3 The frequency of characteristic peak in Admin I and normalized intensities of 2945 cm^{-1} band of composite MP gels with modified starch and dietary fiber

Treatments	Admin I	I2945/1003
C	1669.2 ± 0.94c	5.35 ± 0.11d
M0.5	1669.2 ± 0.94c	5.37 ± 0.17d
M1.0	1669.2 ± 0.94c	5.32 ± 0.13d
M1.5	1669.8 ± 0.54c	5.29 ± 0.10d
M2.0	1669.8 ± 0.54c	5.21 ± 0.05d
D0.5	1669.8 ± 0.54c	5.24 ± 0.05d
D1.0	1670.8 ± 0.51bc	5.07 ± 0.10c
D1.5	1671.4 ± 0.74ab	4.57 ± 0.10b
D2.0	1672.1 ± 0.54a	4.33 ± 0.12a

Different letters (a-g) in a column indicate significant differences ($p < 0.05$) between treatments with same concentration of different polysaccharides

to 82.7% of the total variation. The results indicated the high correlation between microstructure and textural property. The loading plot (Fig. 6B) showed that the first component was positively related with the variables, including strain, J_{max}, lacunary, R%; while negatively related with the variables, including sum, fractal dimension and stress. And the correlation analysis (Fig. 6C) had the same conclusion. Han et al. (2014) studied the relationship between the gel networks and textural property, and found that the compactness of gel networks directly determined the hardness and gel strength of final products. The Fig. 6A showed that the MP gels with modified starch and dietary fiber addition were grouped into three well-differentiated clusters, which suggested that the
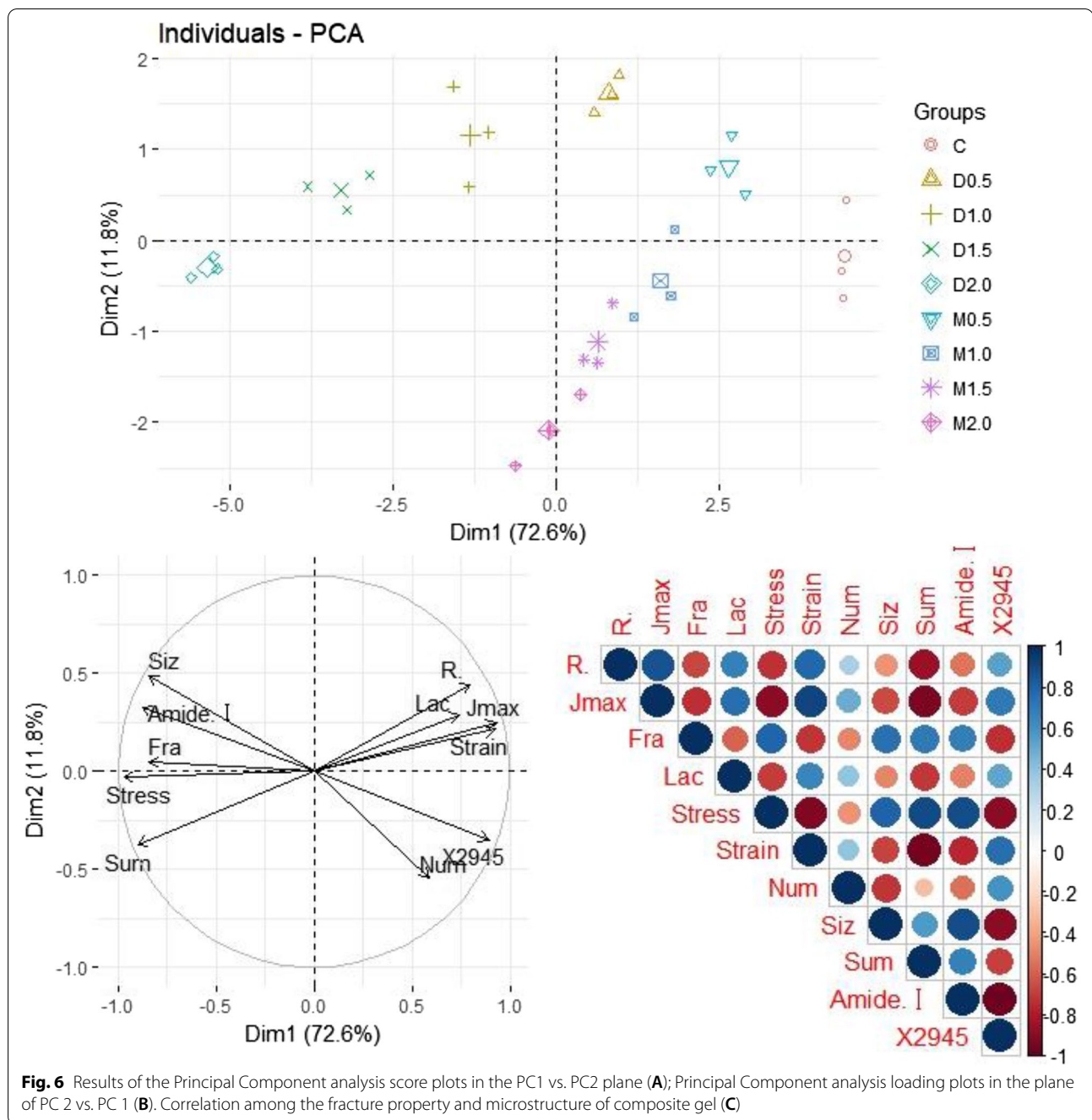
Fig. 6 Results of the Principal Component analysis score plots in the PC1 vs. PC2 plane (**A**); Principal Component analysis loading plots in the plane of PC 2 vs. PC 1 (**B**). Correlation among the fracture property and microstructure of composite gel (**C**)

modified starch and dietary fiber addition significantly changed the microstructure and the textural property of the composite gels.

Mechanistic explanation

The relationships between the myofibrillar protein (MP) gel networks, the spatial distribution of polysaccharides and the fracture property of composite gel were exhibited in Fig. 7.

From the skeleton structure, the MP denatured and aggregated during the thermal process, and formed viscoelastic three-dimensional networks. The PCA result showed that the aggregation of MP was positively related with fracture stress of composite gel system. The Raman spectra reflected that dietary fiber addition could improve the exposure of hydrophobic groups and the aggregation of hydrophobic groups during the thermal process, and the SEM showed that dietary fiber addition

Fig. 7 The modulate relationship between fracture properties and microstructure
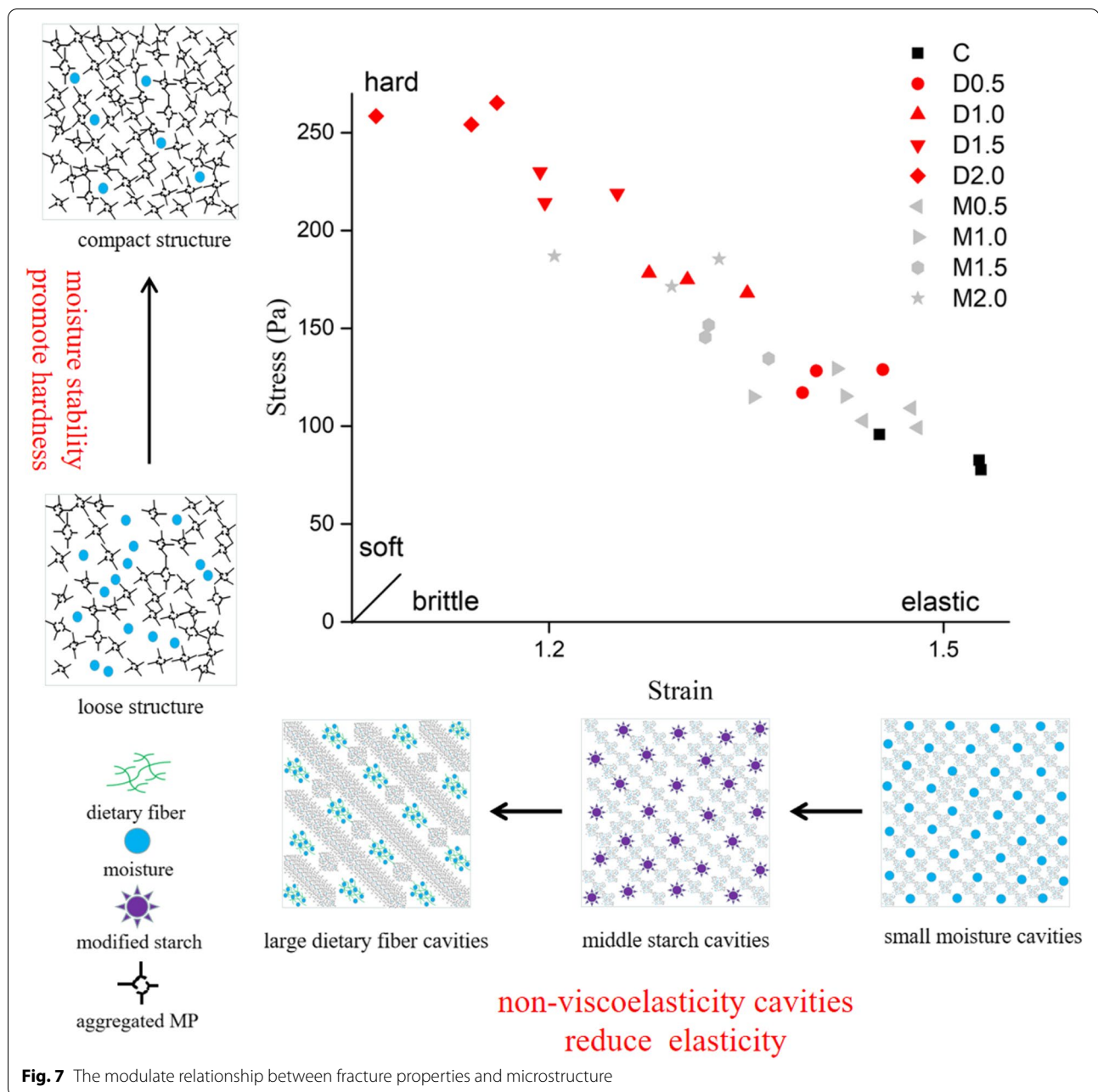
could promote to produce a more homogeneous and compact protein network without numerous moisture channels among it. Although modified starch addition did not improve the aggregation of MP, the gel strength of final gel system still improved. However, the improvement effect was limited and resulted from "swelling effect" of modified starch. Hence, the aggregation of MP molecule determined the fracture stress of final composite gel system.

From the filling structure, the MP was continuing phase and the polysaccharides were disperse phase in the protein-polysaccharide gel system. The polysaccharides were just physical embedded in the MP gel networks and had no direct interaction with MP. The PCA showed that the spatial distribution of polysaccharides was highly related with fracture strain of composite gel system. The modified starch and dietary fiber addition had the same influence on fracture strain of the composite gel. The MP gel networks was an elastic structure, while the polysaccharides cavities had no viscoelasticity. Compare to modified starch, the volume of dietary fiber was much larger. The existence of large volume dietary

Luo *et al. Food Production, Processing and Nutrition* (2022) 4:30

Page 13 of 14

fiber cavities without viscoelasticity would significantly reduce the strain and R% of the composite gel. Hence, the spatial distribution of protein-polysaccharide microstructure determined the fracture stain of final composite gel system.

From the texture level, dietary fiber addition could significantly improve fracture stress and result in the reduction of fracture strain. The MP three-dimensional networks and the spatial distribution of protein-polysaccharide microstructure determine the fracture stress and fracture stress of final composite gel system respectively.

Conclusion

In present study, we found that the MP three-dimensional networks and the spatial distribution of protein-polysaccharide microstructure determined the fracture stress and fracture strain of final composite gel system respectively. The dietary fiber addition could significantly improve the aggregation of protein, leading to a dense and compact gel networks. Hence, the composite gel with dietary fiber addition had the highest stress value and lowest value of J_{max}. However, the dietary fiber had no interaction with myofibrillar protein, and formed large volume cavities with no viscoelasticity, which significantly reduced fracture strain of the composite gel. The fracture stress of treatments with modified starch addition has limited improvement, which resulted from the "swelling effect" of modified starch. Because the modified starch addition did not improve the aggregation of MP.

Authors' contributions
Cheng Luo: Investigation, Writing. Tao Zhang: Investigation, Writing. Xiping Jiang: Software, Formal analysis. Tao Zhang: Software, Formal analysis. Yinji Chen: Writing—Review & Editing. Guanghong Zhou: Review & Editing. Xinbo Zhuang: Conceptualization, Methodology, Writing—Original Draft, Investigation. The author(s) read and approved the final manuscript.

Availability of data and materials
Data available on request from the authors.

Declarations

Ethics approval and consent to participate
Approval for ethics was not needed for the present research.

Consent for publication
Not applicable.

Competing interests
The authors declared that they have no conflicts of interest to this work.

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