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Preparation and Application of Low Molar Mass Konjac Mannan

Machiko Takigami, Panida Prawitwong, Yoshiko Tahara, Ryoji Ohtsuka, Rheo Takahashi* and Shoji Takigami
Research Center for Instrumental Analysis, Gunma University, 5-1, Tenjincho 1-chome, Kiryu, Gunma 376-8515, Japan
Fax: 81-277-30-1141, e-mail:takigami@chem.gunma-u.ac.jp
*Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, 5-1, Tenjincho 1-chome, Kiryu, Gunma 376-8515, Japan
e-mail:rheo@bce.gunma-u.ac.jp

Abstract
Konjac mannan (KM) with high molar mass is preferred to prepare a traditional Japanese food, *Konjac*. Recently physiological functions of KM have been studied extensively and development of KM with lower molar mass is expected to find new application areas. The KM with high molar mass limits the application areas of KM flour. KM with lower molar mass was prepared by acid hydrolysis at elevated temperatures. Except for the molar mass, no chemical structural change was observed in hydrolyzed KM. Molar mass of KM was measured by multi-angle static light-scattering and size exclusion chromatography. Species of acid, acid concentration, hydrolysis time and temperature gave big influence on molar mass of hydrolyzed KM. Citric acid was much more effective to reduce molar mass than acetic acid. Molar mass of KM decreased with increase of acid concentration, hydrolysis time and temperatures. As depolymerization of KM by acid hydrolysis was very rapid above 100°C, it was inferred that not only temperature but also pressure played important role in acid hydrolysis. Changing parameters which affect molar mass, KM with molar mass in wide rage was prepared easily. KM with low molar mass did not form gel in the presence of alkali, and gel-sol transition temperature of KM-Xanthan mixed gels and viscosities of KM decreased with decrease of molar masses.
Key words: Konjac mannan, acid, hydrolysis, molar mass, gel

1. INTRODUCTION

Konjac mannan (KM) is a dietary fiber contained in a tuber of *Amorphophallus konjac* C. Koch. It has been used as foods and food additives in China and Japan for more than 1000 years. Non-thermoreversible elastic gel, a traditional Japanese food *Konjac* is prepared from the KM flour in the presence of alkali. High molar mass KM is essential for the preparation of high quality *Konjac*. Therefore, improvement of KM viscosity has been of interest to the industry. They have developed new species of Konjac plants whose tubers contain much amount of KM with high molar mass and viscosity. However, the high viscosity of KM has limited the usage of KM to *Konjac*, noodles and jellies. Recently, physiological functions of KM are extensively studied including laxation and blood cholesterol attenuation [1-3] because of the resistance of KM to digestion and absorption in the human small intestine. If KM with small molar mass and less viscosity is prepared, the usage of KM will expand to various areas such as supplement for people of aphagia, soft drinks, ready-to-eat materials, meat products, sauces and dressings, bakery products, dairy and desserts. In other words, the user of KM will expand from *Konjac* manufacturers to general consumers. Zhang et al. [4] reviewed the applications of KM and its derivatives in pharmaceutical [5, 6], bio-technical, fine chemical fields etc.

Polysaccharides undergo depolymerization through various methods; γ-ray irradiation [7-9], ultrasonic irradiation [10], chemical [11-13] and enzymatic hydrolysis [14-19]. Slavin and Greenberg [17] reported that the ease of use of partially hydrolyzed guar gum and its clinical effectiveness make it a good choice in clinical nutrition practice. Among the methods to prepare depolymerized polysaccharides, enzymatic hydrolysis seems to be dominant for KM as reported by Kohyama et al. [18] and Yoshimura and Nishinari [19]. They reported gelation kinetics of KMs with different molar masses in the presence of an alkaline coagulant. The gelation time became shorter and rate constant of gelation increased with increasing molar mass or concentration of KM or heating temperature [19]. The range of molar mass of the KMs they used was 2.56×10^5 to 5.96×10^5.

2. EXPERIMENTAL

2.1 Materials

KM flour was a gift from Ogino Shoten. To remove impurities, it was washed with a mixture of ethanol and water, and air-dried. Xanthan was purchased from Sigma-Aldrich, Germany, and dispersed into distilled water, centrifuged, dialyzed against distilled water and freeze dried. Other chemicals used in the study were reagent grade and used without further purification.

2.2 Preparation of low molar mass KM

The KM flour, water and acid were put into an autoclavable plastic bag. The concentration of KM was 3%. The bag was heated in an autoclave at 110, 115 or 121°C. After heating, the flask was kept in the autoclave for 30 min without heating to reduce the pressure. The sample was diluted to 0.5% and centrifuged at 15,000 g for 30min to remove cell wall debris of KM and then dialyzed against deionized

water until the conductivity of the dialysate became that of water. Then the KM solution was freeze-dried.

2.3 FT-IR measurement

FT-IR spectra of the original and hydrolyzed KM were measured by a Magna 560 FT-IR spectrometer equipped with a Continuμm II infrared microscope (Nicolet) to qualify the change of molecular structure of the samples. The FT-IR spectra of the samples were analyzed by the attenuated total reflection (ATR) method.

2.4 Multiangle static light-scattering measurement

Some of the hydrolyzed and freeze-dried KM samples were dissolved into 50mM aqueous $NaNO_3$ solution and filtered with 0.45μm omnipore hydrophilic PTFE membrane filters (Millipore), and molar mass of the KM was measured by multiangle static light-scattering (MALS) using a MALS detector from DAWN EOS (Wyatt Technology) with a vertically polarized Ga-As laser operated at wavelength of 684 nm. The photometer, which was calibrated using pure toluene and aqueous solutions of low molar mass dextran, was connected to a size exclusion chromatography (SEC) column of $GMPW_{XL}$ (Tosoh) and a differential refractive index detector OPTILAB DSP (Wyatt Technology), which was used to determine KM concentration at each position of elution peak. Temperature of the MALS flow cell and the column were controlled at 25±0.1 °C.

Scattered light intensities at scattering angle between 15-163° were measured. The angular dependence of the scattering intensity was analyzed using Berry's square-root plot to determine the radius of gyration (R_G) and molar mass (M) at each position of the peak. 50mM aqueous $NaNO_3$ solution was used as the eluent at the flow rate of 0.5ml/min.

2.5 SEC measurement

Samples were dissolved into purified water and retention times of the samples were measured by HITACHI HPLC equipped with an Intelligent Pump L-6200 and an RI Detector L-7490 at 30℃ to measure the molar masses. Column and eluent used for the measurement were TSK_{gel} $GMPW_{XL}$ (Tosoh) and water, respectively. The flow rate of the eluent was 0.5 ml/min. For the calibration, KMs with different molar masses, which were determined by MALS, were used.

2.6 Determination of acetyl group

The degree of substitution of acetyl groups in the KM sample was determined by titration with NaOH aqueous solution [20].

2.7 Gel formation

Gel forming ability of hydrolyzed KM in the presence of alkali was examined by mixing KM and sodium carbonate solution to be 3% and 0.2%, respectively, in the final concentrations. The mixture in glass tube was boiled for 30 min and cooled. The gel formed in the tube was observed and gel forming ability of hydrolyzed KM was evaluated. .

2.8 Measurement of gel-sol transition temperature

One percent solutions of KM and Xanthan were mixed in the same volume and the mixture was pored into a glass tube with a stainless steel ball. The tube was put into a water bath and the bath temperature was increased from 45 to 65℃ at the rate of 0.5℃/min. The gel-sol transition temperature was measured by the falling ball method. The position of stainless steel ball and temperature were monitored using a video camera and position of the ball was plotted against the bath temperature. A point of intersection of sol and gel tangent lines was determined as gel-sol transition temperature.

2.9 Viscosity measurement

Viscosity of 0.5% aqueous KM solution was measured by a vibrating viscometer, VIBRO VISCOMETER SV-10 (A&D). .

3. RESULTS AND DISCUSSIONS

3.1 Molar mass of KM

Molar masses and polydispersities of the hydrolyzed KM by acetic acid at 121℃ for 30 min were measured by MALS and shown in Table I.

Table I Weight average molar mass of hydrolyzed KM by acetic acid at 121℃ for 30 min and measured by MALS

Acetic acid concentration (mM)	$10^{-3}M_w$ (g/mol)	M_w/M_n
0	631	1.82
5	237	2.27
10	190	1.75
20	106	1.85
50	56.2	2.22

Molar mass decreased drastically at the concentration of 5mM and then decreased gradually with increase of acetic acid concentration.

Weight average molar masses measured by MALS were plotted against retention times measured by SEC as shown in Fig. 1 together with the molar masses of γ-irradiated KMs [8]. Based on the results of MALS and SEC, weight average and number average molar masses of hydrolyzed samples by acetic acid and citric acid at 121℃ for 30 min were determined and shown in Fig. 2. Molar masses of KMs decreased with

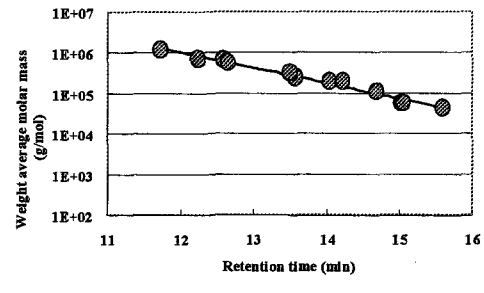

Fig. 1 Calibration curve of KM based on results of MALS and SEC

Molar masses of KM treated with citric acid decreased more than those treated with acetic acid. The results reflect the effectiveness of the acids. Citric acid is tribasic, while acetic acid is monobasic. Furthermore, plot of $1/DP_c - 1/DP_o$, which is related to number of

KM molecules increased by acid hydrolysis, against acid concentration showed that the slope for citric acid was 14 times of that for acetic acid, where DPc and DPo are number average molar mass of KM after and before hydrolysis, respectively (Fig. 3). Therefore, some factors besides polybasicity are responsible for the effectiveness of citric acid. It shows that KM having molar mass in wide range can be prepared easily, changing species of acid and concentration.

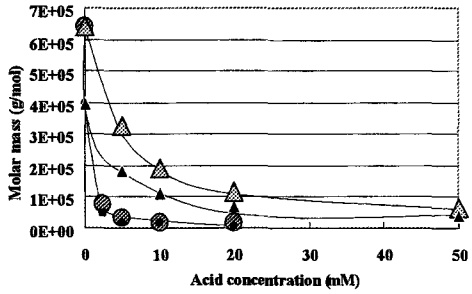

Fig.2 Molar masses of KM treated with acetic acid and citric acid at 121 °C for 30 min.
Mw (△) and Mn (▲)of acetic acid treated KM
Mw (◎) and Mn (●)of citric acid treatedKM

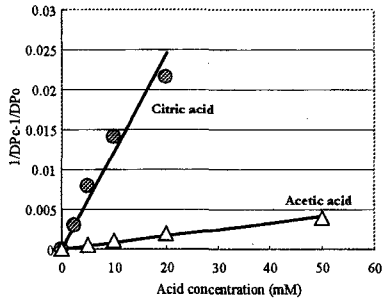

Fig. 3 Hydrolysis of KM by acetic acid and citric acid at 121℃ for 30min

3.2 Gel forming ability of KM

The gel forming ability of KM in the presence of sodium carbonate is summarized in Table II. In appearance, all KM samples formed gel in the presence of alkali, however, KM treated with 50mM acetic acid made just an assembly of KM, actually no gel was formed. With the increase of acetic acid concentration, the gel formed became weaker. It is reported by Gao and Nishinari [21, 22] that acetyl groups contained in KM is responsible for alkaline gel formation in KM. Degree of acetylation of the samples did not change after acid hydrolysis (0.08 for one carbohydrate unit). Besides, FT-IR spectra of the samples did not show any new absorbance after acid hydrolysis. Therefore, we can conclude that acid hydrolysis did not cause any chemical structural change in KM except for molar mass. It means that the high molar mass is essential for stiff gel formation in the presence of alkali.

Table II Gel formation of KM treated with acetic acid at 121℃ for 30 min

Acetic acid concentration (mM)	Feature of gel
0	Stiff gel
5	Fairly stiff gel
10	Weak gel
20	Fragile gel
50	No gel formation

3.3 Gel-sol transition of mixture gel

Mixture gels composed of 0.5% KM and 0.5% Xanthan was prepared and gel-sol transition temperature was measured by falling ball method. The transition temperature, as shown in Fig. 4, decreased with acetic acid concentration with which KM was hydrolyzed. Remarkable reduction in the temperature was observed when 50mM acetic acid was used.

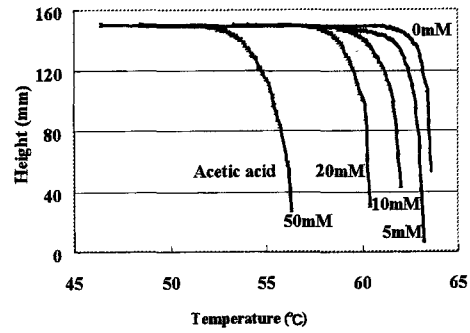

Fig. 4 Gel-sol transition temperature of KM -Xanthan mixed gels
Total polysaccharide concentration : 1 wt%
M/Xanthane:1/1, KM: Hydrolyzed with acetic acid at 121 °C.

Although there are some reports on mechanism of the synergic gel formation of KM and Xanthan, it is clear that molar mass of KM gives effect on the gel formation as shown in Fig. 5.

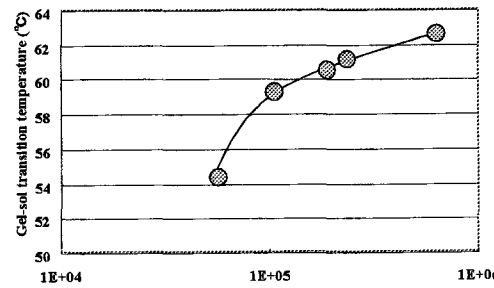

Fig. 5 Gel-sol transition temperature of hydrolyzed KM by acetic acid at 121℃ for 30min.

3.4 Viscosity of KM

The viscosities of 0.5% KM before and after hydrolysis with acetic acid at 121℃ for 30min are shown in Fig. 6 together with that of water. With the increase of acetic acid concentration, viscosity decreased. 0.5% solution of original KM was very viscous and difficult to drink, however, that of KM hydrolyzed with 50mM acetic acid was not viscous at all.

3.5 Effect of temperature on acid hydrolysis of KM

KM hydrolyzed with acetic acid at different temperatures had the different molar masses as shown in Fig. 7. Molar mass decreased with increase of temperature and concentration of acid. The plot of

1/DPt – 1/DPo against temperature, shown in Fig. 8, showed that the slope for samples hydrolyzed with 10mM acid was just twice of that for samples hydrolyzed with 5mM acid. Here DPt expresses number average degree of polymerization after hydrolysis at t °C. The values of (1/DPt-1/DPo) at 120°C were 2.4 and 3.5 times of those at 110°C for samples hydrolyzed with 5mM and 10 mM acetic acid, respectively. Generally speaking, increase of 10 degree in reaction temperature promotes reaction rate twice. The higher hydrolysis rate at elevated temperature indicates that pressure plays an important role in the decomposition reaction. The degree of hydrolysis of KM by 500mM sulfuric acid at 90°C for 15min reported by Okimasu [23] was almost the same with that by 2.5mM citric acid at 121°C for 30min. Therefore, we can say that the application of high temperature and pressure during acid hydrolysis is effective to obtain KM with less molar mass using less amount of acid.

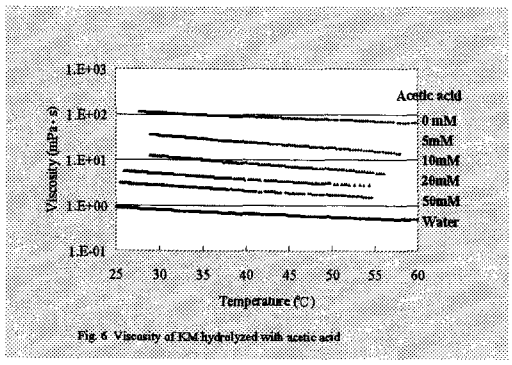

Fig. 6 Viscosity of KM hydrolyzed with acetic acid

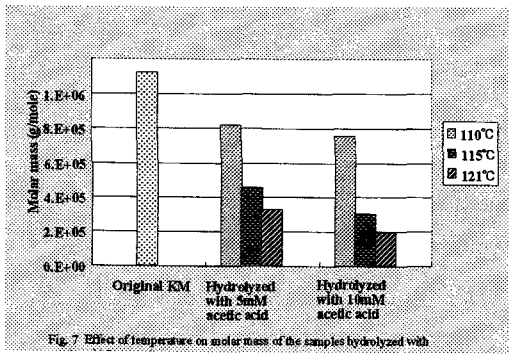

Fig. 7 Effect of temperature on molar mass of the samples hydrolyzed with

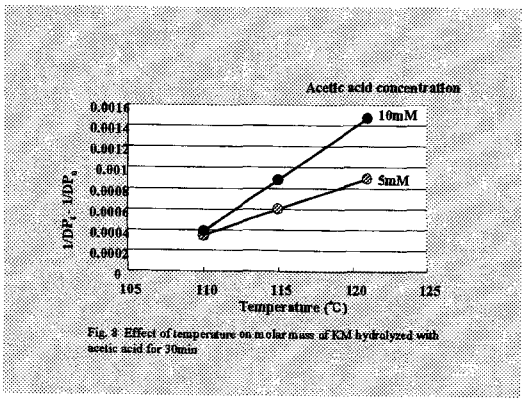

Fig. 8 Effect of temperature on molar mass of KM hydrolyzed with acetic acid for 30min

4. CONCLUSIONS

KM with high molar mass was hydrolyzed by acid at elevated temperatures to give KM with less molar mass. Molar mass of KM decreased with increase of acid concentration, hydrolysis time and temperatures. Citric acid was much more effective to reduce molar mass than acetic acid. Thus KM with various molar mass was easily prepared. KM with low molar mass did not form gel in the presence of alkali, and gel-sol transition temperature of KM-Xanthan mixed gels and viscosities of KM decreased with decrease of molar mass. Using the KM with low molar mass thus prepared, development of new application areas is expected.

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