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An approach for prominent enhancement of the quality of konjac flour: Dimethyl sulfoxide as medium

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ABSTRACT

In this paper, an approach to improve several konjac flour (KF) qualities by dimethyl sulfoxide (DMSO) addition using various concentrations at different temperature levels was proposed. Also, various properties of native and refined KF, including transparency, chemical composition and rheological properties have been investigated. The results showed that the KF refined by 75% DMSO achieved 27.7% improvement in transparency, 99.7% removal of starch, 99.4% removal of soluble sugar, and 98.2% removal of protein as well as more satisfactory viscosity stability. In addition, the morphology structure of refined KF showed a significant difference compared with the native one as observed using the SEM, which is promising for further industrial application. Furthermore, the rheological properties of both native and refined konjac sols were studied and the results showed that DMSO refinement is an effective and alternative approach to improve the qualities of KF in many aspects.

1. Introduction

Konjac is considered not only an important food source but also an abundant hydrophilic colloid with a long history in China, Japan and South East Asia and as a traditional medicine as well (Chua, Baldwin, Hocking, & Chan, 2010). Konjac glucomannan (KGM), being the only glucomannan that could be largely obtained from nature so far, shows the highest viscosity among the vegetable colloids that people have known. Also the distinctive rheological properties of KGM give rise to a wide range of applications in various fields, like food engineering, pharmacy, bio-technology and the fine chemical industry (Shahbuddin, MacNeil, & Rimmer, 2012). However, in actual industrialized process, dissatisfactory KF qualities such as the abnormal low viscosity of the raw KF or the instability of flour quality could lead to a series of unpleasant phenomena, which would seriously disturb the development of the konjac industry. To the best of our knowledge, various KF varieties are the main products in the market, so in a way to enhance the raw material quality of which is a realistic request for the enterprise to make a profit, application requirements to be satisfied and terminal food quality to be improved.

Many approaches have been proposed to improve the quality of the KGM in the KF, and some encouraging achievements have been obtained. A simple centrifugation process was proposed to augment the quality of KGM extracted from KF (Tatirat & Charoenrein, 2011). Furthermore, 92.3% decline of SO_2 content was achieved, 21.4% transparency was improved, along with 34.7% viscosity increase and whiter color after treated by a compound quality improver which was consisted of complex enzyme, aqueous ammonia and a classified catalyst A (Xie, 2002a). Ethanol, lead acetate and hydrogen peroxide have been used to promote the purification of konjac flour, with a conclusion that the purification method was closely related to the structure and the properties of the polysaccharide (Wang, Xia, Li, & Liu, 2005). Furthermore, a lot of attempts have been made to physically or chemically modify KGM to achieve desired properties. KGM that went through carboxymethylation modification achieved 28 times enhancement of the stability of sol, 11 times enhancement of the tensile strength and the considerable boiling water tolerance (Xie, 2002b). Also, Extrusion modification process was shown to be able to reduce molecular weight, water absorption index and viscosity of KGM

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(Tatirat, Charoenrein, & Kerr, 2012). In addition, transplanting of *Amorphophallus konjac* from its native land into the subtropical and tropical zone northward to the northern temperate zone in the northern part of China was proposed to be feasible despite the fact that the KGM content was lower than those in its original environment (Fang & Wu, 2004). Also, a dry-modification with sodium hexametaphosphate was proven to be most effective to refine the raw KF in viscosity, transparency, freeze-thaw stability and fungistasis and tolerance to acids and high temperature. Moreover, KGM was employed to be blended with other hydrocolloids to obtain better properties, such as chitosan, xanthan and curdlan. In view of the immense economic value of konjac in food manufacture, industrial utility, and medicinal use, more approaches need to be proposed to enhance the KF qualities.

Dimethyl sulfoxide (DMSO), with reputation of an all-powerful solvent, is a very simple compound with a long history in practical application and is now well established as a good starch solvent (Gao, Luo, & Luo, 2012) due to its relatively polar nature, capability to accept hydrogen bonds as well as its small and compact molecular structure (Lukasiewicz & Kowalski, 2012). Beyond that, it is also commonly used as an antifreeze and a penetration enhancer in topical pharmaceutical formulation (Michniak-Kohn, 2007). The safety of the clinical use of pharmaceutical-grade DMSO as a penetration enhancer is supported by the numerous data that have accumulated over the past 3 decades demonstrating the favorable safety and tolerability profile (Wu, Zheng, & Song, 2011). In addition, DMSO solvent was proven to be dramatically effective to improve the SN2′ reaction of propragyl mesylates with organozinc reagents, as a result of improvement of solubility of the metal salt, a change of the oligomeric complex structures, and acceleration of reaction (Kobayashi, Naka, Wheatley, & Kondo, 2008). Furthermore, DMSO with various concentrations was reported to be effective to control the conformation of chains. To the best of our knowledge, DMSO has not been reported to be used as a medium to refine the KF in any research.

We have known that DMSO can dissolve starch but not KGM (Chen, Zong, & Li, 2006). The aim of our investigation, therefore, is to propose an approach to enhance the KF qualities, using DMSO as medium, which is very simple and convenient. In this article, the refined KF was characterized by transparency, scanning electron microscope (SEM), chemical composition and rheological measurements.

2. Materials and methods

2.1. Materials

The KF sample was provided through Hubei Yi Zhi Konjac Biotechnology Co. Ltd. (Hubei, China). Other chemicals, including DMSO, absolute ethyl alcohol and dimethicone were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China) and used without further purification. All chemicals were reagent grade.

2.2. Purification of KGM using DMSO

20.00 g KF was added into 120 mL DMSO or its aqueous solutions. The mixtures were refluxed at 80 °C for 4 h under constant stirring and then cooled down to room temperature. The products were collected by vacuum filtration and rinsed with 95% ethanol three times. Finally, the purified KF was dried in a vacuum oven at 80 °C for 2 h. Based on the various concentrations of DMSO, the final products were categorized into three groups coded as D100 (pure DMSO), D88 (88% aqueous solution), D75 (75% aqueous solution), D63 (63% aqueous solution) and D50 (50% aqueous solution) respectively. After that, all 5 groups were prepared into 1% hydrosol for viscosity measurements. The KF of the optimum group was further divided into 5 subgroups coded as W100, W90, W80, W70, and W60, corresponding to the different reflux temperatures of 100 °C, 90 °C, 80 °C, 70 °C, and 60 °C. The control group coded as W was the native KF without any refinement.

2.3. Characterization

2.3.1. Transparency measurement

1% (w/w) KF hydrosol was prepared by dispersing 1 g KF into 99.00 g distilled water and stirring in 30 °C water bath for 2 h. The transmittance of KGM sol was measured at 550 nm against distilled water with a 722E spectrophotometer (Spectrum Shanghai, China).

2.3.2. Morphology studies

The morphology of KF was studied with a JSM-6700F (JEOL, Japan) Scanning Electron Microscope (SEM). Dried KF samples were directly placed on a stub. Thin layers (20–30 nm) gold was sputtered onto the samples to enhance the contrast (Tatirat & Charoenrein, 2011).

2.3.3. Chemical composition analyses

Every KF group was analyzed for contents of ash, starch, soluble sugar and protein according to AOAC procedures (Akesowan, 2007). In addition, KGM content was determined by spectrophotometry (Ouyang & Han, 2002). All analyses were carried out in triplicate. Furthermore, the fishy smell was determined by sensory evaluation. Ten undergraduate students were selected to be panelists who already received training. Samples of each group were presented to panelists in random order. Judges were instructed to evaluate the fishy smell without fixed extremes, and each point was converted to a numerical value from 0, no fishy smell at all to 5, extremely obvious fishy smell (Akesowan, 2007).

2.3.4. Apparent viscosity measurements

Rotational concentric cylinder viscometer (HAAKE RV12, Germany) was employed to measure the apparent viscosity. 1% (w/v) sols prepared under different refinement temperature were filled into the cups and the temperatures were maintained at 30 °C water bath. The viscosities of W100, W90, W80, W70, W60 and W were measured with other conditions constant (Mei et al., 2012). To determine the stability of viscosity, the samples were kept in 30 °C and measured every hour and every day for respectively 3 h and 7 d.

2.3.5. Rheological analyses

The rheological study was performed with an AR2000ex rheometer (TA instruments, USA) with a 40 mm diameter parallel plate geometry and 1.0 mm gap. The samples were 1% (w/w) KGM hydrosols prepared using the method mentioned in Section 2.3.1. All measurements were carried out in triplicate. Flow curves were measured in the range of 0.01–1000 s^{-1} at 25 °C by steady state test. The linear viscoelastic properties of sols were investigated by the storage modulus (G') and the loss modulus (G''), respectively, which could be obtained from oscillatory measurements. The exposed surface of the sample was covered with dimethicone to avoid evaporation of water in the solution (Kim, Takemasa, & Nishinari, 2006). Under the frequency sweep, temperature was fixed at 25 °C and frequency varied from 0.06 to 628 rad/s at a small strain of 2%, while under temperature sweep, frequency was fixed at 6.28 rad/s (1 Hz) and temperature varied from 25 to 90 °C with a constant heating rate of 2.0 °C/min (Moreira, Chenlo, & Torres, 2011).

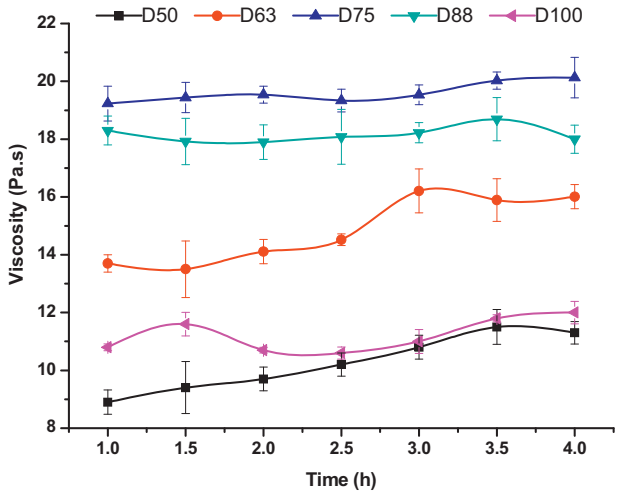


Fig. 1. Effect of DMSO concentrations on viscosities of KGM hydrosols. Each value represents the mean and standard deviation (vertical bars) of three measurements.

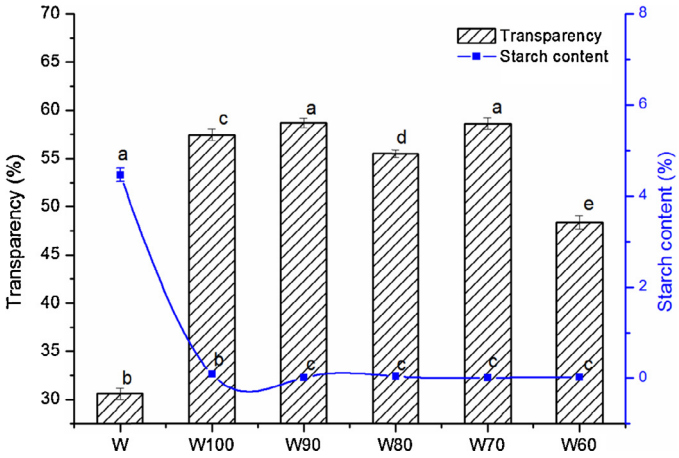


Fig. 2. Transparency of 1.0% KGM hydrosols and starch content of KF of both native and DMSO refined groups. Error bars of transparency indicate mean values ± standard deviations of six replicates. Error bars of starch content indicate mean values ± standard deviations of three replicates.

3. Results and discussion

3.1. Determination of DMSO concentration

To determine the optimum DMSO concentration, the dissolution kinetics of D100, D88, D75, D63 and D50 were demonstrated by evolution of viscosity with time. As shown in Fig. 1, the viscosity of D75 is significantly higher than other groups, though D100 shows a clearly faster swelling speed. D88 and D63 take the second and third places in terms of viscosity. As for the group refined by 50% DMSO, D50 exhibited neither satisfactory swelling speed nor desired viscosity. Taking color and odor into consideration, D75 was chosen as the optimum group. As a consequence, groups coded as W100, W90, W80, W70, and W60 were prepared by 75% DMSO under the temperature of 100 °C, 90 °C, 80 °C, 70 °C, and 60 °C, respectively.

3.2. Transparency

Transparency is considered one of the critical criteria in evaluating the quality of KGM, especially when it is used as a part of films, jellies or suspending agents (Xiao, Gao, Wang, & Zhang, 2000). The transparency of KGM is affected by multiple factors. For example, it decreases with the addition of sodium octenylsuccinate starch or concentrated citric acid, and increases with the addition of cane sugar, sodium chloride or diluted citric acid.

Fig. 2 shows the histogram of KMG transparency for the control plus 5 types of purified KF. Obviously, in this study, all the refined

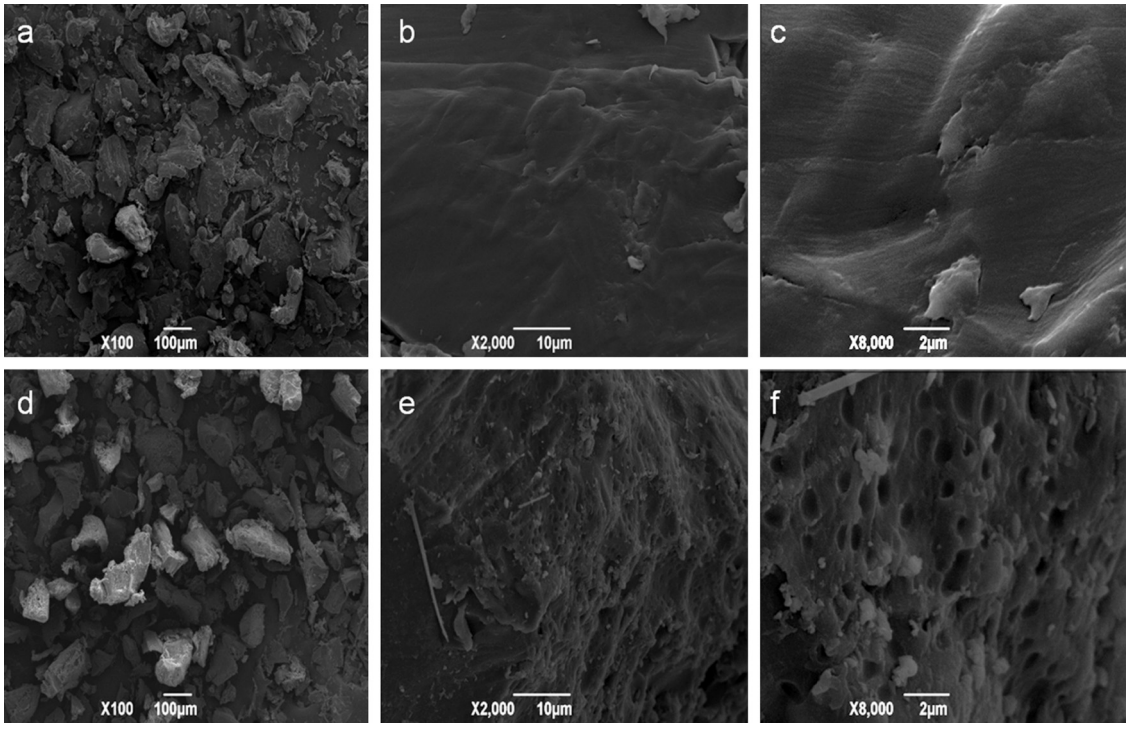


Fig. 3. Scanning electron micrographs of native KF (a), (b) and (c) and KF refined by 75% DMSO at 70 °C (d), (e) and (f).

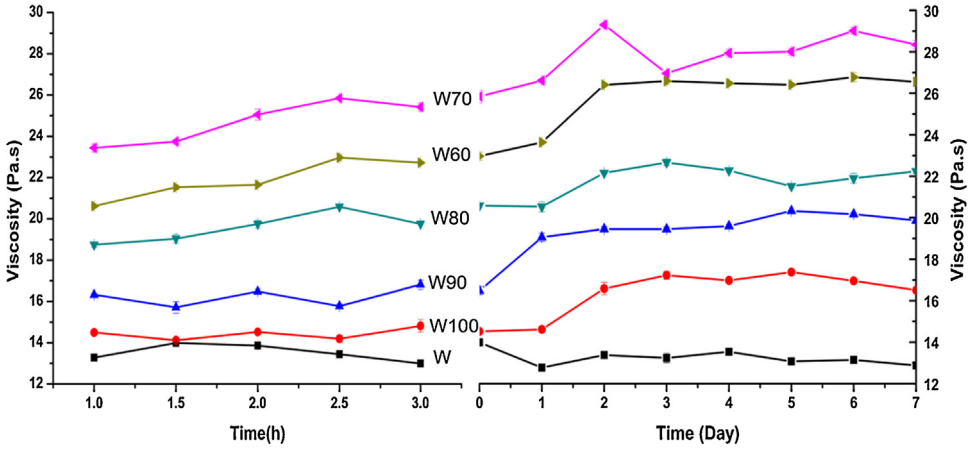


Fig. 4. Apparent viscosities and the stabilities of various KGM hydrosols for 3 h and 7 d. Each value represents the mean and standard deviation (vertical bars) of three measurements.

groups showed higher transparency compared with the control group W ($P < 0.01$). The transparency enhancements for W70 and W90 were the most significant among all groups. It is proven that DMSO-treatment helps the konjac flour to achieve desirable transparency. The starch content of each group is shown by scatter diagram in Fig. 2. All refined groups exhibited significantly lower starch content than the native group. Therefore, the transparency improvement could be attributed to the dissolution of starch in DMSO, which reduces the light scattering, since DMSO is known as an excellent starch solvent (Mukerjea, Mukerjea, & Robyt, 2006). As a result, DMSO treatment could be applied when transparency was the leading criteria.

3.3. Morphology analyses

Native and DMSO-refined KGM morphologies were studied by SEM as shown in Fig. 3. Extreme morphology differences were observed between the two groups. The native KGM (Fig. 3a–c), has a relatively rough surface with a lot of fragments. Fig. 3d–f is the DMSO-refined KGM. In the high magnification image (Fig. 3f), intriguingly, evenly distributed small pits were observed on the surface of the refined konjac granules. These are the vacancies left by the removed starch or starch residual of (Jimenez-Colmenero, Cofrades, Herrero, Solas, & Ruiz-Capillas, 2012), which is consistent with the result of starch content in Section 3.2. As far as we know, there has been no study on the starch within KGM, we propose that KGM starch is a kind of small granule starch just like barley starch (Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2000) and potato starch (Noda et al., 2005). The KGM starch may have an even smaller granule size according to the bore diameter observed in SEM. This is of great potential in applications because the small grain starch plays an important role in such as functional materials, cosmetics and many other recent applications (Lindeboom, Chang, & Tyler, 2004).

3.4. Chemical compositions

The main compositions of konjac flour are presented in Table 1. Soluble sugar and protein content of all five refined groups are much lower compared to the control group W ($P < 0.05$). Meanwhile, KGM contents of all refined groups are clearly higher than the control group ($P < 0.05$), indicating that the DMSO treatment helped to improve the quality of the KF by removing the impurities such as starch, sugar and protein, which is consistent with the conclusion in transparency studies. According to the previous report, there was the positive correlation between the ash content and the odor intensity evaluation (Noguchi et al., 2003). In this study, however, the ash content of all refined groups did not decline significantly ($P > 0.05$) with a less heavy fishy smell than the control group, which was attributed to their higher porosity with strong absorbability (Li, Ji, Xia, & Li, 2012). This is similar to the adsorbent effect of porosity of activated carbon (Kaneko, Ishii, & Ruike, 1992) as well as porous aluminum silicate (Xiong, Li, Bailey, & Xiong, 2012). Furthermore, since the fishy smell was mainly caused by trimethylamine (TMA) (Noguchi et al., 2003), the dissolution effect of DMSO on TMA could be responsible for the alleviation of unpleasant fishy smell, due to its capability to accept hydrogen bonds (Szmant, 1975). This deodorization effect of DMSO aqueous solution is similar to that of ethanol (Jianrong, Donghua, Srzednicki, Kanlayanarat, & Borompichaichartkul, 2008). Moreover, this kind of DMSO-refined konjac flour with porous structure can be used in

Table 1
Main chemical compositions of native and refined KF.

Samples	Ash (%)	Soluble sugar (%)	Protein (%)	KGM (%)[a]	Fishy smell
W	2.70 ± 0.17	1.75 ± 0.09	2.89 ± 0.15	73.01 ± 2.79	5 ± 0.44
W100	2.67 ± 0.09	–	0.05 ± 0.00	91.31 ± 3.01	3 ± 0.70
W90	2.56 ± 0.21	–	0.05 ± 0.01	91.86 ± 3.19	2 ± 0.44
W80	2.68 ± 0.31	0.01 ± 0.00	0.09 ± 0.01	93.15 ± 2.98	3 ± 0.70
W70	2.58 ± 0.15	–	0.12 ± 0.00	93.33 ± 3.72	2 ± 0.44
W60	2.37 ± 0.24	0.01 ± 0.00	0.07 ± 0.00	93.61 ± 1.99	2 ± 0.54

–, no data available.
[a] Dry basis content of KGM.

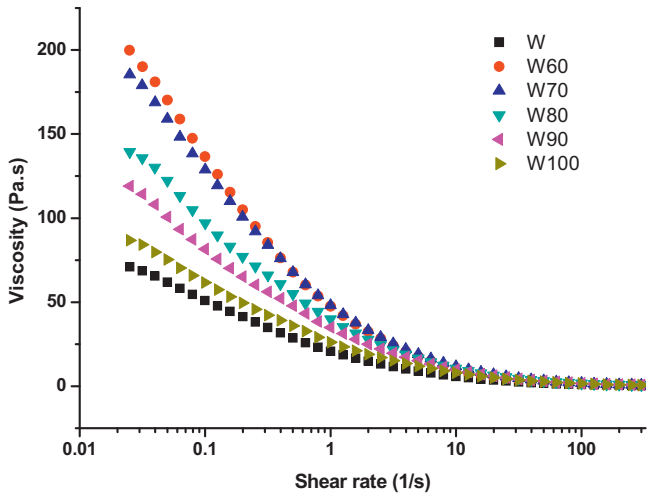


Fig. 5. Flow curves for 1% (w/w) KGM hydrosols at 25 °C.

the adsorption and measurement of heavy metal and odor (Peng, Zhong, Ren, & Sun, 2012).

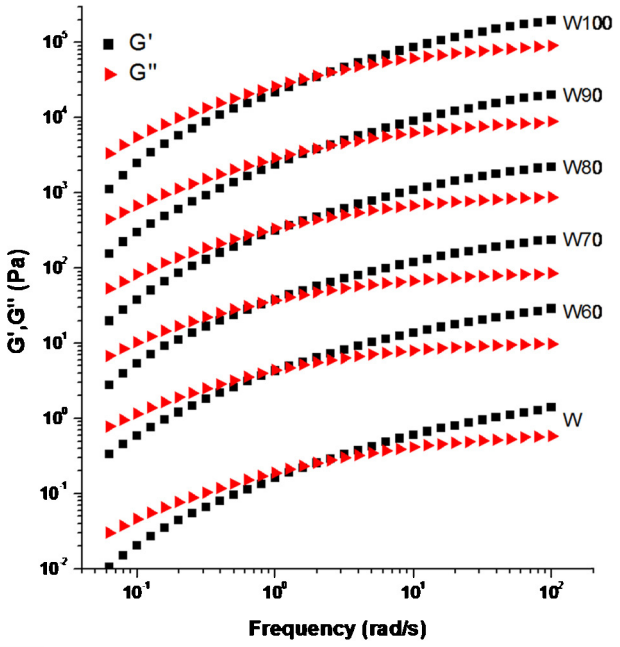
3.5. Apparent viscosity and swelling

The dissolution kinetics, the apparent viscosity and the viscosity stability results are presented in Fig. 4. Compared to the control group, which exhibits the lowest viscosity, the viscosity enhancements of the refining groups are obvious but not perfect. W70 stays in the top position in terms of viscosity, with a margin of 84.73%, followed by W60, W80, W90 and W100. The viscosity enhancement was related to the elimination of starch. Since starch did not interact synergistically with KGM (Yoshimura, Takaya, & Nishinari, 1998), the presence of starch in KF was unfavorable to its viscosity. When the starch was absent, the hydrogen bond network was more ordered, and the intermolecular interaction was stronger, giving rise to higher viscosity. Plus, the viscosity of starch was commonly far smaller than that of KGM (Van Soest, Benes, De Wit, & Vliegenthart, 1996), so higher KGM content can lead to higher viscosity.

The swelling time of the control group W was 1.5 h. The DMSO-refined groups needed a longer swelling time. When the temperature decreases, the swelling time goes up to 2.5 h. This sustained dissolution property is promising for applications that require increasing viscosity over time, like controlled drug delivery systems (Alvarez-Manceñido, Landin, Lacik, & Martínez-Pacheco, 2008). As for the stability, both the control and refined groups behave steadily.

3.6. Rheological measurement

All KGM sols were rheologically characterized at 25 °C at different shear rates. Fig. 5 shows the steady-shear flow curves of all samples. Both the native and refined groups exhibit shear thinning behavior, which is in agreement with previous reports on the behavior of KGM in shear flow (Chua et al., 2012; Tatirat et al., 2012) and that of other polysaccharides like chitosan (Bastos et al., 2010) and xanthan (Choppe, Puaud, Nicolai, & Benyahia, 2010). When the shear rate was low, the formation of new entanglements overwhelmed the disruption of old ones, leading to high viscosities (Kim, Keffer, Kröger, & Edwards, 2008). As the shear rate increased, the entanglements become more likely to be broken and give rise to a decreasing apparent viscosity, which is in accordance with other reports (Alvarez-Manceñido et al., 2006). W60 and W70 exhibited

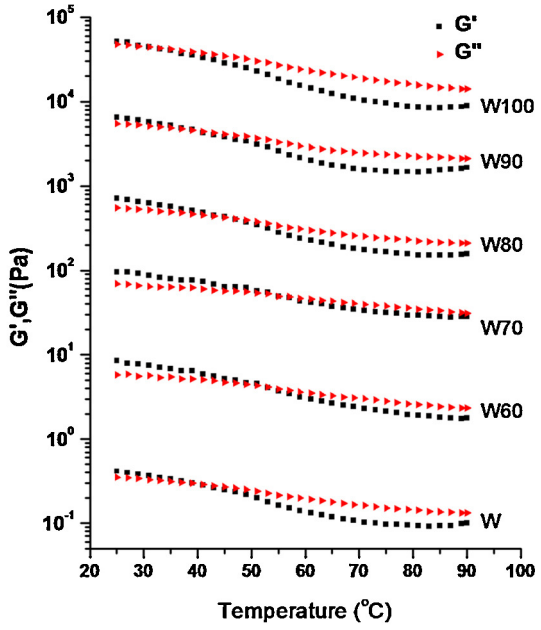


	Crossover moduli (Pa)	Relaxation time (s)
W	25.79	3.09
W60	42.09	5.81
W70	42.27	6.49
W80	37.93	4.69
W90	40.51	2.86
W100	38.73	2.68

Fig. 6. Frequency dependence of storage and loss moduli for 1% (w/w) native KGM and KGM refined by 75% DMSO under various temperatures. The data are shifted along vertical axes by 10^a to avoid overlapping ($a_W = -2$; $a_{W60} = -1$; $a_{W70} = 0$; $a_{W80} = 1$; $a_{W90} = 2$; and $a_{W100} = 3$).

higher viscosities, which was credited to larger entanglement density at 60 and 70 °C (Kohyama, Sano, & Nishinari, 1996).

Viscoelasticity is an important feature of high molecular mass materials (Jimenez-Colmenero et al., 2012). The storage modulus G' is a measurement of elastic components while the loss modulus G' represents viscous components. When $G' > G''$, the sample will be more elastic than viscous, typical gel behavior. When $G' < G''$', the sample will behave like a diluted solution. When $G' = G''$, the sample will behave like a concentrated solution (Peinado, Rosa, Heredia, & Andrés, 2012). Fig. 6 shows the rheological results of the dynamic measurement where the frequency dependence of G' and G'' of various KGM can be observed. Both control group and the DMSO-refined groups show monotonically increasing G' and G'' with frequency, with viscous behavior being in the lead in the low-frequency region and elastic behavior predominating in the high-frequency region, which is in accordance with the previous reports (Ratcliffe, Williams, English, & Meadows, 2013). This was related to the entanglement and disentanglement of the molecular chains. G'' exceeding G' at low frequency was due to the entanglements of the molecular chains being able to disentangle during a long period of oscillation. While at high oscillation frequency, the molecule chains were not fast enough to react to the strain because of its low mobility, giving rise to a solidity rather than liquidity, i.e. $G' > G''$ (Du, Li, Chen, & Li, 2012). Relaxation time, being the reciprocal of crossover frequency, is one of the key parameters that can be used as reference points when comparing with other results (Ratcliffe et al., 2013). Among all groups, W70 exhibited the longest relaxation time, with W60 following closely. Groups refined at 70 °C



	Crossover moduli (Pa)	Crossover temperature (℃)
W	29.68	39.61
W60	41.96	52.51
W70	49.64	54.83
W80	41.95	45.91
W90	45.78	39.45
W100	44.16	31.23

Fig. 7. Temperature dependence of storage and loss moduli for 1% (w/w) native KGM and KGM refined by 75% DMSO under various temperatures. The data are shifted along vertical axes by 10^a to avoid overlapping ($a_W = -2$; $a_{W60} = -1$; $a_{W70} = 0$; $a_{W80} = 1$; $a_{W90} = 2$; and $a_{W100} = 3$).

exhibit more extended relaxation times compared to the control group and other refined groups, which reflects that the groups refined at 70 °C are more likely to exhibit solid-like behavior, indicating a temporary network structures of KGM chains were easier to form after DMSO treatment at 60 and 70 °C (Du et al., 2012). Moreover, the modulus at crossover, which is another key parameter, of the W70 stays in the first position with other refined groups following. Further, the values of G' and G'' of the refined groups as a whole are higher than the control group, and the moduli of the system show a rising trend with decreasing treatment temperature. This is in agreement with the viscosity augment trend.

As shown in Fig. 7, under the temperature program, both G' and G'' decline with the temperature-rise, which is attributed to the decline of hydrogen bonding interactions under heating conditions (Iseki, Takahashi, Hattori, Hatakeyama, & Hatakeyama, 2001). Furthermore, all refined groups possessed much higher system moduli than the control group, demonstrating that polysaccharide network was easier to form after DMSO-treatment. W70 possessed the largest crossover modulus, followed by W60, W80, W90 and W100 without considerable difference. The crossover temperature under the criterion $G' = G''$ was the sol–gel transition temperature. W70 exhibited the highest transition temperature, which meant best thermal stability.

4. Conclusions

In present study, 75% DMSO treatment was effectively proven to achieve an improvement of the KF quality. An enhancement of approximately 27% of transparency can be obtained in the hydrosol of the refined KF. Contents of impurities like starch, soluble sugar and protein of all five refined groups are much lower than the native group. KF with much less fishy smell was obtained after DMSO treatment. Furthermore, the DMSO treatment helps the viscosity to augment, with the maximum being 84.73%. Summarizing above findings, DMSO treatment is an alternative approach which is potentially useful for future studies on improving the KGM qualities.

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