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5   **Ultrasonic degradation kinetics and rheological profiles of a food polysaccharide (konjac**

6   **glucomannan) in water**

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Abstract

This study was carried out to evaluate the effects of power ultrasound (US) on the molecular weight and rheological properties of a food polysaccharide, konjac glucomannan (KGM). Upon the exposure of KGM solution (1% w/v in water) to US at a relatively high power intensity (50 W/cm^2), the apparent viscosity decreased rapidly from about 50 Pa·s to a negligible level within 10-20 min. The intrinsic viscosity ([η]) of KGM solution decreased gradually during the US exposure with a time course closely fitted to the first-order polymer degradation kinetics (random chain scission). The US treatment also caused a significant reduction of particle size (Z_{avg}) of KGM aggregates and changes in the rheological properties including the decrease of storage modulus (G') and loss modulus (G''), and the increase in phase angle (tan δ = G''/G'). Nevertheless, no change in primary structure was detected by Fourier transformation infrared (FT-IR) analysis. The results suggested that high intensity US was an effective means for KGM degradation without significant structural destruction.

Keywords: Polysaccharide; Ultrasonic degradation; Rheological properties

1. Introduction

Power ultrasound (US) has been applied to a variety of physical and chemical processes such as cleaning, degassing, emulsification, dispersion, homogenization, extraction, and various chemical reactions, and especially for clean and safe processing of food and natural products. In addition to the beneficial effects of power US on the processing efficiency that have been widely evaluated in previous studies, recent attention has been paid to the novel and special

38 effects of power US on the property and functionality of products, such as the rheological

39 properties of food hydrocolloids (Ashokkumar, 2015; Soria & Villamiel, 2010; Zhang et al.,

40 2013). In several recent studies, high-intensity US has been applied to the controlled

41 degradation of natural polysaccharides such as xanthan, chitosan, starch, and pectin, leading to

42 changes in water solubility, viscosity and other properties (Czechowska-Biskup et al., 2005;

43 Guo et al., 2014; R. Li & Feke, 2015; Zhang et al., 2013).

44   Konjac glucomannan (KGM) is a water-soluble polysaccharide originated from the tuber of

45 plant *Amorphophallus konjac* C. Koch with an average MW about 1000 kDa, which is

46 composed of β-(1→4) linked D-mannose and D-glucose in a molar ratio of 1.6:1 or 1.4:1

47 (Huang et al., 2002; Nishinari & Takahashi, 2003). KGM is widely used in the food industry,

48 mainly as a gelling and thickening agent in liquid foods owing to its thermo-irreversible

49 property (Herranz et al., 2012). Recently, KGM has been increasingly recognized as a bioactive

50 dietary fiber for relieving constipation, improving gut microbiota, controlling body weight, and

51 lowering blood sugar, cholesterol and the risk of diseases such as type II diabetes, obesity and

52 cancer (Behera & Ray, 2016; Tester & Al-Ghazzewi, 2013).

53   Although the high MW of KGM is important for its application as a thickening agent, lower

54 MW and lower viscosity are more favorable for the bioactive functions. As shown in previous

55 studies, partially degraded or hydrolyzed KGM fractions have higher bioactivities such as

56 antioxidant and probiotic activities (Al-Ghazzewi et al., 2007; Connolly et al., 2010; Wang et

57 al., 2008). Various means have been used for partial depolymerization of KGM and other

58 polysaccharides, such as chemical treatment (Li et al., 2009), enzyme hydrolysis (Jin et al.,

59 2010; Li et al., 2004), ultrasonic treatment (Chen & Qian, 2008; Huang et al., 2006), and γ-

3

60   irradiation (Prawitwong et al., 2007; Xu et al., 2007). The two previous studies (Chen & Qian,

61   2008; Huang et al., 2006) on ultrasonic degradation of KGM have all been on the optimal

62   conditions (e.g. US power, exposure period, temperature and KGM concentration) for rapid

63   reduction of liquid viscosity or intrinsic viscosity.

64   So far only a few preliminary studies have been documented on ultrasonic degradation of

65   KGM and it is of theoretical and practical significance to characterize and quantify the

66   degradation process kinetics and the related functional properties. This study was conducted to

67   investigate the process kinetics of ultrasonic degradation of KGM dissolved in water and the

68   effects of power US on its molecular structure and rheological properties.

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70   **2. Materials and methods**

71   *2.1. Materials and ultrasonic treatment of KGM*

72   Konjac glucomannan (KGM) was purchased from Hubei Konson Konjac Gum Co., Ltd. and

73   used without further purification and pretreatment. The purity of glucomannan was above 90%.

74   All other chemicals were analytical grade obtained from reliable and licensed suppliers. Power

75   US was generated with a Model VCX 750 processor of 20 kHz frequency (Sonics & Materials

76   Inc., Newton, USA). A probe horn with tip diameter of 13 mm was used in this study. For

77   ultrasonic treatment, 150 mL of KGM solution (10 g/L in DI water) was filled in a plastic

78   bottle, and the US probe was inserted into the solution about 3 cm deep. The KGM sample

79   bottle was immersed in ice-water during the treatment to avoid overheating. The US treatment

80   was performed for selected time periods (2, 5, 10, 20, 40 and 60 min) at 40% or 80% amplitude

81   by adjusting the parameters on the controller panel. The corresponding actual US power

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82   intensities (powers per unit area of the probe tip) transmitted into the solution at these two

83   amplitudes were 24 and 50 W/cm^2, respectively, which were obtained by the calibration curve

84   between amplitude and power intensity from our previous study (Cheung et al., 2015). After

85   the US treatment, the KGM sample solution was freeze dried and stored in a desiccator at room

86   temperature before analysis.

87

88   *2.2. Viscosity measurement*

89   Apparent viscosity of KGM sample solutions before and after ultrasonic treatment was

90   measured at 25°C with a rotational viscometer (Lab-line Instruments Inc., Melrose Park, IL,

91   USA). The instrument was operated according to the manufacturer's instruction manual to

92   maintain the torque range of 40-60% by adjusting the rotational speed of spindle for each

93   sample. Intrinsic viscosity [η] was determined by serial dilution method (Yan et al., 2009)

94   measured with an Ubbelohde viscometer (0.5-0.6 mm capillary diameter) at 25 ± 0.1^oC. The

95   freeze-dried samples were dissolved in 0.2 mol/L of NaCl solution, stirred overnight, and then

96   filtered through a Watman No. 1 paper to remove insoluble components. The addition of NaCl

97   to the sample solution was to avoid polymer adsorption on the wall of viscometer capillary.

98

99   *2.3. Molecular weight analysis*

100   The MW distribution of KGM samples was analyzed by gel permeation chromatography

101   (GPC) on a Waters HPLC equipment connected with a Waters refractive index detector (RI)

102   and a Wyatt DAWN laser scattering detector. A solution of 0.9% NaCl containing 0.02% NaN_3

103   was used as the mobile phase flowing at 0.8 mL/min and 30°C. The KGM samples were also

104   dissolved in this solution at 0.05 mg/mL, and centrifuged at 18,000 rpm for 10 min before

105   injection into the GPC system. Average particle size (Z_{avg}) was determined by Zetasizer

106   3000HSA (Malvern Instruments, Ltd.). The KGM samples were diluted 500 times with DI

107   water, centrifuged at 18,000 rpm for 10 min, and then measured at 25°C and a scattering angle

108   of 90°. The chemical structure of KGM was determined by a Nicolet Avatar 360 Fourier

109   transformation infrared (FT-IR) spectrometer with samples blended and pressed into tablets

110   with KBr.

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112   *2.4. Rheological measurement*

113   All the rheological properties including the flow behavior with increasing shear rate and

114   frequency sweep tests were measured with a rheometer (AR2000, TA Instruments, Newcastle,

115   UK) at a constant temperature of 25°C. The KGM sample solution for the rheological

116   measurement was prepared in DI water at 1.0% (w/v) and equilibrated to room temperature for

117   30 min before measurement. Dynamic oscillatory measurements were performed using parallel

118   plates (40 mm diameter, 1 mm gap), with a frequency sweep from 0.1 to 100 rad/s at 20%

119   strain, which was chosen to assure linear viscoelastic conditions. The samples were covered

120   with a thin layer of paraffin oil to prevent moisture loss by evaporation. The storage modulus

121   (G') and loss modulus (G'') as well as the loss tangent (tan δ =G''/G') were recorded.

122

123   **3. Results and discussion**

124   *3.1. Viscosity reduction and kinetics of KGM degradation by US*

125   Fig. 1a shows the apparent viscosity of KGM solution as a function of time during US

treatment at two power levels of 24 and 50 W/cm^2. At both US power levels, the apparent

viscosity of KGM solution decreased dramatically with time. At higher US power (50 W/cm^2)

the apparent viscosity fell steeply from the initial value of about 50 Pa·s to a minimal level

(similar to that of water) within 15 min of US exposure. At lower US power level (24 W/cm^2),

the apparent viscosity dropped rapidly to about 5 Pa·s in the first 20 min and it decreased slowly

in the remaining period to a very low level.

    As shown in Fig. 1b, the intrinsic viscosity [η] of KGM decreased continuously during the

60-min US treatment at both US power levels, and more rapidly at higher power intensity. In

contrast with the sharp drop of apparent viscosity to a very low level in the very early period

of US treatment, the reduction of [η] proceeded gradually and continuously without reaching

a steady value during the 60-min US treatment. This disparity suggests different process

mechanisms for the reduction of apparent viscosity and intrinsic viscosity by the US treatment.

The decrease in [η] was equivalent to the decrease in average MW, thus could be regarded as

an indication of KGM degradation by the US treatment. The reduction of apparent viscosity

was not only attributed to polymer degradation (breakage of polymer chain or glycosidic

linkage) but also to the disruption of aggregation.

    As for other natural polysaccharides (Zhang et al., 2013; Zhou & Ma, 2006), it is assumed

that the US degradation of KGM mainly occurs by the scission of main-chain linkage and the

reaction follows first-order kinetics for random chain session mechanism (the rate proportional

to concentration or total number of bonds) (Wu et al., 2008). The rate constant k is represented

by,

148    $1/M_t - 1/M_0 = kt/m$ (1)

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150   where M_t and M_0 are the weight-average molar mass at time t and zero of US treatment,

151   respectively, and m the molar mass of monomer. With the substitution of Mark-Houwink

152   equation ($[\eta]=k_{MH}\times M^{\alpha}$) into Eq.1, the following relationship between $[\eta]$ and t is derived,

153

154    $[\eta]_t^{-1/\alpha}-[\eta]_0^{-1/\alpha}=(k/k_{MH}^{1/\alpha})t/m$ (2)

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156   where $[\eta]_t$ and $[\eta]_0$ are the intrinsic viscosities at time t and zero, respectively, and k_{MH} and α

157   are the constants of Mark-Houwink equation. Based on Eq. 2, $(k/k_{MH}^{1/\alpha})/m$ can be estimated

158   from the slopes of $([\eta]_t^{-1/\alpha}-[\eta]_0^{-1/\alpha})$ against t in Fig. 2. As the k_{MH} and α used in this study were

159   0.0596 and 0.73 (Li et al., 2006), the degradation rate constant k of KGM to the molar mass of

160   monomer m under ultrasonic treatment at different power intensities are calculated. As shown

161   in Table 1, the experimental data were fitted closely to the linear equation with R^2 values close

162   to unity.

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164   *3.2. Molecular weight distributions*

165   Table 2 shows the molecular weight and polydispersity (M_w/M_n) parameters of KGM after

166   the US treatment. The values of M_w and M_w/M_n decreased with the increase in US power level

167   or treatment time. The results indicate that US treatment caused partial degradation of KGM

168   and the degradation was more significant at a higher US power intensity. The decrease in

169   polydispersity index M_w/M_n implied that the KGM became more uniform in MW distribution

170   with higher US power intensity and longer treatment time.

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3.3. Particle size analysis

Fig. 3 shows the change of Z_{avg} diameter of KGM aggregates in water after US treatment. A notable increase in Z_{avg} diameter immediately appeared after the US treatment for about 2.5 min, and the magnitude of increase was much higher at lower US power of 24 W/cm^2. However, the Z_{avg} diameter exhibited a steady decrease throughout the remaining period (5-60 min) of US treatment, i.e. from 539.4 to 343.6 nm at 24 W/cm^2 and from 411.8 to 276.2 nm at 50 W/cm^2, respectively. The shear forces created when cavitation bubbles collapse is most probably responsible for the changes in structures and properties of polysaccharides and their aggregates in solution (Camino et al., 2009). Although the shear forces produced by power US usually promote the dispersion of polysaccharide aggregates leading to particle size reduction, it can also induce the aggregation of biopolymers such as proteins in aqueous solution through chemical and physical effects on the biopolymer molecules (Stathopulos et al., 2004).

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3.4 Rheological properties of US-treated KGM

As shown in Figs. 4a-b, the native KGM in water exhibited the typical shear-thinning fluid behavior of a decreasing apparent viscosity with shear rate. The shear-thinning property of KGM solution was significantly dampened with 5 min US treatment at both power levels and was completely diminished with US treatment for 40 min at 24 W/cm^2 or 10 min at 50 W/cm^2. Eventually, the US treated KGM solution turned to a Newtonian fluid with a very low viscosity close to that of water. Therefore, power US treatment caused dramatic changes in the rheological properties of KGM solution as reported previously (Jin et al., 2014).

193   Figs. 5a-b present the profiles of storage modulus G' and loss modulus G'' from frequency

194   sweep test of KGM solutions (1% w/v) after various periods of US treatment at 24 and 50

195   W/cm^2. As seen from these two figures, both G' and G'' of KGM solutions were decreased

196   after the US treatment, due probably to the breakage of KGM polymer chain. The tan δ value

197   (tan δ = G''/G') of US treated KGM was much higher than the native KGM (Fig. 5c). Perhaps

198   the native KGM dissolved in water formed a relatively elastic dispersion (solid property) and

199   turned to a viscous dispersion (fluid property) after the US treatment.

200   Overall, the results in this section suggest that the US treatment caused the weakening of the

201   intermolecular interactions such as hydrogen bond and other non-covalent bonds between

202   KGM molecules in an aqueous solution as well as the intramolecular interactions between the

203   KGM polymer chains.

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205   *3.5. Structure characteristics of KGM*

206   Fig. 6 shows the FTIR spectra of native and US treated KGM, all of which had virtually

207   identical characteristic absorption peaks. The broad band at around 3429 cm^{-1} can be assigned

208   to the -OH in KGM, and the stretching and bending vibration absorption peaks at around 2924

209   and 1383 cm^{-1} to C-H (Xu et al., 2007). The peak at 1736 cm^{-1} is attributed to the stretching C

210   = O in acetyl groups (Prawitwong et al., 2007). The intense peak at 1640 cm^{-1} is ascribed to the

211   intramolecular hydrogen bonds and the peak at 1023 cm^{-1} to the stretching C-O, and the

212   absorbance bands at around 874 and 807 cm^{-1} are characteristic of mannose in KGM (Chen et

213   al., 2008). The high similarity of IR spectra of US treated KGM to native KGM implies that

214   the repeating units and primary structure of KGM were not affected by the US treatment.

4. Conclusions

The experimental results have shown several major effects of power US on the properties of KGM dissolved in water, including a dramatic reduction of apparent viscosity, average MW, and aggregate particle size and changes in rheological characteristics. US power and treatment period were two major factors affecting the rate of KGM polymer degradation and the property changes. The time course of degradation followed the first-order kinetics for random chain scission. US treatment did not cause notable destruction of the primary structure of KGM and was only effective for moderate degradation so that the degraded KGM fractions still retained a relatively high average MW in the order of 10^5 with a more uniform MW distribution. Therefore, power US is a useful means for viscosity reduction and partial degradation of KGM. For better understanding of the degradation mechanisms, it is worthwhile to investigate the specific sites of chain cession on the KGM molecule and the contributions of mechanical forces and/or free radicals arising from cavitation activities to the KGM degradation.

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317   Table 1 Degradation kinetic constants and linear regression coefficients R^2 of KGM under

318   different US power intensities.

US power (amplitude)	Rate constant (k/m)	R^2
24 W/cm^2 (40%)	1.035×10^{-5}	0.9881
50 W/cm^2 (80%)	5.481×10^{-5}	0.9951

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321   Table 2 Molecular characteristics of KGM solution treated at different US powers and periods.

US power	24 W/cm^2		50 W/cm^2	
US period	10 min	40 min	10 min	40 min
$M_w \times 10^{-5}$	5.735	5.033	4.096	1.646
M_w/M_n	1.227	1.212	1.275	1.180

322

Figure captions

324   Fig. 1 Effect of US treatment on (a) apparent viscosity and (b) intrinsic viscosity of KGM

325   solution (10 g/L; viscosity measured at 25°C) (Error bars: standard deviations of triplicate

326   measurements).

327   Fig. 2 The relative changes of $[\eta]^{-1/\alpha}$ of KGM solution caused by US treatment at two power
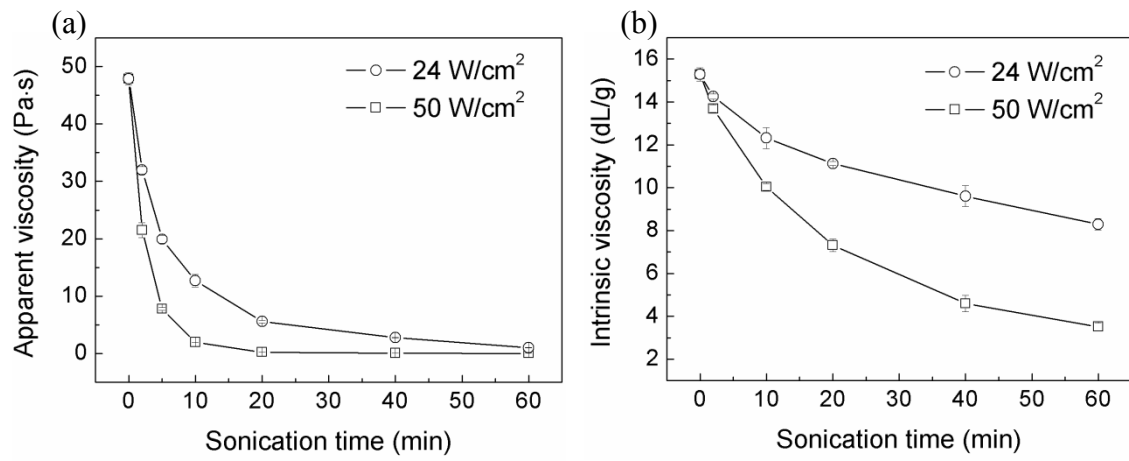
328   intensities.

329   Fig. 3 Variation of Z_{avg} diameters as a function of US treatment time at two power intensities

330   (Error bars: standard deviations of triplicate measurements).

331   Fig. 4 Effect of US treatment on steady-state flow curves (a, b) of KGM solution (1% w/v).

332   Fig. 5 Changes caused by US treatment of (a) storage modulus (G'), (b) loss modulus (G''),

333   and (c) phase angle (δ) of KGM solution with angle frequency.

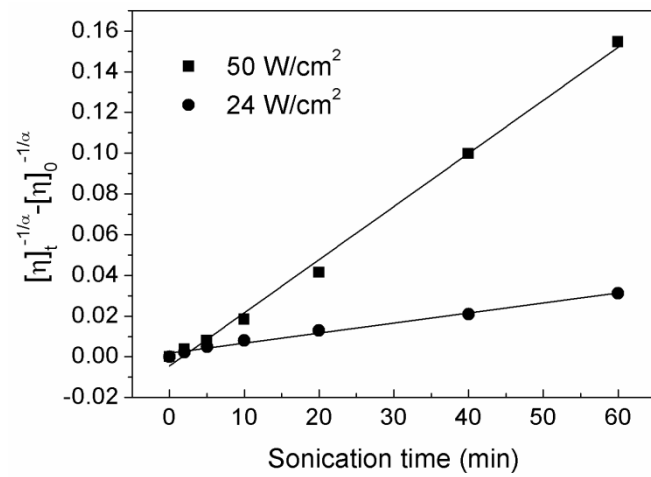334   Fig. 6 FT-IR spectra of native and US-treated KGM.

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Fig. 1 US on solution viscosity

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The y-axis is labeled $[\eta]_t^{-1/\alpha}-[\eta]_0^{-1/\alpha}$ and the x-axis is labeled Sonication time (min). Legend: ■ 50 W/cm^2, ● 24 W/cm^2.

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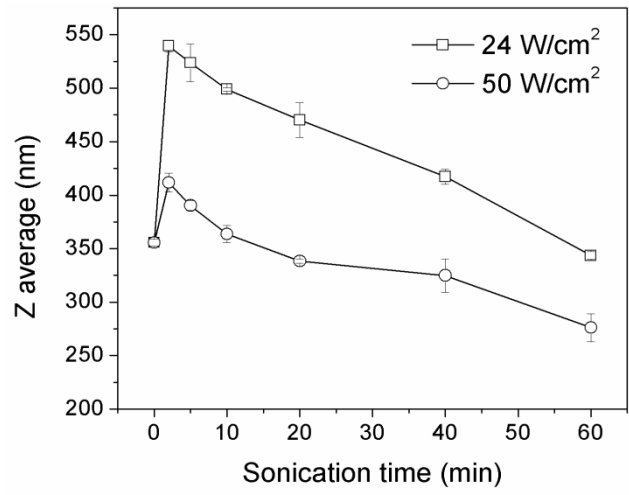Fig. 2 Degradation kinetics of KGM by US
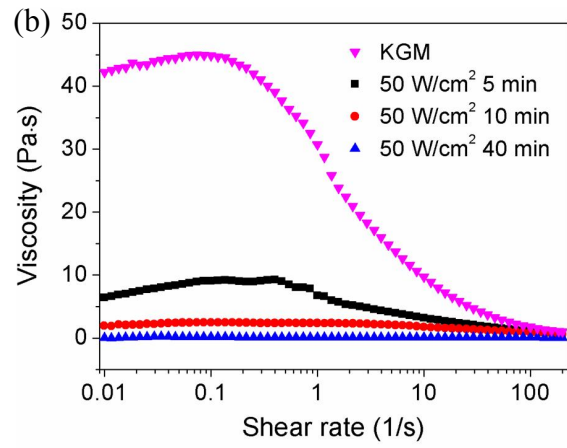
Fig. 3 US on KGM particle size

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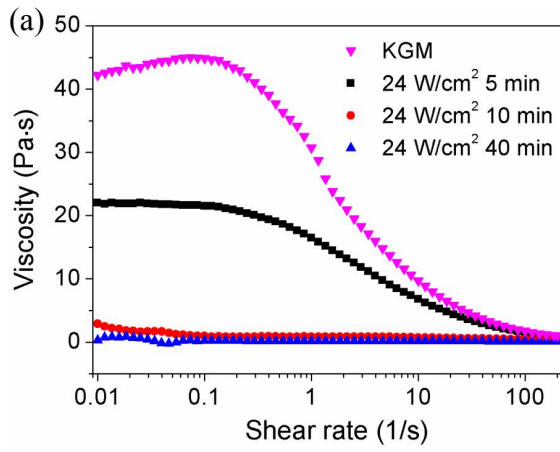
Fig. 4 US on steady-state flow curves
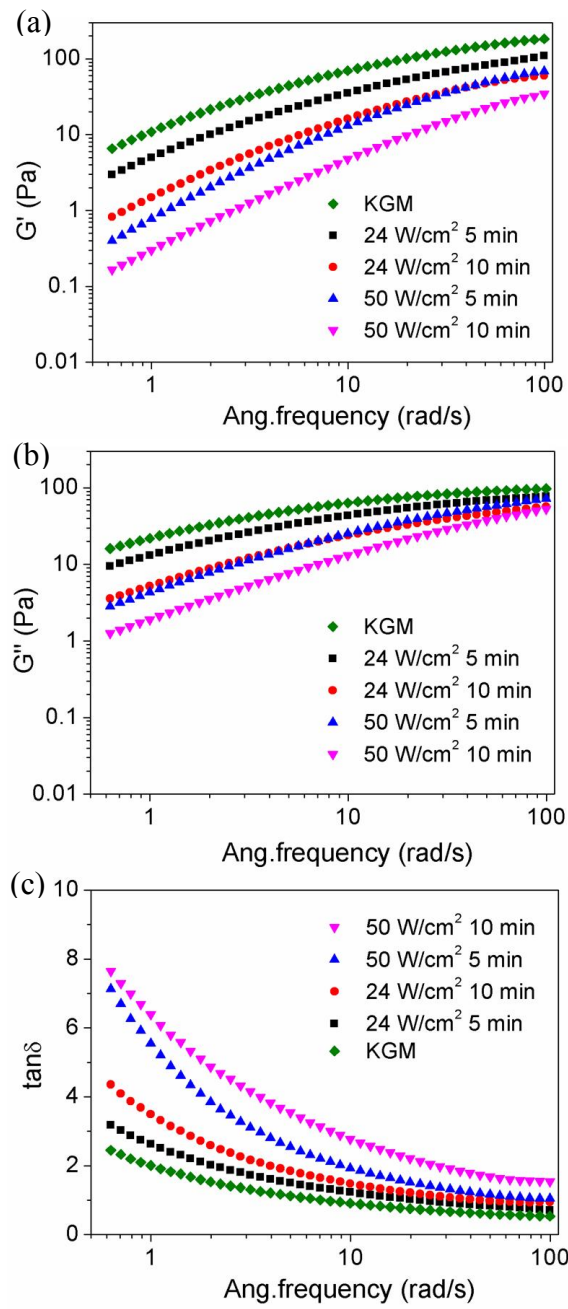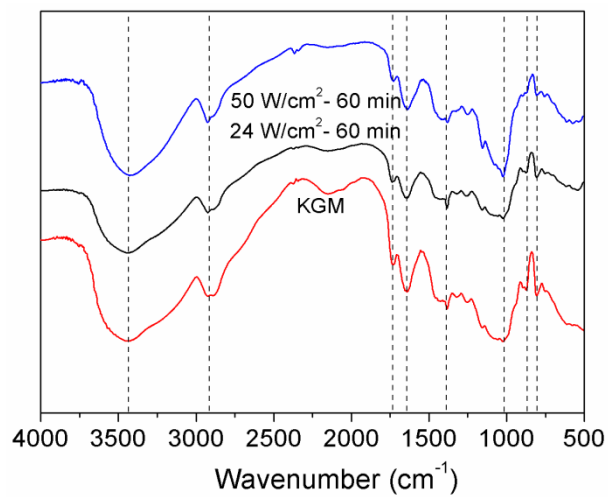
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(a)

(b)

(c)

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Fig. 5 US on rheological properties of KGM

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50 W/cm^2- 60 min

24 W/cm^2- 60 min

KGM

Wavenumber (cm^{-1})

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Fig. 6 FT-IR spectra

Highlights

► Ultrasound (US) was effective for partial degradation of konjac glucomannan (KGM).

► US power and treatment period were major factors affecting the degradation.

► High-intensity US treatment caused dramatic reduction of KGM solution viscosity.

► US degradation of KGM followed the first-order polymer degradation kinetics.