The Effectiveness of Konjac Flour on the Physicochemical and Rheological Properties of the Myofibrillar Proteins of the Common Carp (*Cyprinus Carpio*)

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Abstract: The effects of konjac flour (KF) on the emulsifying properties, turbidity, rheological properties, gel strength, gel water-binding capacity (WBC), and gel microstructure of the myofibrillar protein (MP) of the common carp *(Cyprinus carpio)* were investigated. The results showed that the emulsifying activities and gel strength of MP increased first and then decreased with increasing KF concentrations, achieving the highest values at 0.1% KF (P<0.05). Increased KF concentrations were accompanied by an enhanced gel WBC and increased susceptibility to thermal aggregation (P<0.05). The addition of KF markedly enhanced G' over the entire heating temperature range, and the "peak" G' values of the 2.0% KF sample were 3.6 times than those of the control samples. The reduced G''/G' at the end of the heating process (80°C) revealed that KF addition improved the gel elastic quality and increased the gelling ability of MP. It was determined by observing the gel microstructure that addition of KF reduced empty spaces and produced a more compact and homogeneous MP gel network structure. Overall, these results suggest that KF addition offers an effective approach for improving the MP gel formation ability of the common carp muscle.

Keywords: Common carp, Konjac flour, Physicochemical property, Gel property, Myofibrillar protein.

INTRODUCTION

The common carp (*Cyprinus carpio*) is a popular freshwater fish species found throughout the world, especially in Australia, South America, and Asia, because of its rapid growth, high yield, and high feed efficiency [1]. However, the consumption of common carp is affected by its taste, great quantity of intramuscular small bones, and intense earthy odour [2]. Surimi possesses special gelling properties and is used as a base in gel-based food products and seafood analogues [3, 4].

The functional properties of myofibrillar protein (MP) have a significant association with its protein structure [5, 6]. MP can form a gel reticular structure when the muscle is subjected to heating due to protein denaturation and polymerization, and it also plays a key role in the emulsion and gelation process by aggregating and stabilizing the fat droplets in the gel matrix [7, 8].

Konjac flour (KF) is made from the tubers of various konjac plants (*Amorphophallus konjac C. Koch*). It is a soluble dietary fibre that is similar to pectin in structure and function. KF mainly consists of glucomannan, which is a hydrocolloidal polysaccharide. Glucomannan is composed of mannose and glucose subunits linked by β-1,4 linkages at a molar ratio of 1.6:1. It is a slightly branched polysaccharide having a molecular weight of 200 k to 2000 k [9-11]. KF has been in increasing use as an active material because it can control weight, modify the intestine microbial metabolism, reduce plasma cholesterol, scavenge free radicals, and inhibit tumour genesis and metastasis [12-15]. In addition, KF possesses notable technological properties in its abilities to bind water, form gels, emulsify, and thicken, so it is increasingly used as a potential food additive [10, 16, 17]. KF was used to enhance the textural characteristics of surimi gels at various heating temperatures [18]. Xiong *et al*. [10] showed that KF has good cryoprotective effects on the MP of grass carp during frozen storage.

The gel strength of common carp is usually weaker than that of many marine fishes, such as the Alaska pollock surimi [19]. Some researchers have studied the influence of physical conditions, setting temperature, setting time, and protein concentration on the surimi gel-forming ability [20], while other researchers have focused more on the influence of some of the additive ingredients on the protein gel properties [21, 22]. However, there have been few studies on the influence of KF on the physicochemical and rheological

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properties as well as the gel formation ability of the MP from common carp. The aim of this study was to evaluate the influence of KF on the physicochemical, gel microstructural, and rheological properties of common carp myofibrillar proteins at various heating temperatures.

MATERIALS AND METHODS

Materials

Fresh common carp (body weight 1.5-2.0 kg) was obtained from a local market (Harbin, China). The fish was washed immediately and killed manually. After evisceration and decapitation, the fish was stored at 4°C for approximately 4 h until rigor mortis dissipated. The dorsal muscle of the common carp was cut into small pieces with a knife and then used for MP preparation. KF was obtained from Jiangxi Baiying Biotechnology Co., Ltd (Nanchang, Jiangxi, China). All chemical reagents were of analytical grade.

Myofibrillar Protein Preparation

Myofibrillar protein (MP) was prepared according to the method of Jiang, Zhang, Cai, Hara, Su, & Cao [23]. All of the solutions used for the protein preparation were placed at 4°C to minimize protein denaturation and proteolysis. The protein concentration of MP was adjusted to 40 mg protein/mL. KF was added into the MP solution at concentrations of 0.05, 0.1, 0.15 and 0.2%. KF was not added to the control sample.

Measurement of Emulsifying Properties

The emulsifying ability of carp MP was measured as reported by Pearce and Kinsella [24]. The emulsifying activity index (EAI) and emulsifying stability index (ESI) were used to express the emulsifying ability of MP.

Measurement of Turbidity

The susceptibility to heating aggregation of MP and KF suspensions (1 mg/mL protein in 0.6 M NaCl, 50 mM PIPES buffer) during heating from 30 to 80°C was monitored by determination of the solution turbidity. Turbidity was measured as the absorbance at 600 nm by using the method of Benjakul *et al*. [20].

Dynamic Thermo-Mechanical Analysis

Dynamic thermo-mechanical analysis (DTMA) of the MP solution was evaluated using a Bohlin Gemini II rheometer (Malvern Instruments Limited, Worcester-shire, UK) according to the method of Xia *et al*. [6]. The dynamic rheological properties of the gelling samples were measured in terms of the storage modulus (*G*', the elastic component) and the loss modulus (*G*'', a viscous response to an external force).

Gel Strength

The MP sample (40 mg/mL) was suspended in 50 mM piperazin-N, N'-bis (2-hydroxypropanesulphonic acid) at pH 6.0 containing 0.6 M NaCl. The protein solutions were transferred into 25 × 40 mm (diameter × height) glass vials immediately, and the height of the MP gelling solution was 25 mm. The protein gels were formed at 80°C for 30 min in a water bath. Then, the gels were stored overnight at 4°C. The gel strength of the MP gels was analysed with a texture analyser (Model TA-XT2, Stable Micro Systems Ltd., Godalming, UK). The gels in the vials were compressed with a P/0.5 flat-surface cylindrical probe (12 mm in diameter) measuring at a 20 mm/min cross-head speed. The penetration force was expressed as gel strength.

Gel Water-Binding Capacity

The water-binding capacity (WBC) of the MP gel was measured by the centrifugal method as described by Xia *et al*. [6]. The gel samples (5 g) were centrifuged at 3000 × *g* for 10 min at 4°C. WBC (%) was expressed as the gel weight after centrifugation divided by the gel weight before centrifugation, multiplied by 100.

Gel Microstructure

The microstructure of the MP gels was measured using a scanning electron microscope (SEM) [6]. Gel samples (0.5 × 0.5 × 0.3 cm^3) were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) at 4°C for 2 h. The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50, 70, 80, 90, and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were then observed with a SEM (S-3400N; Hitachi, Tokyo, Japan).

Statistical Analysis

Three independent trials were conducted, and all of the specific assays were performed in triplicate. The data were analysed with the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St. Paul, MN). Significant

differences (P<0.05) among means were identified using the Tukey procedure.

RESULTS AND DISCUSSION

Emulsifying Activity

The emulsifying ability of MP in the absence or present of KF was expressed in Figure **1**. The EAI and ESI increased initially and then decreased with increasing KF levels. The EAI and ESI of the control sample (the MP without KF) were 20.0 m^2/g and 65%, then increased to 26.9 m^2/g and 83.8% at 0.1% KF (P<0.05), respectively, and finally decreased significantly with the further addition of KF (P<0.05). The emulsifying ability is dictated by lipid-protein and protein-protein interactions, and the emulsion activity is influenced by many factors that affect both the continuous and dispersed phases [25]. Protein denaturation could affect the protein emulsifying properties. Campo-Deaño *et al*. [26] suggested that sugars and polyols with low molecular weights, as well as many carboxylic acids, amino acids, and polyphosphates, could improve protein functionality. Kong *et al*. [7] noticed that a cryoprotectant (a mixture of sorbitol and sucrose) had a good protective effect on protein structure and reduced protein oxidation and structural deterioration in common carp surimi. Chen *et al*. [27] revealed that KF possessed the ability to improve the emulsifying activity of meat protein because KF enhanced the binding force between proteins. KF could strengthen the stability of the dispersed system. The viscosity and stability of the system increased when KF was added to the protein emulsifying system, which made it difficult for the protein in the dispersed phase to aggregate. When the addition of KF was more than 0.1%, the EAI and ESI decreases were probably because the balance phase was destroyed by the high KF amount.

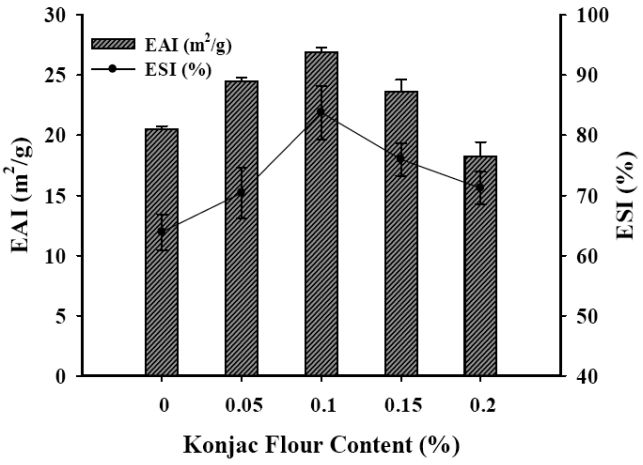

Figure 1: Effect of konjac flour on the emulsifying activity index (EAI) and the emulsifying stability index (ESI) of common carp myofibrillar proteins.

Heat-Induced Protein Aggregation

Turbidity measurements were used to monitor heat-induced MP aggregation. Figure **2** shows the turbidity changes of the MP samples when they were heated. Increases in turbidity when the samples were heated would be due to the aggregation of MP molecules caused by the inter- and intra-molecular interactions of MP [28]. The addition of KF significantly increased the MP turbidity in comparison with the control samples when they experienced the same heating temperature (P<0.05). At 80°C, the turbidity of MP solutions in samples with 0.05, 0.1, 0.15, and 0.2% KF groups increased by 27.3, 23.3, 20.0, and 14.0%, respectively, compared to that of the control (P<0.05). The interactions between proteins and KF may enhance the crosslinking with adjacent proteins and increase protein aggregation. The addition of KF enhanced heat-induced protein aggregation and consequently increased the tendency of these proteins to form aggregates, which may has some role in improving the MP gel strength, as shown below.

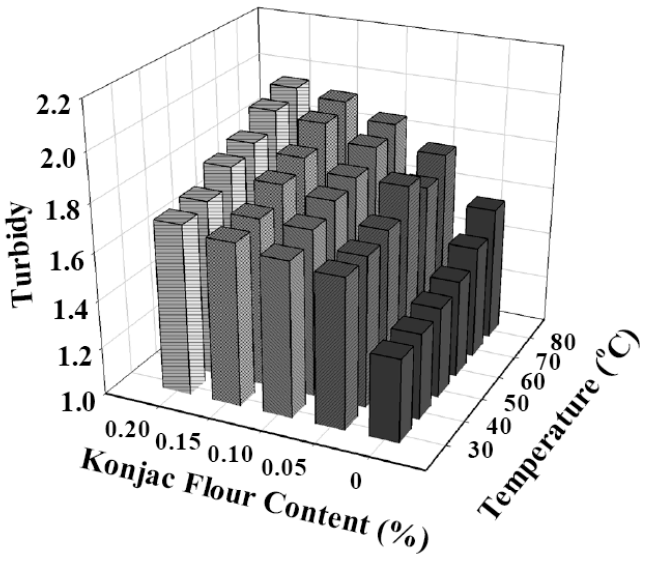

Figure 2: Effect of konjac flour on the turbidity of common carp myofibrillar proteins.

Dynamic Rheological Properties

The DTMA implicated the substantial transformation of the carp MP solution to a three-dimensional protein gel network. The changes in the dynamic rheological properties of protein during thermal gelation are

measured by detecting the *G′* (Figure **3a**) and the *G″* (Figure **3b**). The *G′* of carp MP in all of the samples, which expresses the elastic property of a gelling system, rose at first and attained a maximum value when the temperature reached approximately 43°C. From approximately 43 to 53°C, the *G′* decreased sharply and then increased again until the 80°C. This result was consistent with reports from Li, Kong, Xia, Liu, & Diao [8], and this rheological pattern shows the transitions of light meromyosin and heavy meromyosin. In the first stage, the increase in *G′* was called "gel setting". It was caused by the myosin head subfragment denaturation. Then, the decrease of *G′* (from 43 to 53°C) with an increase in temperature was called "gel weakening," which was assumed to be caused by the helix-to-coil transition of the myosin tail, which may destroy some of the protein network structure. In the second stage, at a peak temperature of 80°C, the increase in *G′* was termed "gel strengthening" [29, 30]. The thermal gelation profile of carp protein was obviously changed by the addition of KF. When the KF concentration was 0.05, 0.1, 0.15, and 0.2%, the first "peak" *G′* values of the MP gel were increased from 21 Pa (no KF addition) to 27, 42, 51, and 89 Pa, respectively. The addition of KF markedly increased *G′* over the entire temperature range. Especially for 0.2% KF samples, the "peak" *G′* values of the protein gel were 3.6 times greater than those of the control samples.

G″ reflects the viscosity of the gelling system, which is another monitoring parameter of the rheological properties. Figure **3b** shows a similar change to *G′* for the first peak by reaching a maximum value when the temperature reached approximately 43°C. From approximately 43 to 53°C, *G″* dropped sharply and levelled off until the temperature reached 80°C. When the heating temperature was higher than 43°C, *G′* was consistently higher than *G″*, especially when the temperature was greater than 53°C. This observation explained the changes into a less viscous or more elastic material during the protein gel formation [29, 31]. At above 53°C, major MP structural changes have already happened and protein cross-linking and continuing molecular interactions would predominate in the viscoelastic gel system [29]. The *G″* of 0.2% KF samples was obviously enhanced at the initiation of heating. When the temperature reached 43°C, the *G″* of the 0.2% KF samples was 61 Pa, which was 2.8 times greater than that of the control samples. The samples with KF possessed higher *G′* and *G″* peak values at 43°C, which suggested that polysaccharides could increase the gelation of MP by different molecular forces. The higher *G′* and *G″* values attained with the addition of KF revealed that KF interacted with proteins in the continuous phase to produce an amorphous network possessing viscoelastic characteristics. The addition of KF may also effectively inhibit the structural changes of MP and enhance the rheological properties of MP. This result was consistent with previous reports in which polysaccharides (chitosans) increased the gelation of salt-soluble proteins by various molecular forces [32].

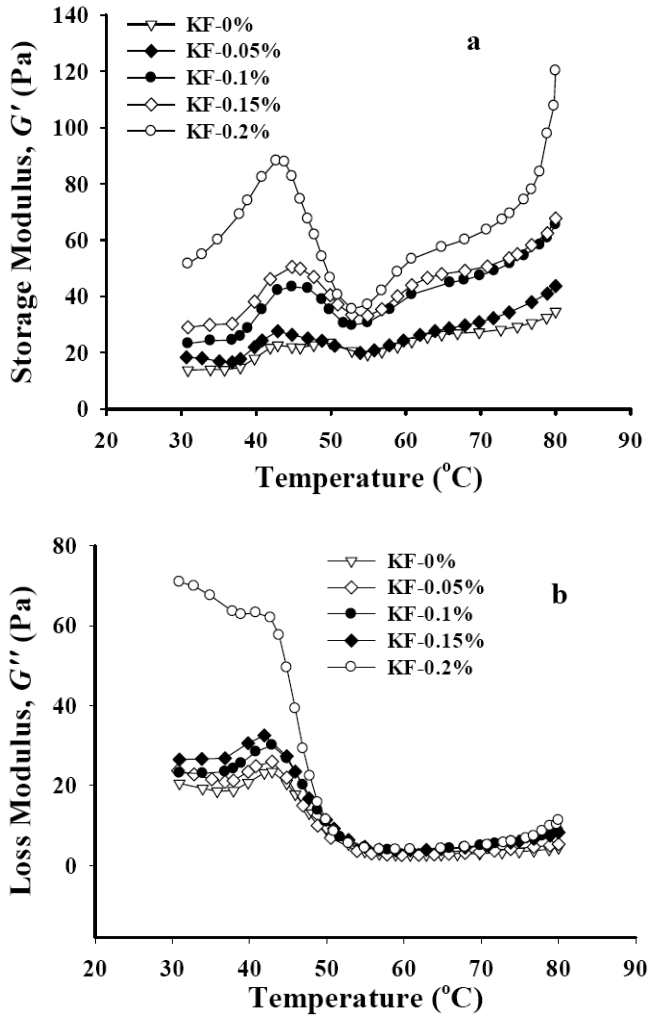

Figure 3: Effect of konjac flour on the storage modulus (*G′*) and the loss modulus (*G″*) of common carp myofibrillar proteins.

The viscoelastic quality of the MP samples was also illustrated by the loss factor (*G″*/*G′*). *G″*/*G′* is a relative distribution of viscosity compared with elasticity during protein gel matrix formation; that is, the lower the *G″*/*G′* value, the higher the elasticity or lower the viscosity of the protein material [33]. As displayed in Figure **3**, the *G′* and *G″* values of the control samples were

approximately 32.2 Pa and 6.0 Pa at the end of heating process, respectively, with the corresponding loss factor being 0.188. When 0.05, 0.1, 0.15, and 0.2% KF was added to the MP sample, the corresponding loss factors were 0.166, 0.156, 0.149, and 0.117, respectively, which indicated that the addition of KF improved the gel elastic quality of MP and increased the gel-forming ability of MP.

Gel Strength and Gel Water-Binding Capacity

The gel-forming ability is very important for the production of high quality surimi and is a critical indicator of protein quality because it can affect the sensory, textural, and water-binding properties of minced meat products. This study showed that the KF concentrations had a significant influence on gel strength (P < 0.05) (Figure **4a**). The MP gel strength increased first and then decreased with increasing KF concentrations, and it reached its highest value at 0.1% KF. The gel strength of samples at 80°C was significant higher than those at 70 and 90°C (P < 0.05) at all KF addition levels (P < 0.05). Surimi protein can form a three-dimensional network when heated [34]. When heated at a lower temperature (70°C), the crosslinking of MP was not completely formed; however, when MP was heated at above 90°C, the protein could not form stable spatial structures because of excessive denaturation [35]. Zhang *et al*. [3] showed that a long period high-temperature treatment can induce protein denaturation and gel texture destruction. The gel properties have a great influence on the functionality of a protein, and they reflect both the ratio of *α*-chains and *β*-components and the amino acid composition [36].

Consistent with the results from the gel strength analysis, increasing KF concentrations caused a significant increase in WBC of carp MP gels (Figure **4b**). The WBC of the MP gel were 92.3, 94.1, and 95.7% for the control samples, and increased to 96.9, 97.5, and 98.8% for the 0.2% KF samples after heating at 70, 80, and 90°C, respectively (P<0.05). Higher heating temperatures could significantly increase the water-binding properties of the carp MP gels (P<0.05). This phenomenon appeared likely because the proteins interacting with KF form stable structures at certain temperatures [37, 38]. KF itself also possessed strong water absorption in the MP gel, and it could interact with proteins to form a more stable structure [39, 40]. Iglesias-Otero *et al*. [41] also have shown that KF could form elastic, strong, and heat-stable gels when heating.

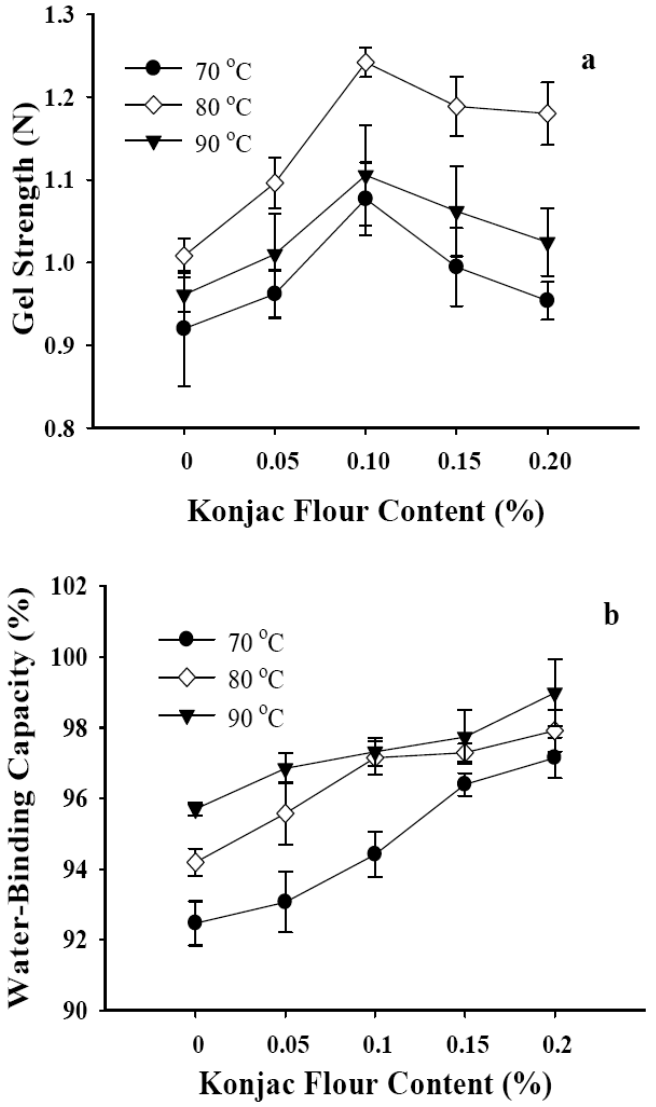

Figure 4: Effect of konjac flour on the gel strength and water-binding capacity of common carp myofibrillar protein gels at different heating temperatures.

Gel Microstructure

The effects of different KF concentrations on the microstructure of carp MP gels are shown in Figure **5**. The MP gel in the control samples exhibited a nonuniform network structure (Figure **5a**), and there were many empty spaces that made the aggregate gel structure into a more coarsely stranded network. The addition of KF reduced the number and size of the empty spaces. With increased KF concentrations, a more homogeneous and compact network structure was observed (Figure **5b-5e**). These improved structural features explained why protein gels prepared with KF exhibited improved textural properties and WBC, as presented above. These results suggest that the addition of KF leads to the formation of a more ordered microstructure with finer strands. KF may be

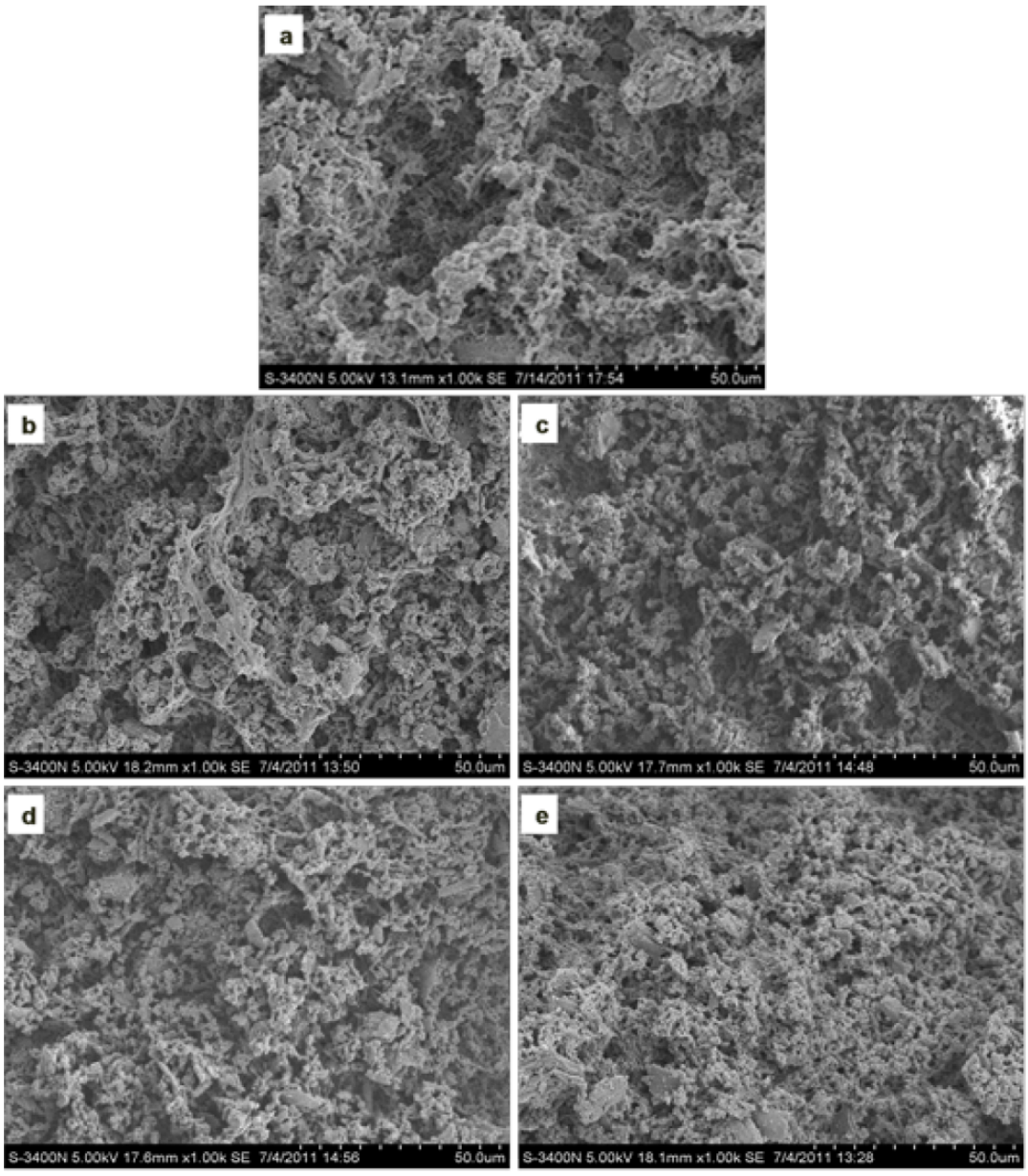

Figure 5: Effect of konjac flour on the microstructure of myofibrillar protein gels (magnification: 1000 ×). **a, b, c, d**, and **e** represent 0, 0.05, 0.10, 0.15, and 0.2% konjac flour addition, respectively.

embedded within the protein structures and/or scattered on the surface, forming a protective shield that stabilizes the protein structure and reduces the deterioration of individual protein molecules [42]. Zhang *et al*. [3] noticed that KF could effectively combine with protein in gel formation by reacting with the polar part of the protein. These results are in agreement with the aforementioned rheological properties.

CONCLUSION

The results of this study demonstrate that KF caused significant changes in the physicochemical and gel properties of the MP in common carp. The EAI, ESI and gel strength of MP increased and then decreased with increasing concentrations of KF. The gel qualities (storage modulus, loss modulus, gel WBC and gel microstructure) were also improved to a certain extent with increasing KF concentrations. These results revealed that KF significantly improved the protein functionality and gel qualities of carp MP. The improved effects of KF on the functionality and gel qualities of MP may be due to its reactivity with proteins by hydrogen, covalent and non-covalent bonding, thereby forming a denser network structure and improving the gel properties.

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