A Manuscript Submitted to

International Journal of Food Engineering

Manuscript 2407

FIRST STEPS IN USING GLUCOMANNAN TO MAKE THERMOSTABLE GELS FOR POTENTIAL USE IN MINCE FISH REESTRUCTURATION

Beatriz Solo-de-Zaldivar, *CSIC*
BEATRIZ HERRANZ, *CSIC*
Javier Borderias, *CSIC*

FIRST STEPS IN USING GLUCOMANNAN TO MAKE THERMOSTABLE GELS FOR POTENTIAL USE IN MINCE FISH REESTRUCTURATION

Beatriz Solo-de-Zaldivar, BEATRIZ HERRANZ, and Javier Borderias

Abstract

A methodology for making thermostable konjac glucomannan (KGM) gels was examined with a view to eventual use with fish mince to make restructured products. A gelation method was developed with 3 and 6 % (w/v) aqueous dispersions of glucomannan (ADG) by adding alkali v/v (NaOH or KOH) up to pH 11.8- 12 and then allowing it to set (1 hr at 30ºC and 4 hrs at 5ºC). Both 3 and 6 % are suitable concentrations and KOH and NaOH (at 0.6 and 1M) are the most suitable alkalis for deacetylation of the glucomannan at pH 11.8- 12.0.

KEYWORDS: Glucomannan, thermostable gels, deacetylation

1. INTRODUCTION

When fish mince is used as a raw material it sometimes lacks functionality as a result of prior processing or because it contains too much fat. It is not possible to make fibre- or myotome-like structures using the conventional restructuring procedure. To avoid these problems, the technological properties of Konjac glucomannan (KGM) offer an interesting, new and unexplored possibility for restructuring this type of products. One way to achieve this would be to make KGM gels that can retain the unfunctional minced muscle. This is an unexploited technology and only a very few papers have been published reporting addition of KGM in powder form as an ingredient (Iglesias-Otero, Borderias et Tovar, 2010; Park, 1996; Xiong et al. , 2009)

KGM is a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. (Nishinari, Williams et Phillips, 1992) that has long been used as a thickener and gelling agent in traditional Asian foods and is generally recognized as safe (GRAS) (Chua, et al., 2010; Nishinari et al., 1992; Thomas, 1997). It is composed of β-(1 - 4) linked D-mannose and D-glucose in a molar ratio of 1.6:1 (Kato and Matsuda, 1969; Katsuraya, et al., 2003; Maeda et al., 1980) and some branches linked to this backbone, although the exact branched position is still debatable (Katsuraya et al., 2003; Nishinari et al., 1992). KGM backbone has a low proportion of acetyl groups (approximately 1 acetyl group per 19 residues) (Kato and Matsuda, 1969; Maeda et al., 1980; Maekaji, 1974), which is assumed to be an important factor promoting the solubility of KGM in water. It is well known that KGM generally forms a thermally irreversible gel in the presence of an alkaline coagulant (Huang et al., 2002; Jacon et al., 1993; Williams et al., 1999; Zhang et al., 2001). The effect of the addition of alkali is to facilitate deacetylation of the KGM chains, although the mechanism is not fully understood. (Huang et al., 2002; Jacon et al., 1993; Williams et al., 2000; Yoshimura and Nishinari, 1999; Zhang et al., 2010).

The aim of this paper is to study the initial steps in the use of KGM to make thermo-irreversible gels strong enough to be used eventually in minced fish restructuring. The paper also examines solubilization conditions, concentrations and types of alkalis used for the deacetylation step in order to make suitable gels.

2. MATERIAL AND METHODS

Purified glucomannan from konjac (KGM ≥ 99%) was purchased from Guinama, Valencia. All chemical reagents were supplied by Panreac, Química S.A., Barcelona and were of reagent grade.

Preparation of glucomannan gels

Aqueous dispersions of glucomannan (AGD) were prepared at 1, 3 and 6% (w/v) total polysaccharide concentration using distilled warm water at 60ºC in a Stephan vacuum cutter machine (Stephan, Universal Machine UC12, Stephan u. Söhne GmbH & Co., Hameln, Germany) for 30 minutes and then left to cool at room temperature. Then, different alkali aqueous solutions were added to these dispersions for the deacetylation step: NaOH, KOH or Na_2CO_3 (Panreac Química, S. A., Barcelona, Spain) at three concentrations: 0.2M, 0.6M and 1M each one until the pH reached 11.8- 12.0 (in the present work the pH was determined by FT-IR as described below). The samples were placed in Petri dishes (1.5 cm thick and 90 cm diameter) then set at 30ºC for 1 hour and then at 5ºC for 4 hours. The gels were neutralized by placing them in a 0.2M citrate-phosphate buffer at pH 5 (gel:buffer proportion 1:10) for 20 hours. In this way heat-stable gels were produced with an approximate pH of 6.5-7.

Analyses

FT-IR spectroscopy analysis:

AGD (3%) was mixed with different amounts of a solution of NaOH 1M to achieve different pHs (6.3, 7.5, 8.2, 10, 11.2 and 12) and analysed by FT-IR. The FT-IR spectrophotometer used to record spectra was a FT-IR / FT-NIR, Spectrum-400, from Perkin-Elmer (USA). The samples were freeze-dried and dispersed in Fluorolube, which only fits absorption bands in IR below 1500 cm^{-1} and therefore does not interfere in the observation of the acetyl bands. The dispersion was carried out in the agate mortar. Once a very fine powder had been obtained, a small quantity was mixed with CaF_2 crystals, they were mounted on the supports and transmission measured in the FTIR. In all cases, IR spectrum were recorded by accumulation of at least 32 scans, with a resolution of 2 cm^{-1} in the 4000 - 500 cm^{-1} range. Measurements were carried out in triplicate. The spectra was normalized to the acetyl band area. In this way the pH of the sample was associated with the relative intensity of the acetyl bands and hence to the degree of acetylation of the glucomannan. The spectral data were processed with the Grams /AI (Thermo Electron Corporation, Waltham, MA) software, which includes baseline correction, smoothing (with a nine-point Savitsky-Golay function) to reduce the noise, and band area measurement.

pH:

The pH was determined in quadruplicate using a pH meter (Thermo-Orion 720, Spain) with a specific electrode for direct measuring on the mixture (Thermo-Orion Sure-flow 9165BNWP, Spain).

Water Binding Capacity (WBC):

Samples were cut into small pieces, weighed (approx. 2g) and then placed in a centrifuge tube (10 mm diameter) with filter paper as absorber (Whatman nº1). Samples were then centrifuged in a Heraeus Multifuge 3 Plus centrifuge (USA) for 10 min at 3000×g at room temperature. Water Binding Capacity (WBC) was expressed as % water retained per 100 g water present in the sample prior to centrifuging. Measurements were carried out in triplicate.

Colour:

The surface colour of the samples was evaluated on a colorimeter (Minolta. CR-400 Konica-Minolta, Japan) (D65/2º), using CIELab scale (Lightness, L*; redness, a* and yellowness, b*).Whiteness index was determined using the following formula: $100 - [(100-L^*)^2 + a^{*2}b^{*2}]^{1/2}$ (Park, 1995). Colour determinations were carried out after neutralizing time. Determinations were performed on five points per formulation.

Puncture test:

Gels (cylindrical samples of diameter 3 cm x height 3.5 cm) were penetrated to breaking-point using a TA-XT plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a rod with spherical tip (5 mm diameter (P/5S)). Cross-head speed was 1mm/s and a 5 Kg load-cell connected to the crosshead of the Texture Analyzer was used. Breaking force [N] and breaking deformation [mm] were determined in the force-deformation curves. Puncture tests were carried out on gels kept at room temperature. All determinations were carried out at least in quadruplicate.

Statistical analysis:

Analysis of variance (One–way ANOVA) was carried out to evaluate the statistical significance ($p < 0.05$). Statistical analysis was performed using Statgraphics Plus version 5.0.

3. RESULT AND DISCUSSION

KGM dispersion conditions

First at all it is very important to get a homogeneous solution of glucomannan. Glucomannan forms highly viscous solutions when dissolved in water (Kök et al., 2009; Shinzato et al., 1996) but it is not always easy to obtain a homogeneous

dispersion. Hence, different concentrations of glucomannan (1-6%) were studied at various temperatures within the range reported in the literature (50-100ºC) and times (30- 90 min) in order to obtain a homogeneous solution without lump formation at the lowest temperature and in the shortest time possible. The best dispersion conditions were 30 min at 60ºC with continuous stirring in a homogenizer

After that, the next step was to determine the pH level that would assure complete deacetylation so as to convert the glucomannan solution into a thermo-stable gel

Determining the deacetylation pH by analysis of the acetyl bands measured by FTIR.

To determine the pH level that would assure deacetylation of the KGM, FTIR analysis was performed in AGD at different pHs. A solution of 1M NaOH was added to different 3% AGD aliquots to obtain a range of diverse pHs (6.3, 7.5, 8.2, 10, 11.2 and 12). This range of pH values was based on the one described by Thomas (1997), who reported pH values of 9-10, and Kohyama and Nishinari (1990), who reported 11.3- 12.6 for KGM gelation. In this case other lower pHs were checked to observe the gradual disappearance of acetyl groups as a function of pH.

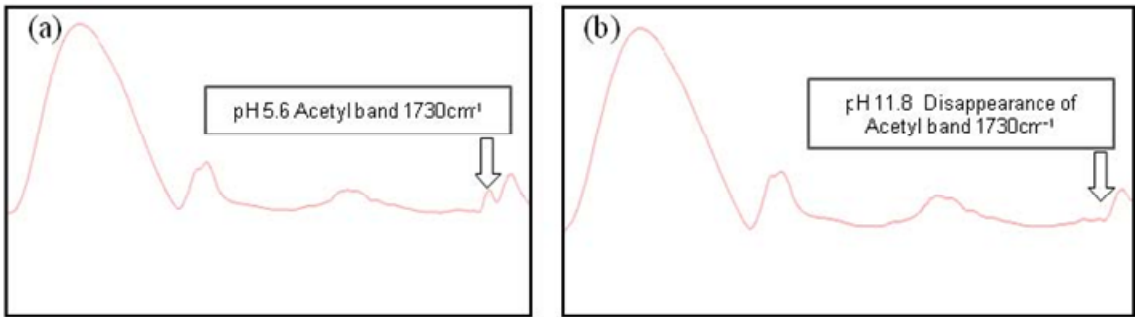

Figure 1. IR IR spectrum of glucomannan at: (a) pH native (pH 5.6) with acetyl band at 1730 cm-1 and (b) pH of gelation (pH 11.8) without acetyl band.

As shown in the spectrum in Figure 1a, the band at 1730 cm^{-1} is visible in glucomannan dispersions at pH = 5.6 (before alkali addition) and practically disappears when the dispersion reaches a pH value of 11.8 (Figure 1b). These results agree with the findings of other authors (Maekaji, 1974; Jacon et al. 1993; Zhang et al, 2001), who assigned this peak to the C=O group. One may therefore

assume that this peak corresponds to the acetyl group and its removal from the KGM backbone initiates the polymer chain interactions to form a gel (Zhang et al., 2001). Figure 2 shows the regresion line of sample pH in relation to the normalized area of the above-mentioned band at 1730 cm^{-1}. This figure shows that the area of the mentioned band is nil from pH 11.8.

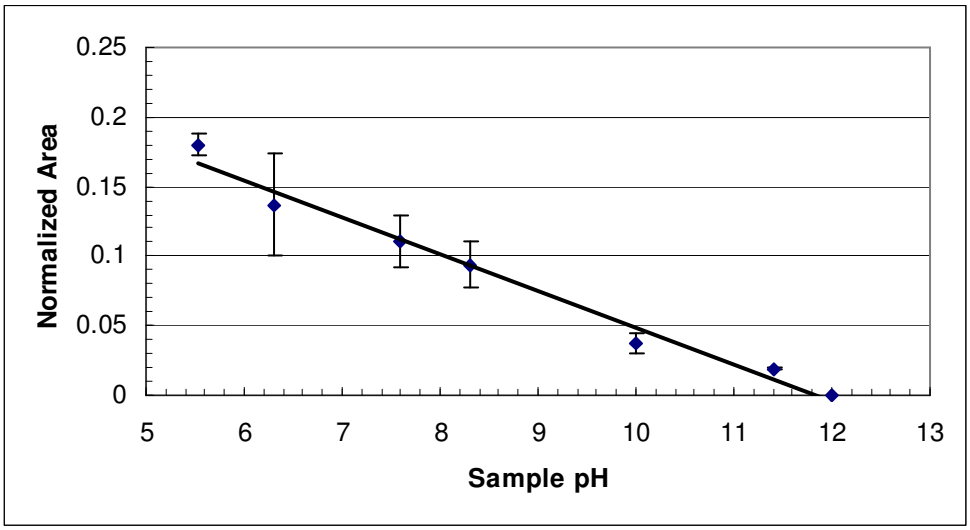

Figure 2. Lineal regression between pH and relative intensity of the acetyl bands.

Selection of type and concentration of alkali added for deacetylation:

Various types of alkaline solutions at different concentrations (0.2, 0.6 and 1M) were added at 1, 3 and 6% (w/v) AGD until the pH reached 11.8- 12.0, to obtain a gel of a firmness and texture similar to that of whole fish muscle. As a guideline, various alkalis were selected (KOH, NaOH and Na_2CO_3) from Thomas (1997), which describes the relative gel strength of different glucomannan gels with different alkalis added at different pHs.

Table 1 shows the breaking force (N) and breaking deformation (mm) of these gels at 1 day of chilled storage. Glucomannan gels were more rigid when made at a concentration of 6% than 3% as shown by the significant differences ($p<0.05$) in breaking force values (Table 1A), but breaking deformation was similar in all gels (Table 1B). On the other hand, the concentration of alkali did not significantly influence the breaking force of gels with 6% glucomannan. Breaking force values

Table 1. Breaking force (N) and breaking deformation (mm) in gels at 1, 3 and 6% of glucomannan (w/v) deacetylated with NaOH, KOH and Na_2CO_3 at different concentrations.

A.

	Breaking force (N)		
Concentration of AGD*	**1%**	**3%**	**6%**
Type of alkali & Concentration			
0.2M NaOH	NG**	NG**	NG**
0.6M NaOH	NG**	0.86 ± 0.20 d	4.1 ± 1.0 a
1M NaOH	NG**	1.37 ± 0.39 c	4.11 ± 0.73 a
0.2 M KOH	NG**	NG	NG**
0.6 M KOH	NG**	1.04 ± 0.16 c,d	3.50 ± 0,55 b
1M KOH	NG**	0.94 ± 0.10 c,d	3.7 ± 1.1 a,b
0.2M Na_2CO_3	NG**	NG**	NG**
0.6M Na_2CO_3	NG**	NG**	0.441 ± 0.03 d
1M Na2CO_3	NG**	NG**	1.12 ± 0.29 c,d

*AGD: Aqueous glucomannan dispersion.
**NG: Not gel formation.
a-d Different letters indicate significant differences ($p < 0.05$) among gels.

B.

	Breaking deformation (mm)		
Concentration of AGD	**1%**	**3%**	**6%**
Type of alkali & concentration			
0.2M NaOH	NG**	NG**	NG**
0.6M NaOH	NG**	8.68 ± 0.81 e,f	9.4 ± 1.3 c,d,e
1M NaOH	NG**	8.9 ± 1.8 d,e,f	9.85 ± 0.79 b,c
0.2M KOH	NG**	NG**	NG**
0.6 KOH	NG**	9.49 ± 0.48 b,c,d	9.49 ± 0.70 b,c,d
1M KOH	NG**	8.33 ± 0.57 f	8.49 ± 0.34 f
0.2M Na_2CO_3	NG**	NG**	NG**
0.6M Na_2CO_3	NG**	NG**	10.80 ± 0.76 a,b
1M Na2CO_3	NG**	NG**	11.3 ± 1.4 a

*AGD: Aqueous glucomannan dispersion.
**NG: Not gel formation.
a-f Different letters indicate significant differences ($p < 0.05$) among gels.

were higher in glucomannan gels made at a concentration of 3% with NaOH at 1M than at 0.6M. Breaking deformation, was only affected by alkali concentration

($p<0.05$) at both glucomannan concentrations in gels made with KOH. Breaking deformation was higher at 0.6M than at 1M KOH concentration.

All the gels were translucent with high whitness values except the ones made with Na_2CO_3, which had a light brown colour (Table 2). This high whiteness value is important because it can be transmited to the mince. European consumers consider whiteness a quality factor for white fish products (Ang, 1993).

Table 2. Whitenes index of gels at 1, 3 and 6% of glucomannan (w/v) deacetylated with NaOH, KOH and Na_2CO_3 at different concentrations.

	Whitenes Index		
Concentration of AGD*	**1%**	**3%**	**6%**
Type of alkali & Concentration			
0.2M NaOH	NG**	NG**	NG**
0.6M NaOH	NG**	48.0 ± 1.6 c	52.13 ± 0,66 a
1M NaOH	NG**	44.95 ± 0.48 d	50.5 ± 1.3 a,b
0.2 M KOH	NG**	NG**	NG**
0.6 M KOH	NG**	40.4 ± 1,7 f	49,2 ± 1,2 b,c
1M KOH	NG**	42,8 ± 1,6 e	50,89 ± 0.72 a
0.2M Na_2CO_3	NG**	NG**	NG**
0.6M Na_2CO_3	NG**	NG**	31.33 ± 0.95 g
1M $Na2CO_3$	NG**	NG**	32.27 ± 0.81 g

*AGD: Aqueous glucomannan dispersion.
**NG: Not gel formation.
a-g Different letters indicate significant differences ($p < 0.05$) among gels.

Gels made with 3 and 6 % glucomannan deacetylated by 0.6 and 1 M solutions of NaOH and KOH were therefore chosen on the basis of their mechanical properties and colour. Gels made with N_a2CO_3 as the alkali were not suitable for our purpose because they were very soft at the same glucomannan concentration and also sticky, and their colour was too dark

The water binding capacity (WBC) measured in gels made by alkalinization with KOH and NaOH is shown in Figure 3. WBC values were around 70, except for gels containing 1M NaOH, where the value was almost 80%, and 1M KOH gels, whose values were slightly lower; however, there were no significant differences

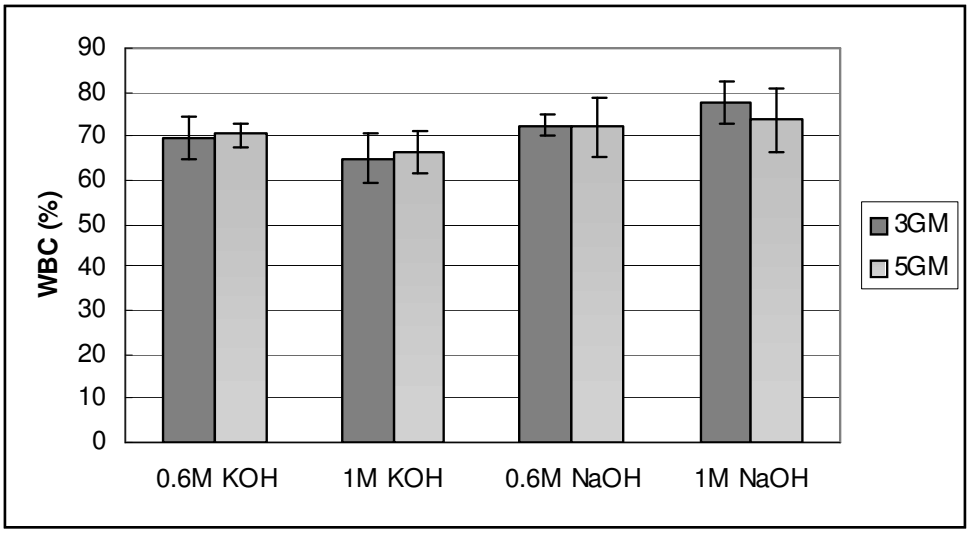

Figure 3. WBC (%) (b) of the different gels elaborated with NaOH and KOH at 0.6N and 1N. 3GM= 3% glucomannan gels, 5GM= 5% glucomannan gels.

($p<0.05$) among any of the gels. The extraordinarily high WBC of KGM has already been reported by some authors. One gram of KGM is reportedly able to absorb up to 200 ml of water (Maeda et al., 1980; Wen et al., 2008).
Briefly then, based on the power of different added alkalis to deacetylate glucomannan, KOH and NaOH are suitable gelling agents at concentrations of 0.6M and 1M to be used.

4. CONCLUSION

Aquous dispersions of glucomannan at concentrations of 3 and 6 % made following the procedure reported above are suitable for deacetylation with 0.6M KOH and 1M NaOH at pH 11.8- 12.0. This produces thermostable gels with an appropiate texture for structural formation when used in combination with fish mince.

REFERENCES

Ang, J. F. (1993). Reduction of fat in fried batter coatings with powdered cellulose. *Journal of the American Oil Chemists' Society, 70,* 619-622.

Chua, M., Baldwin, T.C., Hocking, T. J., & Chan, K. (2010). Traditional uses and potential health of *Amorphophallus konjac* K. Koch ax N.E.Br. *Journal of Ethnopharmacology, 12,* 268-278.

Huang, L.,Takahashi, R., Kobayashi, S., Kawase, T., & Nishinari, K. (2002). Gelation behavior of native and acetylated konjac glucomannan. *Biomacromolecules, 3,,* 1296-1303.

Iglesias-Otero, M. A., Borderias, J., & Tovar, C. A. (2010). Use of konjac glucomannan as additive to reinforce the gels from low quality squid surimi. *Journal of Food Engeenering, 101,* 281- 288.

Jacon, S. A., Rao M. A., Cooley, H. J., & Walter, R. H. (1993). The isolation and characterization of a water extract of konjac flour gum. *Carbohydrate Polymers, 20,* 35-41.

Kato, K., & Matsuda, K. (1969). Studies on the chemical structure of konjac mannan. *Agricultural and Biological Chemistry, 33,* 1446-1453.

Katsuraya, K., Okuyama, K., Hatanaka, K., Oshima, R., Sato, T., & Matsuzaki K. (2003). Constitution of konjac glucomannan: chemical analysis and 13C NMR spectroscopy. *Carbohydrate Polymers, 53,* 183-189.

Kök, M. S., Abdelhameed, A. S., Ang, S., Morris, G. S., & Harding, S. E. (2009). A nobel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan. *Food Hydrocolloids, 23,* 1910–1917.

Kohyama, K., & Nishinari, K. (1990). Dependence of the specific volume of konjac glucomannan on pH. In G. O. Phillips and D. J. Wedlock & P. A. Williams (Eds.), *Gums and Stabilisers for the Food Industry, 5,* (p. 459). Oxford: IRL Press.

Maeda, M., Shimahara, H., & Sugiyama, N. (1980). Studies of mannan and related-compounds .5. detailed examination of the branched structure of konjac glucomannan. *Agricultural and Biological Chemistry, 44,* 245-252.

Maekaji, K. (1974). Mechanism of gelation of konjac mannan. *Agricultural and Biological Chemistry, 38,* 315-321.

Nishinari, K., Williams, P. A., & Phillips, G. O. (1992). Review of the physico-chemical characteristics and properties of konjac mannan. *Food Hydrocolloids, 6,* 199-222.

Park, J. W. (1995). Surimi gel colors as affected by moisture-content and physical conditions. *Journal of Food Science, 60,* 15-18.

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

Shinzato, C., Broussalis, A. M., & Ferraro, G. E. (1996). Glucomanano: Un aporte a su control de calidad. *Revista de la Asociación Argentina de Farmacia y Bioquímica Industrial (SAFYB) 35,* 26-31.

Thomas, W. R. (1997). Konjac gum. In Imeson A, (Ed.), *Thickening and gelling agents for foods* (pp.169-179). London: Blackie Academic & Professional.

Wen, X., Wang, T., Wang, Z. Y., Li L., & Zhao, C. S. (2008). Preparation of konjac glucomannan hydrogels as DNA-controlled release matrix. *International Journal of Biological Macromolecules, 42,* 256-263.

Williams, M. A., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. *Biomacromolecules, 1,* 440-450.

Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., Geng, S., Geng, S., Chen, M., Corke, H., & Cai, Y. Z. (2009). Effect of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (*Ctenopharyngodon idella*) . *Food chemistry, 116,* 413- 418.

Yoshimura, M., & Nishinari, K. (1999). Dynamic viscoelastic study on the gelation of konjac glucomannan with different molecular weights. *Food Hydrocolloids, 13,* 227-233.

Zhang, F.Y., Zhou Y.M., Sun,Y.-Q., Ye, X.Y., & Huang, J.Y. (2010). Preparation and characterization of Chitosan/Konjac glucomannan/CdS nanocomposite film with low infrared emissivity. *Materials Research Bulletin, 45,* 859-862.

Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A. K., Foster, T. J., & Norton, I. T. (2001). Gelation behaviour of konjac glucomannan with different molecular weights. *Biopolymers, 59,* 38-50.