



## Study on swelling model and thermodynamic structure of native konjac glucomannan

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**Abstract:** We investigated the higher structure of konjac glucomannan (KGM) in the amorphous state and solution using a laser particle size analyzer and a water activity meter. The results show that the thermodynamic structures of native KGM were primarily composed of the lamella structure units, which involve both granular crystalline and amorphous regions, and that the connection zones of such units contained both loose and tight aggregation regions. The value of surface tension ( $\sigma$ ) of native KGM, resting with the density of its hydroxyl groups' self-association, was an important parameter to analyze the higher structures of native KGM in the thermodynamic swelling model of native KGM.

**Key words:** Swelling model, Thermodynamic structure, Konjac glucomannan (KGM), Higher structure

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### INTRODUCTION

Konjac glucomannan (KGM) is an  $\alpha$ -(1,4) linked polysaccharide with a D-glucosyl (G) and D-mannosyl (M) backbone, possibly lightly branching through  $\beta$ -(1,6) glucosyl units (Katsuraya *et al.*, 2003). The M:G ratio was reported to be approximately 1.6:1 (Ratcliffe *et al.*, 2005; Kato and Matsuda, 1969; Koroskenyi and McCarthy, 2001). The short side branches and acetyl groups in konjac exist randomly at the C-6 position of the sugar units. Gelation occurs through a 3D hydrogen-bounded network (Tye, 1991). The 3D-structure of KGM crystalline state is a two-fold helix stabilized by intramolecular O-3---O-5' hydrogen bonds, with the O-6 rotational position gt (Yui and Ogawa, 1992). The higher structure of native KGM reveals actinomorphic micelle distribution and includes no quite ordered structure. The degree of crystallization of native KGM is up to 16.1% (Pang *et al.*, 2003), which in-

dicates that the higher structure of native KGM is mainly on the amorphous state. Absence of order within polymer molecules of native KGM leads to poor quality X-ray diffraction patterns. The details of higher structure of native KGM are still unknown. The X-ray diffraction method is the most important tool to determine the 3D-structure of carbohydrate. It can produce high-quality X-ray diffraction data and pose a particularly acute molecular structure. However, this method is limited, because it can only determine the molecular structure in solid, but in the amorphous state or solution. Recent rapid development in nuclear magnetic resonance (NMR) and calculation chemistry not only compensates for the deficiency of the X-ray method in solid state, but also reveals the conformations and interactions of carbohydrates in solution. In addition, dynamic structure analyses of carbohydrates and polysaccharides are also available. When the three methods are combined, they undoubtedly become more powerful tools (Ogawa, 1997). In the present study, we systematically analyzed the dynamic structures of native KGM

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in the amorphous state and in solution using swelling dynamics model and thermodynamic theory in order to offer theoretical guidance for its application.

## MATERIALS AND METHODS

### Materials

Native KGM was supplied by Guangzhou M&S Food Technology Ltd., China. Ethanol and other chemicals were all obtained from Chemical Reagent Company, Shanghai, China. All other chemicals were of extra-pure grade or analytical grade.

### Methods

#### 1. Theoretical description

A binary solvent system (water and ethanol) was designed in order to study and control the swelling behavior of native KGM at different water activities. In this experiment, we established a swelling model to simplify the ternary system, which was composed of native KGM and the binary solvent. The assumptions were based on the following: (1) The interaction between ethanol hydroxyl groups with native KGM hydroxyl groups can be negligible. (2) The change of native KGM's chemical potential is equal to the change of native KGM's surface free energy during the course of swelling. (3) The experimental data have been determined at infinite dilution of native KGM in ternary system, and the affection of native KGM on the liquid-liquid equilibrium of binary solvent system can be negligible.

According to the thermodynamics theory, if the difference of chemical potential between native KGM and swelling medium is larger than zero ( $\Delta\mu > 0$ ), the native KGM will absorb water and swell until  $\Delta\mu = 0$ ; When swelling equilibrium, the relationship between the surface tension of the native KGM and the partial potential of water can be described by Eq.(1):

$$n_w d\mu_w + n_p d\mu_p = 0, \quad (1)$$

where  $\mu_w$  is the partial potential of water,  $n_w$  is the molar number of water,  $n_p$  is the molar number of native KGM, and  $\mu_p$  is the partial potential of native KGM.

According to the assumption (2),  $n_p d\mu_p = -w_p \sigma dA$ , where  $A$  denotes the specific surface area of native

KGM;  $\sigma$  is defined as the surface tension of native KGM and is a variable parameter, which is mainly affected by the dispersion force and the density of hydroxyl groups' self-association of native KGM; and  $w_p$  is the mass of native KGM. Then Eq.(1) can be written as Eq.(2):

$$n_w d\mu_w - w_p \sigma dA = 0. \quad (2)$$

Undoubtedly, when the formula  $\mu_w = \mu_w^\circ(T, P) + TR \ln a_w$  is substituted into Eq.(2), Eq.(2) transforms into Eq.(3):

$$\sigma dA = (n_w RT) \cdot da_w / (a_w w_p), \quad (3)$$

where  $R$  is the universal gas constant,  $T$  the temperature, and  $a_w$  water activity. Then Eq.(3) transforms into a definite integral Eq.(4):

$$\int_{A_1}^{A_2} \sigma dA = \frac{n_w RT}{w_p} \ln(a_{w_2} / a_{w_1}), \quad (4)$$

where  $A_1$  and  $A_2$  are the specific surface areas at water activities  $a_{w_1}$  and  $a_{w_2}$ , respectively. When  $a_{w_2} - a_{w_1}$  is quite close to zero,  $\sigma$  can be taken as a constant parameter  $\bar{\sigma}$ , and Eq.(4) transforms into Eq.(5):

$$\bar{\sigma} = \frac{n_w RT}{(A_2 - A_1)w_p} \ln(a_{w_2} / a_{w_1}). \quad (5)$$

#### 2. Water activity measured in the ternary system by a water activity meter

The water activity ( $a_w$ ) was measured using an Aqua Lab water activity meter (Model 3TE, Decagon devices, Pullman, WA, USA). For all measurements, the volatile sample block was used as an accessory to the instrument. With this sample block, the water activity can be determined in the presence of other semi-volatile components. Experimental errors for the volatile sample block are  $\pm 0.015 a_w$ . To correct errors from instrument drifts and offset, the performance of the sample block was frequently controlled and readjusted with reference samples. All measurements were performed at 298 K. Eight samples were obtained with ethanol of various concentrations (10%, 16%, 20%, 25%, 30%, 33%, 40%, and

50%), and then the native KGM was added to produce the eight ternary systems in the ratio of 0.01 g KGM/100 g water, respectively. The water activities of the eight ternary systems were estimated after the swelling equilibrium.

3. Specific surface area of native KGM determined by a laser particle size analyzer

The specific surface area of native KGM in the different ratio of ethanol and distilled water was determined by a laser particle size analyzer (BT-9300, Dandong Baite Instrument Co., Ltd., Dandong, China) with a time resolution of 15 s after swelling equilibrium at 298 K.

4. Optical micrograph of native KGM in swelling procedure

An optical micrograph of the swelling native KGM in different water activities was obtained by an Olympus BH-2 light microscope (original magnification 100 $\times$ ) and the difference of water swelling state was investigated.

### Data analysis

This swelling model, an average value of surface tension  $\bar{\sigma}$  difference-integration model Eq.(5), can transform swelling curve of native KGM into a 1D feature vector, the numerical solution of  $\bar{\sigma}$  obtained using subdomain method, so it can implement classification of swelling curve using classical clustering method. Through clustering calculation of swelling curve from  $\bar{\sigma}$ , it is shown that swelling curve can be classified accurately and efficiently using a 2-segment model and square sum of deviations in the swelling model Eq.(5). Moreover, the segment model can be used to analyze the thermodynamic layer structure of native KGM and the uniformity of the densities of hydroxyl groups' self-association further. In order to reduce experimental error, at least five replicate tests were performed.

## RESULTS AND DISCUSSION

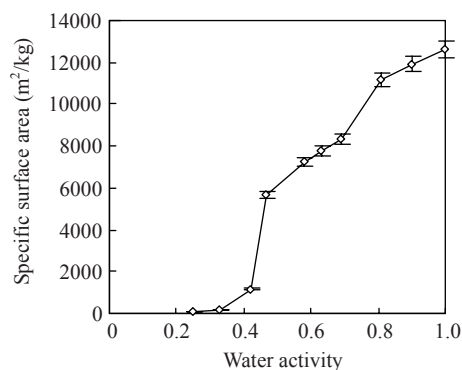
### Water activity

The swelling kinetics of native KGM was studied when it was suspended in water-ethanol binary solvent. The specific surface area of native KGM changes was monitored as one of the functions of water activity until equilibrium was observed. The

data of specific surface area of native KGM in different ambient water activities obtained in the experiment are shown in Table 1. The variation of water activity affected by specific surface area is shown in Fig.1.

**Table 1** Water activity and specific surface area of native KGM (298 K)

Sample No.	Ethanol contents (% , v/v)	Water activity	Specific surface area of native KGM (m <sup>2</sup> /kg)
1	50	0.25	85 $\pm$ 3
2	40	0.33	143 $\pm$ 5
3	33	0.42	1170 $\pm$ 35
4	30	0.47	5861 $\pm$ 180
5	25	0.58	7220 $\pm$ 220
6	20	0.63	7761 $\pm$ 240
7	16	0.69	8353 $\pm$ 260
8	10	0.81	11178 $\pm$ 340
9	5	0.90	11910 $\pm$ 360
10	0	1.00	12639 $\pm$ 380



**Fig.1** The plot of specific surface areas of native KGM with different water activities of native KGM, in which KGM was swollen in water-ethanol binary solvent and specific surface area was defined as an thermodynamic characterization of native KGM

### Optical micrograph of native KGM in swelling process

In swelling process, water can plasticize the natural polymer or form stable bridges through intermolecular hydrogen bonds. In addition, thermal properties of cellulose and water are markedly influenced through their interaction (Kennedy *et al.*, 1989; Hatakeyama and Hatakeyama, 1998; Hatakeyama *et al.*, 2000). Depending on the polymer characteristics and the physical associations between water and polymers, cellulose is considered as a structurally heterogeneous polymer, consisting of

paracrystalline and amorphous domains. Water can interact with the amorphous domains, but it is excluded almost completely from the crystalline regions (Princi *et al.*, 2005). Water molecules can break intermolecular hydrogen bonds among cellulose chains to make the intermolecular distance when they were diffused into the amorphous region. Therefore, water absorbed can cause an increase in cellulose chain mobility by opening the intermolecular space and allowing more water molecules to influx and form hydrogen bonds with cellulose. At saturation, water absorption in the amorphous domains leads to the swelling and the formation of trapped water.

The swelling process of native KGM, as shown in optical micrographs (Fig.2), indicated that three steps might be involved in the procedure. The first step is that the hydrogen bonds of loose aggregation region between lamellar structure units of native KGM were broken by water, within the range of water activity at 0.25~0.42 (Figs.2a~2c). At saturation, water absorption in the loose aggregation region between lamellar structures units leads to swelling and forming the trapped water. The gap of native KGM surface can be observed in Figs.2a~2c. The second step is that the hydrogen bonds of tight aggregation region between lamellar structure units of native KGM were reestablished by water, in the range of water activity at 0.47~0.69 (Figs.2d~2f). In the range of water activity at 0.47~0.69, water molecules might break intermolecular hydrogen bonds among lamellar structure units when they diffuse into the tight

aggregation region between lamellar structures units, increasing the distance between lamellar structures units. Therefore, water absorbed might cause an increase in lamellar structures units' mobility by opening the space between lamellar structures units and allowing more water molecules to enter and form hydrogen bonds with lamellar structure units. The single lamellar structure units can be observed clearly in Fig.2f. The third step is the decomposition of the amorphous regions of lamellar structure units (Figs.2g~2h). The hydrogen bonds among amorphous regions in lamellar structure units were broken by water (in the range of water activity at 0.81~1.0). Water absorbed caused an increase of the distance between granular crystalline by opening the intermolecular space and allowing more water molecules to enter and to form hydrogen bonds with cellulose chains. The decomposition of lamellar structure units by water can be observed, while granular crystalline will not be decomposed by water.

### Swelling model

#### 1. Thermodynamic characterization of native KGM swelling model

Using experimental data of  $A_1$ ,  $A_2$ ,  $a_{w_1}$ ,  $a_{w_2}$ ,  $n_w$ ,  $w_p$  and  $R$  for certain numbers ( $n_w/w_p=555.55$  mol/g) at room temperature ( $T=298$  K), when  $a_{w_2} - a_{w_1}$  is the smallest one,  $\sigma$  can be taken as a constant parameter  $\bar{\sigma}$ , the swelling model can be obtained from Eq.(5).

Since the specific surface area of native KGM is

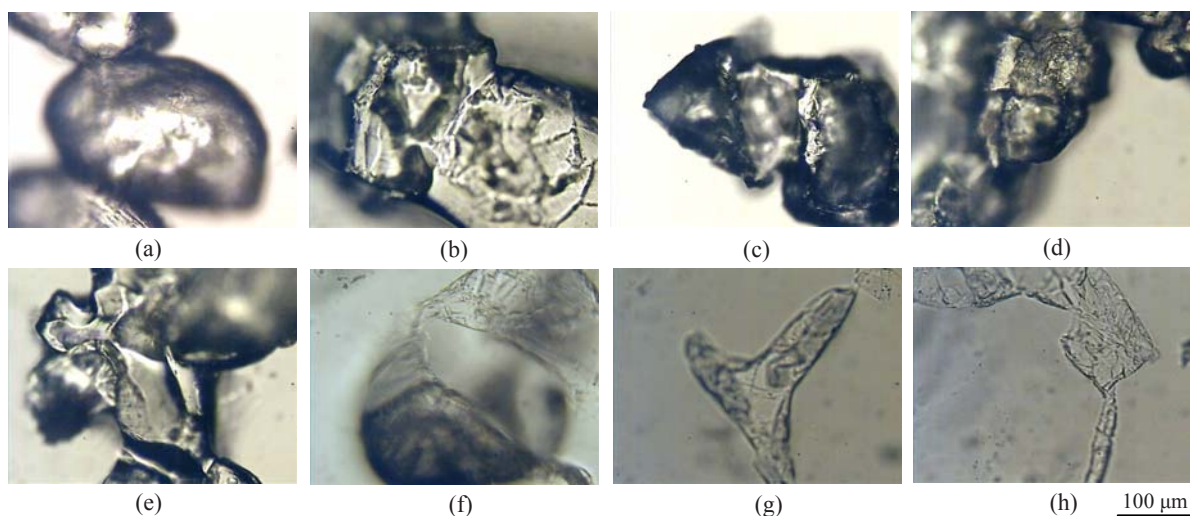
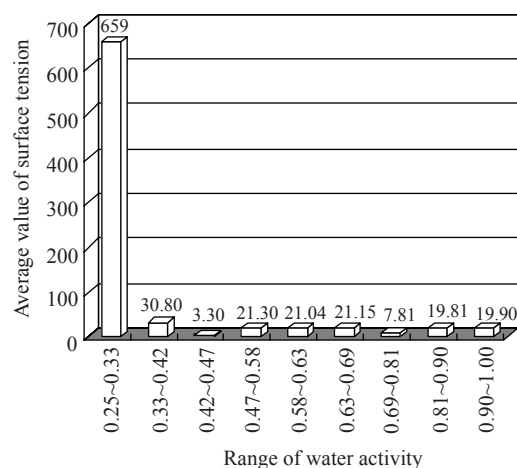


Fig.2 Optical micrograph showing the swelling process of native KGM with different water activity ( $a_w$ ). (a)  $a_w=0.25$ ; (b)  $a_w=0.33$ ; (c)  $a_w=0.42$ ; (d)  $a_w=0.47$ ; (e)  $a_w=0.58$ ; (f)  $a_w=0.69$ ; (g)  $a_w=0.81$ ; (h)  $a_w=0.90$

a measurable parameter, it is possible to evaluate the average value of surface tension in different ranges of water activities from relations established through Eq.(5). Fig.3 indicates that when water activity at the range of 0.25~0.33, the average value of surface tension of native KGM was very high. It suggests that the specific surface area of native KGM has less change at the range of water activity 0.25~0.33, which indicates that the number of activated water molecules to break the hydrogen bonds between lamellar structures units (loose aggregation region) is fewer at the range of water activity 0.25~0.33. At the range of water activity 0.33~0.42, the average value of surface tension of native KGM was 30.8. In this swelling process, the hydrogen bonds of loose aggregation region between lamellar structure units were broken almost completely by active water molecules. At the range of water activity 0.42~0.47, the average value of surface tension of native KGM was 3.3. In this swelling process, the specific surface area of native KGM increased rapidly at the range of water activity 0.42~0.47, which implied that the distances between lamellar structures units (loose aggregation region) in native KGM rapidly enlarged with the increase of water activity. The potential change of water partly compensates for the energy loss of native KGM molecule conformation changes. At the range of water activity 0.47~0.69, the average value of surface tension of native KGM was approximately up to 21. In this process, it indicates that the intermolecular hydrogen bonds between lamellar structure units (tight aggregation region) were broken by water molecules and that the density of hydroxyl groups' self-association among lamellar structure units was uniform. At the range of water activity 0.69~0.81, the average value of surface tension of native KGM was 7.81. The distances among lamellar structure (tight aggregation region) rapidly enlarged with the increase of water activity. The partial potential change of water compensates for the energy loss of KGM molecule conformation changes. At the range of water activity 0.81~1.0, the average value of surface tension of native KGM was approximately 19.8. In this swelling process, water molecules could interact with the hydrogen bonds of amorphous regions in lamellar structure, but they were excluded almost completely from the granular crystalline in lamellar structure. At the ranges of water activities 0.42~0.47 and

0.69~0.81, Fig.3 shows that the distance between lamellar structures (loose aggregation region in native KGM and tight aggregation region in native KGM) increased rapidly after the hydrogen bonding and that at the same short range van der Waals forces had been broken by water. This is the reason of the rapid increase of the specific surface area of native KGM.

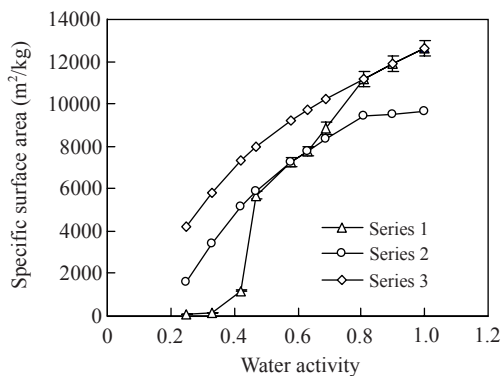


**Fig.3** Graphic representation of the average value of surface tension ( $\bar{\sigma}$ ) in different range of water activity when  $a_{w_2} - a_{w_1} \rightarrow 0$  as a thermodynamic characterization of native KGM swelling model (KGM swollen in water-ethanol binary solvent)

2. Comparison of the data of swelling model with experimental data

In this work, the data of swelling model (Eq.(5)) were series 2 and 3, with  $\bar{\sigma}$  as constant parameters 21.15 and 19.85, respectively. The results were used to test the uniformity of the densities of hydroxyl groups' self-association among lamellar structure units and the densities of hydroxyl groups' self-association in the amorphous regions of lamellar structure. From a thermodynamic point of view, the results could be used to analyze the thermodynamic layer structure of native KGM based on the variety of the surface tension ( $\bar{\sigma}$ ) of native KGM. Based on the results shown in Fig.4, the densities of hydroxyl groups' self-association in the amorphous regions of lamellar structure are uniform, but the densities of hydroxyl groups' self-association among lamellar structure units are not quite uniform because of the different aggregation degree between lamellar structure units. These phenomena are based on the actinomorphic distribution of lamellar structure units in

native KGM. The experimental data at the ranges of water activity 0.47~0.69 and 0.81~1.0 fit the data of swelling model (Eq.(5)) series 2 and series 3, respectively. This indicates that native KGM exhibits the thermodynamic layer structure.



**Fig.4 The interaction of water activity and the specific surface area of KGM**

Series 1: the experiment data of native KGM while swelling equilibrium at different water activity; Series 2: the data of swelling model (Eq.(5)) while  $\bar{\sigma}$  as a constant parameter 21.15; Series 3: the data of swelling model (Eq.(5)) while  $\bar{\sigma}$  as a constant parameter 19.85

### Thermodynamic structures analysis

From the thermodynamic point of view, the swelling behavior depends on the chemical structure and physical properties of the materials, and also on the ambient water activity. On the contrary, the formation of higher structure in native KGM is a dehydration process, which displays in reverse swelling order.

Granular crystalline is first formed in the process of formation of higher structure in native KGM. Its formation primarily depends on the conformation of cellulose chains. In this process, cellulose chains must overcome the hampering of the large entropic barriers because of the need to arrange a large number of monomers linked to each other sequentially. The large entropic barriers are defined as activity energy. Therefore, one of the major factors that affect the formation of granular crystalline is ambient temperature of native KGM in dehydration process, which can be the explanation for the decrease of the viscosity of dried native KGM products and the increase of the crystallization degree in dried native KGM products (dried at above temperature 80 °C).

The second step is to form lamellar structure as a supramolecule. If the lamellar structure forms in a

quite ordered way, KGM molecules must have overcome large entropic barrier hamper and steric effects. At the constant temperature of 70 °C, based on the Ostwald step rule, when the transformation goes from an unstable (or metastable) state to a stable one, the system does not go directly to the most stable conformation but prefers to reach intermediate stages having the closest free-energy to the initial state (Ostwald, 1897; Larini and Leporini, 2006). Therefore, the lamellar structure will be composed of granular crystalline and amorphous region.

The third step is the formation of native KGM, and the lamellar structures are associated with each other through hydrogen bonding to form native KGM. The connection zones between lamellar structures are divided into loose aggregation region and tight aggregation region owing primarily to the entropic barrier and energy barrier. The hydrogen bonds of loose aggregation region were broken by water molecules at the range of water activity 0.25~0.42, and then, the hydrogen bonds of tight aggregation region were broken by water molecules at the range of water activity 0.47~0.69.

### CONCLUSION

Based on the thermodynamic swelling model of native KGM, the experimental data of BT-9300 laser particle size analyzer, and optical micrographs, we can deduce that the thermodynamic structures of native KGM are primarily composed of the lamella structure units. We observed that the connection zones between lamellar structures contained both loose aggregation region and tight aggregation region, and that the lamella structure units contained both granular crystalline and amorphous region. In the swelling processes of native KGM, the value of surface tension of native KGM depended on the density of hydroxyl groups' self-association of native KGM, namely, aggregative state. When the water activity increased, the hydrogen bonds of loose aggregation region between lamella structure units were broken by water molecules. Then the hydrogen bonds of tight aggregation region between lamella structure units were broken by water molecules, and also the hydrogen bonds of amorphous region in lamella structure units were broken by water molecules. The

changes of the average value of surface tension of native KGM reflect the thermodynamic structures of native KGM, which is an important parameter to analyze the higher structures of native KGM in the thermodynamic swelling model of native KGM.

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