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Carbohydrate Polymers 69 (2007) 29–40

Carbohydrate Polymers

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Preparation and characterization of a quaternary ammonium derivative of konjac glucomannan

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Received 1 June 2006; received in revised form 16 August 2006; accepted 29 August 2006
Available online 27 October 2006

Abstract

Cationic derivatives of konjac glucomannan (KGM) were prepared by reacting KGM with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) in presence of sodium hydroxide. The chemical structures of derivative were characterized by use of elemental analysis, FTIR and ^{13}C NMR spectroscopy and static light scattering technique. The results revealed that the degree of substitution (DS) of quaternized konjac glucomannan (QKGM) could be controlled by adjusting the weight ratio of CHPTAC to KGM and the degradation of KGM molecules occurred during quaternization. Thermogravimetric analysis showed that the thermal stability of QKGM was lower than that of KGM. Rheological properties of KGM and QKGM were evaluated in steady and dynamic shear. The flow data were fitted fairly well by the Cross equations and various parameters were calculated. Dynamic shear tests demonstrate that highly modified KGM behaved like dilute solution, while unmodified KGM displayed entanglement network behavior. Significant shear-thinning effects and deviations from the Cox–Merz rule are observed in present study. *In vitro* antimicrobial activity assessment exhibited QKGM showed pronounced inhibitory effect against the three bacteria and four fungi investigated, but no *Pseudomonas aeruginosa* and *Aspergillus niger* inhibition. The improved functionalities of the derivative might be explained by its polycationic characteristics.

Keywords: Konjac glucomannan; Quaternization; Rheological property; Antimicrobial activity

1. Introduction

In past decades considerable attention has been devoted to the investigation of new applications of natural polymer because of their unique properties. Nowadays, a number of studies showed that natural polymer and their derivatives were widely used as new functionalities materials in biochemical, industrial, medical, and controlled release fields. Konjac glucomannan (KGM) is a high-molecular weight water-soluble non-ionic natural polysaccharide isolated from the tubers of the amorphophallus konjac plants and the main crop in mountainous areas of China. Chemically it consists of β-l,4 linked D-mannose and D-glucose in a molar ratio of 1.6:1 (Kato & Matsuda, 1969) with about 1 in 19 units being acetylated (Maekaji, 1978). KGM has long been used as a health food in China and Japan. Since KGM is regarded as a non-calorie food, the role of which has been displayed in weight loss and cholesterol reduction. The Food Chemicals Codex in the United States only listed Konjac flour as food additives (Zhang, Xie, & Gan, 2005). Moreover, characters of low cost, excellent film-forming ability and good biocompatility, biodegradability, as well as gel-forming properties entitle KGM to be a novel polymer material, which make natural KGM exhibits promising application in various fields like packing and preservatives material (Liu & Xiao, 2004) control release material (Wang & He, 2002) and wood adhesives (Umemura, Inoue, & Kawai, 2003).

Rapidly growing demand for materials and products from renewable resources made it worthwhile to develop new functional properties of KGM through physical or

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doi:10.1016/j.carbpol.2006.08.024

chemical modifications. Since these modifications may impart new or distinctive physical or chemical properties to it, many new uses have been found in the derivatives of KGM. Previously, several KGM derivatives were prepared by graft (Xiao, Gao, Li, & Zhang, 1999), carboxymethylation (Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002), palmitoylation (Tian, Dong, & Chen, 1998), sulfation (Zhang, Gan, Xie, & Xiao, 2005) and then their properties and application were assessed. The applications of KGM have been also extended greatly from food and food additives to various fields, such as colon-specific delivery (Liu, Hu, & Zhuo, 2004), field-flow fractionation (Benincasa, Cartoni, & Fratte, 2002), ion exchange and adsorption (Luo & Feng, 2004) etc.

With a view to prepare value added products, the exploitation of new chemical modification of KGM is attractive. It is reported that quaternization is an efficient means of awarding properties of commercial value on natural polysaccharides (Geresh, Dawadi, & Arad, 2000). So far, quaternization have been widely applied to a variety of polysaccharides (Haack, Heinze, Oelmeyer, & Kulicke, 2002; Groth & Wagenknecht, 2001; Xu, Du, Huang, & Gao, 2003). However, quaternization of konjac glucomannan is little reported. In order to tailor new properties of konjac glucomannan, in this study, quaternized derivatives of KGM were prepared by reacting KGM with CHPTAC. As a polymer having quaternary ammonium salt group, QKGM possess potential antimicrobial properties. Additionally, it has some advantages over conventional small molecular antimicrobial agents because it is more stable against volatilization, dissolution and diffusion on the surface of the material to be protected. As we know, the antimicrobial capability is as indispensable as the good film-forming ability for preservation and coating film material. In previous report (Liu & Xiao, 2004; Pang, Xie, Zhang, Xu, & Tian, 2004), the antimicrobial ability of KGM film material is improved by blending with a polymer with antimicrobial properties or using small molecular antimicrobial agents as additive. So, such chemical modification of KGM is able to provide practical applications in preservatives and packing fields.

The aim of this work was to prepare a KGM derivative by chemical modification, specifically, quaternization, and then to gain insight into its structural and physical or chemical properties. The results reported in this article may contribute to finding further applications of QKGM in pharmaceutical, bio-technical and fine chemical industry fields.

2. Experimental

2.1. Materials

CHPTAC was purchased from commercial (Guofeng fine chemical Co. Ltd, Shandong, China) and applied as etherifying reagents without further purification. KGM was purchased from Huaxianzi konjac Corp., Shiyan, China. The content of glucomannan is above 95% according to proclaims of its KGM was purified according to our previous method (Xiao, Gao, & Zhang, 2000) before being used. Other chemicals were of reagent grade.

2.2. Synthesis of the derivative

KGM (1 g) was dissolved in deionized water to prepare a certain concentration aqueous dispersions, and then 30 wt% aqueous NaOH was poured into the mixture. After being stirred vigorously at room temperature for 1 h, various amounts of CHPTAC aqueous solution was dropped into the alkali-KGM solution through pressure-equalizing dropping funnel. The whole reaction systems maintain 2.0 wt% KGM aqueous dispersion concentration and a molar ratio of NaOH/CHPTAC of 1.1, after that stirring was continuous for 16 h at 30 °C. The solution was then neutralized with 10 wt% hydrochloric acid to a pH of about 7. Reaction product QKGM was precipitated with acetone, and then washed twice with 80% acetone. The crude product was dispersed in doubly distilled water again, and was purified by dialysis through a 10,000–8000 molecular weight cut-off dialysis tubing until free of chloride ions. The dialyzed product was freeze-dried and white powders were obtained. The dried samples were stored in vacuum desiccators over P_2O_5 for further analysis. Experiments were conducted under different reaction conditions with the aim of making primary discussion about optimum reaction parameters.

2.3. Characterization of the products

Nitrogen contents of the products (N %) was measured by elemental analyzer (EA, Heraeus Co., Germany). The degree of substitution (expressed as DS which is defined as the number of hydroxyl (OH) groups substituted per repeating structural unit of the KGM backbone) was determined by nitrogen contents and calculated according to the following Eq. (1) :

$$\mathrm{DS} = 162 \times \mathrm{N}\ \%/(14.01 - 151.5 \times \mathrm{N}\ \%) \quad (1)$$

IR spectra were recorded using a Nicolet 170SX (USA) Fourier transform infrared (FTIR) spectrometer. The samples were prepared as KBr pellets and were scanned against a blank KBr pellet background. Spectra were signal averaged over 16 scans with a resolution of $4\,\mathrm{cm}^{-1}$ at room temperature.

The ^{13}C NMR spectrum for the QKGM sample was recorded on INOVA-600 spectrometer (Varian Inc., USA) operating at 600 MHz (150 MHz for carbon). The sample was dissolved in D_2O at 30 °C and the concentration of the solution was about 4 wt%. Chemical shifts were expressed in δ (ppm) relative to the resonance.

The weight-average molecular weight (M_w) and the polydispersity index (M_w/M_n) of natural and modified KGM were determined using a multi-angle laser light scattering (MALLS) photometer (Dawn DSP, Wyatt Technology Corporation) operated in the chromatography mode. The helium neon laser of the MALLS has an operating wavelength of 632.8 nm. The samples were eluted with 0.5 M NaCl and the operating flow rate of the mobile phase was 0.5 ml/min.

2.4. Thermal analysis

Thermogravimetric analysis was conducted with Netzsch STA 449C instrument (Germany) under a nitrogen atmosphere with a flow capacity of 30 ml/min. The scan was carried out at a heating rate of 10 °C/min from 0 °C to 600 °C. The sample weight was about 8–10 mg.

2.5. Rheological measurements

The rheological properties were determined with a Haake Rheostress 600 rheometer (Germany) using a cone-plate mode (cone angle 2°, diameter 35 mm). The measuring device is equipped with a temperature unit that gives a very good temperature control over an extended time. To understand in detail the sample viscosity properties under steady shear, the data were fitted to the well-known Cross models (Eq. 2). The Cross equation is often used to describe shear-thinning behavior of polysaccharide systems.

$$\eta_a = \eta_\infty + (\eta_0 - \eta_\infty)/[1 + (a\dot{\gamma})^n] \quad (2)$$

where η_a is the apparent viscosity at a given shear rate $\dot{\gamma}$; η_0 and η_∞ are zero and infinite shear rates viscosities, respectively; a is a structural relaxation time, whilst n is an exponent.

For dynamic shear properties determination, all oscillatory shear experiments were performed within the linear viscoelastic region, where the dynamic storage modulus (G') and loss modulus (G'') are independent of the strain amplitude. Dynamic oscillatory measurement, in the range 0.1–100 Hz, as well as steady shear measurements, in the range 1–1000 s^{-1} were carried out on 1.5% (w/w) KGM and QKGM aqueous dispersions. All rheological properties were determined at a temperature of 25 ± 0.1 °C.

2.6. Antimicrobial activity assessments

In vitro antimicrobial activity of KGM and QKGM was assessed against four strains of bacteria (*Shigella flexneri*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*) and five fungal strains (*Trichoderma viride*, *Penicillium chrysogenum*, *Aspergillus niger*, *Aspergillus terreus*, *Aureobicidium pullulans*) by zone of inhibition. The prepared suspensions of microorganisms were inoculated onto nutrient medium plate's surfaces with a sterile cotton swab. The filter paper discs disinfected of 6 mm in diameter was impregnated with sterile distilled water and then covered with 2 mg sample power, followed by lying on the plates. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 60 h for fungi. Blank sterile filter paper discs and sterile water were used as control in this study.

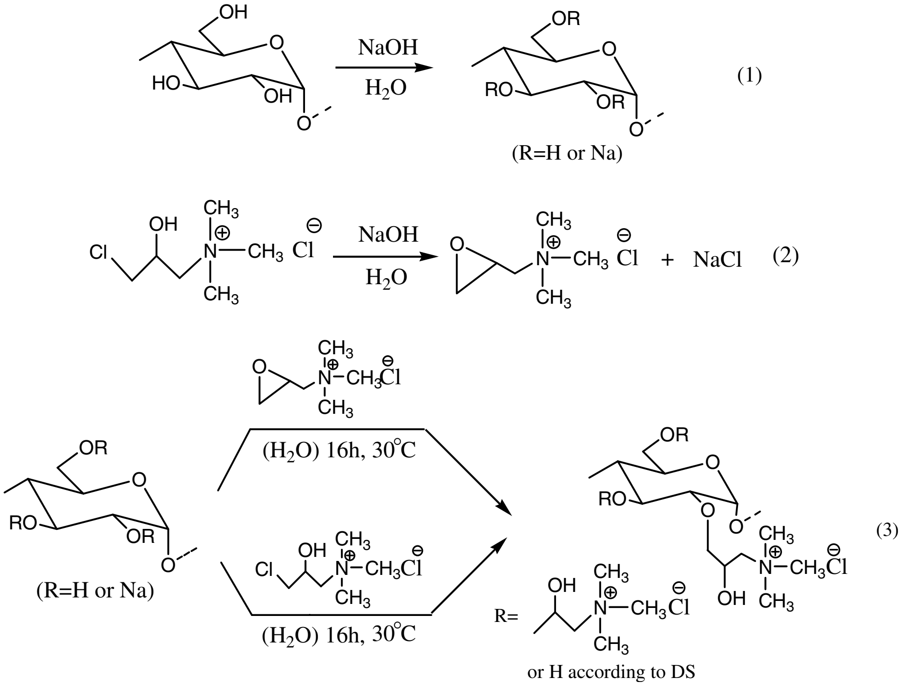
3. Results and discussion

3.1. Synthesis of the derivative

The quaternization of KGM using CHPTAC as etherifying agent under the catalytic action of sodium hydroxide undergo several steps reaction: The sodium hydroxide reacts with the hydroxyl groups of the KGM to yield sodium alkoxide groups; epoxide is produced in situ from CHPTAC by applying a stoichiometric amount of a base; QKGM is then formed through reaction between the KGM sodium alkoxide and epoxide or CHPTAC. The reaction was represented in Scheme 1.

To obtain primary insight on optimum reaction parameters, some details should be considered: First, in low concentration sodium hydroxide solution, alkali-KGM easily forms insoluble products due to deacetylation if its sodium hydroxide is neutralized; quaternization will not successfully proceed in heterogeneous reaction system. Second, the sodium hydroxide can hydrolyze CHPTAC and produce the diol at high amount; KGM is also degraded in such condition. On account of above reasons, appropriate amount sodium hydroxide is necessary to activate the KGM, at the same time, the hydrolysis of the CHPTAC and degradation of KGM should be controlled to as low as possible extent. Accordingly, a series of experiments were performed with 2.0 wt% KGM aqueous dispersion and a molar ratio of NaOH/CHPTAC of 1.1 to determine the exact quantity of CHPTAC at given weight of KGM for an optimal reaction, which is different from quaternization of other polysaccharides (Geresh et al., 2000; Haack et al., 2002) because of addition of excess sodium hydroxide. The degree of quaternization (expressed as DS value) under various reaction conditions was showed in Table 1.

Pure KGM dose not show any significant existence of nitrogen according to the results of elemental analysis (the date without listed) and DS value is zero. Whereas, attributing to the presence of quaternary ammonium salt group on KGM backbone, there is a considerable percentage of nitrogen in the QKGM. When The CHPTAC weight is 2 g and 4 g, the DS value of products is zero. It is because, in such two reaction conditions, the concentration of sodium hydroxide solution is lower than others, alkali-KGM formed insoluble products when sodium hydroxide amount became less and less with CHPTAC solution dropped into, which made quaternization unproductive. From Table 1, we can see, with higher weight ratio of CHPTAC to KGM, higher DS value can be expected, which gets optimized at 22 g CHPTAC. However, further increase in amount of CHPTAC beyond 22 g presented ineffective to enhancement of the DS value. It probably indicated that there is absence of available position in KGM backbone for quaternization. It showed that the



Scheme 1. Reaction scheme for the quaternization of konjac glucomannan with CHPTAC.

Table 1
Effect of the amount of CHPTAC on the extent of quaternization. (The molar ratio of NaOH to CHPTAC is 1.1)

KGM (g)	Sample code	CHPTAC(g)	DS
1		2	0
1		4	0
1		6	0.034
1	QKGM8	8	0.067
1		10	0.115
1		12	0.169
1		14	0.241
1		16	0.253
1		18	0.266
1		20	0.279
1	QKGM22	22	0.349
1		28	0.333
1	QKGM30	30	0.348

DS value can be simply controlled by adjusting the weight ratio of CHPTAC to KGM.

3.2. Characterization of the derivative

FTIR is a very useful tool for studying the molecular structure of polymers. Fig. 1 presents the IR spectra of KGM, CHPTAC and QKGM (QKGM22 as example). The natural KGM, stretching vibration modes of O–H groups is a broad band and occurs at about 3336 cm^{-1}. The band at 2922, 1376 and 1062 cm^{-1} assigned to stretching vibration of $–CH_2–$ groups. The peak at 1735 cm^{-1} due to the C=O groups in pure KGM. The intense peak at 1647 cm^{-1} is attributed to the in-plane deformation of the water molecule (Zhang et al., 2001). In the case of CHPTAC, the broad band at 3175 cm^{-1} is assigned for O–H stretching vibration. The peak of 1149 and 720 cm^{-1} ascribed to bending peaks of $–CH_2–$. A band at 693 cm^{-1} is ascribed to stretching vibration peaks of C–Cl. The infrared bands of the QKGM appeared in close proximity to KGM. The broad peak at 3359 cm^{-1} is due to the O–H stretching vibration. The peaks of 2922, 1375 and 1059 cm^{-1} referenced as stretching vibration peaks of $–CH_2–$ groups. The peak of 1735 cm^{-1} disappeared due to removal of the acetyl side groups from the backbones of KGM during reaction. The most striking difference between KGM and QKGM spectra was the peak of QKGM positioned at 1479 cm^{-1}, which corresponds to the methyl groups of ammonium (Loubaki, Ourevitch, & Sicsic, 1991). That peak was not detected in the infrared spectrum corresponding to the KGM. The IR spectrum shows evidence of the introduction of the quaternary ammonium salt group on KGM backbone. The characterization peaks of all samples and their fragment are summarized in Table 2.

The assignments of ^{13}C NMR peak suggested in native konjac were based on a previous report of the characterization of purified native KGM by ^{13}C NMR work (Crescenzi et al., 2002; Katsuraya et al., 2003).

The peak at 60.5 ppm attribute to the pendant methylene carbon (C6), at the lower field $\delta = 101.6$ ppm it is due to the Cl. δ value from 69.1 to 77.5 ppm corresponds to the remaining carbon atoms present in the basic konjac backbone. ^{13}C NMR spectra of QKGM (QKGM22 as example) and CHPTAC ($CDC1_3$ as solvent) are showed in Fig. 2. The peak at the lower field $\delta = 100.4$ ppm corresponds to the Cl; the peak at 60.3 ppm was assigned to the

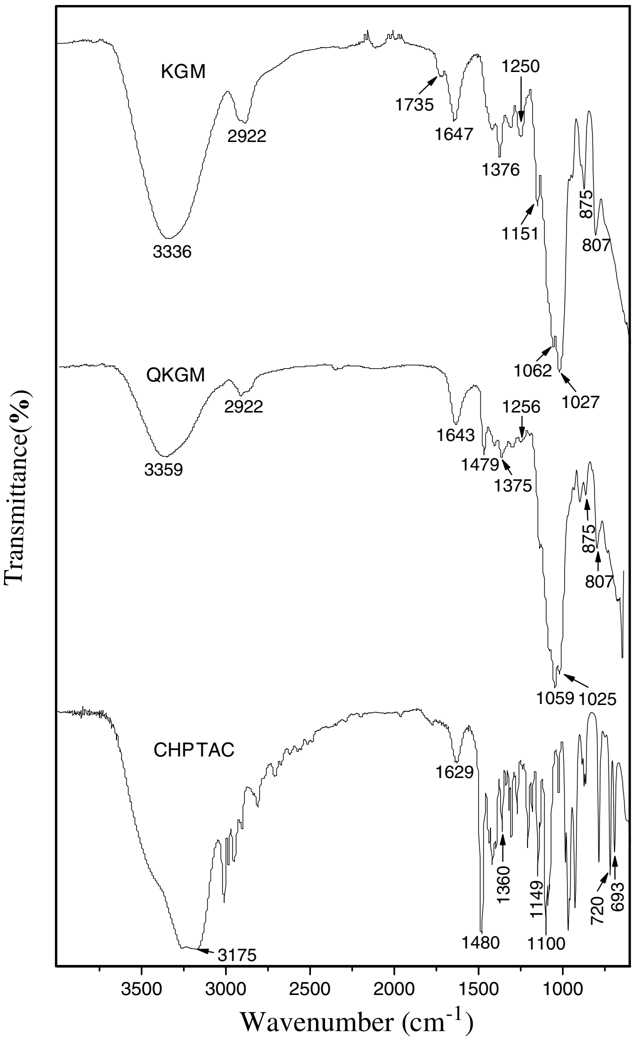


Fig. 1. FTIR spectra of natural, quaternized konjac glucomannan and CHPTAC.

Table 2
FTIR Analysis of KGM, QKGM and CHPTAC

Sample code	Characterization peaks (cm^{-1})	Fragment
KGM	3336	–OH
	2922, 1376, 1062	$-CH_2-$
	1735	C=O
	1151,1027	C–O–C
CHPTAC	3175	–OH
	1149, 720	$-CH_2-$
	1480	$-CH_3$
	1360, 1100	C–O, –OH
	693	C–Cl
QKGM	3359	–OH
	2922, 1375, 1059	$-CH_2-$
	1479	$-CH_3$
	875, 807	Mannose ring

pendant methylene carbon C6. The rest of carbon atoms present in the basic polymer backbone were determined by the wider signals between 70.2 and 78 ppm. The signals of the 2-hydroxypropyltrimethylammonium chloride group were determined at 73.4, 65.4 and 68.3 ppm for C7, C8 and C9, respectively. A relatively sharp high intensity signal at 54.60 ppm in the spectrum of QKGM was observed, it is worth noting that above sharp signal was not appear in ^{13}C NMR spectrum of native KGM. The chemical shift value of this sharp new signal was consistent with that expected from a quaternary ammonium *N*-methyl carbon nucleus (C10); the results in this work are somewhat similar to previous report (Haack et al., 2002).

The weight-average molecular weight (M_w) of samples was obtained by static light scattering measurements and showed in Table 3. Clearly, the molecular weight of QKGM decreases with increase of the CHPTAC amount in reaction, and is 6.07×10^5, 4.28×10^5 and 3.67×10^5 for QKGM8, QKGM22 and QKGM30, respectively. In case of KGM, the molecular weight is 9.80×10^5, which indicated degradation of KGM molecules occur during quaternization. This may be attributed to the degradation effect of sodium hydroxide on KGM molecules as mentioned already, because more CHPTAC incur more excess sodium hydroxide. The degradation of KGM molecular in other chemically modified treatment was also found (Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002; Gao & Zhang, 2001).

3.3. Thermal studies

The characteristic TG and DTG curves for KGM and QKGM (QKGM22 as example) were presented in Fig. 3 and the primary thermogram of KGM and QKGM were obtained at a scan rate of 10 °C/min in an inert atmosphere. Both of samples involve only two steps of degradation, after the first stage loss of moisture at low temperature, the major weight loss occurred. In the second step, KGM begins to decompose at 250 °C, rapidly loses 70.6% of its weight up to 335 °C, which could be attributed to a complex process including degradation of the saccharide rings and disintegration of macromolecule chains of KGM. Beyond 335 °C, the weight loss is slow and gradual up to 600 °C leaving about 13.1% residual weight. The maximum rate of weight loss occurs at 312 °C. However, in the case of QKGM, the decomposition in the second step commences at 242 °C and proceeds at a faster rate up to 295 °C; and at this temperature, QKGM loses 53.5% of its original weight. It may be due to decomposition of polymer chain carrying quaternary ammonium groups, beyond this temperature, degradation proceeds at a very slow rate up to 600 °C. In this degradation stage, the temperature at the maximum rate of weight loss occurs is 273 °C. The overall degradation leaves about 7% residue at 600 °C.

As can be seen from the TG and DTG plot of two samples, there is only about 30% weight loss of KGM until a temperature of 290 °C is reached, while the overall weight loss of QKGM is about 60%. In addition, initial decomposition temperature (T_i) and the temperature at the maximum rate of weight loss (T_{max}) of QKGM are much

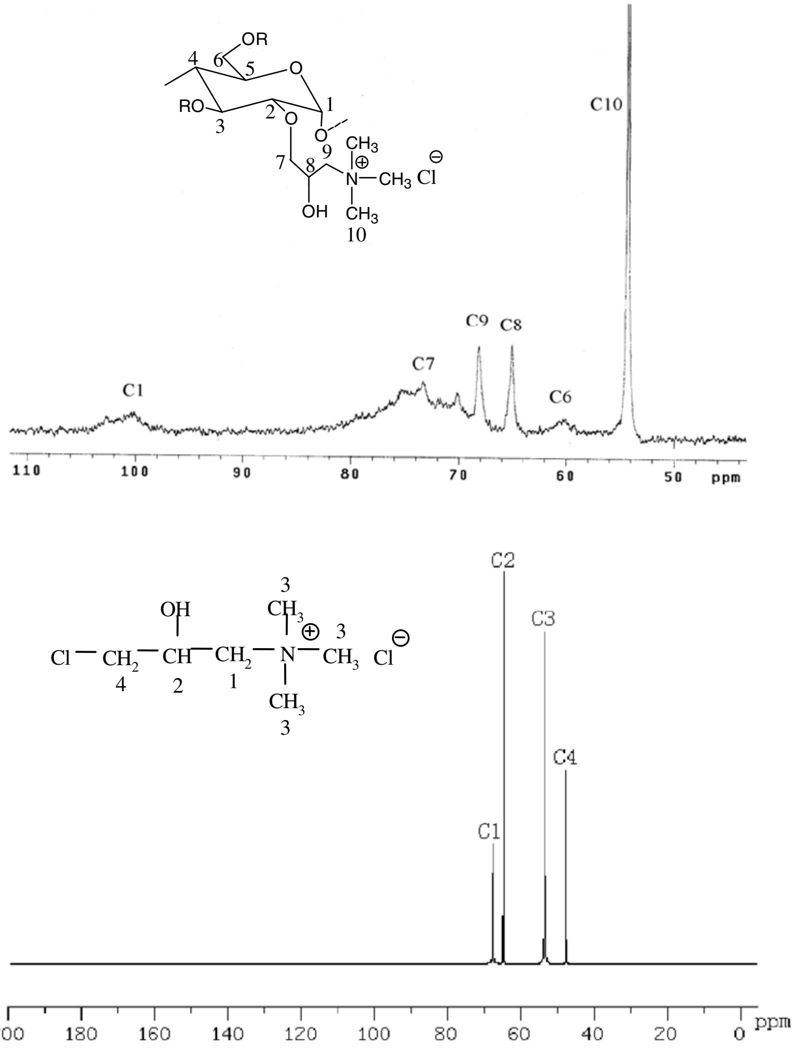


Fig. 2. ^{13}C NMR spectra of quaternized konjac glucomannan and CHPTAC.

Table 3
Molecular weight of sample obtained from static light scattering measurement

Sample code	KGM	QKGM8	QKGM22	QKGM30
Molecular weight (M_w)	9.80×10^5	6.07×10^5	4.28×10^5	3.67×10^5
Polydispersity (M_w/M_n)	1.361 ± 0.016	1.325 ± 0.014	1.074 ± 0.015	1.086 ± 0.014

lower than that of KGM, and indicating that the second degradation stage of KGM takes place at a higher temperature than the corresponding stage of the QKGM. Namely, the quaternization lowers the thermal stability of QKGM. Previous report (Baudrion, Perichaud, & Coen, 1998; Spinelli, Laranjeira, & Favere, 2004) also revealed that substitution reaction of quaternary ammonium groups led to significant decrease of the thermal stability of polymer. The data obtained from TG curves can be analyzed in more detail by Broido equation (Broido, 1969), which can give the activation energy (E_a) of reaction in selected temperature ranges. Thermogravimetric (TGA and DTG) data of the KGM and QKGM and their activation energy are tabulated in Table 4. The activation energy of KGM and QKGM is 134.2 and 118.5 kJ/mol, respectively. This indicates that the QKGM is less stable than natural KGM itself.

3.4. Rheological analysis

3.4.1. Steady shear properties

Fig. 4 showed the apparent viscosity versus shear rate in the range of 1–1000 s^{-1} for all samples dispersion in water. A typical non-Newtonian pseudoplastic behavior was exhibited. All samples showed marked shear-thinning behavior, i.e. decreasing viscosity with increasing shear rate under steady shear conditions. This shear-thinning behav-

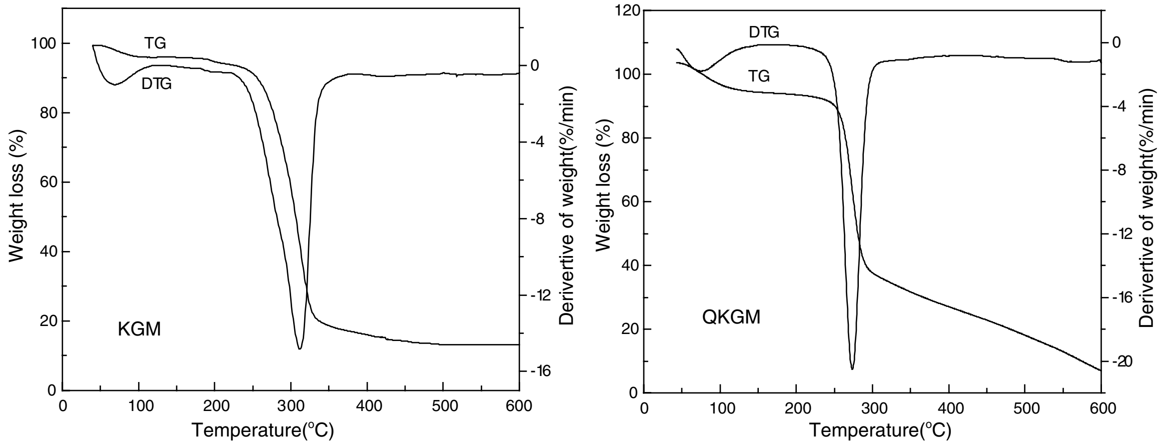


Fig. 3. TG and DTG curves for natural and quaternized konjac glucomannan.

Table 4
Thermogravimetric (TGA and DTG) data of the natural and quaternized konjac polysaccharides in nitrogen atmosphere

Sample code	Decomposition stage	Temperature range (°C)	DTG maximum (°C)	Weight loss (%)	Char yield at 600 °C	Activation energy (kJ/mol)
KGM	1	40–117	68	4.2	–	–
	2	250–335	312	70.6	13.10	134.2
QKGM	1	43–141	75	5.5	–	–
	2	242–295	273	53.8	6.92	118.5

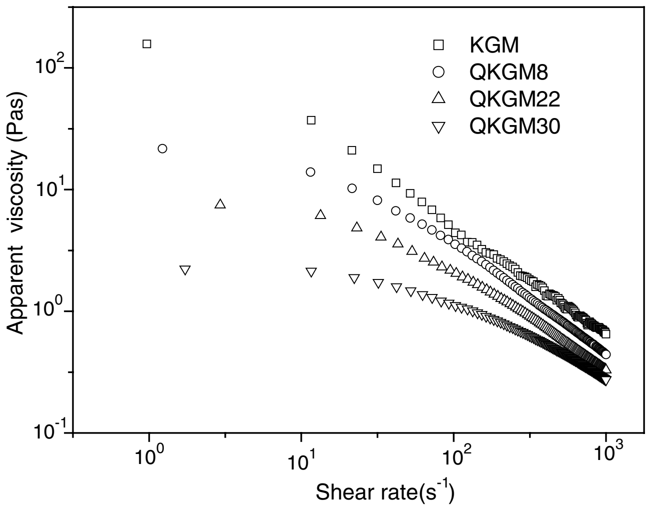


Fig. 4. Shear rate dependence of viscosity for natural and quaternized konjac glucomannan.

ior can be demonstrated by breaking of an entangled polysaccharide molecule network during shearing, as represented by previous report (Bhandari, Singlhal, & Kale, 2002; Morris, 1989). Namely, in an entangled network system, when the shear rate is increasing, the rate of disentangle becomes greater than the rate of re-entanglement of long chain molecules. The result is less intermolecular resistance to flow and a lower apparent viscosity.

From Table 5, the steady shear curves were well fitted by Cross model with high correlation coefficients R^2 (0.99).

Table 5
Value of the rheological parameters derived using the Cross model for sample solution at 25 °C

Sample	η_0 (Pa s)	η_∞ (Pa s)	a (s)	n	R^2
KGM	214.4	0.303	0.389	1.04	0.99
QKGM8	23.6	−0.028	0.061	0.95	0.99
QKGM22	8.3	0.048	0.031	0.96	0.99
QKGM30	2.4	0.029	0.011	0.89	0.99

The zero shear viscosity η_0 of four samples was also calculated using the Cross equation, it is 214.4, 23.6, 8.3 and 2.4 Pa S for KGM, QKGM8, QKGM22 and QKGM30, respectively. The result indicated that the corresponding zero shear viscosity decrease with enhancement of DS value. Above phenomena are compatible with the observed decrease in molecular weight as a function of DS value, as mentioned earlier. Due to the scission of polymer chains and decline of molecular weight, the number of effective entanglement couplings will be reduced and lead to easy flow and low apparent viscosity. Such dependence of the rheological properties of macromolecular dispersions on molecular weight has been also reported in other polysaccharide study (Zhang et al., 2001; Arendt & Kulicke, 1998; Geresh, Adin, Yarmolinsky, & Karpasas, 2002). Exponent value (n) of the Cross equation also displayed in Table 5, according to Morris, Cutler, Ross-Murphy, and Rees (1981), Typical flexible random coil polysaccharide solutions and non-structured systems usually show exponent values n of 0.76 or less for Cross mode, while the exponent value was higher (more 0.82) indicated decrease in the flexibility of the molecules and increase in stiffer conformation. However, in this study, the exponent *n* value is 1.04, 0.95, 0.96 and 0.89 for KGM, QKGM8, QKGM22 and QKGM30, respectively. It indicated that natural and quaternized KGM hold the inflexible backbone conformation and rigid intermolecular hydrogen bond, though it is destroyed to some extent with the increase the extent of quaternization.

3.4.2. Dynamic shear properties

Fig. 5 depicted changes in storage (G') and loss modulus (G'') as a function of angular frequency (ω) for all samples at 25 °C. We note that, for four types of sample, the magnitudes of G' and G'' increased with increasing angular frequency (ω), this tendency is in good agreement with those found for other macromolecular dispersions.

When the frequency increased, the curves of storage modulus G' and loss modulus G'' approach, then cross over each other, ω values at the intersected point of G' and G'' is 0.215, 7.743 and 21.54 $\mathrm{rad\,S^{-1}}$ for solution KGM, QKGM8 and QKGM22, respectively. In case of QKGM30 sample solutions, G'' was always higher than G' throughout the frequency domain studied; the expected cross-over can hardly be seen due to instrumental limitations. This observable fact showed that KGM, QKGM8 and QKGM22 behaved like entanglement network system, while QKGM30 displayed liquid-like behavior and a more elastic behavior at lower DS value. In addition, the values of the dynamic modulus are higher for the KGM than for the QKGM. This effect is associated with the relatively lower molecular weight of the latter, and coincides with the steady shear properties discussed above. As described previously (Yoshimura & Nishinari, 1999), the increasing molecular weight will induce longer molecular chains connecting junction zones, which can increase the number of elastically active chains and enhance the dynamic modulus of polymers.

In general, the introduction of ionic group into the polymer chain encourage enhancement of viscosity of solution, due to expansion of coil chain by the electrostatic repulsion and the osmotic pressure (Kobayashi et al., 2002). Such as earlier report (Geresh et al., 2000), quaternized polysaccharides possess higher elastic modulus value than that of unmodified natural polymer. However, in our study, the viscosity and dynamic modulus of QKGM was lower than that of KGM. The probable cause is that the molecular

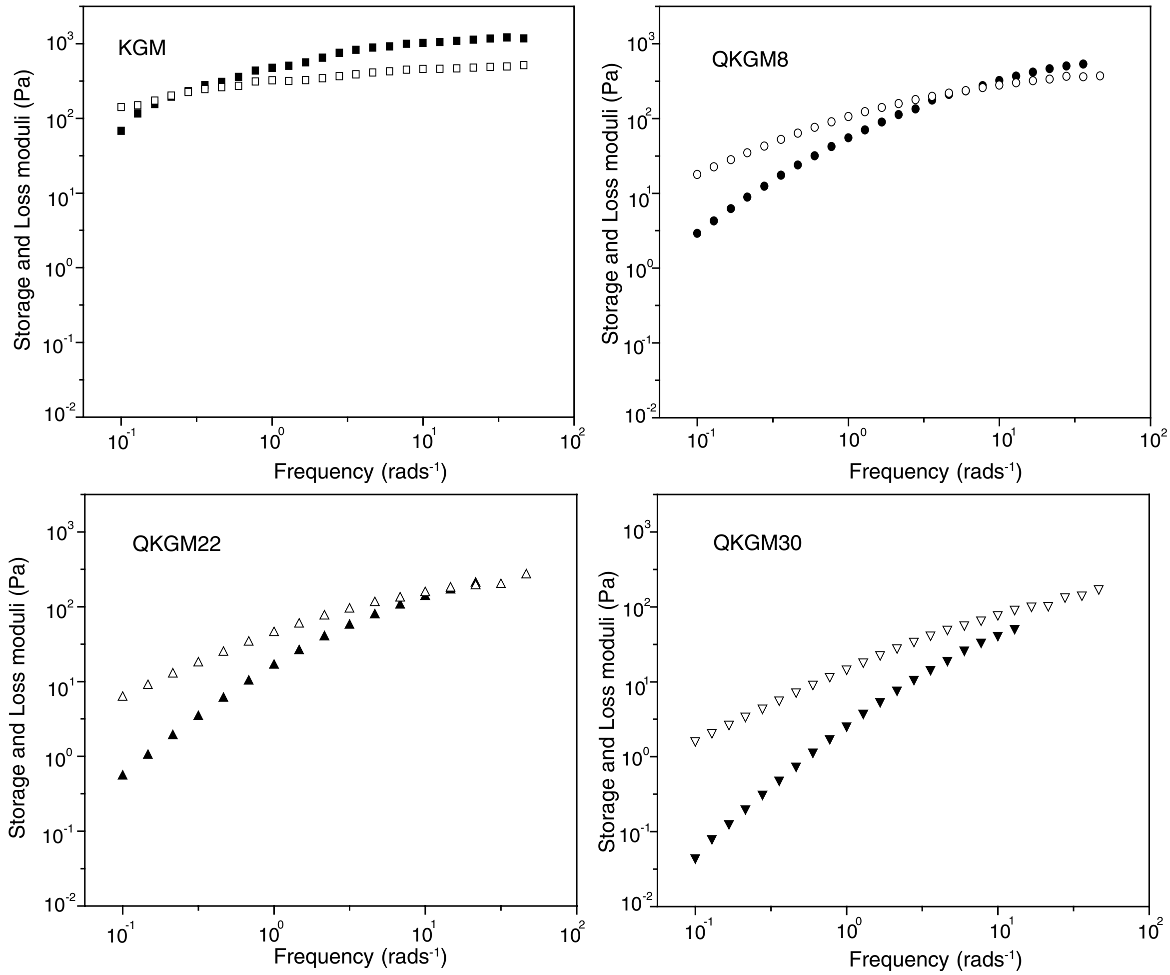


Fig. 5. Frequency dependence of G' (close sysmbols) and G'' (open symbols) for natural and quaternized konjac glucomannan.

weights exert more influence on rheological properties than the coil chain expansion induced by the electrostatic repulsion of cationic group and the osmotic pressure owing to the ions in the system. Namely, the electrostatic repulsion of cationic group was negligibly small in this study. So, compared with QKGM22, QKGM30 hold lower viscosity and dynamic modulus values, though their DS value is similar.

3.4.3. Applicability of Cox–Merz rule

For many flexible non-associating polymer solutions, the frequency dependence of complex viscosity (η^*) and the shear rate dependence of apparent viscosity (η_a) are found to be virtually identical, when the same numerical values of ω and $\dot{\gamma}$ are compared. This empirical correlation is known as the Cox–Merz rule (Eq. 3) (Cox & Merz, 1958):

$$| \eta^* | (\omega) = \eta_a(\dot{\gamma})|_{\omega=\dot{\gamma}} \tag{3}$$

However, this rule is not obeyed by macromolecule dispersions with either association or high density entanglements. Because the rate of disruption of exiting intermolecular entanglement enhances with increasing shear rate and dramatic structural reorganizations of the network in associating polymer systems may evoked by shear forces. At the same time, when moderate shear rates are imposed on this system, it is possible that the topological constraints are strengthened (Groot & Agterof, 1995; Baldursdottir, Kjoniksen, Karlsen, Nystrom, & Roots, 2003), while oscillatory shear does not have this effect.

Fig. 6 presents that, in order to examine the applicability of the Cox–Merz rule, η^* and η_a of pure KGM and quaternized KGM samples are plotted against ω and $\dot{\gamma}$, respectively. It was observed that the magnitudes of η_a were higher than those of η^*, indicating that the Cox–Merz rule was not applicable to KGM and QKGM samples. However, generally, the complex viscosity values are higher than the apparent viscosity values. Because most departures from the Cox–Merz rule are attributed to structure decay due to the effect of the strain deformation applied to the polymer system (Chamberlain & Rao, 1999).

Perhaps the behavior from Fig. 6 (i.e. $|\eta^*| < \eta_a$) was due to the highly stiff chain structure of the KGM and QKGM, as mentioned earlier. As far as QKGM, the electrostatic

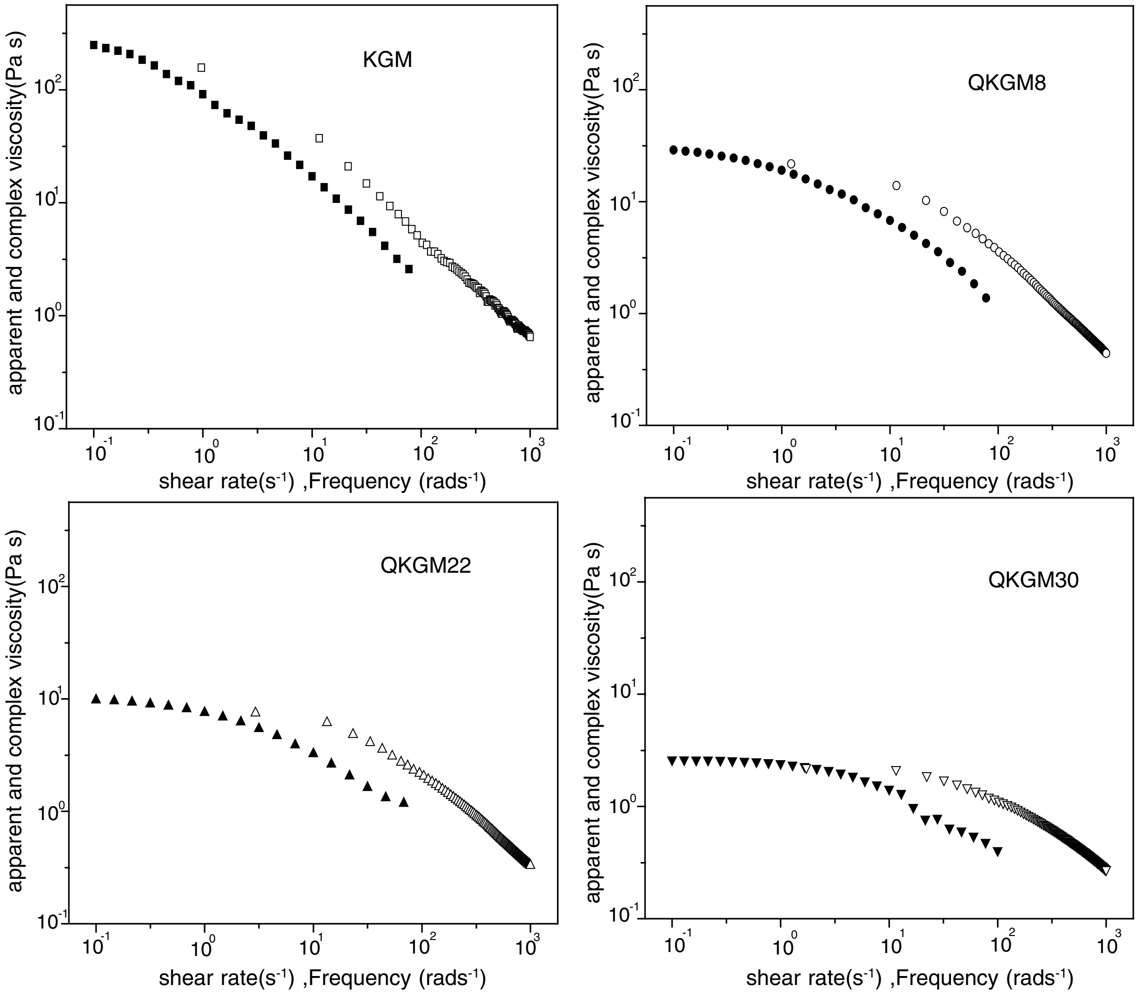


Fig. 6. Comparison of steady flow viscosity (η_a: open symbols) and complex viscosity (η^*: close symbols) for natural and quaternized konjac glucomannan.

repulsion of cationic group maybe also responsible to this phenomenon.

Similar departures from the Cox–Merz rule (i.e. $|\eta^*| < \eta_a$) were also found in other polysaccharide study (Chamberlain & Rao, 1999; Lopes da Silva, Goncalves, & Rao, 1994; Da Silva, Oliveira, & Rao, 1998), and were considered to be due to the highly branched structure of the starch, two-phase system of pectin micro-aggregates dispersed in solvent and heterogeneous nature of the starch dispersions, respectively. Moreover, we note, in other pure KGM rheological properties study (Jacon, Rao, Cooley, & Walter, 1993), the complex viscosity is higher than the apparent viscosity (i.e. $|\eta^*| > \eta_a$) We considered this difference is assigned to origin and purification method of KGM.

Table 6
Antibacterial and antifungal activity of natural KGM and quaternized KGM

Microorganisms	Antibacterial and antifungal activity			
	QKGM8	QKGM22	KGM	Control
Bacteria				
S. flexneri	+	–	–	–
S. aureus	–	+	–	–
S. typhimurium	–	+	–	–
P. aeuruginosa	–	–	–	–
Fungi				
T. viride	+	+	–	–
P. chrysogenum	+	+	–	–
A. niger	–	–	–	–
A. terreus	–	+	–	–
A. pullulans	+	+	–	–

'–': no inhibition; '+': inhibition.

3.5. Antimicrobial activity assessment

Antimicrobial activity of the QKGM is considered to be one of the important properties linked directly to the possible applications. Natural polysaccharides were seldom reported in the literature for having antimicrobial activity with the exception chitosan. Since some microbe are accesses to growth in surfaces of various natural polysaccharides material or have implication in human infections and diseases, which warrant a greater emphasis on the development of active polysaccharides material. Presently, some natural polysaccharides material with antimicrobial activity was obtained by blending with chitosan or reductive animation (Moller, Grelier, Pardon, & Coma, 2004; Shin, Lee, Lee, & Lee, 2005).

The capabilities of KGM and QKGM in inhibiting the growth of the tested microbes are listed in Table 6. Clearly, KGM is devoid of antimicrobial activity, whereas QKGM showed inhibitory effect against the three bacteria and four fungi investigated. For antibacterial assay, Inhibition zone (image without showed) was observed for QKGM8 against *S. flexneri* and for QKGM22 against *S. aureus* and *S. typhimurium.* However, *P. aeruginosa* was not susceptible to both samples.

In case of antifungal test, it was found that fungal strains were more susceptible to the quaternized KGM than the bacterial strains. QKGM22 proved to be effective against four of

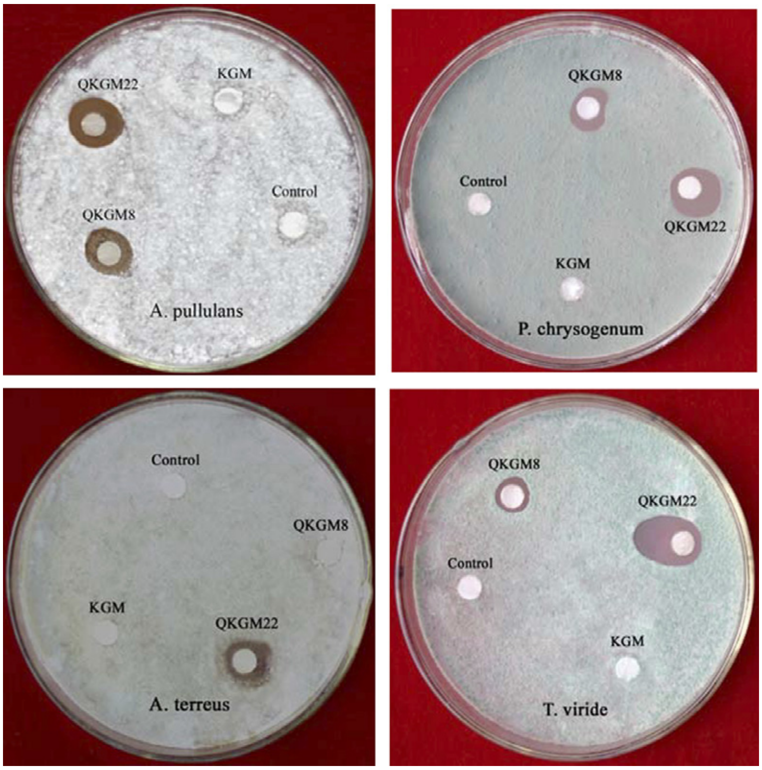


Fig. 7. Effect of natural and quaternized konjac glucomannan on growth of four fungal strains.

the five fungi examined, while QKGM8 only showed active against the three fungi tested. But both were ineffective against *A. niger.* The image in Fig. 7 shows growth inhibition zone of four fungal strains in the presence of KGM and QKGM. As shown in Fig. 7, the higher zone of inhibition was recorded at QKGM22 than QKGM8 for the entire four fungal strains. This result implies that high DS value causes gain of antifungal activity. In addition, the size of inhibitory zones associated with QKGM22 is similar for *T. viride, P. chrysogenum, A. terreus* and *A. pullulans*, which indicated four fungal strains, are parallel susceptivity to QKGM22.

The exact antimicrobial mechanism of the QKGM on test microorganisms is probably complex, and its elucidation was largely beyond the scope of this study. Since major component of cell walls of bacteria is peptidoglycan and teichoic acid or lipoprotein and lipopolysaccharide, while major constituent of cell walls of some fungi is chitin and glucan, difference of inhibiting effect of QKGM on growth of bacteria and fungi tested may be due to the difference of structure and component of cell between bacteria and fungi. As noted previously, chitosan showed better antimicrobial efficacy against bacteria than fungi, because it is one of component of the cell walls of some fungi. However, an important point about antibacterial mechanism to be proposed might be associated with the interaction of quaternary ammonium salt group with cell membrane of bacteria (Peng, Han, Liu, & Xu, 2005). As regards antifungal mechanism, it might be due to direct interference of nutrilite synthesis or blocking of fissiparism. Since data on the toxicology is lacking, further investigations may be required for QKGM.

4. Conclusions

A derivative of KGM with quaternary ammonium functionality is synthesized and optimal conditions for the quaternization are defined. The new derivative is evaluated in terms of thermal, rheological analysis and the suppression of the growth against bacteria and fungi. The results showed that the thermal stability of QKGM is lower than that of KGM and the degradation of the polysaccharide molecule occurs during quaternization. Significant shear-thinning effects and deviations from the Cox–Merz rule are observed in rheological analysis The molecular weight of QKGM exerts more influence on their rheological properties than electrostatic repulsion of cationic group. Compared with the KGM, QKGM displayed pronounced inhibitory effect on growth of four fungal strains and three bacterial strains investigated. Such new properties open new possibilities for commercial applications of KGM. So, further investigation is recommended to broaden the fields of applications of QKGM.

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