Effects of heat treatment at two temperatures on the myosin cluster of bighead carp for gel formation

Li Yuan, Yan-ai Liu, Jing Ge, Xue-ping Feng & Rui-chang Gao

To cite this article: Li Yuan, Yan-ai Liu, Jing Ge, Xue-ping Feng & Rui-chang Gao (2017) Effects of heat treatment at two temperatures on the myosin cluster of bighead carp for gel formation, CyTA - Journal of Food, 15:4, 574-581, DOI: 10.1080/19476337.2017.1321045

To link to this article: https://doi.org/10.1080/19476337.2017.1321045

© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 13 Jun 2017.

Submit your article to this journal

Article views: 439

View Crossmark data
Effects of heat treatment at two temperatures on the myosin cluster of bighead carp for gel formation

Li Yuan, Yan-ai Liu, Jing Ge, Xue-ping Feng and Rui-chang Gao
School of Food and Biological Engineering, Jiangsu University, Zhenjiang, China

ABSTRACT
The α-helix of myosin decreased and the diameter of the myosin clusters increased monotonically with increasing heating time at 50 and 90°C. The clusters that formed during heating at 90°C were much larger than those that formed at 50°C. With increasing heating time, the G’ of the myosin heated at 50°C increased, but it decreased in the myosin heated at 90°C. The rheology data suggested that myosin heated at 50°C exhibited better gel formation than myosin that was heated at 90°C. The myosin gel network formed at 50°C was more consistent and homogeneous, whereas the gel formed at 90°C was irregular and sturdy. Myosin heated at 50°C tended to form small, open clusters via heads, and the tails were outstretched, whereas the clusters that formed via myosin heads and tails at 90°C were larger and closed.

1. Introduction
Surimi products are made of muscle myofibrils, which contain actin and myosin. Myosin, which is composed of two globular ‘heads’ and a fibrous domain, the ‘rod’ (Lanier, Carvajal, & Yongsawatdigul, 2004), plays an important role in the gelation of muscle proteins (Wicker, Lanier, Knopp, & Hamann, 1989; Yarnpakdee, Benjakul, Visessanguan, & Kijroongrojana, 2009; Yongsawatdigul & Park, 2003). During heating, myosin molecules first form globules via head–head interactions and subsequently form the strands and cross-links of the gel network via tail–tail interactions (Sharp & Offer, 1992). The thermal gelation of myosin generally involves both denaturation and aggregation. Denaturation is the process in which myosin undergoes unfolding and other conformational changes (Hermannson, 1979; Yarnpakdee et al., 2009). Denaturation exposes myosin surfaces with great reactivity, which leads to protein–protein interactions in the process of ‘aggregation’ (Lanier et al., 2004). As a consequence, a three-dimensional network structure is formed. The thermal denaturation and aggregation of fish myosin has been widely studied among cold water species (Beas, Wagner, Crupkin, & Anon, 1990; Chan, Gill, & Paulson, 1992; Sano, Ohno, Otsuka-Fuchino, Matsumoto, & Tsuchiya, 1994; Visessanguan, Ogawa, Nakai, & An, 2000). Yongsawatdigul and Park (2003) noted that the denaturation and aggregation of fish myosin depends on the temperature of the fish habitat. Freshwater fish is an important substitute for sea fish, which is becoming scarce for use in producing surimi. However, the mechanism for forming gels from freshwater fish muscle is still unclear.

Bighead carp (Aristicthys nobilis), a kind of freshwater fish, is one of the four main species of Chinese carp, and it has high nutritional value. However, bighead carp is rarely utilized in the surimi production industry due to its poor gelation properties. Some papers have reported the denaturation and aggregation patterns of freshwater fish. Ding, Liu, Rong, and Xiong (2014) reported the heat-induced denaturation and aggregation of yellowcheek carp myosin at a low temperature, whereas Liu, Zhao, et al. (2010) studied the relationship between the gel properties and secondary structure of silver carp myosin over a range of pH values. However, as far as we know, no study has focussed on the myosin clusters that form during heating, which are correlated with changes in turbidity, structure, size, and rheological properties in response to temperature. Furthermore, the effects of myosin clusters on gel formation are not clear.
Myosin molecules aggregate and form a three-dimensional network when exposed to temperature over 60°C (Lanier et al., 2004). To determine the effects of aggregation on the rheological properties of freshwater fish myosin, we investigated a low temperature (50°C), at which the rate of the aggregation is lower, and a high temperature (90°C), at which the rate of aggregation is higher.

The objective of this study was to investigate the difference between the aggregations of bighead carp myosin induced by low and high temperatures and the effects on aggregation on the gel rheological properties. As consequence, it may be true that the quality of surimi produced from bighead carp could be improved by controlling the state of myosin clusters via heating treatment.

2. Materials and methods

2.1. Materials

Live bighead carps were purchased from Auchan supermarket at Xuefu Road, Zhenjiang, China, in July of 2015. The carps were put in bags filled with water and oxygen and kept in a polystyrene box filled with crushed ice and were then taken to the laboratory within 30 min. The carps were stunned using knife-blade, slaughtered, and then cut in half through the dorsal flesh. All steps were performed at a temperature below 10°C. All chemicals used were of analytical grade.

2.2. Preparation of myosin

Myosin was prepared according to the method of Park and Lanier (1989) with some modifications. The dorsal muscle of fresh bighead carp (50 g) was minced by hand and mixed with 500 mL solution A (0.10 M KCl, 20 mM Tris–HCl buffer, pH 7.5). Next, the mixture was homogenized using a Waring blender (T18, IKA Co., Staufen, Germany). Then, the homogenate was incubated for 15 min at 4°C and centrifuged at 3000g for 6 min (Avanti J-26XP, Beckman Coulter, Germany). The precipitate was dissolved in 500 mL solution B (0.45 M KCl, 5 mM β-mercaptoethanol, 0.2 M Mg(COO)₂, 1 mM EGTA, and 20 mM Tris-maleate buffer, pH 6.8) and adenosine 5′-triphosphate was added to a final concentration of 10 mM. The resulting solution was set at 4°C for 90 min, and the mixture was centrifuged at 12,000g for 13 min at 4°C. The supernatant was slowly diluted with 100 times its volume of 1 mM KHCO₃ and stored at 4°C for 25 min. The mixture was centrifuged at 12,000g for 13 min and the precipitate was dissolved in 2.5 times its volume of solution C (0.5 M KCl, 5 mM β-mercaptoethanol, and 20 mM Tris–HCl buffer, pH 7.5) and incubated at 4°C for 10 min. The mixture was diluted with five times its volume of 1 mM KHCO₃ and MgCl₂ was added to a final concentration of 10 mM. Then, the mixture was diluted overnight at 4°C and centrifuged at 17,000g for 25 min. The precipitate obtained was dissolved in 0.5 M NaCl, 20 mM Tris–HCl buffer (pH 7.0) and then centrifuged at 5000g for 15 min. The myosin solutions were stored at 4°C and used within 3 days of preparation. Myosin purity was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis to be over 90% (Figure 1). All solutions used for myosin preparation were maintained at 4°C.

![Figure 1. SDS-PAGE of myosin from dorsal muscle of bighead carp. Lane 1 was loaded with 5 µL of protein and shows the premixed protein marker (high). Lane 2 was loaded with 20 µL of protein and shows the extracted myosin. MHC: Myosin heavy chain; MLC: myosin light chain.](image-url)

2.3. Sample preparation

The myosin solution (pH 7.0) was heated at 50 or 90°C for 3, 6, 9, 12, 15, 30, and 60 min, and at 50°C for 90 min. The protein concentration was determined according to the Biuret method (Wan, Hong, & Xi, 1993) using bovine serum albumin as a standard.

2.4. Circular dichroism spectrum

The extracted myosin was diluted to a protein concentration of 0.1189 mg/mL with 0.5 M KCl, 20 mM Tris–HCl (pH 7.0). The circular dichroism (CD) spectrum was measured using a Biologic MOS-450 spectropolarimeter (Biologic Co. Ltd., Claix, France) and a 1-cm path-length quartz cell. Molecular ellipticity was determined in the range from 190 to 250 nm using a mean residue weight of 110 g/mol. The cell temperature was increased from 5 to 50/90°C at a rate of 2°C/min and held for the designed time as described in Section 2.3, respectively. A magnetic stirring an accessory to CD spectrum was used in order to prevent the formation of gel of myosin. The percentage of α-helix was calculated according to the following equation (Wu & Yang, 1976):

\[ \alpha - \text{Helix} = \frac{-\theta_{222} + 3000}{39,000} \times 100 \]  

where \( \theta_{222} \) is the ellipticity at 222 nm.

2.5. Dynamic light-scattering measurement

The diameter of the myosin molecules was determined using the Zeta potential and a particle-size analyser (NanoBrook 90 Plus PALS, Brookhaven Instruments, New York).
York, USA), equipped with a 5-mW He–Ne ion laser (λ =640 nm). The myosin concentration was adjusted to 0.5 mg/mL. The scattering angle was 90°, the elapsed time was 2 min, and the temperature was set to 25°C. Three dynamic light-scattering (DLS) measurements were collected for every sample. Optical density measurements were also collected to determine the diameter and distribution of myosin by cumulative analysis. All treatments were measured in triplicate.

2.6. Turbidity measurement

Turbidity was measured according to the method of Han, Xu, Lin, and Zhou (2004) with some modifications. The myosin solution was diluted to 1 mg/mL with 0.5 M NaCl–Tris–HCl buffer (pH 7.0). The absorbance at 340 nm, which reflects turbidity, was monitored at room temperature (25°C). All treatments were measured in triplicate.

2.7. Rheological measurement

Rheological measurements were performed on an MCR 302 rotational rheometer (Anton Paar (China) Co. Ltd., Graz, Austria) using the PP50 rotor and a 40-mm parallel steel plate geometry with a 1-mm gap. The myosin concentration was adjusted to 15 mg/mL. Excess sample was used to prevent evaporation. The constant oscillation frequency was 0.1 Hz, and the amplitude gamma was 2%. The myosin samples were heated from 30 to 90°C at 1°C/min, and G′, G″, and η were recorded.

2.8. Scanning electron microscopy

The gels were prepared by heating myosin (25 mg/mL) at 50 and 90°C for 60 min. The samples of myosin gel for scanning electron microscopy (SEM) were made according to the method of Hayakawa et al. (2012) with some modification.

2.9. Data analysis

The Gel-Pro Analyzer (Version 4.0, Media Cybernetics, Inc., Rockville, MD, USA) was used to determine myosin purity. The results were analysed using Excel (Version 2007, Microsoft, Redmond, WA, USA) and Statistical Analysis System (SPSS Inc., Chicago, IL, USA). A one-way ANOVA was used to evaluate differences between treatments, and the differences were considered significant at P < 0.05.

3. Results and discussion

3.1. Effect of temperature on the α-helix of myosin

Myosin conformation was determined by CD to explore the effect of heating temperature on the unfolding of fish myosin. Myosin is composed of two globular head regions and a rod-like tail portion comprising a coiled-coil of α-helices (Harrington & Rodgers, 1984). The gelation model of myosin is more complex than that of globular proteins. Heat treatment induced drastic conformational changes, including the loss of its α-helical structure. The change in the α-helix content of myosin is shown in Figure 2. The α-helix content tended to fluctuate from 3 to 12 min (data not shown). However, the α-helix content decreased as heating time was extended, suggesting that the α-helical structure of myosin became more unfolded with increasing heating time. Heating at 50°C for 90 min decreased the α-helix content from 75.27% to 34.29%, and the content markedly decreased to 16.74% after heating at 90°C for 60 min. These results indicate that the higher temperature has a more pronounced influence on the unfolding of bighead carp myosin. The α-helices of native myosin of silver carp unfolded with increasing temperature (Liu, Zhao, Xiong, Xie, & Liu, 2007). Liu, Xiong, Zhang and Peng (2010) reported that the melting temperatures of myosin were 40 and 55°C. The rate of the decrease in α-helix content slowed with extended heating time, suggesting that temperature rather than heating time is the limiting factor for myosin thermal unfolding. In addition, a greater extent of unfolding may promote the exposure of hydrophobic residues and intermolecular hydrophobic interactions, thereby allowing the formation of more cross-links (Ferry, 1948).

3.2. Effect of temperature on myosin the diameter of aggregates

The diameters of these clusters were measured during heating at 50 and 90°C (Figure 3). The DLS data showed that the diameter of the clusters increased monotonically with heating time at both temperatures. The clusters that formed during heating at 90°C were much larger than those that formed at 50°C, indicating that temperature plays a major role in the increase of diameter with heating time at low protein concentration (0.5 mg/mL). The size of the myosin clusters that formed at 50°C increased slowly with the heating time; however, the size of the myosin clusters formed at 90°C rapidly increased. The DLS data suggested that myosin unfolding was slow at 50°C and that the energy was not enough for myosin aggregation, therefore, at 50°C, the diameter of the myosin clusters was small and increased slowly. However, at 90°C, the rate of myosin aggregation was rapid enough to form myosin larger clusters at 90°C due to the sufficient energy for aggregation provided by high temperature. In addition, the intermolecular interactions in the sample heated at 90°C were stronger than those within the sample at 50°C. Temperature may affect the self-association...
of dissociated myosin monomers or oligomers (Shimada, Takai, Ejima, Arakawa, & Shiraki, 2015). The authors of that study also suggested that heat-denatured myosin molecules form soluble oligomers at 0.30 M NaCl and bind to soluble filaments, most likely via the myosin heads. The rate of aggregation was highly dependent on temperature (Pouzet, Nicolai, Visschers, & Weijers, 2005), which resulted in different rate of myosin unfolding.

3.3. Effect of temperature on the turbidity of fish myosin solutions

The changes in the turbidity of the myosin solutions heated at two temperatures were monitored (Figure 4). Turbidity reflects the size and number of the insoluble particles in suspension. An increase in particle diameter following aggregation increases light scattering, and increasing absorbance reflects increasing turbidity. The turbidity of the myosin solution heated at 50°C gradually increased as the heating time increased. At 90°C, the turbidity increased to a maximum (0.946) at 6 min and then decreased with continued heating. The turbidity of the myosin solution at 50°C was greater than that at 90°C. Both the size and amount of myosin clusters increased as the heating time was extended. The DLS data indicated that at low concentrations (0.5 mg/mL), myosin that was thermally denatured at 90°C aggregated to form larger clusters compared with those formed by denaturation at 50°C. At higher concentrations (1 mg/mL), aggregation continued until large clusters precipitated, leading to a decrease in turbidity. More large clusters precipitated at 90°C, attenuating light scattering and decreasing turbidity as heating time increased.

The results indicated that the shape and size of the formed clusters differed between the samples heated at 50 and 90°C. Bighead carp myosin that was heated at 50°C formed soluble small open clusters that most likely formed via the myosin heads, with the myosin tail extending out of the clusters. Bighead carp myosin that was heated at 90°C formed insoluble large closed clusters via both the myosin heads and the myosin tail. Consequently, the myosin clusters that formed at 50°C did not precipitate, whereas those that formed at 90°C did precipitate.

The increased turbidity of the heated myosin solution resulted from the formation of myosin aggregates (Gill, Chan, Phongchareon, & Paulson, 1992). Aggregation occurred as unfolded protein molecules interacted via hydrogen bonds, hydrophobic interactions, disulphide linkages, and electrostatic interactions (Oakenfull, Pearce, & Burley, 1997). Because no endogenous transglutaminase activity was detected in this study, no ε-(γ-glutamyl) lysine cross-links were involved in the aggregation process, indicating that the clustering of myosin was caused primarily by aggregation.

3.4. Effects of temperature on the rheological properties of fish myosin

The storage modulus (G′) and the loss modulus (G″) are used to characterize the elasticity and viscosity of a system, respectively, and δ represents the ratio of fluidity to elasticity (Egelandsdal, Martinsen, & Autio, 1995; Reed & Park, 2011). The rheological properties of a gel are usually characterized by these indexes. Figure 5 presents the changes in the rheological properties of bighead carp myosin solutions during heating at 50 and 90°C.

Figure 5(a–d) shows that G′ and G″ of the natural myosin (0 min) exhibited three stages: first, G′ increased in the range of 30–45.6°C; next, G″ decreased as the temperature increased to 70°C; and finally, G′ increased again when temperature increased from 70 to 90°C. Meanwhile, δ decreased at temperatures below 41°C and then increased until the temperature reached 42.1°C. Upon further heating, δ decreased rapidly and then increased from 44°C. When heated over 48°C, a decrease in δ was observed until 80°C, and then a weak increase was shown. A similar pattern of δ was reported in threadfin bream actomyosin by Yongsawatdigul and Park (2003), except that only one increase exhibited at 41°C, instead of two increases at 41 and 44°C, which were observed in our study. Reed and Park (2011) reported that the changes in G′ and δ of the natural myosin from tilapia were also similar to those of the myosin from bighead carp. G′ and G″ of bighead carp myosin measured in our study were
lower than those of silver carp myosin (Liu et al., 2007); however, these values were the same as those of Pacific whiting surimi (Yin & Park, 2015), indicating that the gel network structure of bighead carp myosin may be weaker than that of silver carp myosin but similar to that of Pacific whiting surimi. The increase in $G'$ and $G''$ and the decrease in $\delta$ of the unheated sample in the range of 30–45.6°C may be due to the cross-linking of myosin filaments, leading to a sol-to-elastic transformation of the network (Egelandsdal, Fretheim, & Samejima, 1986). $G'$ and $G''$ subsequently decreased and reached a minimum at approximately 51°C, which may have resulted in a large increase in protein mobility due to the helix-to-coil transformation of myosin (Sano, Noguchi, Tsuchiya, & Matsumoto, 1988) and, at this temperature range, the presence, and high activity of endogenous proteolytic enzymes in the muscle (Cao, Wu, Hara, Weng, & Su, 2010). Furthermore, hydrogen bonding decreased with heating, thereby weakening $G'$ (Lanier et al., 2004). Upon further heating, $G'$ slightly increased from 51 to 83°C and then rapidly increased. The increase in $G'$ above 83°C was likely caused by the increase in hydrophobic interactions that occurred with progressive unfolding myosin, which became the dominant driver of aggregation (Liu, Zhao, et al., 2010). In addition, strong disulphide bonds formed at 70–80°C (Liu, Zhao, Xie, & Xiong, 2011), and an irreversible protein network structure developed (Liu et al., 2007). In our study, we observed that the temperatures corresponding to the peak values of $G'$ and $G''$ were lower than those reported by Liu, Xiong, et al. (2010), which may have resulted from the different fish species and culture season. These results of our work were similar to those
found in a study of silver carp myosin (Liu et al., 2007). Those authors reported that G′ of the silver carp paste started to increase from 5°C, reaching the first peak at 35°C. Subsequently, G′ dropped and reached a minimum at 51°C; the same result was observed in bighead carp. Finally, G′ increased until 90°C.

The rheological properties of the myosin heated at two temperatures were different from those of natural myosin. The G′ and G″ of myosin that were heated at 50°C began to increase at 30°C and formed small peaks between 34 and 35°C, these peaks increased with the extension of heating time. The rate of increase in G′ gradually increased with heating time, increasing from 0.115 Pa°C at 3 min to 0.302, 0.377, 0.509, 0.547, 0.658, 0.868, and 0.947 Pa°C at 6, 9, 12, 15, 30, 60, and 90 min, respectively. This higher rate of increase in the rheological properties was likely due to the greater extent of myosin unfolding in samples that were heated for longer periods prior to the rheological measurement, resulting in the increased formation of intermolecular bonds. G′ and G″ transiently decreased from 34 to 36°C, indicating that the intermolecular bonds were unstable and easily broken. G′ and G″ increased rapidly again from 36 to 45.6°C, which may have been due to the interactions between the unfolded myosin molecules (Lanier et al., 2004). G′ and G″ decreased when the temperature was over 45°C during the experiment. This stage in surimi processing is termed ‘modori,’ which refers to the weakening of the gel. The modori rate represents the slope of G′ versus temperature (45–70°C). The modori rates of the solutions that were heated for 3, 6, 9, 12, 15, 30, 60, and 90 min were 0.233, 0.046, −0.127, −0.253, −0.257, −0.456, −0.405, and −0.489 Pa°C, respectively. This result indicated that the modori rate of myosin increased with the extension of heating. The decrease in G′ and G″ of myosin in the range of 45–70°C likely indicated that the rate of bond formation associated with protein aggregation was slower than the rate of bond disruption caused by thermal denaturation (Stone & Stanley, 1994). G′ and G″ increased again from 70 to 90°C, which were similar to those of natural myosin. These increases were attributed to both the increasing number of cross-links between the myosin aggregates and the loss of redundant denatured myosin from the existing gel network, strengthening the gel matrix (Xiong, 1997). Compared to those of the natural myosin, G′ and G″ of the heated myosin were lower. However, the heating time of the myosin before denaturation had an influence on the variation of G′ and G″. Longer heating times were associated with higher G′ and G″. The δ of the myosin heated at 50°C first decreased then increased, subsequently decreased, increased again in the temperature range of 40–45.6°C, and finally decreased when the temperature exceeded 45.6°C. In the case of myosin of yellowcheek carp (Elopichthys bambusa), G′ and G″ decreased first and then rapidly increased from 10 to 45°C, while δ increased first and then decreased (Ding et al., 2014). Those authors suggested that the changes in G′, G″, and δ were induced by the denaturation and/or slight aggregation of the myosin but not by gelation. Therefore, our data agreed with their suggestion.

The rheological properties of the samples heated at 90°C are shown in Figure 5(b,d,f). Compared to those of natural myosin, G′ and G″ of the heated myosin were also lower. However, the heating time at 90°C also had an effect on the variation of G′ and G″, which was the opposite effect to that observed in the samples heated at 50°C. Longer heating times were associated with lower G′ and G″ values. All samples at 90°C started to form a gel, as indicated by an increase in G′ and G″ from 30 to 45°C. Gelatinization was interrupted at temperatures above 45°C, where gel degradation led to a decrease in G′ and G″. Stronger interactions between denatured myosin molecules/clusters caused the gelatinization to continue from 73 to 90°C. However, the samples that were heated for 3 min at 90°C demonstrated rheological properties that differed from those of samples that were heated at 90°C in the range of 47–59°C. The samples that were heated for 3 min began to form a gel again in this temperature range, which may have resulted from the further unfolding of myosin rather than aggregation into clusters.

Higher values of G′ represent better gelatinization and gel quality (Thawornchinsombo & Park, 2007). Natural myosin has a natural microstructure and therefore shows the highest G′ during determination. The preheated myosin unfolded before being determined on the rotational rheometer. However, the extent of myosin unfolding was different due to differences in temperature. Protein unfolding exposes buried hydrophobic residues, causing the proteins to aggregate into self-similar polymers depending on temperature (Wang et al., 2016). Liu et al. (2007) suggested that unfolding of the myosin α-helical structure was beneficial for gelation. As myosin unfolds, hydrophobic interactions, disulphide linkages, and other chemical bonds lead to aggregation and gel formation (Lanier et al., 2004; Liu et al., 2007; Yongsawatdigul & Park, 2003). The unfolding of myosin increased with the heating time at 50°C due to insufficient energy, resulting in an unfolded microstructure that easily aggregated. By contrast, the aggregation of myosin at 90°C increased with the heating time, resulting in larger aggregates with reduced tendencies further aggregate. In addition, the endogenous protease in this species used in our work exhibited a higher activity at 90°C than that at 50°C (data not shown), which induced the hydrolysis of myosin. Therefore, the myosin that was heated at 50°C had a higher value of G′ than the myosin that had been heated at 90°C. As discussed above, the myosin clusters that formed at 50°C were small due to the aggregation of the myosin heads (Sharpe & Offer, 1992), which resulted in the myosin tails stretching out of the cluster. These small clusters associated with each other through the myosin tails when the temperature increased during the rheology experiment. Myosin that was thermally unfolded at 50°C could cross-link and aggregate in an ordered pattern to form a loose three-dimensional network. Therefore, the preheated bighead carp myosin molecules at 50°C still have excellent ability to form a fine gel network. Pre-unfolded myosin that aggregated slowly may form a gel with good rheological properties. Furthermore, longer preheating time was associated with stronger gel-forming abilities because the extent of unfolding increased with heating time.

Myosin that was thermally unfolded at 90°C may have excessively accumulated to form larger clusters (Figure 3) due to the higher temperature. The myosin molecules aggregated not only via the heads but also via the tails (Sharpe & Offer, 1992). Therefore, the myosin clusters were large and closed. As a consequence, the myosin did not have
the ability to further form a fine network during the rheology experiment because the myosin tails in the cluster were cross-linked. Furthermore, the ability of myosin for forming network was weaker due to the time of preheating. These findings were attributed to the different structures of the myosin clusters formed at 50 and 90°C, which were caused by the different aggregation rates.

3.5. Scanning electron microscopy

Figure 6 shows SEM of the three-dimensional microstructure of the myosin gel heated at the two temperatures for 60 min. Myosin formed a cellular gel network under both at 50 and 90°C. The myosin gel structure induced at 50°C was more exquisite and homogeneous and presented with a fluffy, flocculent texture. Furthermore, the dense myosin network structure was clear and the pores of the gel were smaller. However, the myosin gel formed at 90°C had more irregular pores that were bigger than those within the gel formed at 50°C. The myosin molecules were closely aggregated and had no obvious fine mesh structure.

As discussed above, the myosin molecules unfolded, aggregated, and formed cluster during heat treatment. However, the myosin clusters formed at 50 and 90°C were different due to different rates of myosin unfolding and aggregation. The clusters assembled at 50°C were small and open, presenting with myosin tails that extended out of the cluster. Therefore, the clusters formed an exquisite, homogeneous network via the myosin tail. However, the network was weak because the aggregation was not strong enough to form sufficient bonds. By contrast, the myosin molecules heated at 90°C formed larger, closed clusters due to the rapid rate of aggregation. As a consequence, the myosin clusters closely aggregated and formed a sturdy network via intermolecular bonds, such as hydrogen bonds, hydrophobic interactions, covalent bonds, and ionic linkages (Lanier et al., 2004). The results suggested that the state of myosin clusters formed during heat treatment play a very important role in the quality of myosin gel.

4. Conclusions

Bighead carp myosin lost their α-helix structure and formed different clusters during heat treatment at 50 and 90°C. The diameter of the myosin clusters increased with heating time at different speeds under both temperatures. Compared to the myosin heated at 90°C, the myosin heated at 50°C had a higher value of $G'$. Therefore, $G'$ and $G''$ increased with an increase in heating time at 50°C but decreased at 90°C. The myosin clusters that formed at 50°C were smaller, and the myosin tails were stretched out, resulting in a stronger ability to further form fine gels with exquisite and homogeneous microstructure. By contrast, the large myosin clusters that formed at 90°C were closed and resulted in a sturdy gel with larger, irregular pores. These results indicated that the gel microstructure was affected by the clusters, which were controlled by the heating temperature. Therefore, the temperature should be carefully controlled in the production of freshwater fish surimi products. This study was performed to provide a theoretical basis for improving surimi products.

Acknowledgements

This study is funded by the National Nature Science Foundation of China: [Grant Numbers. 31471611 and 31671882] and by Jiangsu University ‘youth backbone teacher training project’ also is supported by the priority academic programme development of Jiangsu higher education institutions (PAPD).

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: [Grant Numbers 31471611 and 31671882].
