

Effects of γ -irradiation on Properties of Konjac Mannan

Panida Prawitwong, Shoji Takigami, Rheo Takahashi* and Glyn O. Phillips**

Technical 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, Kiryu, Gunma 376-8515, Japan

** Phillips Hydrocolloids Research Centre, NEWI, Mold Rd., Wrexham, LL 2AW, UK

Konjac mannan (KM) is a water soluble glucomannan with high molar mass. KM aqueous solutions have extremely high viscosities and give irreversible gels in the presence of alkali. Here, the effects of γ -rays irradiation on the molar mass distribution and viscosity of KM were studied when irradiated under reduced pressure. The changes in properties after irradiation were investigated using ESR, FT-IR, SEC-MALS and viscometrically. Free radical yields increased with absorbed dose. No significant new chemical groups were introduced into the structure as a result of the irradiation. Molar mass, R_G and intrinsic viscosity of the irradiated KM decreased rapidly with dose up to 10 kGy and then gradually. The Mark-Houwink-Sakurada equation determined for the KM was $[\eta] = 5.30 \times 10^{-4} M^{0.78}$. The α value showed that KM molecules were solvated in the form of random coils in water.

Key words: konjac mannan, γ -irradiation, molar mass, viscosity, radius of gyration

1. INTRODUCTION

Konjac mannan (KM) is a neutral glucomannan derived from tubers of *Amorphophallus Konjac* C. Koch. It is mainly cultivated in Japan and found also in other Asian countries. It is composed of β -1, 4 linked β -D-glucose and β -D-mannose backbone [1,2] with some branches on the C-6 of glucosyl units [3]. The glucose:mannose ratio is approximately 1:1.6 [4,5]. It contains some acetyl groups in the main chain. Deacetylation occurs in the presence of alkali and a thermally stable gel is formed [6]. The solubility of KM in water is low due to strong hydrogen bonding in its structure. Therefore it was difficult to estimate its molar mass by viscometry. Chemicals modification [7], using a solvent other than water [8], sonication [9] and application of heat under pressure [10] have been used for the improvement of water solubility. However, there was concern that such severe treatments might cause main chain scission. High energy radiation to polysaccharides can cause main chain scission [11,12].

The object of this study is using γ -radiation to prepare KM with lower molar mass but increased solubility. For the KM thus obtained, the Mark-Houwink-Sakurada equation was determined and the effects of radiation on chemical structure of KM were investigated.

2. EXPERIMENTAL

2.1 Materials

To remove impurities, commercial konjac flour (Akagi ohdama species) was washed with aqueous methanol solution several times before air-drying. The

degree of substitution (DS) of acetyl groups in the KM, determined by the Kunz method [13], was 0.08. Other chemicals were reagent grade and used without further purification.

2.2 γ -rays irradiation

The purified KM was irradiated with ^{60}Co γ -rays under reduced pressure at room temperature. The absorbed dose was varied from 0.5 to 100 kGy.

2.3 ESR measurements

ESR measurements on the γ -irradiated KM were carried out using a JES-RE2X ESR spectrometer (JEOL) at room temperature. The modulation frequency and the microwave power were 100 kHz and 1 mW. The relative intensity of the radicals was determined by the double integration method.

2.4 FT-IR measurements

Fourier transform infrared (FT-IR) measurements of the irradiated KM were carried out using a Magna 560 FT-IR spectrometer equipped with a Continuum infrared microscope (Nicolet). The FT-IR spectra of the samples were analyzed by the attenuated total reflection (ATR) method.

2.5 Molar mass measurement

Molar mass of KM samples was determined by a multiangle static light-scattering (MALS) using a MALS detector from DOWN 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. The temperature of the MALS flow cell and the column were controlled at $25 \pm 0.1^\circ\text{C}$.

The KM sample solutions were filtered through a $0.45 \mu\text{m}$ omnipore hydrophilic PTFE membrane filter (Millipore). Scattered light intensities at scattering angles 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 at each position of the peak. 3mM aqueous NaN_3 solution was used as both solvent and eluent at 0.5 mL/min .

2.6 Viscosity measurement

The intrinsic viscosity $[\eta]$ of the irradiated KM aqueous solution was measured using an Ubbelohde viscometer at $25 \pm 0.1^\circ\text{C}$ at varying solution concentrations. The flow time of distilled water was 941.99 sec .

3. RESULT AND DISCUSSIONS

3.1 ESR measurements

ESR spectra of the irradiated KM on the day of irradiation are shown in Fig.1. The original KM showed a very weak singlet spectrum due to the radical formed by the mechanical manufacturing process of the flour when the KM was prepared by crushing the dried konjac tuber. Main chain scission could have occurred during this process. The KM irradiated at 0.5 kGy showed a doublet spectrum. As absorbed dose increased, the doublet component increased and new peaks appeared outside of the doublet. The new peaks became more prominent as irradiation continued over 5 kGy when the intensity of the peaks increased with the dose. However, the shape of the spectra did not change with the dose. The ESR spectra of irradiated KM are quite similar to those of γ -irradiated cellulose [14]. The ESR spectra showed superimposed patterns, and it can be inferred that there are at least three kinds of radicals produced in the irradiated KM. Radicals resulting from the main chain scission give rise to a singlet in the ESR spectrum, due probably to an alkoxy radical and a three line spectrum, which can be attributed to a C1 and/or C4 radical [15]. Another dominant radical shows up in the spectrum as a doublet and increases in concentration as irradiation dose is increased. Overall the behaviour is as generally observed in other polysaccharide radiation induced chain scission processes.

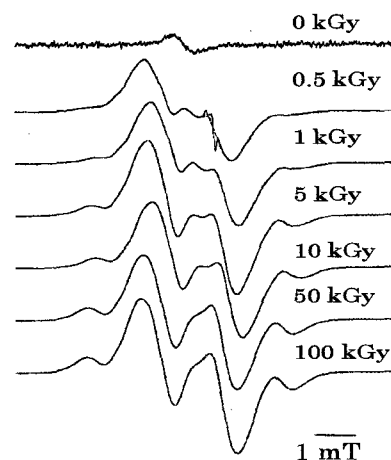


Fig.1 ESR spectra of γ -irradiated KM.

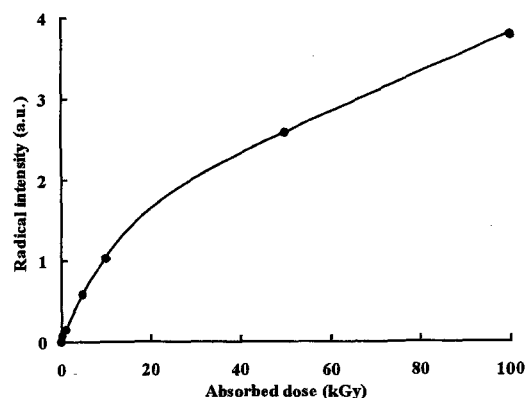


Fig.2 Relationship between radical intensity and absorbed dose for irradiated KM.

The radical intensity is given as the ratio of intensity to that of original KM. Fig. 2 shows the relationship between the relative radical intensity and the absorbed dose. The radical intensity increased rapidly up to 10 kGy and then increased linearly with increase of the absorbed dose.

3.2 FT-IR spectra

FT-IR spectra of the irradiated KM were measured by the ATR method. Fig. 3 shows a typical FT-IR spectrum of the irradiated KM and the absorbed doses are given on the spectra. The KM samples show no noticeable difference on the FT-IR spectra. A small peak assigned to the $\text{C}=\text{O}$ stretching vibration of acetyl groups is observed at 1730 cm^{-1} for all samples [6]. To examine influence of γ -irradiation on the elimination of acetyl groups, the ratio of absorbance at 1154 cm^{-1} assigned to $\text{C}-\text{O}-\text{C}$ anti-symmetry stretching vibration of the pyranose ring to the absorbance of $\text{C}=\text{O}$ was determined [16]. The ratio did not change with the absorbed dose. Accordingly, it was concluded that no significant new chemical groups were introduced into the structure as a result of the irradiation.

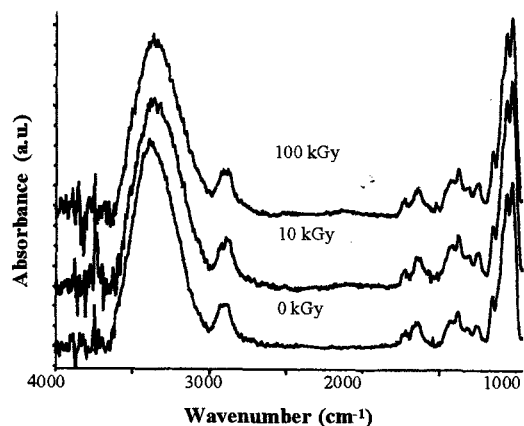
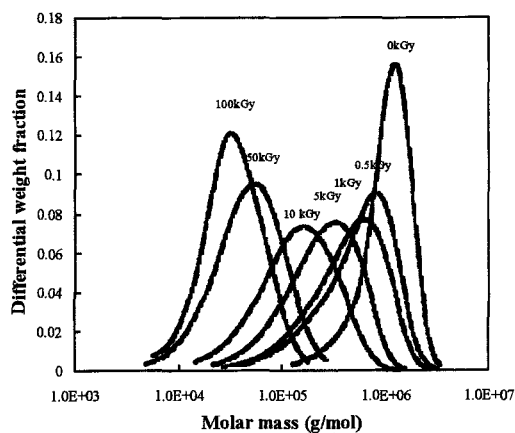
Fig. 3 FT-IR spectra of KM and γ -irradiated KM.

Fig. 5 Molar mass distribution of irradiated KM.

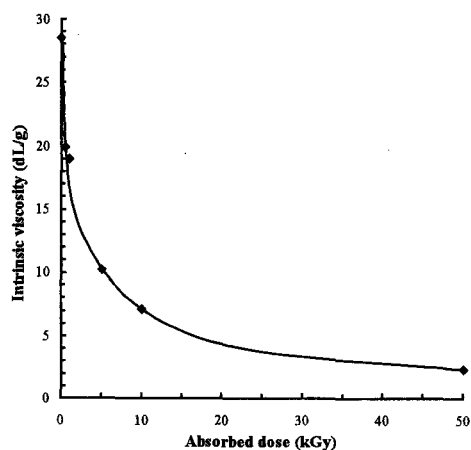
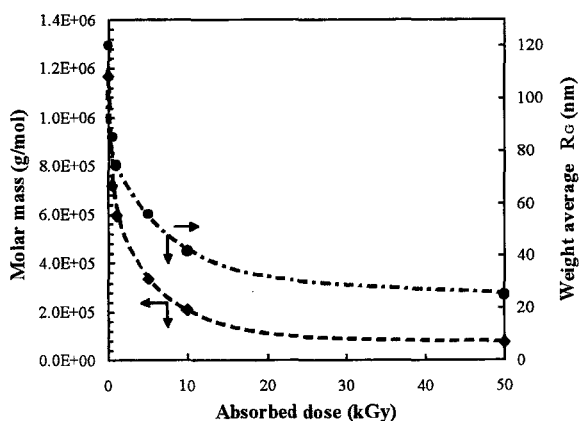


Fig. 4 Effect of absorbed dose on intrinsic viscosity of irradiated KM.

3.3 Molar mass and viscosity

The intrinsic viscosity of the original KM aqueous solution was 28.45 dL/g and the Huggins constant was 0.77 when measured at 25°C. Fig. 4 shows the relationship between the intrinsic viscosity of irradiated KM and the absorbed dose. The intrinsic viscosity decreased significantly with increasing absorbed dose up to 10 kGy and then decreased gradually. The values of Huggins constant for irradiated KM were in the range of 0.31 to 0.52.

To determine the absolute molar mass, SEC-MALS measurements were carried out using 3mM aqueous NaN_3 as both solvent and eluent. SEC chromatograms of the irradiated KM are shown in Fig. 5. The chromatogram shifted to lower molar mass with absorbed dose, but the width of peak hardly changed. It is inferred that the decrease of molar mass is caused by the main chain scission of KM induced by the irradiation.

Fig. 6 Effect of absorbed dose on molar mass and weight average R_G of irradiated KM.

The weight average molar mass (M_w) and the radius of gyration (R_G) calibrated to weight average were determined and they are plotted against the absorbed dose (Fig. 6). The values of M_w and R_G of original KM were 1.17×10^6 and 120.3 nm, respectively. The values of both M_w and R_G decrease with increase of the dose and the behaviour is consistent with the changes in intrinsic viscosity (Fig. 4).

Fig. 7 shows the log-log plot of the intrinsic viscosity vs. M_w for the irradiated KM. The logarithmic value of intrinsic viscosity increased linearly with log M_w and obeys the Mark-Houwink-Sakurada equation as follows,

$$[\eta] = 5.30 \times 10^{-4} M^{0.78} \quad (1)$$

It is known that the α value (exponent value) is related to shape of the polymer and nature of solvent. The α value we obtained is slightly higher than proposed values for aqueous methyl KM (0.74) [17] and for enzymatic degraded KM (0.69) measured in cadoxen solution [18].

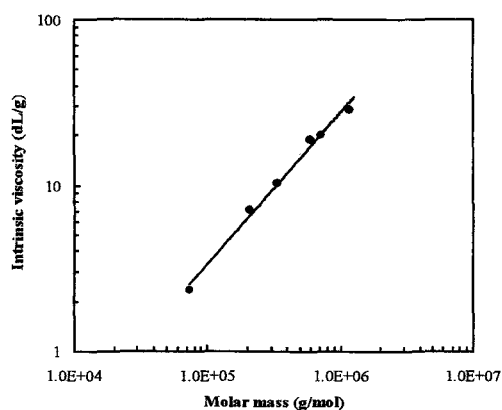


Fig. 7 Relationship between intrinsic viscosity and molar mass of irradiated KM.

To confirm the accuracy of the α value, $\log R_G$ is plotted against $\log M_w$ for the irradiated KM (Fig. 8). As seen in Fig. 8, the linear relationship is obtained according to the relationship

$$R_G = kM^v \quad (2)$$

where k and v are constants. The values of k and v were determined to be 2.77×10^{-2} and 0.60, respectively. In general, the α value is related to the v

$$\alpha = 3v - 1 \quad (3)$$

In this study, the calculated α value is 0.79. Since the calculated α value accords with the experimental value within experimental error, we consider that α value of 0.78 we obtained to be reasonable, which indicates that KM molecules are solvated in the form of a random coil in water. The slightly higher α value is presumed to be due to the branch structure of KM.

4. Conclusion

γ -irradiation process is a useful method to prepare KM with the desired molar mass. The core chemical structure of KM is not significantly changed by the process and no new chemical groups are introduced. The weight average molar mass, R_G , and the intrinsic viscosity decrease with increase of the absorbed dose. The relationship between the molar mass and the intrinsic viscosity is given by Mark-Houwink-Sakurada equation and the α -value is 0.78. KM molecules are solvated in the form of random coils in water.

5. References

- [1] K. Nishinari, P.A. Williams and G.O. Phillips, *Food Hydrocolloids*, **6**, 199-222 (1992).
- [2] S. Takigami, "Handbook of Hydrocolloids", Ed. by G.O. Phillips and P.A. Williams, Woodhead Publishing, Cambridge (2000) pp. 413-424.

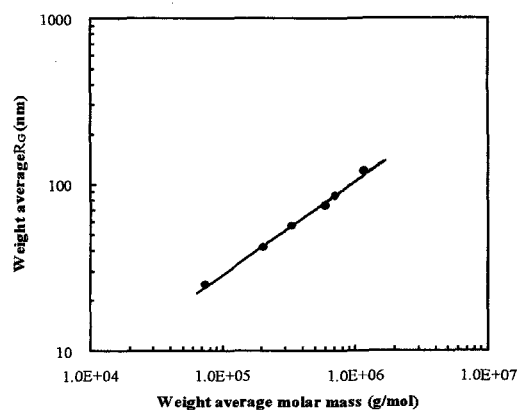


Fig. 8 Relationship between weight average R_G and weight average molar mass of irradiated KM.

- [3] K. Katsuraya, K. Okuyama, K. Hatanaka, R. Oshima, T. Sato and K. Matsuzaki, *Carbohydr. Polym.*, **53**, 183-89 (2003).
- [4] H. Shimahara, H. Suzuki, N. Sugiyama and K. Nisizawa, *Agric. Biol. Chem.*, **39**, 293-99 (1975).
- [5] M. Maeda, H. Shimahara and N. Sugiyama, *Agric. Biol. Chem.*, **44**, 245-52 (1980).
- [6] K. Maekaji, *Agric. Biol. Chem.*, **38**, 315-21 (1974).
- [7] N. Kishida and S. Okimasu, *Agric. Biol. Chem.*, **42**, 669-70 (1978).
- [8] K. Kohyama, Y. Sano and K. Nishinari, *Food Hydrocolloids*, **10**, 229-38 (1996).
- [9] S.M. Clegg, G.O. Phillips and P.A. Williams, "Gums and Stabilisers for the Food Industry 5", Ed. by G.O. Phillips, P.A. Williams and D.J. Wedlock, Pergamon Press, Oxford (1990) pp. 463-71.
- [10] I. Ratcliffe, P.A. Williams, C. Viebke and J. Meadows, *Biomacromolecules*, **6**, 1977-86 (2005).
- [11] Y. Kusama, E. Kageyama, M. Shimada and Y. Nakamura, *J. Appl. Polym. Sci.*, **20**, 1679-88 (1976).
- [12] J.M. Wasikiewicz, F. Yoshii, N. Nagasawa, R.A. Wach and H. Mitomo, *Radiation Physics and Chemistry*, **73**, 287-95 (2005).
- [13] M.L. Wolfrom and A. Thomson, "Methods in Carbohydrate Chemistry vol.1", 2nd ed, Ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York (1964) pp. 448-49.
- [14] M. Shimada, Y. Nakamura, Y. Kusama, O. Matsuda, N. Tamura and E. Kageyama, *J. Appl. Polym. Sci.*, **18**, 3379-86 (1974).
- [15] Y. Nakamura, Y. Ogiwara and G.O. Phillips, *Polymer Photochemistry*, **6**, 135-59 (1985).
- [16] H. Zhang, M. Yoshimura, K. Nishinari, M.A.K. Williams, T.J. Foster and I.T. Norton, *Biopolymers*, **59**, 38-50 (2001).
- [17] N. Kishida, S. Okimasu and T. Kamata, *Agric. Biol. Chem.*, **42**, 1645-50 (1978).
- [18] K. Kohyama, H. Iida and K. Nishinari, *Food Hydrocolloids*, **7**, 213-26 (1993).