Thermal gelling properties and mechanism of porcine myofibrillar protein containing flaxseed gum at various pH values

Li-hua Pan, Mei-qin Feng, Jian Sun, Xing Chen, Xing-lian Xu and Guang-hong Zhou

ABSTRACT

Flaxseed gum (FG) was reported to enhance the water holding capacity and thermal stability of myofibrillar protein (MP). However, the role of pH in the interaction of MP and FG is still unclear. The aim of this study was to evaluate gelling properties and physicochemical properties of MP-FG gel at various pH values, to explore the mechanism. The results reveal that higher pH value could increase the water holding capacity of MP-FG gels and the highest gel strength is achieved at pH 6.5. Raman spectroscopic analysis reveals that decreasing the pH from 7.5 to 5.5 induces the partial transformation of α-helices into β-sheets. At the same pH value, addition of FG also leads to the transformation of α-helices into β-sheets, affecting the formation of gel. The storage modulus (G') values and gelation rates during gelation process gradually declines with the increasing pH, which might be conducted to form a fine dense network.

Las propiedades térmicas de gelificación y el mecanismo de la proteína miofibrilar porcina que contiene goma de linaza con diferentes valores pH

RESUMEN

Se declaró que la goma de linaza (FG) mejora la capacidad de retención del agua y la estabilidad térmica de la proteína miofibrilar (MP). Sin embargo, el papel del pH en la interacción de MP y FG aún no está claro. El objetivo de este estudio fue evaluar las propiedades de gelificación y las propiedades fisicoquímicas del gel MP-FG con diferentes valores pH para explorar su mecanismo. Los resultados revelan que un valor pH mayor podría aumentar la capacidad de retención de agua de los geles MP-FG, además la mayor fuerza del gel se consiguió con un pH 6.5. El análisis espectroscópico Raman reveló que reducir el pH de 7,5 a 5,5 induce a la transformación parcial de helices α a laminas beta β. Con el mismo valor pH, la adición de FG también conlleva a la transformación de helices α a laminas beta β, afectando la formación de gel. Los valores del módulo de almacenamiento (G') y el índice de gelificación durante el proceso de gelificación se reducen gradualmente con el incremento del pH, lo cual puede conducir a la formación de una densa red.

Introduction

Recently, hydrocolloids, such as κ-carrageenan, chitosan and locust bean gum, derived from a variety of plants and microorganisms, have been applied as one of the most effective fat substitutes for developing low-fat meat products and to impart desirable binding characteristics, textures and appearance in ground meat products (Zhou et al., 2014). An understanding of the gelation properties of porcine MPs containing hydrocolloids additives is beneficial for the development of comminuted meat products as well as maintaining quality in meat products.

Flaxseed gum (FG) is one of the hydrocolloids, which has been used extensively in the food industry. FG is a heteropolysaccharide containing anionic polysaccharide and neutral polysaccharide (Qian, Cui, Wu, & Goff, 2012). It has shown considerable potential in the meat product due to its good hydrophilicity and emulsibility. It was found in previous studies that, FG significantly enhanced the water holding capacity (WHC) of MPs (Sun, Li, Xu, & Zhou, 2011) and salt-soluble meat protein (SSMP) gels (Chen, Xu, & Wang, 2007), as well as increased the emulsification properties of soybean protein isolate (SPI) (Wang, Li, Wang, & Adhikari, 2011). Addition of FG increased thermal stability of SSMP, suggesting that an interaction between FG and SSMP could have occurred (Chen et al., 2007). The results obtained from the study of adding destabilizer to SSMP gels indicated that electrostatic interaction seemed to be the main force involved in the formation and stability of protein–polysaccharide gel. Sun and other researchers (2011) found that the improvement effect of FG on WHC of heat-induced MP gel was concentration dependent, which was achieved by the formation of a finer gel network, lower relaxation time, and stronger electrostatic attraction.

However, most of studies were conducted in simple characterization of the appearance of MPs system containing FG, and few researches focused on the mechanism under the phenomenon. In the comminuted meat systems, the heat-induced gelation of MP results in the formation of a 3-dimensional network structure that holds water and gives rise to a unique texture of meat products. During thermal gelation, the interactive effects of MP, the predominant factor in controlling the extent of formation and behavior of the matrix, were supposed to depend upon pH (Hong, 2011).
Materials and methods

Materials

FG (powder, purity 99.8%) was provided by Sinkiang Luqi Biotechnology Ltd. (Sinkiang Province, China). The viscosity of 10 g/L FG solution was 17,000 mPa.s at 25°C. The fresh pork *longissimus dorsi* was purchased from SuShi Meat Corporation through a local supermarket.

Extraction of MPs

MPs were extracted from pork *longissimus dorsi* at 4°C using a modified procedure reported by Doerscher, Briggs, and Lonergan (2004). At first, 200 g pork *longissimus dorsi* was ground by a Waring grinder (Modelnr 8010ES, Waring Commercial, New Hartford, Conn., U.S.A.) for 7 sec at 2,000 rpm twice. The paste was mixed with 800 mL cold extracting solution (100 mmol/L KCl, 20 mmol/L potassium phosphate, 2 mmol/L MgCl₂, 1 mmol/L EGTA, pH 7.0) homogenized for 1 min at 10,000 rpm by a homogenizer (T25 digital Ultra-Turrax, IKA, Germany) and centrifuged at 1500 × g for 15 min. The procedure was repeated twice. After that, the sediment was treated with extracting solution and homogenized. Ten g/Kg Triton X-100 was then added and stirred for 15 min. The extraction was centrifuged at 1500 × g for 10 min with an agitator. The extraction was centrifuged at 2000 × g for 10 min, which was repeated twice. The final step was to mix the sediment with 4 volumes of 100 mmol/L NaCl solution, which was centrifuged at 2,000 × g for 10 min. The resulting sediment was regarded as MPs. MPs purity was checked using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Figure 1) and the protein concentration was measured using the Biuret method (Gornall, Bardawill, & David, 1949). MPs were stored at 4°C and tested within 3 days.

Preparation of MP-FG gels

FG was added to MP to make MP-FG sols with an isolation buffer as the protein extraction (MP concentration of 4%, and FG concentration of 0 or 0.4%). After standardizing all sols in 0.6 mol/L NaCl, they were stirred and homogenized. The pH of MP-FG sols was adjusted to 5.5, 6.0, 6.5, 7.0 and 7.5 respectively using 0.5 mol/L HCl or NaOH solution. The samples were stored at 4°C prior to measurements.

Preparation of MP-FG gels

MP-FG sols prepared as described above were heated in a water bath from 20°C to 80°C and kept at 80°C for 20 min. The obtained gels were stored at 4°C overnight (12 h) prior to determinations of WHC and scanning electron microscopy (SEM) images.

Dynamic rheological measurements

The dynamic oscillatory measurements were performed using a method described by Westphalen, Briggs, and Lonergan (2005) with slight modifications on a rheometer (Anton Paar, Physica MCR 301, Austria) in oscillatory mode. A 25 mm-parallel steel plate geometry with a 500 µm gap was used. A oscillation constant frequency of 0.1 Hz and a strain of 0.3% were applied to monitor the storage modulus (G'). The temperature was increased from 20°C to 80°C at a 2°C/min rate and decreased from 80°C to 20°C at a 4°C/min rate for sample treating. All data were collected from triplicate treatments.
**Raman spectroscopic analysis**

The Raman spectrum of each sample was measured on an FRA 106/S FT-Raman spectrometer equipped using a modified procedure from Li, Kang, Zhao, Xu, and Zhou (2014) and Wang et al. (2015). The spectra were obtained in the range of 400 to 3,600 cm\(^{-1}\). Each spectrum of the samples was obtained under the following conditions: three scans, 30 sec exposure time, 2 cm\(^{-1}\) resolution, sampling speed 120 cm\(^{-1}\) min\(^{-1}\), and data collection every 1 cm\(^{-1}\). The spectra were smoothed, baseline-corrected, and normalized against the phenylalanine band at 1003 cm\(^{-1}\). The secondary structures of each sample were determined as percentages of α-helix, β-sheet, β-turn, and unordered conformations (Alix, Pedanou, & Berjot, 1988).

**WHC**

The WHC was measured using the protocol described by Shao, Zou, Xu, Zhou, and Sun (2015). Five g gel in centrifuge tube was centrifuged at 10,000 x g at 4°C for 10 min. The WHC was the percentage of the gel’s weight retained after centrifugation relative to its initial weight. All data were collected in triplicate.

**Gel strength**

The strength of the gels was analyzed using a TA.XT Plus Texture Analyzer (TA.XT Plus, Stable Micro Systems, UK) at ambient temperatures (approximately 20°C). The gel was subjected to a compression test using a cylindrical probe (P/0.5 in., aluminum) at a trigger type button with a 1.5 mm s\(^{-1}\) pretest speed, a 1.0 mm s\(^{-1}\) test speed, a 1.0 mm s\(^{-1}\) posttest speed, a 4.0 mm distance and a −5 g trigger force. Peak load after compressing were recorded. The maximum sustained compression force was described as the gel strength (Zhou et al., 2014). The experiments were conducted in eight replicates.

**SEM measurements**

The samples were fixed in a 0.1 M phosphate buffer (pH 7.0) containing 25 ml/L glutaraldehyde at 4°C for 2 days as described by Han, Zhang, Fei, Xu, and Zhou (2009). The SEM observations were performed on a SEM (S-3000, Hitachi Science System Ltd., Hitachinaka, Japan) with an accelerating voltage of 7 kV. Two fields from each treatment were examined, and one of the two was presented.

**Statistical analysis**

Statistical analysis was conducted using Statistical Analysis System for windows (SAS 8.2, SAS Inst. Inc., Cary, N.C., U.S.A., 2000). All data are expressed as mean ± standard error. And the experiments were conducted at least for triplicates.

**Results and discussion**

**Effects of pH on the WHC of thermal MP-FG gel**

It can be seen from Table 1 that, addition of FG to the MP gel formulation significantly (P < 0.05) improves the WHC of MP gel, even in that prepared at a low pH. Similar results were also observed in our previous study, and the maximum WHC improvement was achieved with addition of 0.4% FG (Sun et al., 2011), which is also the additive amount in present study. With the addition of FG, the WHC of MP gel around the pl point (pH 5.5) of the protein is significantly improved to be similar to that under the pH value between 7.0 and 7.5.

The WHC indicates a protein’s capability to bind water and is generally used to evaluate the quality and yield of meat products. When pH is adjusted to the pl point, the net charge on the protein is zero and most proteins will aggregate (Huff-Lonergan & Lonergan, 2005), which would make negative influence on the network formation, resulting in a reduction in the amount of water that could be attracted and held by the MP gel matrix. The results obtained in present study means that the addition of FG can effectively overcome the loss of the WHC of protein gel caused by the approaching of pH value to its pl point.

In addition, the WHC of the MP-FG gel is increased significantly (P < 0.05) from 86.80 % to 98.73% when the pH value increases from 5.5 to 7.5 (Table 1). This phenomenon is similar with the effect of pH on the WHC of thermal MP gel, a lower pH value is associated with a lower WHC (Bertram, Kristensen, & Andersen, 2004). An increase of pH away from the pl of MP leads to more charged groups on the surface of the protein, rendering MPs better solubility and mobility, thus more surrounding water would be exposed to protein sites via hydrogen bonding. In addition, the electrostatic interaction between MP and FG may be enhanced due to

| FG (%) | 5.5   | 6.0   | 6.5   | 7.0   | 7.5   |
|--------|-------|-------|-------|-------|-------|
| 0      | 48.62 ± 0.55e | 60.14 ± 0.33d | 65.22 ± 0.09c | 77.36 ± 1.00b | 95.97 ± 0.44a |
| 0.4    | 86.80 ± 1.72*c | 88.35 ± 1.48*c | 95.21 ± 1.78*b | 96.64 ± 0.14*a | 98.73 ± 0.13*a |

1. Data are expressed as the mean ± SD, n = 3.
2. * in the column indicate MP and MP-FG are statistically significant differences at P < 0.05; T-test.
3. a-e Different letters in the same row of the sample with adding 0% or 0.4% FG indicate statistically significant differences at P < 0.05; one-way ANOVA and Duncan’s multiple range test.
4. Los datos están expresados como promedio ± SD, n = 3.
5. * en la columna indica que MP y MP-FG son diferencias estadísticamente significativas a P < 0.05; test T.
6. a-e Las distintas letras en la misma fila de la muestra añadiendo 0% o 0.4% de FG indican diferencias estadísticamente significativas a P < 0.05; ANOVA de un único sentido y test de rango múltiple de Duncan.
higher pH could facilitate improving WHC of MP-FG gel.

**Effects of pH on the gel strength of thermal MP-FG gel**

Different gel strength caused by addition of FG under different pH conditions are shown in Table 2. The effect of pH seemed to be different for each system (with or without flaxseed gum). It can be seen from Table 2 that, among MP samples, the highest gel strength appeared at pH 6.0, and the maximum gel strength of MP-FG occurred at pH 6.5. The gel strength declines significantly with movement of the pH away from 6.0 or 6.5 (P < 0.05). When pH is lower than 6.5, the MP-FG gels show lower gel strength than MP gels. However, the gel strength of MP-FG is higher than that of MP as pH raised to 7.0 and 7.5. The change of gel strength of MP-FG gel is insignificant (P > 0.05) when pH value increased from 5.5 to 6.5, which is in consistence with the effects of pH on the WHC of MP-FG gel.

Variations in gel strength as affected by pH cannot be fully explained by differences in protein solubility, which might also be ascribed to the change of myosin isoforms (Morita, Choe, Yamamoto, Samejima, & Yasui, 1987) and the different interactions between protein-protein and protein-FG (Chen et al., 2007; Lesiów & Xiong, 2003). It is expected that increasing pH away from the pl point can enhance the protein solubility via electrostatic repulsion force. The high solubility of protein is thought to only alter the quantity of the effective gelling components, rather than affecting the specific protein bonds in the gel matrix. This hypothesis is supported by the observation that the proteins form substantially weaker gels as the pH is raised from 6.0 to 7.0 (Xiong, 1994). Thus, we propose that the different gel strength of MP-FG induced by pH is ascribed to the protein-protein and protein-FG interactions that form the gel structures with various texture properties.

On the basis of the results of the effects of pH on the WHC and gel strength of MP-FG gel, the following research will focus on the gel samples prepared under pH 5.5, 6.5 and 7.5 conditions to explore the possible gelling mechanism of the MP-FG gel influenced by pH.

**Effects of pH on the rheological properties of MP-FG gel**

Dynamic rheological tests have been used extensively to study heat-induced gelation of MPs. The viscoelastic measurement of the storage modulus (G’) is used to characterize the MP gel. The G’ value estimates the stored energy of the elastic portion (Cao, Xia, Zhou, & Xu, 2012). G’ in Table 3 is defined as the initial G’ before heating. And the rate of gelation from 60 to 80°C is extracted from the slope of the storage modulus versus time (Westphalen et al., 2005), which is associated with the degree of protein unfolding and aggregation. In consequence, it would affect gel properties.

The effect of pH on rheological properties of MP-FG is shown in Figure 2 and Table 3. As pH increases from 5.5 to 7.5, the G’ decreases. At each pH level, addition of 0.4% FG...

---

**Table 2.** Gel strength of myofibrillar protein (MP) containing 0 or 0.4% flaxseed gum (FG) as affected by different pHs (5.5, 6.0, 6.5, 7.0, and 7.5).

| FG (%) | pH 5.5 | pH 6.0 | pH 6.5 | pH 7.0 | pH 7.5 |
|--------|--------|--------|--------|--------|--------|
| 0      | 32.45 ± 1.91c | 42.90 ± 6.58a | 35.89 ± 2.56b | 24.32 ± 1.00d | 20.18 ± 1.19e |
| 0.4    | 22.89 ± 1.69c | 23.74 ± 1.65c | 33.05 ± 4.74a | 28.60 ± 1.96b | 22.03 ± 1.75c |

1. Data are expressed as the mean ± SD, n = 8.
2. * in the column indicates MP and MP-FG are statistically significant differences at P < 0.05; T-test.
3. a-e Different letters in the same row of the sample with adding 0% or 0.4% FG indicate statistically significant differences at P < 0.05; one-way ANOVA and Duncan’s multiple range test.

**Table 3.** G’ and gelation rate of myofibrillar protein (MP) containing 0 or 0.4% flaxseed gum (FG) as affected by different pHs (5.5, 6.5, and 7.5).

| FG (%) | pH 5.5 | pH 6.0 | pH 6.5 | pH 7.5 |
|--------|--------|--------|--------|--------|
| G’ (Pa) | 219.33 ± 17.90b | 271.00 ± 12.29a | 346.33 ± 29.143a | 206.00 ± 6.00b |
| Gelation rate (Pa/°C) | 35.07 ± 4.46a* | 206.00 ± 6.00*b | 165.00 ± 10.00c | 346.33 ± 29.143*a |

1. Data are expressed as the mean ± SD, n = 3.
2. * in the column indicates MP and MP-FG are statistically significant differences at P < 0.05; T-test.
3. a-c Different letters in the same row of the sample with adding 0% or 0.4% FG indicate statistically significant differences at P < 0.05; one-way ANOVA and Duncan’s multiple range test.

---
generates a higher $G_0$ value as we could found in Table 3, indicating that an electrostatic interaction between MP and FG occurs before heating (Chen et al., 2007). At pH 5.5, the $G_0$ of MPs and MP-FG exhibits insignificant differences ($P > 0.05$) in Table 3. The negative and positive charges inside protein molecules are approximately equal under this pH condition close to pl point and the electrostatic interactions between them are weak. As the pH (6.5, 7.5) deviates from the pl point, the interactions are enhanced, thus significantly higher $G_0$ is detected in MP-FG (Table 3).

The gelation rate from 60 to 80°C of MP-FG are significantly different compared with MP, indicating FG has a noteworthy impact on the mixture. On the other hand, as the pH value increases from pH 5.5 to pH 7.5, the gelation rate of MP-FG decreases from 19.62 Pa/C to 9.87 Pa/C, respectively. The same phenomenon was also observed in porcine myosin gels (Liu, Zhao, Xiong, Xie, & Qin, 2008) and fish myosin gels (Liu et al., 2010). It seems that MPs at lower pH always possess a higher gelation rate during the heating stage (Liu et al., 2008; Westphalen et al., 2005). The results probably arise from the closer association of proteins at lower pH or the increased strength of hydrophobic interactions and disulphide bonds (Riebroy, Benjakul, Vissessanguan, Erikson, & Rustad, 2008; Westphalen et al., 2005). Slow gelation rate might give rise to full unfolding and aggregation which is conducive to the formation of the orderly gel network with good gel properties.

**Effects of pH on the secondary structure of MP and MP-FG in sol condition**

The secondary structural contents of proteins in MP-FG samples, as affected by pH, are shown in Table 4. The $\alpha$-helical content of MP-FG decreases markedly (lower than 40%) as the pH decreases from 6.5 to 5.5, and there is no significant difference on the secondary structures $\Theta$-$\alpha$-helix, $\beta$-sheet, $\beta$-turn and unordered between pH 6.5 and 7.5. At different pH values, addition of FG varies the secondary structure of MP (Table 4). At the pl point (pH 5.5), the presence of FG has no significant effects ($P > 0.05$) on the secondary structures of MP. When the pH value is set at 6.5, addition of FG to MP significantly ($P < 0.05$) reduces the content of $\alpha$-helical structure (Table 4). At the same time, the content of $\beta$-sheet structure increases. The changes in protein conformation occur in the presence of polysaccharides, which might be caused by the promoted interactions between MP and FG (Turgeon, Schmitt, & Sanchez, 2007).

Hydrogen bonds between the carbonyl oxygen (–CO) and amino hydrogen (NH–) of a polypeptide chain mainly account for the stabilization of $\alpha$-helix structure (Sano, Ohno, Otsuka-Fuchino, Matsumoto, & Tsuchiya, 1994). Electrostatic interactions between amino acids also contribute to the stability of the secondary structures (Satoh, Nakaya, Ochiai, & Watabe, 2006). As the pl point of myosin is about pH 5.5 (Foegeding, Lanier, & Hultin, 1996), reducing the pH value from 7.5 to 5.5 might weaken the electrostatic attraction among protein-protein and protein-FG via charge neutralization, and destabilize the hydrogen bonds. The changes in electrostatic interactions and hydrogen bond stability could in turn lead to the reduction of $\alpha$-helix content (Liu et al., 2008). The $\beta$-sheet content of MP-FG significantly increases as pH decreases from 6.5 to 5.5 (Table 4). Therefore, there might be a $\alpha$-helical to $\beta$-sheet transition with reducing pH from 6.5 to 5.5, probably originating from the conformational changes of myosin (Liu et al., 2010, 2008).

Protein gel properties are closely related to the changes in its conformation. Both the unfolding of $\alpha$-helic and the formation of $\beta$-sheets play an important role in MP gel formation (Liu et al., 2010; Villamonte, Jury, Jung, & de Lamballerie, 2015). It was suggested that $\alpha$-helic exhibited a negative effect on $G'$ at 90°C whereas $\beta$-sheets exhibited positive effects on $G'$ at 90°C (Liu et al., 2010). $\beta$-sheets were an important conformational component of the aggregated-state secondary structure of globulin derived from common buckwheat (Choi & Ma, 2007). In the present study, formation of

---

**Table 3. Secondary structure of myofibrillar protein (MP) containing 0 or 0.4% flaxseed gum (FG) as affected by different pHs (5.5, 6.5, and 7.5).**

| pH  | FG (%)  | MP | MP-FG | MP | MP-FG |
|-----|---------|----|-------|----|-------|
| 5.5 | 0       | 79.29 ± 4.89 | 73.62 ± 7.96 | 61.78 ± 6.93a | 61.78 ± 6.93a |
| 6.5 | 0.4     | 73.62 ± 7.35 | 69.29 ± 5.08 | 61.78 ± 6.93a | 61.78 ± 6.93a |
| 7.5 | 0       | 61.78 ± 6.93 | 56.83 ± 8.46 | 61.78 ± 6.93a | 61.78 ± 6.93a |

1. Data are expressed as the mean ± SD, n = 3.
2. $^*$ in the column indicate MP and MP-FG are statistically significant differences at $P < 0.05$.
3. Differences at the same row of the sample with adding 0% or 0.4% FG indicate statistically significant differences at $P < 0.05$; one-way ANOVA and Duncan’s multiple range test.

---

**Table 4. Secondary structure of myofibrillar protein (MP) containing 0 or 0.4% of goma de linaza (FG) afectada por diferentes pHs (5.5, 6.5, 7.5).**

| pH  | FG (%)  | MP | MP-FG | MP | MP-FG |
|-----|---------|----|-------|----|-------|
| 5.5 | 0       | 34.83 ± 5.83 | 79.29 ± 4.89 | 34.83 ± 5.83 | 79.29 ± 4.89a* |
| 6.5 | 0.4     | 35.62 ± 4.70 | 67.35 ± 5.84 | 61.78 ± 6.93a | 61.78 ± 6.93a |
| 7.5 | 0       | 35.07 ± 4.46a | 6.34 ± 1.29b | 61.78 ± 6.93a | 61.78 ± 6.93a |

1. Los datos están expresados como promedio ± SD, n = 3.
2. $^*$ en la columna indica que MP y MP-FG son diferencias estadísticamente significativas a $P < 0.05$.
3. Un orden diferente de letras en la misma fila indica que MP y MP-FG tienen diferencias estadísticamente significativas a $P < 0.05$; ANOVA.

---

**Table 4. Secondary structure of myofibrillar protein (MP) containing 0 or 0.4% of goma de linaza (FG) afectada por diferentes pHs (5.5, 6.5, 7.5).**

| pH  | FG (%)  | MP | MP-FG | MP | MP-FG |
|-----|---------|----|-------|----|-------|
| 5.5 | 0       | 34.83 ± 5.83 | 79.29 ± 4.89 | 34.83 ± 5.83 | 79.29 ± 4.89a* |
| 6.5 | 0.4     | 35.62 ± 4.70 | 67.35 ± 5.84 | 61.78 ± 6.93a | 61.78 ± 6.93a |
| 7.5 | 0       | 35.07 ± 4.46a | 6.34 ± 1.29b | 61.78 ± 6.93a | 61.78 ± 6.93a |

1. Los datos están expresados como promedio ± SD, n = 3.
2. $^*$ en la columna indica que MP y MP-FG son diferencias estadísticamente significativas a $P < 0.05$.
3. Un orden diferente de letras en la misma fila indica que MP y MP-FG tienen diferencias estadísticamente significativas a $P < 0.05$; ANOVA.
\(\beta\)-sheets occurs simultaneously with the unfolding of \(\alpha\)-helical structures as pH value shifts from 6.5 to 5.5 (Table 4). Thus, it can be observed from Figure 2 that a higher G' of MP-FG gel appeared at lower pH of 5.5, which could be attributed to the increased content of \(\beta\)-sheets.

Regarding to the WHC of the myosin gel, it was found in previous studies that plenty of \(\alpha\)-helices prior to heating were beneficial for the WHC of fish myosin gel and the WHC of porcine myosin gel was negatively correlated with the \(\beta\)-sheet fraction prior to heating (Liu et al., 2010). However, this correlation couldn’t be found when comparing the case of MP gel with that of MP-FG gel. Although MP has higher content of \(\alpha\)-helices (Table 4), its WHC is significantly lower than that of MP-FG gel at the same pH condition of pH 6.5 (Table 1). The electrostatic interactions between MP and FG and the strong hydrophilicity of FG might lead to the enhanced WHC.

Effects of pH on the SEM of MP-FG gel

The 3-dimensional network structure of a gel is an important determinant of WHC and gel strength. Figure 3 shows the structure of the MP or MP-FG gels at 3000 \(\times\) magnification, affected by different pH (5.5, 6.5 and 7.5) treatments.

The addition of FG enhances the density of the MP matrix and eliminates some of the cavities, forming a smooth, continuous and uniform gel matrix (Figure 3). In MP-FG gel samples, there are some membrane structures (marked with arrows) distributing throughout the surface or interacting with the protein matrix (Figure 3 (b1-b3)). We attributed these visible structures to FG matrix in MP-FG gel. Some hydrocolloid (locust bean, guar, xanthan and carboxymethyl-cellulose) had also been observed to disperse on or inside gel matrix of meat protein by SEM (Montero, Solas, & Pérez-Mateos, 2001; Pérez-Mateos, Solas, & Montero, 2002). It is proposed that the denser network induced by FG may be caused by the electrostatic interaction between MP and FG and the physical entrapment effect.

Irrespective of the FG addition, the matrix of the MP-FG gel demonstrated distinctive appearances under different pH treatment. As displayed in Figure 3 (b1), the MP-FG gel exhibits a network with many large cavities and coarse cross-linked strands at pH 5.5. When pH is increased to 6.5 and 7.5 (Figure 3 (b2,b3)), a slightly denser structure with small cavities and more cross-linkages is revealed. In addition to the influence of pH on the hydration, mobility and solubility of proteins, it is considered that the interaction possibly exists between FG and meat protein (Chen et al., 2007). Higher pH environment might cause enhanced electrostatic interaction between MP and FG, thus forming a denser structure. In addition, the changes in the microstructure MP-FG gels at different pH levels might be explained by the gelation rate (Liu et al., 2010; P.; Wang, Xu, Huang, Huang, & Zhou, 2014). When pH is increased from 5.5 to 7.5, the gelation rate of MP-FG decreases from 19.62 Pa/°C to 9.87 Pa/°C, respectively (Table 3). It could be presumed that low gelation rate is beneficial for formation of a fine dense

**Figure 3.** SEM images (×3000) of myofibrillar protein (MP) gels containing 0 or 0.4% flaxseed gum (FG) as affected by different pHs (5.5, 6.5 and 7.5); A1: MP, pH5.5; A2: MP, pH6.5; A3: MP, pH7.5; B1: MP-FG, pH5.5; B2: MP-FG, pH6.5; B3: MP-FG, pH7.5. Arrows marked: membrane structures.

**Figura 3.** Imágenes SEM (×3000) de geles de proteína miofibrilar (MP) que contienen 0 o 0,4% de goma de linaza (FG) afectados por diferentes pH (5,5; 6,5; 7,5); A1: MP, pH5,5; A2: MP, pH6,5; A3: MP, pH7,5; B1: MP-FG, pH5,5; B2: MP-FG, pH6,5; B3: MP-FG, pH7,5. Flechas marcadas: estructuras de membranas.
gel network. Hence, higher pH value leads to a denser gel network for MP-FG.

It was well reported that a fine, uniform structure with numerous small pores would benefit for possessing greater WHC. The addition of FG and elevation of pH can both produce high dense network with small pores, and this change is more conducive to water immobilization, hence improving the WHC (Table 1). Although it is complicated to interpret the relationship between gel strength and network structure of MP-FG gel, some investigations might lead us further inside into the mechanism. As described above, there appears to be less interaction between MP and FG at the pH of 5.5. Then a physical entrapping effect is supposed to be the main role in taking part in the MP gel matrix where the FG absorbed water via hydrogen bonding, FG would interfere with the formation of MP gel network through entanglement (Xiong & Blanchard, 1993), inducing gel network with thin cross-linked strands (Figure 3 (b1)), which consequently results in a decreased gel strength of MP-FG at pH 5.5 (Table 2). As pH increases to 6.5, a strong electrostatic interaction between MP and FG might occur, transforming gel into a highly interactive matrix system that is more resistant to deformation (Feng & Xiong, 2003; Sun et al., 2011). Then the gel strength of MP-FG at pH 6.5 is expected to be significantly higher than that at pH 5.5 (Table 2). However, further pH increment decreases the gel strength of MP-FG (Table 2), which we speculate to be attributed to the thin cross-linked strands (Figure 3) and high water retention of gel network that weakens its gel strength.

Conclusions

Different responses to pH are found on the thermal gelation process of MP-FG complex. Gel WHC shows a significant increase at high pH values. Maximum gel strength of MP-FG gel is observed at pH 6.5. Roman spectroscopy analysis shows that the addition of FG to MP leads to a α-helical to β-Sheet transition under the pH conditions away from the pl, which plays an important role in the MP-FG gelation process. Decreasing gelation rate coupled with increasing pH of MP-FG facilitates the formation of a fine dense gel network, hence enhancing WHC and weakening gel strength. These results suggest that pH adjustment is necessary for MP-FG system to meet the textural and WHC requirements for meat processing.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the [National Natural Science Foundation of China] under Grant number [31401516] and [Fundamental Research Funds for the Central Universities] under Grant number [Y0201400114].

References

Alix, A., Pedanou, G., & Berjot, M. (1988). Fast determination of the quantitative secondary structure of proteins by using some parameters of the Raman amide I band. Journal of Molecular Structure, 174, 159–164. doi:10.1016/0022-2860(88)80151-0
Bertram, H.C., Kristensen, M., & Andersen, H.J. (2004). Functionality of myofibrillar proteins as affected by pH, ionic strength and heat treatment—a low-field NMR study. Meat Science, 68(2), 249–256. doi:10.1016/j.meatsci.2004.03.004
Boyer, C., Joandelt, S., Roussilhes, V., Culliol, J., & Ouali, A. (1996). Heat-Induced gelation of myofibrillar proteins and myosin from fast- and slow-twitch rabbit muscles. Journal of Food Science, 61(6), 1138–1143. doi:10.1111/j.1532-6835.1996.tb10948.x
Cao, Y., Xia, T., Zhou, G., & Xu, X. (2012). The mechanism of high pressure-induced gels of rabbit myosin. Innovative Food Science & Emerging Technologies, 16, 41–46. doi:10.1016/j.ifset.2012.04.005
Chen, H., Xu, S., & Wang, Z. (2007). Interaction between flaxseed gum and meat protein. Journal of Food Engineering, 80(4), 1051–1059. doi:10.1016/j.jfoodeng.2006.08.017
Choi, S. M., & Ma, C. Y. (2007). Structural characterization of globulin from common buckwheat (Fagopyrum esculentum Moench) using circular dichroism and Raman spectroscopy. Food Chemistry, 102(1), 150–160. doi:10.1016/j.foodchem.2006.05.011
Doenscher, D., Briggs, J., & Lonergan, S. (2004). Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar protein gels. Meat Science, 66(1), 181–188. doi:10.1016/S0309-1740(03)00082-2
Feng, J., & Xiong, Y.L. (2003). Interaction and functionality of mixed myofibrillar and enzyme-hydrolyzed soy proteins. Journal of Food Science, 68(3), 803–809. doi:10.1111/j.1532-6835.2003.tb08246.x
Foegeding, E., Lanier, T., & Hultin, H. (1996). Characteristics of edible muscle tissues (Vol. 3). New York, NY: Marcel Dekker.
Gornall, A.G., Bardawill, C.J., & David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry, 172(2), 751–766.
Han, M., Zhang, Y., Fei, Y., Xu, X., & Zhou, G. (2009). Effect of microbial transglutaminase on NMR relaxometry and microstructure of pork myofibrillar protein gel. European Food Research and Technology, 228(4), 665–670. doi:10.1007/s00217-008-0976-x
Hong, G.P., Min, S.-G., & Chin, K.B. (2012). Emulsion properties of pork myofibrillar protein in combination with microbial transglutaminase and calcium alginate under various pH conditions. Meat Science, 90(1), 185–193. doi:10.1016/j.meatsci.2011.06.023
Huff-Lonergan, E., & Lonergan, S.M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71(1), 194–204. doi:10.1016/j.meatsci.2005.04.022
Jang, H.-S., & Chin, K.-B. (2011). Emulsifying and gelling properties of pork myofibrillar protein as affected by various NaCl levels and pH values. Korean Journal for Food Science of Animal Resources, 31(5), 727–730. doi:10.5851/Kosfa.2011.31.5.727
Jiang, J., & Xiong, Y.L. (2013). Extreme pH treatments enhance the structure-reinforcement role of soy protein isolate and its emulsions in pork myofibrillar protein gels in the presence of microbial transglutaminase. Meat Science, 93(3), 469–476. doi:10.1016/j.meatsci.2012.11.002
Lesliow, T., & Xiong, Y.L. (2003). Chicken muscle homogenate gelation properties: Effect of pH and muscle fiber type. Meat Science, 64(4), 399–403. doi:10.1016/S0309-1740(02)00206-1
Li, K., Kang, Z.-L., Zhao, -Y.-Y., Xu, X.-L., & Zhou, G.-H. (2014). Use of high-intensity ultrasound to improve functional properties of batter suspensions prepared from PSE-like chicken breast meat. Food and Bioprocess Technology, 7(12), 3466–3477. doi:10.1007/s11947-014-1358-y
Liu, R., Zhao, S.-M., Liu, Y.-M., Yang, H., Xiong, S.-B., Xie, B.-J., & Qin, L.-H. (2010). Effect of pH on the gel properties and secondary structure of fish myosin. Food Chemistry, 121(1), 196–202. doi:10.1016/j.foodchem.2009.12.030
Liu, R., Zhao, S.-M., Xiong, S.-B., Xie, B.-J., & Qin, L.-H. (2008). Role of secondary structures in the gelation of porcine myosin at different pH values. Meat Science, 80(3), 632–639. doi:10.1016/j.meatsci.2008.02.014
Montero, P., Solas, T., & Pérez-Mateos, M. (2001). Pressure-induced gel properties of fish mince with ionic and non-ionic gums added. Food Hydrocolloids, 15(2), 185–194. doi:10.1016/S0268-005X(00)00064-3
Morita, J.-I., Choe, I.-S., Yamamoto, K., Samejima, K., & Yasui, T. (1987). Heat-induced gelation of myosin from leg and breast muscles of chicken. Agricultural and Biological Chemistry, 51(11), 2895–2900. doi:10.1271/bbb1961.51.2895
Pérez-Mateos, M., Solas, T., & Montero, P. (2002). Carrageenans and alginate effects on properties of combined pressure and temperature
in fish mince gels. *Food Hydrocolloids*, 16(3), 225–233. doi:10.1016/S0268-005X(01)00086-8

Qian, K.Y., Cui, S.W., Wu, Y., & Goff, H.D. (2012). Flaxseed gum from flaxseed hulls: Extraction, fractionation, and characterization. *Food Hydrocolloids*, 28(2), 275–283. doi:10.1016/j.foodhyd.2011.12.019

Riebroy, S., Benjakul, S., Visessanguan, W., Erikson, U., & Rustad, T. (2008). Comparative study on acid-induced gelation of myosin from Atlantic cod (Gardus morhua) and burbot (Lota lota). *Food Chemistry*, 109(1), 42–53. doi:10.1016/j.foodchem.2007.12.008

Sano, T., Ohno, T., Otsuka-Fuchino, H., Matsumoto, J.J., & Tsuchiya, T. (1994). Carp natural actomyosin: Thermal denaturation mechanism. *Journal of Food Science*, 59(5), 1002–1008. doi:10.1111/j.1365-2621.1994.tb08177.x

Satoh, Y., Nakaya, M., Ochiai, Y., & Watabe, S. (2006). Characterization of fast skeletal myosin from white croaker in comparison with that from walleye pollack. *Fisheries Science*, 72(3), 646–655. doi:10.1111/j.1444-2906.2006.01195.x

Shao, -J.-J., Zou, Y.-F., Xu, X.-L., Zhou, G.-H., & Sun, J.-X. (2015). Effects of NaCl on water characteristics of heat-induced gels made from chicken breast proteins treated by isoelectric solubilization/precipitation. *CyTA - Journal of Food*, 14(1), 145–153. doi:10.1080/19476337.2015.1071432

Smith, D.M. (2001). Functional properties of muscle proteins in processed poultry products. In A.R. Sams (Ed.), *Poultry meat processing* (pp. 181–195). Boca Raton, FL: CRC Press.

Sun, X.D., & Holley, R.A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. *Comprehensive Reviews in Food Science and Food Safety*, 10(1), 33–51. doi:10.1111/j.1541-4337.2010.00137.x

Turgeon, S., Schmitt, C., & Sanchez, C. (2007). Protein-polysaccharide complexes and coacervates. *Current Opinion in Colloid & Interface Science*, 12(4), 166–178. doi:10.1016/j.cocis.2007.07.007

Villamonte, G., Jury, V., Jung, S., & de Lamballerie, M. (2015). Influence of xanthan gum on the structural characteristics of myofibrillar proteins treated by high pressure. *Journal of Food Science*, 80(3), C522–C531. doi:10.1111/1750-3841.12789

Wang, P., Xu, X., Huang, M., Huang, M., & Zhou, G. (2014). Effect of pH on heat-induced gelation of duck blood plasma protein. *Food Hydrocolloids*, 35, 324–331. doi:10.1016/j.foodhyd.2013.06.015

Wang, Y., Li, D., Wang, L.-J., & Adhikari, B. (2011). The effect of addition of flaxseed gum on the emulsion properties of soybean protein isolate (SPI). *Journal of Food Engineering*, 104(1), 56–62. doi:10.1016/j.jfoodeng.2010.11.027

Wang, Z., Liang, J., Jiang, L., Li, Y., Wang, J., Zhang, H. . . . Sui, X. (2015). Effect of the interaction between myofibrillar protein and heat-induced soy protein isolates on gel properties. *CyTA - Journal of Food*, 1–8. doi:10.1080/19476337.2015.1011240

Westphalen, A.D., Briggs, J.L., & Lonergan, S.M. (2005). Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. *Meat Science*, 70(2), 293–299. doi:10.1016/j.meatsci.2005.01.015

Xiong, Y.L. (1994). Myofibrillar protein from different muscle fiber types: Implications of biochemical and functional properties in meat processing. *Critical Reviews in Food Science & Nutrition*, 34(3), 293–320. doi:10.1080/10408399409527665

Xiong, Y.L., & Blanchard, S.P. (1993). Viscoelastic properties of myofibrillar protein-polysaccharide composite gels. *Journal of Food Science*, 58(1), 164–167. doi:10.1111/j.1365-2621.1993.tb03235.x

Zhou, Y.-Z., Chen, C.-G., Chen, X., Li, P.-J., Ma, F., & Lu, Q.-H. (2014). Contribution of three ionic types of polysaccharides to the thermal gelling properties of chicken breast myosin. *Journal of Agricultural and Food Chemistry*, 62(12), 2653–2662. doi:10.1021/jf4053812