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Effect of molecular characteristics of *Konjac* glucomannan on gelling and rheological properties of *Tilapia* myofibrillar protein

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ABSTRACT

Konjac glucomannan (KGM) is an important gelling agent in composite gels. This study aimed to investigate the effects of KGM molecular characteristics (molecular weight, size and conformation) on gelling properties of *Tilapia* myofibrillar protein (TMP). In this work, TMP composite gels were prepared under neutral pH with varying KGM (native KGM, 10 kGy-KGM, 20 kGy-KGM, and 100 kGy-KGM) of different molecular characteristics. Native KGM, 10 kGy-KGM, and 20 kGy-KGM exerted negative effect on gel strength or whiteness of TMP gels. Interestingly 100 kGy-KGM improved gelling properties and whiteness of TMP gels. Such effects presented by varying KGM were attributed the physical filling behaviors and the interaction between KGM and TMP. These behaviors or interactions are resulted from different molecular size and conformation. Smaller molecular size (root-mean square radius, Rz 20.2 nm) and approximated spherical conformation in 100 kGy-KGM enhanced its interaction with TMP and maintained its compact and smooth structure, but the larger molecular size (Rz ≥ 40.2 nm) and random coil conformation in other KGMs inhibited part of actins from gelling and deteriorated the network structure. Our study provided principle knowledge to understand the structure-functions relationships of KGM-TMP composite gels. These results can be used to provide theoretical guidance for surimi gel processing.

1. Introduction

Surimi is a composite gel made of fish protein and other food hydrocolloids. Conventionally, the fish protein for surimi is obtained from white-muscle marine species such as Alaskan *Pollock*, *Hake*, and *Cod*, etc (Chanarat & Benjakul, 2013). Due to the overfishing of marine white-muscle fishes, the stock for these marine fishes has been dramatically decreased (Buamard & Benjakul, 2015; Moreno, Herranz, Pérez-Mateos, Sánchez-Alonso, & Borderías, 2016). So, attempts have been made by researchers to find new species with high quality myofibrillar protein and favorable gel forming ability from freshwater fish (Andres-Bello, Iborra-Bernad, Garcia-Segovia, & Martinez-Monzo, 2013; Mahawanich, Lekhavichitr, & Duangmal, 2010; Wu, Yuan, Chen, Liu, Ye, & Hu, 2015). With rapid growth, high yield and excellent processing property of muscle, *Tilapia* has attracted the attention of seafood manufacturers, and has been tried to use as a major freshwater fish to produce surimi (Duangmal & Taluengphol, 2010; Reed & Park, 2011).

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Generally, surimi mainly consists of salt-soluble myofibrillar proteins after removing fat and water-soluble sarcoplasmic proteins from minced fish meat through washing with cold water (Ramirez, Uresti, Velazquez, & Vazquez, 2011). So, myofibrillar protein is a dominant functional ingredient in fish product and has profound effect on the processing and textural properties of surimi gel (Reed, Guilford, & Park, 2011). Nevertheless, for freshwater fishes the gelling properties of myofibrillar proteins alone are still poor and cannot meet customer expectations (Murthy, Panda, & Shamasundar, 2011). Thus, there is a need to improve the gelling properties of myofibrillar protein from freshwater fish.

Comparing to the moderate gel-forming capacity of single protein, composite gels made of protein and polysaccharide present excellent gelling properties and unique gel structure (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014). The desired textural properties and gel structure of composite gels might be achieved by adjusting processing environment (pH, ionic strength, and temperature) and component level. So, composite gels have been widely applied on surimi processing to improve its mechanical properties and achieve desired gelling texture (Sarker, Elgadir, Ferdosh, Akanda, Manap, & Noda, 2012). Several polysaccharides, such as starch, carrageenan, and pectin are typically used to improve the textural properties of surimi gels (Hu et al., 2015; Ramirez et al., 2011).

Konjac glucomannan (KGM), a plant polysaccharide derived from the tuber of *Amorphophallus Konjac* C. *Koch*, is composed of glucose and mannose at 1:1.5–1:1.6 molar ratio with 5–10% acetyl substitution (Gong, Liu, & Liu, 2006; Jin et al., 2014). Besides the excellent gelling properties and healthy bioactivity of dietary fiber, KGM shows its unique cryoprotective effect on fish myofibrillar proteins and significant increase in water-holding capacity, breaking force and deformation of surimi gels (Xiong et al., 2009). Several researches have been reported about its improvement on gel properties of low-quality surimi (Liu, Wang, & Ding, 2013). KGM not only reinforces gel harness in *Whiting* and *Pollock* surimi (Park, 1996; Zhang, Xue, Li, Wang, & Xue, 2015), but also significantly improves gel properties of low-quality *Squid* and *Grass Carp* surimi (Iglesias-Otero, Borderias, & Tovar, 2010; Xiong et al., 2009).

Most of the previous researches focus on the alkaline addition, although it improves significantly the gelling properties of KGM in surimi gels. The operation of removing residual alkaline is tedious and may cause loss of flavors and nutrition in practical production (Iglesias-Otero et al., 2010). Therefore, it is necessary to develop a non-alkaline processing method for surimi industry. On the other hand, gelling properties of composite gels are also profoundly determined by the chemical composition and molecular characteristics (e.g. molecular weight, size and conformation) of polysaccharides (Joshi et al., 2014). However, there is no report so far on the effect of molecular characteristics of KGM (varying KGM) on the gelling properties of surimi composite gel.

The aim of this work is to find suitable formulation for KGM-*Tilapia* myofibrillar protein (TMP) composite gels without addition of alkaline and to evaluate the effect of varying KGM on the gelling properties of composite gels.

Based on the preparations of varying KGM by γ-irradiation degradation in our previous researches (Jian et al., 2013; Xu, Sun, Yang, Ding, & Pang, 2007), KGM-TMP composite gels were made in different levels and temperature of heating without addition of alkaline in this work. Then the effect of KGM molecular characteristics on the rheological and textural properties (gel strength, water-holding capacity and whiteness) of composite gels was fully evaluated. Finally, the mechanism for the effects presented by varying KGM was fully elucidated by combination of dynamics rheological measurement, microstructure observation, and electrophoresis.

2. Materials and methods

2.1. Raw materials and chemicals

2.1.1. KGM

KGM was purchased from Shaotong Shanai *Konjac* Development Co. (Yunnan, China) with purity up to 95%, which was extracted from the corm of *Amorphophallus konjac* C. *Koch*. After being weighed, washed and removal of epidermis, the corm was sliced into pieces with 2–3 mm thickness. Then, the corm slices were immersed in 1% (w/v) sodium bisuphite for 1 min, followed by oven-drying (120 °C, 40 min) and continuous drying at 60 °C until constant weight reached. Afterwards, the dried slices were ground and sieved (425 μm aperture) to obtain crude *konjac* flour. The crude *konjac* flour was further purified by wind-sifting to produce KGM.

After purchase, KGM was further purified by alcohol sedimentation according to our previous study (Jian, Yao, Wang, Guan, & Pang, 2010). The detailed purification methods were as follows. KGM was washed thrice with five volumes (v/w, based on KGM weight) of 50% ethanol containing 0.1% sodium azide so as to remove water-soluble impurity. Then, the sample was dissolved in distilled water to form a 0.6% (w/v) hydrosol, followed by centrifugation (16,000 r/min, 20 min) at 4 °C using Avanti J-E with rotor JLA-16.250 (Beckman, USA) in order to remove the fibrin and other insoluble impurities. Afterward, amylum and protein was removed by enzymatic hydrolysis and Sevag method respectively. Then the hydrosol was precipitated by adding the same volume of 95% ethanol (V/V), and the precipitate was fully washed with absolute ethanol and aether. Finally, the sample was freeze-dried and used for the following experiments.

After purification, the chemical composition of KGM was further determined. The content of ash, protein, and acetyl was 1.23% ± 0.02%, 1.60% ± 0.23%, and 1.62% ± 0.18% respectively. The molar ratio of mannose/glucose was about (2.3 ± 0.27):1.

Referred to the method of our previous study (Xu et al., 2007), the degraded KGM were made from the above purified KGM (100 g, previously sealed in polyethylene bags) by γ-irradiation with a ^{60}Co source at Radiation Center of Guangdong Province at room temperature in dosages of 10 kGy, 20 kGy, and 100 kGy respectively. Purified KGM and its degraded products from γ-irradiation were labeled as native KGM, 10 kGy-KGM, 20 kGy-KGM or 100 kGy-KGM according to their irradiation dosage.

Based on our previous researches (Jian, Siu, & Wu, 2015; Xu et al., 2007), the molecular characteristics (weight-average molecular weight, root-mean-square radius, and molecular conformation) of varying KGMs were determined by gel permeation chromatography with on-line multi-angle laser light scattering (GPC-MALLS). The GPC-MALLS system consisted of a Waters e2695HPLC system and a Waters 2414 refractive index detector, and a Wyatt DAWN HELEOS II detector (18 angles). Sample solution (0.2 mg/mL) was injected into the system at 100 μL. Two GPC columns were used in a series for the separation, TSK-GEL G4000PWxl and G5000PWxl, (Tosoh Bioscience, Tokyo, Japan). Sodium nitrate solution (50 mM with 0.02% w/v sodium azide) was used as the mobile phase at a flow rate of 0.4 mL/min and 25 °C. Normalization was performed with dextran standards (Sigma Aldrich, Mw 30 kDa). Both GPC data and MALLS data were collected and analyzed using Astra 6.0 software package. In calculating molecular weight, the value of dn/dc was set to 0.147 mL/g, referring to literature (Xu et al., 2013).

The weight-average molecular weight (*Mw*) of native KGM, 10 kGy-KGM, 20 kGy-KGM and 100 kGy-KGM are 923.8 ± 25.9 kDa, 307.8 ± 1.6 kDa, 169.0 ± 0.40 kDa and 53.0 ± 0.16 kDa, respectively. Their root-mean-square radius (Rz) is 108.5 ± 1.84 nm, 53.4 ± 0.37 nm, 40.2 ± 0.24 nm and 20.2 ± 0.67 nm, respectively.