Food Chemistry 116 (2009) 413–418

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Effects of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (*Ctenopharyngodon idella*)

Guangquan Xiong [a,b,c], Wei Cheng [a,b], Lixiu Ye [a,b], Xin Du [a,b], Ming Zhou [a,b], Ruotai Lin [a,b], Shengrong Geng [a,b], Mingli Chen [a,b], Harold Corke [c], Yi-Zhong Cai [c,*]

[a] *Institute of Agricultural Products Processing and Nuclear Agricultural Technology, Hubei Academy of Agricultural Sciences, Wuhan 430064, China*
[b] *Innovative Center of Hubei Agricultural Sciences and Technologies, Wuhan 430064, China*
[c] *School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China*

ARTICLE INFO

*Article history:*
Received 7 November 2008
Received in revised form 3 February 2009
Accepted 18 February 2009

*Keywords:*
Konjac glucomannan
Grass carp
Surimi
Protein denaturation
Physicochemical properties
Frozen storage

ABSTRACT

The cryoprotective effect of konjac glucomannan (KGM) on myofibrillar protein from grass carp (*Ctenopharyngodon idella*) during frozen storage at −18 °C and the influence of five levels of KGM (0%, 0.5%, 1%, 1.5%, and 2%) on texture properties, water-holding capacity, and whiteness of grass carp surimi gels were investigated. KGM as a novel cryoprotectant could significantly mitigate the decrease in salt extractable protein (SEP), $Ca^{2+}$-ATPase activity, and total sulphydryl and active sulphydryl contents of myofibrillar protein during frozen storage. KGM at the level of 1% showed the same good cryoprotective effect as a conventional cryoprotectant (10% sucrose–sorbitol, 1:1, w/w). As the levels of KGM increased, breaking force and deformation of grass carp surimi gels increased significantly. Water-holding properties of the surimi gels are improved with the increasing addition of KGM, but the whiteness decreased and the colour became darker. The optimum addition level of KGM was suggested to be 1%.



## 1. Introduction

Grass carp (*Ctenopharyngodon idella*) is a popular species of freshwater fish in China because of its rapid growth, high yield, and low price. It can be processed as surimi-based products, which is one of its major processing approaches. Surimi is a heating elastic gel food that is made from fresh or frozen fish with addition of salt and starch. Myofibrillar protein is a dominant functional ingredient in fish surimi. Low-temperature frozen storage is a widely used method for long-term storage of surimi products. However, during frozen storage, surimi may lose its functional properties as a result of myofibrillar protein denaturation (Shenouda, 1980), such as decrease of water-holding capacity, protein solubility and gel-forming ability. In order to inhibit or mitigate the protein denaturation during frozen storage, cryoprotectants are normally added into surimi during its processing (Park, 2005; Tyre & Chong, 1992). The mixture of sucrose and sorbitol has commonly been used as cryoprotectants in the surimi industry. Though this commercial blend has an excellent cryoprotective effects on inhibiting the protein denaturation, it could cause excessive sweet taste and high caloric value in surimi products. Moreover, it could affect the taste of surimi products and limit its consuming population (e.g., diabetic patients and obesity subjects). Therefore, other cryoprotectants with reduced sweetness and caloric value have been widely investigated and used in surimi products, such as protein hydrolysates, starch hydrolysates, polyalcohol, and oligosaccharides (Auh, Lee, Kim, Yoon, & Park, 1999; Herrera & Mackie, 2004; Hossain et al., 2004; Sych, Lacroix, Adambounou, & Castaigne, 1990; Zhang, Yamashita, & Nozaki, 2002).

Konjac glucomannan (KGM) is a high-molecular weight water-soluble non-ionic polysaccharide extracted from tubers of the konjac plant (*Amorphophallus* Blume ex Decne in the family Araceae) (Li, Xie, & Kennedy, 2006; Pang, 2003). It has the structure of a linear random copolymer of $\beta(1 \rightarrow 4)$ linking D-mannose and D-glucose in the ratio of 1.6:1 (number of D-mannose/number of D-glucose), with approximately one in nineteen of the sugar units being acetylated (Katsuraya et al., 2003; Li et al., 2006). KGM could not be hydrolysed by digestive enzymes in the human upper gastrointestinal tract and is therefore considered as a non-calorie indigestible dietary fibre. Its function was reported to prevent and treat constipation (Zhang et al., 1990), regulate lipid metabolism, improve glucose metabolism, and reduce the risk of developing diabetes and heart disease (Huang et al., 1989; Peng, Zhang, Zhang, & Wu, 1994; Vuksan et al., 1999; Zhang, Huang, Wang, Hong, & Peng, 1989). KGM can be used as various kinds of food additives because of its good water absorptivity, gel-forming ability, stability, emulsifiability, thickenabilty, and film-forming properties

* Corresponding author. Tel.: +852 22990314; fax: +852 28583477.
*E-mail address:* caiyizhonghk@yahoo.com (Y.-Z. Cai).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.02.056

(Dave, Sheth, McCarthy, Ratto, & Kaplan, 1998; Pang, 2003; Zhang, Xie, & Gan, 2005).

Konjac flour was introduced into the surimi made from sea fish (Alaska Pollock and Pacific whiting) to investigate its ability to maintain fracture properties of the surimi gels against various temperatures (5, 25, 55, or 75 °C) (Park, 1996). However, so far nobody has reported the cryoprotective effect of konjac glucomannan (KGM) as a cryoprotectant in surimi and systematically investigated the influence of KGM on physicochemical properties of surimi products. The objectives of this study were to investigate the cryoprotective effect of KGM on myofibrillar protein from freshwater fish (grass carp) surimi during frozen storage at −18 °C and to evaluate the influence of different levels of KGM on the physical properties of the grass carp surimi gels (e.g., textural properties, water-holding capacity and whiteness). This work may provide a scientific basis for commercial application of KGM as an effective and healthy cryoprotectant in the surimi products made from freshwater fishes.

## 2. Materials and methods

### 2.1. Materials and chemicals

Fresh grass carp (*Ctenopharyngodon idella*) was purchased from a local supermarket (Wellcome in Hong Kong). Konjac glucomannan (KGM) was obtained from Wuhan Qiangsheng Food Co., Ltd. (Wuhan, China). $Ca^{2+}$-ATPase test kit and active sulphydryl and total sulphydryl test kit were purchased from Institute of Nanjing Jiancheng Bioengineering (Nanjing, China). Potato starch and other chemical reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

### 2.2. Preparation of myofibrillar protein

One kilogram of grass carp fish meat was added with 10 times of volume of Tris–maleate buffer (50 mM KC1–20 mM Tris–maleate, pH 7), and then homogenized. The homogenate was centrifuged at 9110*g* using an Avanti J-25 Beckman centrifuge (Beckman Coulter, Inc., New York, NY, USA) at 4 °C for 30 min. Supernatant was removed and precipitate was washed twice. The precipitate was added with Tris–maleate buffer (0.6 M KC1–20 mM Tris–maleate, pH 7), and then homogenized. The homogenate was extracted at 4 °C for 60 min and centrifuged at 9110*g* using an Avanti J-25 Beckman centrifuge (Beckman, New York, NY, USA) at 4 °C for 30 min. The obtained supernatant was myofibrillar protein solution for use in this study. Myofibrillar protein solution was mixed with 1% KGM as a cryoprotectant and without any cryoprotectants (common control). Addition of 10% sucrose–sorbitol (1:1, w/w) (commercial cryoprotectant) in myofibrillar protein solution was used as a positive control in this study. The mixture solutions were stored at −18 °C for 30 days. The relevant parameters such as SEP content, $Ca^{2+}$-ATPase activity, and total sulphydryl and active sulphydryl contents were determined every 5 days. Tests were performed in triplicate per sample.

### 2.3. Determination of salt extractable protein (SEP) content

Myofibrillar protein solutions at different frozen storage periods were centrifuged at 9110*g* for 50 min at 4 °C. The protein concentration of the supernatant was determined according to the Biuret method (Gornall, Burdawill, & David, 1949). Briefly, sample was diluted 10 times with distilled water. One millilitre of the diluted sample was added with 4 ml of Biuret reagent, and then kept at room temperature for 30 min. Absorbance was measured at 540 nm using a U-1800 HITACHI Spectrophotometer (Hitachi, Ltd., Tokyo, Japan). SEP content was expressed as the percentage of protein concentration of myofibrillar protein solutions to the initial protein concentration.

### 2.4. Determination of $Ca^{2+}$-ATPase activity

Sample was diluted 10 times with buffer solution (0.6 M KC1–20 mM Tris–maleate, pH 7). $Ca^{2+}$-ATPase activity of myofibrillar protein was determined according to the instruction of the $Ca^{2+}$-ATPase kit. Briefly, 100 μl of the diluted sample and chemical reagent of the $Ca^{2+}$-ATPase kit were mixed and incubated at 37 °C for 10 min. The reaction mixture was centrifuged at 2280*g* for 5 min and the supernatant was obtained. Then, 150 μl of supernatant and chemical reagent of the $Ca^{2+}$-ATPase kit were mixed and incubated at 25 °C for 5 min. The absorbance value was recorded at 636 nm. $Ca^{2+}$-ATPase activity of myofibrillar protein was expressed as μmol (pi) $mg^{-1}$(pro) $min^{-1}$ (pi = inorganic phosphorus, pro = protein).

### 2.5. Determination of total sulphydryl content and active sulphydryl content of myofibrillar protein

Total sulphydryl content of myofibrillar protein was determined according to the instruction of the corresponding kits. Briefly, 100 μl of sample and the corresponding chemical reagent of the sulphydryl kit were mixed and incubated at 37 °C for 15 min. Absorbance was measured at 412 nm.

Active sulphydryl content of myofibrillar protein was determined using NTSB (2-nitro-5-thiosulphobenzoate) according to the method of Benjakul, Visessanguan, Thongkaew, and Tanaka (2003). Briefly, 100 μl of sample were added with 3 ml of freshly prepared NTSB assay solution (pH 9.5). The mixture was incubated in dark at 25 °C for 25 min. Absorbance was measured at 412 nm. Total sulphydryl content and active sulphydryl content of myofibrillar protein were expressed as mol $g^{-1}$ (pro) (pro = protein).

### 2.6. Surimi gel preparation

Grass carp were washed thoroughly with chilled water. Head, scale, and viscera of fish were removed and meat was picked manually. Picked meat was carefully washed with ice water (1:2 w/w) twice. Meat was centrifuged at 9110*g* for 10 min at 4 °C to remove surface water, and then frozen and storage at −18 °C until use. The frozen meat was thawed at room temperature for 2 h, and then cut into pieces, chopped and blended at 0–10 °C for 1 min to obtain the homogeneous paste by a Kenwood FP510 food processor (Kenwood Limited, Hants, UK). The homogeneous paste (500 g) was mixed with 20 g of sodium chloride (accounting for 2% in total surimi sol) and blended for 2 min, then added with different levels of KGM (0%, 0.5%, 1.0%, 1.5%, and 2.0%, equal to 0, 5, 10, 15, and 20 g) and potato starch (9%, w/w). The moisture content was adjusted to about 78% with ice water (380 g) and the mixture was continuously blended for 2 min to obtain the homogeneous sol. The sol (the surimi paste with KGM, salt, starch, and iced water) was stuffed into a plastic tube (length = 120 mm, inner diameter = 20 mm) and was centrifuged at 5000*g* for 30 min to remove the supernatant. The surimi sol in the tube was heated to surimi gel at 90 °C in a water bath for 25 min, and then was cooled to room temperature with iced water. Finally, the surimi gels were stored at 4 °C in a freezer overnight for determining texture, whiteness and water-holding capacity.

### 2.7. Texture analysis

Texture analysis of surimi gels was carried out according to the method of Benjakul, Visessanguan, and Srivilai (2001). The samples

of surimi gels were taken out from the freezer and equilibrated at room temperature for 2 h, and then cut into pieces (2.5 cm in length). Breaking force (gel strength) and deformation (elasticity/deformability) of surimi were determined by using a TA-XT2 texture analyzer (Stable Micro systems, Surrey, UK) equipped with a cylindrical plunger (5 mm diameter; 60 mm/min depression speed).

### 2.8. Determination of whiteness

The colour of surimi gels was determined with a Minolta CR-300 Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan) according to the method of Benjakul et al. (2001) by measuring the $L^*$ (lightness), $a^*$ (redness/greenness), and $b^*$ (yellowness/blueness) values. The whiteness was calculated by the following:

$$\text{Whiteness} = 100 - [(100 - L^{*2}) + a^{*2} + b^{*2}]^{1/2}$$

### 2.9. Determination of water-holding capacity

The water-holding capacity of surimi gels was determined according to the method of Ng (1987). Briefly, the surimi gels were cut with length of 0.5 cm and weighed (expressed as $\times$g). Two pieces of Whatman filter papers were placed above the surimi gels and three pieces of the filter papers were placed under them. The samples were given up to 5 kg mass pressure for 2 min, and then weighed (expressed as Z g). The water-holding capacity was expressed as expressible water and calculated by the following:

$$\text{Water-holding capacity (WHC)} = (X - Z) \times 100\%/X$$

### 2.10. Statistical analysis

All experiment data were calculated as mean ± standard deviation (SD). Differences between means of data were compared by least significant difference (LSD) ($p < 0.05$) calculated using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

## 3. Results and discussion

### 3.1. Effect of KGM on the salt extractable protein (SEP) content of myofibrillar protein from grass carp during frozen storage

The changes in the SEP contents of the myofibrillar protein samples added with KGM (1%, w/w) and commercial cryoprotectant (10% sucrose–sorbitol mixture, 1:1, w/w) and without cryoprotectant (control) were observed during the frozen storage at −18 °C for 30 days. Fig. 1 shows that there are significant differences ($p < 0.05$) in SEP contents between with KGM and the control, but basically no significant differences in SEP contents between two cryoprotectants (KGM and sucrose–sorbitol mixture).

It was found that SEP in all tested samples of myofibrillar protein decreased along with extension of frozen storage. However, the decrease rate of SEP in the samples with cryoprotectants was significantly slower than in the control, especially during initial storage. SEP contents of KGM, sucrose–sorbitol mixture, and control at 5 days were 84.1%, 85.6%, and 60.1%, respectively, while those at 30 days were 75.2%, 70.2%, and 40.1%, respectively. The results suggested that addition of KGM and sucrose–sorbitol mixture as cryoprotectants had good protective effect on protein denaturation of grass carp, compared with the control. The present study had the similar change tendency to previous studies (Sych et al., 1990; Zhou, Benjakul, Pan, Gong, & Liu, 2006). The decrease in SEP was a primary indicator of protein denaturation during frozen storage resulting from the formation of hydrogen and/or hydrophobic bonds, as well as disulfide bonds and ionic interactions (Auh et al., 1999; Sych et al., 1990; Tyre & Chong, 1992).

Moreover, from Fig. 1, the SEP decrease of KGM was close to that of the sucrose–sorbitol mixture at initial storage period (5 days) and slightly slower than that of the sucrose–sorbitol mixture after 10 days. This suggested that the protective effect of KGM as a novel cryoprotectant on myofibrillar protein from grass carp surimi during frozen storage was at least comparable to that of sucrose–sorbitol mixture (a conventional cryoprotectant) widely used in the surimi industry, especially when the storage time was extended.

### 3.2. Effect of KGM on the $Ca^{2+}$-ATPase activity of myofibrillar protein from grass carp during frozen storage

During frozen storage, protein denaturation could normally result in the decrease of the ATPase activity of myofibrillar protein from fish surimi. Therefore, the decrease in $Ca^{2+}$-ATPase activity is also a primary indicator of protein denaturation during frozen storage and it has been widely used as an indicator of fish or surimi protein denaturation (MacDonald & Lanier, 1994). Fig. 2 shows the changes of the $Ca^{2+}$-ATPase activity of myofibrillar proteins added with 1% KGM or 10% sucrose–sorbitol mixture as cryoprotectants during the frozen storage at −18 °C. The results showed that there were significant differences ($p < 0.05$) in the $Ca^{2+}$-ATPase activity of

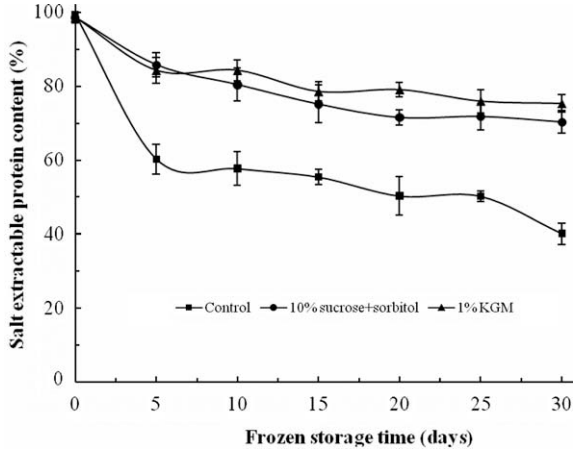


**Fig. 1.** Salt extractable protein content change of grass carp myofibrillar protein with and without cryoprotectants during frozen storage at −18 °C. Bars represent standard deviation from three replications.

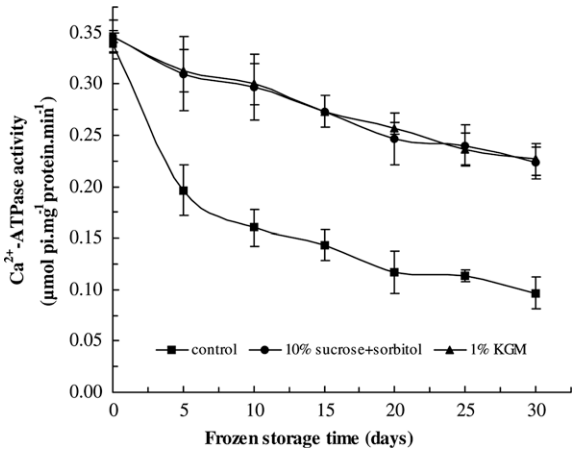


**Fig. 2.** $Ca^{2+}$-ATPase activity change of grass carp myofibrillar protein with and without cryoprotectants during frozen storage at −18 °C. Bars represent standard deviation from three replications.

myofibrillar protein with cryoprotectants (KGM and sucrose–sorbitol mixture) and without cryoprotectants (control). The $Ca^{2+}$-ATPase activity of the control decreased quickly at initial stage. Its $Ca^{2+}$-ATPase activity at 5 days was 0.24 μmol (pi)·$mg^{-1}$(pro)·$min^{-1}$ and decreased by 42.8%, while its $Ca^{2+}$-ATPase activity at 30 days was 0.10 μmol (pi)·$mg^{-1}$(pro)·$min^{-1}$ and decreased by 71.4%, compared to its corresponding initial value (0.34 μmol (pi)·$mg^{-1}$(pro)·$min^{-1}$). However, the decreasing speeds of the $Ca^{2+}$-ATPase activity of the samples with two cryoprotectants were significantly mitigated. The $Ca^{2+}$-ATPase activity of the treated samples was significantly higher than that of the control. During storage of 5 days, the $Ca^{2+}$-ATPase activity of the samples with KGM or sucrose–sorbitol mixture was 0.31 μmol (pi)·$mg^{-1}$(pro)·$min^{-1}$ and decreased by only 11.4%, compared with their corresponding initial values (0.35 μmol (pi)·$mg^{-1}$(pro)·$min^{-1}$). During storage of 30 days, the $Ca^{2+}$-ATPase activity values of the samples with KGM or sucrose–sorbitol mixture were 0.22 and 0.23 μmol (pi)·$mg^{-1}$(pro)·$min^{-1}$ and decreased by 37.1% and 34.3%, respectively, compared to their initial values. This suggested that the cryoprotectants tested in this study could mitigate the decrease in the $Ca^{2+}$-ATPase activity and had better cryoprotective effect on myofibrillar protein from grass carp. This was similar to previous results with other cryoprotectants (MacDonald & Lanier, 1994; Ramirez, Martin-Polo, & Bandman, 2000; Zhou et al., 2006). Furthermore, no obvious difference in mitigating the decrease in the $Ca^{2+}$-ATPase activity of myofibrillar protein was observed between 1% KGM and 10% sucrose–sorbitol mixture, indicating that KGM as a novel cryoprotectant could be in replace of sucrose–sorbitol mixture (commercial cryoprotectant) for use in the surimi processing.

### 3.3. Effect of KGM on the total sulphydryl and active sulphydryl contents of myofibrillar protein from grass carp during frozen storage

Fig. 3 shows the effect of 1% KGM as a novel cryoprotectant and 10% sucrose–sorbitol mixture as a commercial cryoprotectant on the total sulphydryl and active sulphydryl contents of grass carp myofibrillar protein during frozen storage at −18 °C. From Fig. 3A, during the storage of 30 days, the total sulphydryl content of the control sample was $6.2 \times 10^{-5}$ mol $g^{-1}$(pro) and decreased by 31.9%, while the total sulphydryl contents of KGM and sucrose–sorbitol mixture were $7.1 \times 10^{-5}$ and $7.3 \times 10^{-5}$ mol $g^{-1}$(pro) and decreased only by 22.0% and 19.8%, respectively, in comparison with their initial values. The result suggested that the decrease in the total sulphydryl contents of KGM and sucrose–sorbitol mixture was obviously slower than that of the control, especially during extension of storage.

The decreasing tendencies of active sulphydryl contents of grass carp myofibrillar protein with and without cryoprotectants during frozen storage were similar to their total sulphydryl contents (Fig. 3B). Throughout the storage of 30 days, the active sulphydryl content of the control sample (decreased by 45.8%) reduced more rapidly than those of the samples added with KGM (decreased by 30.5%) and sucrose–sorbitol mixture (decreased by 32.2%).

Sulphydryl groups (the most reactive functional groups) in myofibrillar protein were readily oxidised to disulfide groups during frozen storage and freeze-thawing process, therefore resulting in an obvious decrease in total sulphydryl content and active sulphydryl content (Benjakul et al., 2003; Ramirez et al., 2000). Addition of the cryoprotectants in myofibrillar protein from fish could inhibit the oxidisation of the protein sulphydryl groups and the formation of the disulphide bonds during frozen storage (Jiang, Hwang, & Chen, 1988; Ramirez et al., 2000). Our results showed that KGM as a cryoprotectant could effectively mitigate the decrease in the total sulphydryl and active sulphydryl contents of myofibrillar protein from grass carp, in comparison with the control. Moreover, the results in Fig. 3A and B also indicated that KGM as a novel cryoprotectant had similar cryoprotective effect to the sucrose–sorbitol mixture.

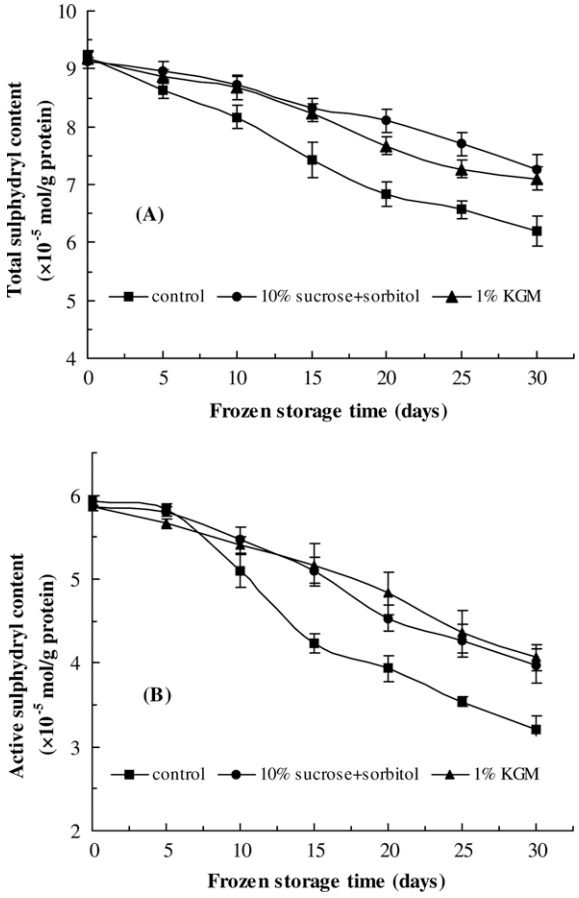


**Fig. 3.** Total sulfhydryl content change (A) and active sulfhydryl content change (B) of grass carp myofibrillar protein with and without cryoprotectants during frozen storage at −18 °C. Bars represent standard deviation from three replications.

Generally, the positive effects of KGM on SEP, $Ca^{2+}$-ATPase activity, total sulphydryl and active sulphydryl contents of myofibrillar protein from grass carp were associated with the chemical structure of KGM which is a water-soluble non-ionic polysaccharide with a large number of hydroxyl groups. During frozen storage of myofibrillar protein, active hydroxyl groups on D-glucose and D-mannose in KGM molecules of KGM molecules could reduce the formation of disulfide bonds, hydrogen bonds, and hydrophobic bonds so as to alleviate protein molecule aggregation and to prevent protein denaturation. In addition, KGM molecules had good water absorptivity because of lots of hydroxyl groups and could enhance the transformation of free water into bound water amongst myofibrillar protein molecules to reduce the temperature of eutectic point and the ice crystal amount and to form incompletely frozen area, which might cause the decrease of protein molecule aggregation and the mitigation of protein denaturation during frozen storage.

### 3.4. Effect of different KGM contents on the texture properties of grass carp surimi gels

From Table 1, there were significant differences ($p < 0.05$) in the breaking force and deformation values of the grass carp surimi gels added with five levels of KGM (0%, 0.5%, 1.0%, 1.5%, and 2.0%, w/w). The breaking force and deformation of the gel sample without KGM (control) were 297.8 g and 6.8 mm, while those of the gel

**Table 1**
Effect of different levels of konjac glucomannan (KGM) on the textural properties, colour parameters, and water-holding capacity of grass carp surimi gels[a].

| KGM (%) | Breaking force (g) | Deformation (mm) | $L^*$ | $a^*$ | $b^*$ | Water-holding capacity (%) |
|---|---|---|---|---|---|---|
| 0.0 | $297.8 \pm 5.91^{D}$ | $6.80 \pm 0.35^{C}$ | $74.6 \pm 1.45^{A}$ | $-3.18 \pm 0.41^{A}$ | $0.14 \pm 0.03^{C}$ | $5.33 \pm 0.30^{A}$ |
| 0.5 | $325.2 \pm 13.53^{C}$ | $7.08 \pm 0.40^{C}$ | $74.3 \pm 3.62^{A}$ | $-3.07 \pm 0.47^{A}$ | $0.70 \pm 0.05^{AB}$ | $5.18 \pm 0.11^{A}$ |
| 1.0 | $335.9 \pm 11.95^{C}$ | $8.13 \pm 0.44^{B}$ | $74.1 \pm 0.95^{AB}$ | $-2.86 \pm 0.07^{A}$ | $0.81 \pm 0.18^{A}$ | $4.95 \pm 0.21^{AB}$ |
| 1.5 | $369.3 \pm 6.41^{B}$ | $9.06 \pm 0.17^{A}$ | $73.7 \pm 1.10^{AB}$ | $-2.77 \pm 0.05^{A}$ | $0.56 \pm 0.07^{B}$ | $4.56 \pm 0.24^{BC}$ |
| 2.0 | $422.3 \pm 11.27^{A}$ | $9.50 \pm 0.28^{A}$ | $70.3 \pm 0.44^{B}$ | $-2.90 \pm 0.13^{A}$ | $0.04 \pm 0.02^{C}$ | $4.11 \pm 0.20^{C}$ |

[a] Values are given as mean ± SD (standard deviation) from three replications. Different upper case letters indicate the significance of difference amongst mean values in the same column at $p < 0.05$.

sample with the highest level of KGM (2%) were 335.9 g and 8.13 mm, respectively. As the levels of KGM increased, breaking force and deformation of the surimi gels made from grass carp increased significantly ($p < 0.05$). The result suggested that KGM could affect the textural properties of the surimi gels to increase the gel-forming ability and improve both the gel strength and elasticity. Zhou, Zeng, Liu, Huang, and Chen (2004) reported the similar result that KGM could improve gel properties of *Aristichthy nobilis* surimi. Therefore, KGM will have potential as a good improver of the gel properties for use in the surimi processing. However, more than 2% KGM added could easily cause the surimi gels to be too hard because of KGM's strong hygroscopicity. In addition, higher levels of KGM significantly reduced the whiteness of the surimi gels (see next section and Table 1). Thus the optimum addition amount of KGM was suggested to be 1%. This was the reason why 1% KGM as a cryoprotectant was selected for preparation of myofibrillar protein from grass carp to test its cryoprotective effect during frozen storage in this study.

### 3.5. Effect of different KGM contents on the colour parameters, whiteness and water-holding capacity of grass carp surimi gels

Addition of different levels of KGM affected the colour parameters ($L^*$, $a^*$, and $b^*$) of the grass carp surimi gels (Table 1). Along with increasing levels of KGM addition, the $L^*$ values decreased and the $a^*$ values had a increasing tendency, but the $b^*$ values did not clearly show an identical tendency. Whiteness is calculated by the formula = $100 - [(100 - L^{*2}) + a^{*2} + b^{*2}]^{1/2}$. It is one of the most important quality indexes for surimi products (Park, 2005). Fig. 4 shows the effect of different levels of KGM on the whiteness of the surimi gels made from grass carp. Basically, as the levels of KGM increased, the whiteness of the surimi gels decreased. Statistical analysis showed a highly negative non-linear relationship between the levels of KGM and the whiteness ($Y = -2.9714X^2 + 1.6229X + 5.438$; $R^2 = 0.99$) (Fig. 4). At ⩽1% level of KGM, the decrease in the whiteness of the surimi gels was not obvious (without significant differences, $p < 0.05$), but at more than 1% level of KGM, the whiteness decreased significantly ($p < 0.05$) (1.5% KGM for 74.1 and 2% for 70.0), in comparison with the control (0% KGM for 78.8). It was considered to be acceptable that the whiteness value of the surimi gels was over 75 (Simpson, Morrissey, Kolbe, Lanier, & MacDonald, 1994). Considering that whiteness is an important quality factor, we suggested that the optimum amount of KGM should be 1% (the corresponding WHC = 77.9 in this study) in the commercial use for grass carp surimi processing.

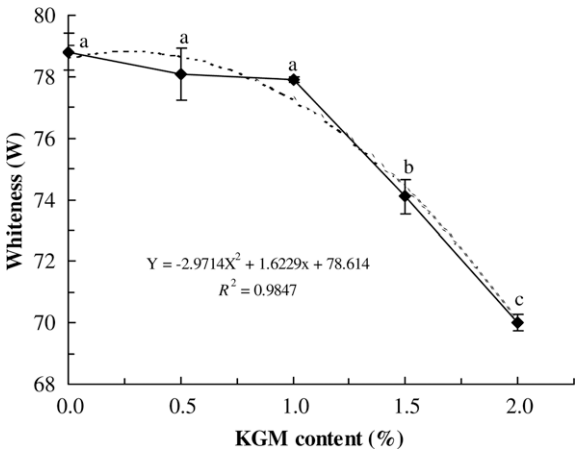


**Fig. 4.** Effect of different levels of KGM on the whiteness ($W$) of grass carp surimi gels. $W = 100 - [(100 - L^{*2}) + a^{*2} + b^{*2}]1/2$. A dotted line indicates a negative non-linear relationship between KGM concentrations and surimi gel whiteness. Bars represent standard deviation from three replications. The different letters on the bars between different KGM contents indicate their significant differences ($p < 0.05$).

Water-holding capacity (WHC) is a physical parameter of surimi gel properties. Higher WHC values indicate higher expressible water in the surimi gel (Ng, 1987), suggesting that the surimi gel has lower water-holding properties. The WHC values of the surimi gels made from grass carp with different levels of KGM are shown in Table 1. Correlative analysis showed a highly negative linear relationship between the levels of KGM and the water-holding capacity ($Y = -0.612X + 5.438$, $R^2 = 0.96$). As the levels of KGM increased, the WHC values of the surimi gels decreased obviously. Higher levels of KGM could significantly ($p < 0.05$) enhance the water-holding properties of the surimi gels, in comparison with the control (0% KGM). It was likely due to the strong water absorptivity of KGM in the surimi gels.

## 4. Conclusions

KGM at the level of 1% (w/w) could effectively mitigate the protein denaturation of grass carp surimi during storage at −18 °C and showed similar positive effects to a commercial cryoprotectant (10% sucrose–sorbitol mixture, 1:1, w/w) on SEP, $Ca^{2+}$-ATPase activity, total sulphydryl and active sulphydryl contents of myofibrillar protein from grass carp. This suggested that KGM as a novel cryoprotectant significantly reduced its usage amount in surimi products to avoid excessive sweet taste and high caloric value caused from the conventional cryoprotectant (sucrose–sorbitol mixture). Additionally, different levels of KGM (0%, 0.5%, 1%, 1.5%, and 2%) had various effects on textural properties, colour parameters (especially whiteness), and water-holding capacity of the surimi gels made from grass carp. Addition with higher levels of KGM in the surimi could significantly increase the breaking force and deformation of the surimi gels and improved their water-holding properties, but when the levels of KGM was over 1% the whiteness of the surimi gels decreased obviously. The optimum addition level of KGM was suggested to be 1% for the grass carp surimi processing. Therefore, KGM may be used as an alternative cryoprotectant and quality improver in surimi products because of its non-calorie, low dosage, and good cryoprotective effect as well as improving gel properties. However, the relevant sensory evaluation is required to

conduct and further studies are also needed to investigate the mechanism of action of KGM and the synergic effect of KGM with other ingredients (e.g. starch) in surimi gels.

## Acknowledgements

This work was supported by The University of Hong Kong Seed Funding for Basic Research and China International Science and Technology Cooperation Funding Programme.

## References

Auh, J. H., Lee, H. G., Kim, J. W., Yoon, H. S., & Park, K. H. (1999). Highly concentrated branched oligosaccharides as cryoprotectant for surimi. *Journal of Food Science, 64*, 418–422.

Benjakul, S., Visessanguan, W., & Srivilai, C. (2001). Porcine plasma protein as proteinase inhibitor in bigeye snapper (*Priacanthus tayenus*) muscle and surimi. *Journal of the Science of Food and Agriculture, 81*, 1039–1046.

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2003). Comparative study on physiochemical changes of muscle proteins from some tropical fish during frozen storage. *Food Research International, 36*, 787–795.

Dave, V., Sheth, M., McCarthy, S. P., Ratto, J. A., & Kaplan, D. L. (1998). Liquid crystalline, rheological and thermal properties of konjac glucomannan. *Polymer, 39*, 1139–1148.

Gornall, A. G., Burdawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reagent. *Biological Chemistry, 177*, 751–766.

Herrera, J. R., & Mackie, I. M. (2004). Cryoprotection of frozen stored actomyosin of farmed rainbow trout by some sugars and polyols. *Food Chemistry, 84*, 91–97.

Hossain, M. A., Alikhan, H. M. A., Ishihara, T., Hara, K., Osatomi, K., Osaka, K., & Nazaki, Y. (2004). Effect of proteolytic squid protein hydrolysate on the state of water and denaturation of lizardfish myofibrillar protein during freezing. *Innovative Food Science and Emerging Technologies, 5*, 73–79.

Huang, C. Y., Zhang, M. Y., Peng, S. S., Hong, J. R., Wang, X., Jiang, H. J., et al. (1989). Effect of konjac food on the blood glucose level in diabetics. *Acta Nutrimenta Sinica, 12*, 360–366 (in Chinese).

Jiang, S. T., Hwang, D. C., & Chen, C. S. (1988). Effect of storage temperature on the formation of disulfides and denaturation of milkfish actomyosin (*Chanos chanos*). *Journal of Food Science, 53*, 1333–1335.

Katsuraya, K., Okuyama, K., Hatanaka, K., Oshima, R., Satoc, T., & Matsuzaki, K. (2003). Constitution of konjac glucomannan: Chemical analysis and $^{13}C$ NMR spectroscopy. *Carbohydrate Polymers, 53*, 183–189.

Li, B., Xie, B. J., & Kennedy, J. F. (2006). Studies on the molecular chain morphology of konjac glucomannan. *Carbohydrate Polymers, 64*, 510–515.

MacDonald, G. A., & Lanier, T. C. (1994). Actomyosin stabilization to freeze—thaw and heat denaturation by lactate salts. *Journal of Food Science, 59*, 101–105.

Ng, C. S. (1987). Measurement of free and expressible drips. In H. Hasegawa (Ed.), *Laboratory manual on analytical methods and procedures for fish and fish products* (pp. 1–2). Singapore: Southeast Asian Fisheries Development Center.

Pang, J. (2003). Progress in the application and studies on functional material of konjac glucomannan. *Chinese Journal of Structural Chemistry, 22*, 633–642.

Park, J. W. (1996). Temperature-tolerant fish protein gels using konjac flour. *Journal of Muscle Food, 7*, 165–174.

Park, J. W. (2005). *Surimi and surimi seafood* (2nd ed.). Boca Raton, FL: Taylor & Francis/CRC Press.

Peng, S. S., Zhang, M. Y., Zhang, Y. Z., & Wu, Z. H. (1994). Effect of the refined konjac meal on cell aging of brain, liver and cardiovascular tissues in rats. *Acta Nutrimenta Sinica, 16*, 280–284 (in Chinese).

Ramirez, J. A., Martin-Polo, M. O., & Bandman, E. (2000). Fish myosin aggregation as affected by freezing and initial physical state. *Journal of Food Science, 65*, 556–560.

Shenouda, S. Y. K. (1980). Protein denaturation in frozen fish. *Advances in Food Research, 26*, 275–311.

Simpson, R., Morrissey, M. T., Kolbe, E., Lanier, T. C., & MacDonald, G. A. (1994). Effects of varying sucrose concentrations in Pacific whiting (*Merluccius productus*) stabilized mince used for surimi production. *Journal of Aquatic Food Product Technology, 3*, 41–52.

Sych, J., Lacroix, C., Adambounou, L., & Castaigne, F. (1990). Cryoprotective effects of lacticol, palatinit and polydextrose on cod surimi proteins during frozen storage. *Journal of Food Science, 55*, 356–360.

Tyre, C. L., & Chong, M. L. (1992). *Surimi technology*. New York, NY: Marcel Dekker Inc.

Vuksan, V., Jenkins, D. J. A., Spadafora, P., Owen, R., Vidgen, E., Brighenti, F., et al. (1999). Konjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes – A randomized controlled metabolic trial. *Diabetes, 22*, 913–919.

Zhang, M. Y., Huang, C. Y., Peng, S. S., Wang, X., Hong, J. R., Zhang, C. W., et al. (1990). The effect of refined konjac meal on colonic function of the subject with constipation. *Acta Nutrimenta Sinica, 12*, 185–190 (in Chinese).

Zhang, M. Y., Huang, C. Y., Wang, X., Hong, J. R., & Peng, S. S. (1989). The influence of konjac food on human lipid metabolism. *Acta Nutrimenta Sinica, 11*, 25–30 (in Chinese).

Zhang, Y., Xie, B., & Gan, X. (2005). Advance in the applications of konjac glucomannan and its derivatives. *Carbohydrate Polymers, 60*, 27–31.

Zhang, N., Yamashita, Y., & Nozaki, Y. (2002). Effect of protein hydrolysate from Antarctic krill meat on the state of water and denaturation by dehydration of lizard fish myofibrils. *Fisheries Science, 68*, 672–679.

Zhou, A. M., Benjakul, S., Pan, K., Gong, J., & Liu, X. (2006). Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. *Food Chemistry, 96*, 96–103.

Zhou, A. M., Zeng, Q. X., Liu, X., Huang, W. H., & Chen, Y. Q. (2004). Study on the improvement of gel properties of *Aristichthy nobilis* surimi. *Journal of South China Agricultural University, 25*, 104–107 (Natural Science Edition) (in Chinese).