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# Effects of konjac glucomannan on heat-induced changes of physicochemical and structural properties of surimi gels

Tao Zhang, Zhaojie Li, Yuming Wang, Yong Xue *, Changhu Xue *

*Department of Food Science and Engineering, Ocean University of China, Qingdao 266003, PR China*

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ABSTRACT

This work demonstrated the protective effects of konjac glucomannan (KGM) on the physicochemical and structural properties of surimi gels subjected to 120 °C. The $T_2$ relaxation of LF-NMR which was used to detect water mobility, changed more obviously with the addition of deacetylated KGM than with the native, which indirectly implied that deacetylated KGM more pronouncedly influenced the protein structure. Hydrophobic interaction, ionic, hydrogen, and disulfide bonds, were sheltered by deacetylated KGM to greater extent than by native KGM. Raman spectra showed that the reduction of the main random-coil secondary protein structure at 120 °C was more significantly mitigated by deacetylated KGM. The interactions between protein and native or deacetylated KGM were investigated by X-ray diffraction, finding that protein and polysaccharide complexes might be formed. The microstructure of the mixed gels, determined by confocal laser scanning microscopy, demonstrated native and deacetylated KGM helped protein aggregation recover to uniform distribution.

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## 1. Introduction

Surimi, with a high content of salt-soluble myofibrillar proteins, can be processed to be gelled food product because of the unique gelling properties. Surimi is a good choice because of its convenience and beneficial health effects (Kristinsson & Rasco, 2000; Regenstein, 1984). So it has potential application in ready-to-eat (RTE) seafood products, for which the demand is increasing since RTE products can be directly consumed without secondary processing (Lorentzen, Ytterstad, Olsen, & Skjerdal, 2010). In order to obtain the RTE food, it is necessary to undergo the sterilization process. For which process, high-temperature treatment (120 °C) is the most common method to form products with good bactericidal properties, allowing them to be stored at room temperature and extending their shelf life.

Myofibrillar proteins are the main contributors imparting textural attributes and functional properties to surimi foods. Defining the performance of myofibrillar proteins during heat-induced gelation is beneficial in maintaining quality and developing processed meat products and processes (Westphalen, Briggs, & Lonergan, 2006). The functional behavior of myofibrillar proteins is manifested by their ability to produce three-dimensional (3D), viscoelastic gel matrices to bind water and form cohesive and strong membranes on the surfaces of fat globules in emulsion systems. These functional properties are the major factors contributing to palatability or sensory perception (tenderness, juiciness, and mouthfeel) of processed meat products (Khoury, 2004). During heat-induced processes, unfolding (exposure of hydrophobic parts), aggregation, and formation of a network are intertwined (Alting, 2003). The process begins with myosin (the main component of myofibrillar proteins) losing its non-covalently stabilized structure (α-helix) due to heating, followed by inter-molecular association (turbidity), finally developing into a rigid structure stabilized by covalent (disulfides) and non-covalent interactions (Ziegler, Foegeding, & John, 1990).

Konjac glucomannan (KGM) is a high-molecular weight water-soluble non-ionic polysaccharide extracted from tubers of the konjac plant, and has been approved in Europe and the FDA as a kind of food additive. It has the structure of a linear random copolymer of β-(1 → 4) linking D-mannose and D-glucose in the ratio of 1.6:1, with approximately one in nineteen of the sugar units being acetylated (Kato & Matsuda, 1969). The gelation mechanism of KGM alone has been under investigation since the early studies on the polysaccharide (Maekaji, 1974), now it is well-known that the removal of acetyl groups on the molecular chains of konjac glucomannan upon addition of an alkaline coagulant and heating results in the formation of a thermo-irreversible gel. KGM offers great potential for applications in food technology because of its good water absorptivity, gel-forming ability, stability, emulsifiability, thickenabilty, and film-forming properties, and is increasingly being studied and used in protein foods. Chin, Go, and Xiong (2009) found that konjac flour could improve the textural and water retention properties of transglutaminase-mediated, heat-induced porcine myofibrillar protein gel. Xiong et al. (2009) confirmed that KGM significantly increased the breaking force and deformation of surimi gels from grass carp, and was used as a cryoprotectant to significantly mitigate the decrease in the salt extractable protein (SEP), $Ca^{2+}$-ATPase activity, as well as the total and active sulfhydryl contents of myofibrillar proteins during frozen storage. Liu et al. (2013) studied

* Corresponding author.
*E-mail address:* xueyong@ouc.edu.cn (Y. Xue).

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the Influence of konjac glucomannan on gelling properties and water state in egg white protein gel, which found that the egg white–KGM blending gel (KGM at 0.06 wt.%) formed higher water retention capacity and porous microstructure, improving gel strength of egg white gel.

Protein and polysaccharide complexes have previously been introduced as gelling systems (Leloup, Colonna, & Ring, 1987). Nonspecific protein–polysaccharide interactions are responsible for the attractive and repulsive forces that induce complex formation or immiscibility of biopolymers (Doublier, Garnier, Renard, & Sanchez, 2000). For example, polysaccharides expands during heating, and thus affects the thermal stability of the protein, when the length of the polysaccharide chain exceeds a certain range, long-chain polysaccharide steric effects prevent protein aggregation. Among the many applications, protein–polysaccharide interactions play a significant role in the structure and stability of many processed foods, which receives increasing attention in recent years. High-pressure homogenization-induced dissociation of casein micelles in the presence of a non-ionic polysaccharide leads to the formation of stable casein–polysaccharide aggregates (Ye & Harte, 2014). The complexation of ovalbumin and gum Arabic, dominated by electrostatic attractive forces and certain pH values, provides very promising structures, textures, and shelf-life stability (Niu et al., 2014). The formation of a protein–water–polysaccharide complex involves some changes in the composition, structure, and functional properties of the protein particles, as proteins and polysaccharides possess a large number of ionizable side chain groups and differ in shape, size, conformation, flexibility, and net charge at a given pH and ionic strength. It has been shown that the interactions of proteins with polysaccharides, and of various proteins with one another and with water, govern the solubility of biopolymers, their ability to form viscoelastic gels, and their behavior at interfaces (Tolstoguzov, Damodaran, & Paraf, 1997). Protein–carbohydrate interactions determine the functional properties of foods in which proteins are the major ingredient, especially processed meat and fish products. Different hydrocolloids could improve the mechanical and functional properties of surimi and restructured fish gels (Lee, Wu, & Okada, 1992; Gómez-Guillén, Borderías, & Montero, 1997; Park, 2000; Ramírez, Barrera, Morales, & Vázquez, 2002). Among them, hydrocolloids from different sources mixed with myofibrillar proteins of fish products have been increasingly studied (Ramírez, Uresti, Velazquez, & Vázquez, 2011).

In our previous study, the effect of deacetylation of KGM on the textural properties of surimi gels exposed to a high-temperature (120 °C) treatment was investigated. Deacetylation of KGM effectively mitigated protein denaturation of Alaska Pollock surimi during high-temperature treatment, and showed positive effects on the gel strength, as high degree of deacetylation of the added KGM could significantly increase the breaking force and deformation of the surimi gels (Zhang, Xue, Li, Wang, & Xue, 2015). However, the relationship between the functional properties of gels and the changes in protein structure with the addition of KGM is not well understood, and the influence of deacetylated KGM on the structure of surimi gels is still unknown. The objectives of this study were to investigate the effect of deacetylated KGM on the physicochemical and structural properties of surimi gels exposed to a high-temperature (120 °C) treatment and to further improve the understanding of the mechanisms of protein–polysaccharide interactions between myofibrillar protein and KGM.

## 2. Materials and methods

### 2.1. Materials

Frozen Alaska Pollock surimi (grade AAA) was purchased from Jincan Foods Co., Ltd. (Qingdao, Shandong, China). The surimi was maintained at −20 °C until use. Native KGM was purchased from Qingdao Qiuyang Biological Co., Ltd. All of the chemicals used were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

### 2.2. Preparation of KGM samples with different degree of deacetylation

According to the method of Du, Li, Chen, and Li (2012), KGM (30 g) was mixed with 150 mL of 50 vol.% ethanol in a water bath shaker at 150 rpm and 40 °C for 30 min. A predetermined amount of NaOH was added immediately into it. The deacetylation reaction was controlled at 40 °C for 24 h. The crude product was then immersed into twice the volume of 50 vol.% ethanol, and this was repeated several times until the sample pH became neutral. Afterward, the product was dehydrated gradually with 75 vol.% aqueous ethanol and 95 vol.% aqueous ethanol, and pure ethanol. The final products were obtained by vacuum-drying at 40 °C. By changing the amount of NaOH, a series of deacetylated products with different DDs were obtained.

### 2.3. Determination of different degrees of deacetylation of KGM

The degrees of deacetylation of KGM were determined by titration according to the method of Chen, Zong and Li, (2011). Briefly, dried sample of powered KGM was placed in a 100 mL Erlenmeyer flask with a stopper and 75% ethanol (10 mL) was added. The mixture was stirred at 40 °C for 30 min, followed by adding NaOH with swirling. Then the reaction was kept at 40 °C for 24 h with stirring. The excess alkali was back titrated with 0.1 M HCl using phenolphthalein as an indicator. A blank (to which no KGM sample had been added) was titrated in parallel. The content of acetyl ($\omega_0$) was calculated according to the following equation:

$$\omega_0 = \frac{(V_2 - V_1) \times N_{HCl} \times M_{acetyl}}{m_s} \times 100\% \quad (1)$$

where $V_2$ is the volume of hydrochloric acid consumed for the blank in liters, $V_1$ represents the volume of hydrochloric acid consumed for the sample in liters, $N_{HCl}$ stands for the normality of the hydrochloric acid, $M_{acetyl} = 43$ g/mol and $m_s$ is the mass of the sample in grams.

By changing the amount of NaOH and controlling reaction time, different DDs of KGM were obtained, and the DD can be calculated as following:

$$DD = \frac{\omega_0 - \omega}{\omega_0} \times 100\% \quad (2)$$

where $\omega_0$ is the whole content of acetyl of native KGM, Ω is the content of acetyl when they were partially removed by controlling reaction conductions.

Thus, different DDs of KGM samples were obtained and coded as Da0 (native KGM), Da1 (DD/% = 65.84 ± 0.34), and Da2 (DD/% = 98.29 ± 0.17). All the data are averages of experiments performed at least in duplicate.

### 2.4. Preparation of KGM–surimi samples

Frozen surimi (250 g) was partially thawed at 4 °C for 4 h and then cut into small pieces (about 3 cm cubes) according to the procedure of Zhang et al. (2015). The frozen surimi cubes were chopped at a speed of 1500 rpm for 4 min in a Stephan vertical vacuum cutter (Model UM 5, Stephan Machinery Co., Germany). The double-walled chopping bowl was continuously circulated with a cooling medium (ethanol:water, 95:5) to maintain the sample below 4 °C during chopping. Native or deacetylated KGM (degrees of deacetylation were 0% and 98.29%, respectively, 2 wt.%) was added after swelling, together with sodium chloride (3 wt.%), and mixed with the surimi at a speed of 1800 rpm for 2.5 min under 0.5 bar pressure to remove air pockets that developed during chopping. The surimi sol was stuffed into plastic casings (3 cm i.d.), and both ends were sealed tightly. The surimi samples were then equilibrated at 4 °C for 4 h before the measurements.

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