

Deacetylation of Konjac Glucomannan by Mechanochemical Treatment

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ABSTRACT: This article presents the experimental results on deacetylation of a konjac glucomannan (KGM) powder with alkali in mechanochemical (MC) treatment by a dry vibrating mill. It is indicated that the acetyl group of the KGM powder could be removed by means of the MC method. In addition, some characteristics, such as gel rheol-

ogy, particle size/conformation, water absorbency, and thermal behavior, of the deacetylated KGM are analyzed. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 1566–1573, 2008

Key words: deacetylation; mechanochemistry; grinding; Konjac glucomannan

INTRODUCTION

Konjac glucomannan (KGM), a kind of natural material, is regarded as a noncalorie food, and one of the primary benefits of traditional foods made from konjac flour is to supply indigestible high quality dietary fibers. Besides, the KGM has been widely used for modification of carbohydrate metabolism in diabetics, reduction of plasma cholesterol, scavenging with free radical for isolated islets, and inhibition of tumor genesis/metastasis as well. This natural material can be also used for the preparation of composite materials, edible film, coating/packaging film, biodegradable film, and controlled release matrix.^{1–7} In the molecularity, the KGM is a neutral polysaccharide derived from the tubers of *amorphophallus konjac* C. Koch, consisting of β -1, 4-linked D-glucose, and D-mannose units with a glucose: mannose ratio of around 1 : 1.6.⁸ There are some branching points at the C-3 position of the glucomannan backbone. The backbone of KGM possesses 5–10% of the acetyl-substituted residues.^{9,10} An acetyl group is attached to one per 19 sugar residues.¹¹ It has been proved that the presence of this group confers solubility on the glucomannan in aqueous solution (it should be noted that unsubstituted β -1, 4-linked glucomannan is insoluble in water).¹² Figure 1 shows its structural representation.

Some studies^{5,11,13–20} have been carried out to improve the properties of the KGM by alkaline treatment for deacetylation. The chemical deacetylation of the KGM gels with alkaline occurs, which can form a heat-stable gel. This should be because that the deacetylation of the KGM causes the association between acetyl free regions of the backbone, leading to the formation of junction zones, and a three dimensional network. In addition, the alkali treatment at pH > 11 results in the ionization of hydroxyl groups and increased solvation, promoting solubility.¹⁸ It was shown that the deacetylation generated by the addition of alkali is a triggering step leading to the gelation.¹⁹ However, the gelation mechanism for the KGM has not been fully comprehended.^{11,20} The deacetylation of the KGM gels has been performed by chemical methods in solvents (preferably in water) by alkali treatment so far, leading to the complicate procedure and wastewater treatment. Recent attention to solid-phase reaction by the mechanochemical (MC) treatment has been increased due to the growing economical and ecological requirements.²¹ This MC technology for processing the initial or intermediate products changes the quality of characteristics of the final material, so that it is possible to obtain a product of higher quality with the advanced properties. It has been also proved that the MC treatment is a simple and effective process without the subsequent treatment of waste.²¹ The purpose of this study is to investigate the deacetylation of KGM by the MC treatment in a vibrating mill. In addition, the characteristics, such as gel rheology, particle size/conformation, water absorbency, and thermal behavior, of the deacetylated KGM products are analyzed.

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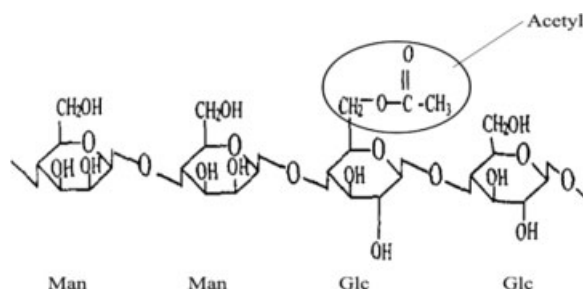


Figure 1 The structural representation of konjac glucomannan.

MATERIALS AND METHODS

Materials

A KGM powder, supplied by Tianshan Food, Yongshan, China, was used as a sample material in this study. All chemicals used are analytical reagents.

Equipment and procedure

For the deacetylation in a MC treatment, the KGM powder was dried in an electrical oven for 3 h at 60°C. The KGM powder with 2% NaOH was treated in a model M-3 dry vibrating mill (the mill volume: 1.8 L; manufactured by Beijing Hongding Basic Technology and Development, China). Figure 2 shows the structure of the mill. The stainless steel balls with various sizes of $\Phi 15$, $\Phi 8$, and $\Phi 6$ mm (in the volume ratio: 28 : 42 : 30) were selected as grinding media. The media load was 80 vol % of the grinding chamber volume. The treatment duration in the vibrating mill was 5, 10, 15, 20, and 25 min. After dry grinding, each powdered sample was first put into 50% absolute ethyl alcohol, and the suspension was neutralized to pH = 7 with 6 mol/L of hydrochloric acid, and then was filtrated. The wet particulate material after filtration was dried at 60°C in an electric oven. The dry powdered samples were used for the subsequent analysis.

Measurements

Fourier transform infrared spectra

After drying, Fourier transform infrared spectra of the KGM powder samples were recorded in the wave number range of 400–4000 cm^{-1} on a Fourier transform infrared spectra (FTIR) spectrometer (Nexus, Thermo Nicolet, USA) with diffuse reflectance attachment. The disc sample of KBr/powder in the spectrometer served as a window during the FTIR studies.

Degree of acetylation

To determine the degree of acetylation in the KGM samples before and after the MC treatment, 1 g of the KGM powder with 50 mL of 75% ethanol was mixed in a 250-mL conical flask. The mixed suspension was heated to 50°C and stirred for 30 min, and then was cooled down to room temperature. The suspension with 40 mL of 0.5 mol/L KOH solution in the conical flask was sealed for 72 h to perform the deacetylating reaction under stirring; After the reaction, the excess of alkali was back nitrated with 0.5 mol/L hydrochloric acid with phenolphthalein as an indicator. The titration process was executed twice within 2 h. The titration process for each sample was repeated for three times and the repeated results were calculated to obtain the average value. The degree of acetylation (%) in the sample was calculated by the following formula

$$\% \text{ acetyl} = \frac{(\bar{V}_a - \bar{V}_b) N_{\text{HCl}} M_{\text{acetyl}}}{m_s} \times 100\% \quad (1)$$

where \bar{V}_a is the average value of the volume of hydrochloric acid consumed for the blank in liters, \bar{V}_b is the average value of the volume of hydrochloric acid consumed for the sample in liters, N_{HCl} is the molarity of the hydrochloric acid, M_{acetyl} is the molecular mass of acetyl, and m_s is the weight of the sample in grams.²²

Particle characterization

Particle size/formation analysis of the sample before and after the MC treatment in the mill was carried out in a model BT9300S laser particle size analyzer and a model BT-1600 image analyzer (Better Instrument, China), respectively.

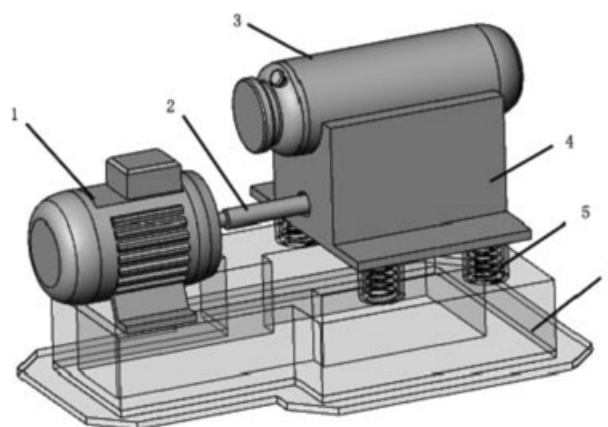


Figure 2 Schematic structure of dry vibrating mill; 1-electromotor, 2-elastic coupling, 3-barrel, 4-vibrator, 5-springs, 6-bearing.

TABLE I
Rate of Deacetylation at Various MC Treatment Durations

Treatment duration (min)	0	5	10	15	20	25
\bar{V}_b (mL)	34.63	35.14	35.29	35.54	35.75	36.02
\bar{V}_a (mL)			36.55			
% Acetyl ^a	4.13	3.03	2.71	2.17	1.72	1.07
Rate of deacetylation (%)	0	26.63	34.38	47.46	58.35	74.09

^a Calculated in eq. (1).

Rheological behavior

The rheological measurements on the gels with 1.0 wt % of the KGM samples were performed in a model R/S-SST 2000 Controlled stress rheometer (Brookfield, USA), using a CC45 rotating spindle/stationary system.

Water absorbency

Water absorbency of the nondeacetylated and deacetylated samples was determined by the following method: 0.1 g of dry sample was placed into a centrifuge tube of 50 mL, and then weighed (W_1). After adding 30 mL deionized water, the gel was allowed to stand for swelling completely. The gel was then dewatered in a centrifuge at 3000 rpm for 5 min. The remaining (W_2) was weighed when the supernatant was removed. The weight difference was determined as the amount of water absorbed in the samples.

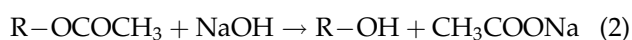
Thermal analyses

The thermal analysis of the KGM samples was carried out with a model STA 449C thermal analysis instrument (NETZSCH, Germany). The samples of 5–8 mg in a crucible with a reference sample of α - Al_2O_3 were analyzed in the N_2 gas atmosphere at a heating rate of 10 K/min in a temperature range of 293–673 K.

RESULTS AND DISCUSSION

Deacetylation by mechanochemical treatment

Deacetylation of the KGM powder was carried out by a MC treatment in a vibrating mill with NaOH as a modifier. Under such conditions, a saponification reaction between the KGM ($\text{R}-\text{OCOCH}_3$) and alkali (i.e., NaOH) occurs according to the following equation:



where R is the remaining group of the KGM.

Table I shows the rate of deacetylation and the degree of acetylation in the KGM samples with respect to the effect of the MC treatment duration. Clearly, the rate of deacetylation increases and the degree of acetylation decreases with increasing the treatment duration.

Also, Figure 3 shows FTIR spectra of the KGM samples before and after various durations of MC treatment. The FTIR spectra show two characteristic absorption bands of the mannose near 805 and 879 cm^{-1} because of the basic unit of the mannan in the nondeacetylated sample. It could be concerned that they are caused by a D-mannose residue in the polymers irrespective of the configuration and the position of the mannosidic linkage.²³ These two absorption bands were also observed for the KGM samples treated mechanochemically. It is indicated that the backbones of KGM samples before and after MC treatment have the same backbone structure. For the KGM, a stretching vibration of $-\text{OH}$ groups shows a broad band around 3434 cm^{-1} . The absorption bands near 2924, 1376, and 1069 cm^{-1} indicate the stretching vibration of $-\text{CH}_2-$ groups. The $\text{C}=\text{O}$ groups show the absorption at 1733 cm^{-1} , indicating the existence of carbonyl of acetyl groups. The absorption band at 1640 cm^{-1} is attributed to the intramolecular hydrogen bonds.^{24,25} However, the absorption band of acetyl groups becomes smaller with increasing the treatment duration in the range of 0–20 min, and then disappears when the KGM is treated for 25 min. Meanwhile, the stretching band of hydroxyl around 3434 cm^{-1} becomes larger with increasing the MC treatment duration. This could be partly due to the deacetylation.²⁶

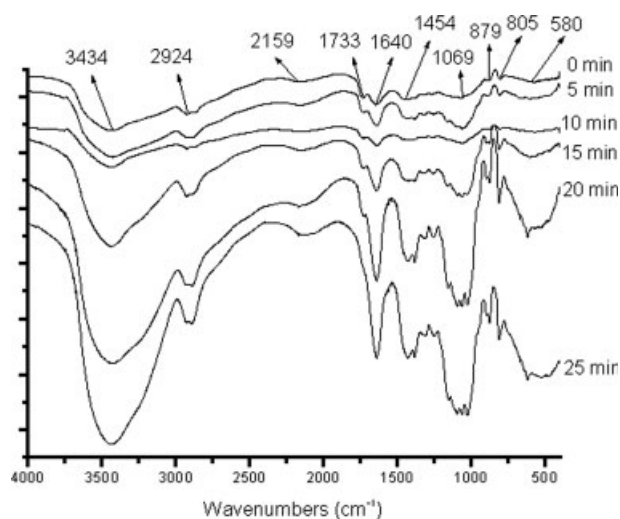


Figure 3 FTIR spectra of KGM powders with and without mechanochemical treatment.

Characteristics of products before and after deacetylation

Figures 4 and 5 show the rheological behavior of KGM gels before and after deacetylation in the MC treatment by grinding. The shear-thinning appears for each KGM gel. In general, the shear-thinning

phenomenon is due to the structural changes of the KGM gel with respect to the effect of shear rate. Shear-thinning is particularly common to systems containing asymmetric colloids, i.e., macromolecule polymers. Asymmetric particles disturb the flow lines to a greater extent when they are randomly

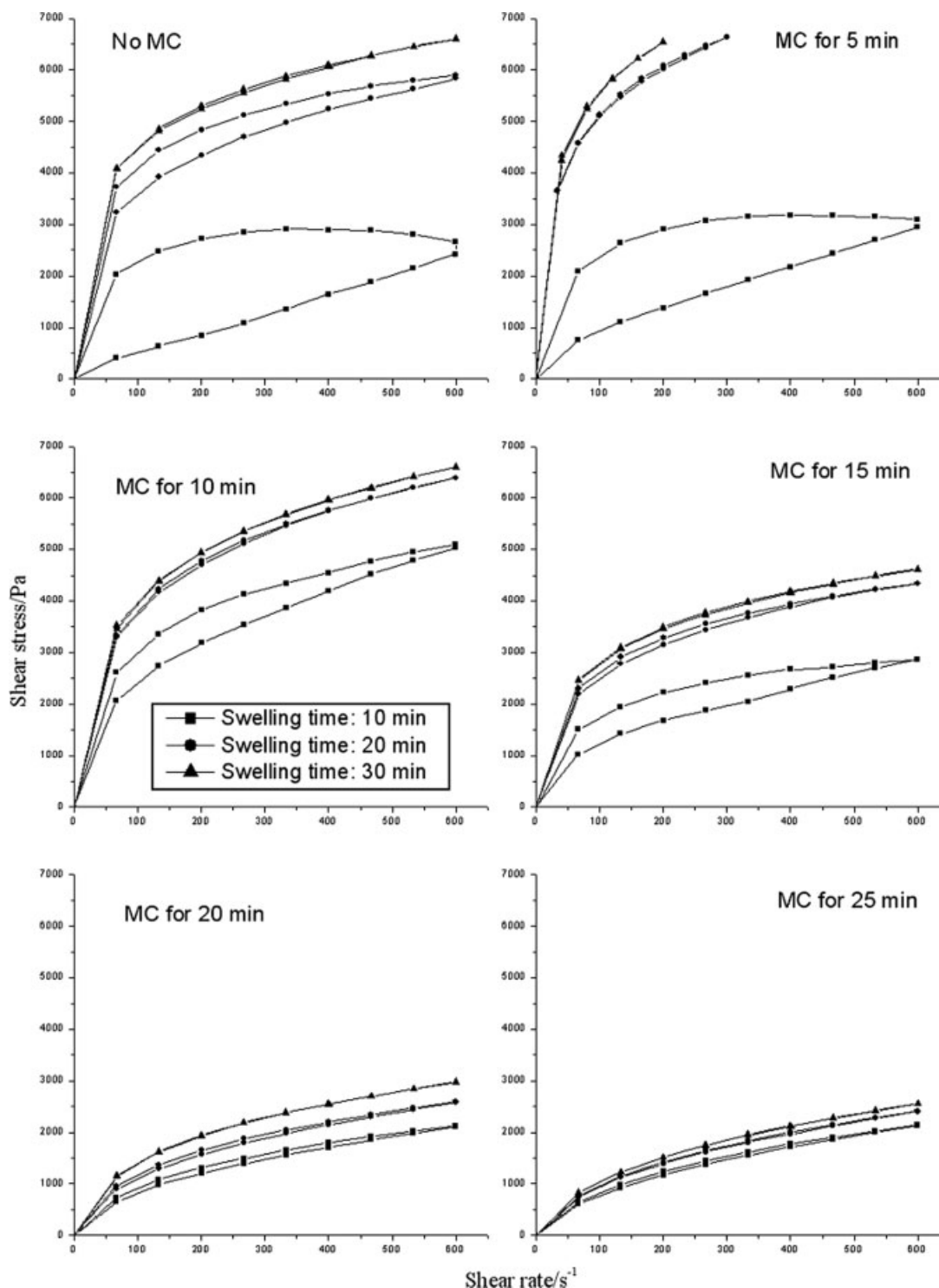


Figure 4 Thixotropic hysteresis loops for the KGM gels before and after deacetylation in MC treatment by grinding with respect to the swelling time.

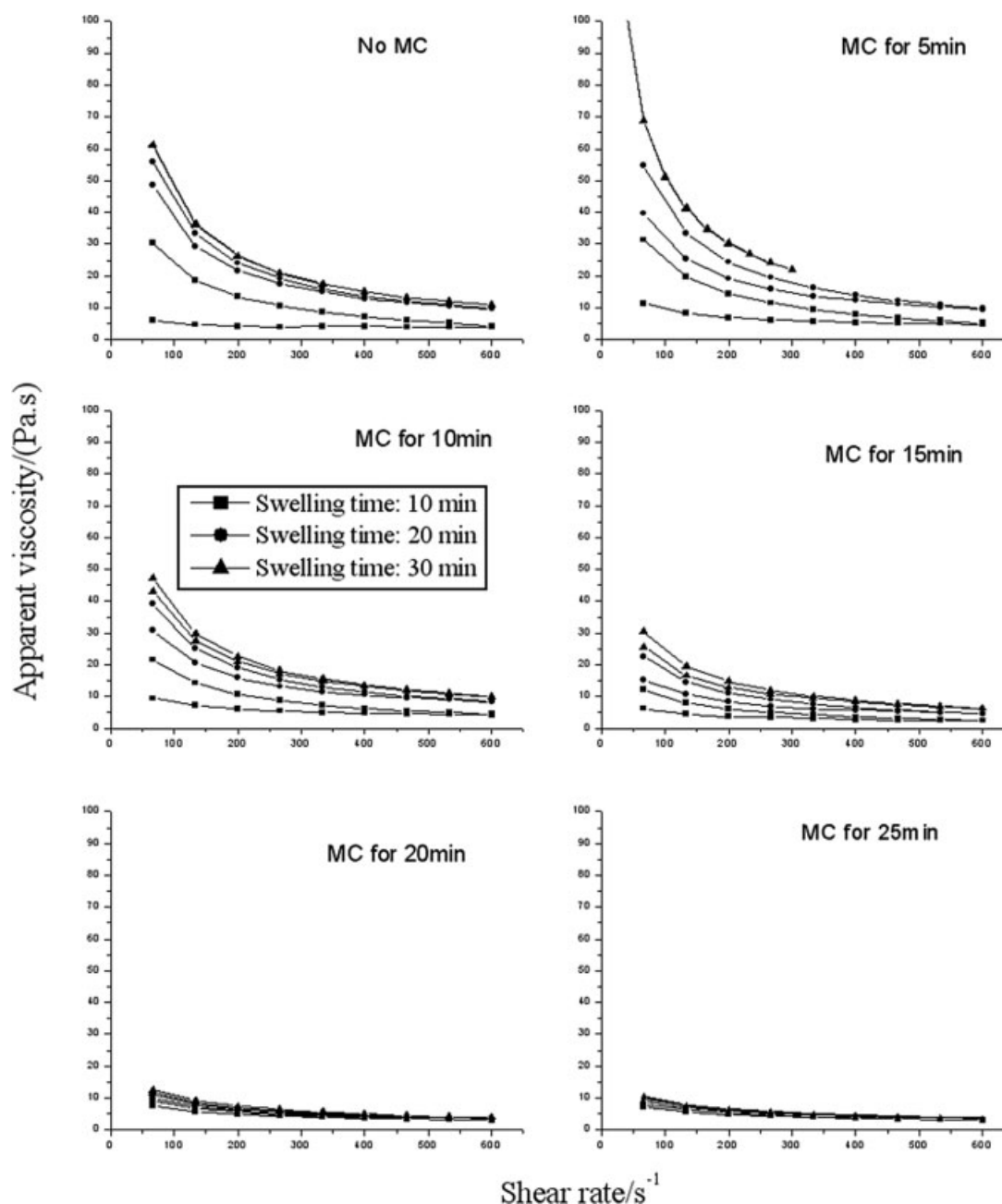


Figure 5 Apparent viscosities of KGM gels at various swelling time with respect to the duration of the MC treatment in milling.

orientated at low velocity gradients rather than when they have been aligned at high velocity gradients. In addition, the colloid interaction and the solvent immobilization are favored when prevailing over the random orientation.²⁷

The majority of the food gels show a time-dependent flow behavior.²⁸ A thixotropic hysteresis loop can be obtained by measuring the nonequilibrium shear stress as the shear rate is first increased and then decreased in a standard way. The area of the thixotropic hysteresis loop indicates the extent of thixotropy of colloids. Thixotropic behavior means

the reduction in structural strength during the shear load phase and the more or less rapid, but a complete structural regeneration during the subsequent rest phase.²⁹ Figure 4 shows the thixotropic hysteresis loops for the KGM gels before and after deacetylation in the MC treatment by grinding with respect to the swelling time. Clearly, the area of thixotropic hysteresis loop decreases gradually with increasing swelling time, and disappeared in the end, indicating that the KGM gel after the deacetylation loses the thixotropic property. Also, it is seen that the area of thixotropic hysteresis loop decreases with

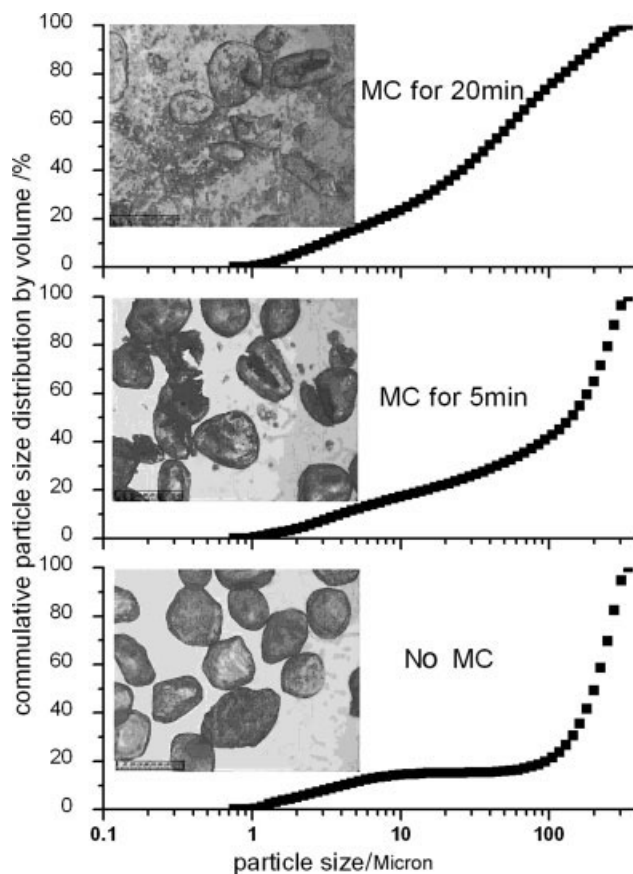


Figure 6 Particle size/formation of KGM before and after MC treatment.

increasing the duration of the MC treatment. This could be since the acetyl group in the KGM reduced after the deacetylation. The two- or three-spiro hydrocarbons of the KGM are fractured by the MC treatment. Thus, the hydrogen bonding interaction of the deacetylated KGM chains increases after eliminating the repulsive forces of the main chains. The molecular structure becomes denser, which is favorable to the formation of the structural network. Also, the chains length becomes shorter after fractured after the MC treatment. These may result in a significant decrease of the thixotropy in the deacetylated KGM. A previous work¹⁹ indicated that the mechanical treatment of starch could reduce the thixotropy.

Figure 5 shows a relation between the apparent viscosity of KGM gel and the MC treatment duration. It is interesting to see that the KGM gel obtained by the MC treatment for 5 min possesses the maximum apparent viscosity. This illustrates that a moderate MC treatment is beneficial to increase the viscosity of the KGM gel. It could be mainly due to the effect of particle size (Fig. 6).³⁰ It is also noticed that the rate of deacetylation in the sample treated for 5 min is only 26.63%, indicating that the deacetylation in this case could not be effec-

tive to decrease the gel viscosity. However, the prolonged MC treatment after 5 min will lower the viscosity of the KGM gel. It is known that the deacetylated KGM gel possesses a lower viscosity since the molecules of the deacetylated KGM vary from the semiflexible straight chain into the elastic microsphere, and cause the unsymmetry, indicating the formation of an ordered structure.¹⁴ Therefore, the deacetylation of the KGM in the MC treatment by prolonged milling can lower the apparent viscosity of the KGM gel. Deacetylation dominates over the reduction of the apparent viscosity rather than the physical changes (i.e., particle size/conformation). Figure 6 shows that the MC treatment for 20 min gives a finer KGM product with the rough particle surface. However, the reduction may be not simply due to deacetylation and may also relate to the other chemical changes of the deacetylated KGM (i.e., the gelation and the aggregation kinetics), which may appear indistinguishable. Williams et al.¹⁹ discussed that the addition of alkali into the KGM clearly plays an important solubilizing role in the gelation of konjac mannan in addition to facilitating the deacetylation of the chain. Figure 5 also shows that the viscosity of the KGM gels treated in the MC treatment increases with increasing the swelling time when the KGM was treated by the MC before 20 min. However, this variation appears insignificant for the gels of the KGM treated for over 20 min.

Table II shows the water absorbency of the KGM samples before and after the deacetylation. Clearly, the nondeacetylated KGM has the lowest water absorbency, compared to the deacetylated KGM. Also, the MC treatment of the KGM produced an increase in the water absorbency. This is partly due to the higher rough surface area available and a loss of the highly ordered structure after the MC treatment (Fig. 6). This may be also attributed to the increased amount of the hydroxyl (OH^-) of the deacetylated KGM, resulting in the increased water absorbency. Cheng et al.⁵ concerned that the alkaline deacetylation of the KGM molecules could permit greater intermolecular interactions with water.

Thermal stability analysis for the original KGM sample and the deacetylated KGM sample after the

TABLE II
Water Absorbency of the KGM Samples Before and After Deacetylation

Treatment duration (min)	0	5	10	15	20
W_1	13.101	12.581	12.559	12.589	13.619
W_2	15.850	15.633	15.790	16.093	17.879
Water absorbency	2.75	3.05	3.23	3.50	4.26
Relative water absorbency (%)	100	110	117	127	131

TABLE III
Thermal Properties of Original and Deacetylated KGM

Sample	DSC				TGA			
	Endothermic peak		Exothermic peak		1st Mass loss stage		2nd Mass loss stage	
	Temperature (°C)	Heat flow (mW/mg)	Temperature (°C)	Heat flow (mW/mg)	Temperature (°C)	Mass loss (%)	Temperature (°C)	Mass loss (%)
Original KGM	88.9	-0.3758	294.1	2.571	30-185	-7.64	200-390	-51.97
Deacetylated KGM	72.1	-0.5547	305.8	5.603	30-185	-7.27	200-390	-61.41

MC treatment by 25 min grinding was performed by the DSC and the TGA. The results are shown in Table III. There are two kinds of crystals in the KGM powder. One is the KGM-H₂O crystallite and the other is the KGM-KGM crystallite. The both are formed by hydrogen bonds. Figure 7 shows the DSC curves of the original KGM sample and the deacetylated KGM sample after the MC treatment by 25 min grinding. It is seen that the endothermic peak was due to a loss of hydroxyl group of KGM as water molecules. The reaction is as follow: $\text{KGM-H}_2\text{O} \xrightarrow{\Delta} \text{KGM} + \text{H}_2\text{O}$. The exothermic peak was attributed to the disintegration of the intermolecular side chains of the KGM. The reaction is as follow: $\text{KGM} \xrightarrow{\Delta} \text{C} + \text{H}_2\text{O}$. In the process of the MC treatment, the acetyl in the molecular chains of the KGM was substituted by OH⁻, and the hydrogen bond energy increased between the molecular chains. The deacetylated KGM shows a higher temperature of exothermic peak, which indicates that their crystalline domains and hydrogen bonding interaction enhance the thermal stability of the KGM.^{31,32} From the TGA curves showed in Figure 8, there were two corresponding mass loss stages to the DSC curves indicating the loss of hydroxyl group of KGM and the disintegration of the intermolecular side chains of the KGM. The deacetylated KGM shows a larger

mass loss, compared to the original KGM due to the higher water absorbency.

SUMMARY

A deacetylated KGM powder can be prepared with alkali by a MC treatment in a dry vibrating mill. An increase in the treatment duration led to an increased rate of deacetylation from the KGM. FTIR analysis showed that the absorption band of acetyl groups at 1733 cm⁻¹ became smaller with increasing the treatment duration in the range of 0–20 min, and then disappeared when the KGM was treated by the MC for 25 min.

The measurement of the KGM gel rheology indicated that the MC treatment could reduce the thixotropy in the deacetylated KGM. The deacetylated KGM by a prolonged milling showed a lower apparent viscosity. The viscosity of the KGM gels increased with increasing the swelling time when the KGM was treated before 20 min, but this variation appears insignificant for the gels of the KGM treated for over 20 min. The deacetylated KGM showed a higher temperature of exothermic peak, which indicated that their crystalline domains and hydrogen bonding interaction could enhance the

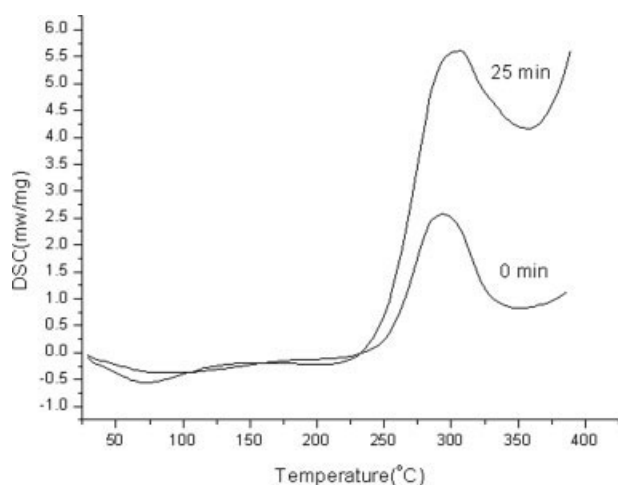


Figure 7 DSC curves of original and deacetylated KGM samples.

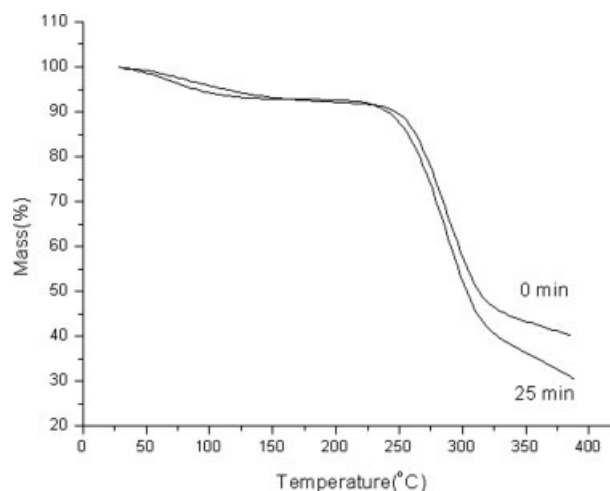


Figure 8 TGA curves of original and deacetylated KGM samples.

thermal stability. The deacetylated KGM has the higher water absorbency.

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