

Study on Sorbed Water in Konjac Mannan

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Konjac mannan (KM) is a water soluble glucomannan with high molar mass. The solubility of KM in water is low due to its strong structural hydrogen bonding and the aqueous solution shows extremely high viscosity. Here, the phase transition behavior of sorbed water in KM-water system with various water content (Wc) was investigated using differential scanning calorimetry (DSC). At least five types of sorbed water can be identified in the KM-water system: 1) non-freezing water, 2) three types of freezing bound water designated W_{fb1} , W_{fb2} and W_{fb3} , and 3) free water. Glassy water was also found in with Wc less than 1.6. The proportion of each type of water changed with increasing Wc. The equivalent value of non-freezing water per pyranose ring was ca. 6.6 (mol/mol). W_{fb1} and W_{fb2} were categorized as freezing bound water that strongly received interaction from KM molecules at lower Wc region and transferred to W_f in the high Wc region. W_{fb3} was strongly bound water, maintaining interaction between KM chains; the equivalent value of W_{fb3} per pyranose ring was ca. 0.7 (mol/mol). Most of the sorbed water in the system with high Wc was held as W_f . The system with lower Wc exhibited lyotropic liquid crystalline (LC) behavior, but not thermotropic LC property. The endothermic transition observed at temperatures over 0 °C should reflect conformation change of KM induced by breakdown in hydrogen bonding.

Key words: konjac mannan, DSC, sorbed water, glassy water, liquid crystalline

1. INTRODUCTION

Konjac mannan (KM) is a water soluble glucomannan, with the ratio of β -D-glucose (G) to β -D-mannose (M) between 1 to 1.6 [1, 2]. KM has side chains and the branching positions are considered to be the C3 of M [3] and/or C3 of both G and M [2]. Recently, it was reported that KM has a branching point at C6 of the glycosyl units with β -C1 link C6 of glycosyl unit as the branching group [4]. Acetyl group also exists in the main chain. The solubility of KM in water is low due to strong hydrogen bonding in its structure. KM aqueous solution has extremely high viscosity and give irreversible gels in the presence of alkali [5, 6, 7]. Information about the state of water in water swollen KM is sparse.

In polysaccharide hydrogels, three types of water have been distinguished: non-freezing water, freezing bound water and free water [8, 9, 10]. When examined by a differential scanning calorimeter (DSC), the free water shows an endothermic peak on melting at a temperature identical with pure water. The freezing bound water promotes interaction between polymer molecules and melts below 0 °C. The non-freezing water is strongly associated with the polymer and shows no the first-order transition.

In this study, the phase transition behavior of sorbed water in KM-water system is measured by DSC and the nature of each type of sorbed water investigated by changing the water content. The state of sorbed water in relation to the water holding ability of KM and the liquid crystal properties of water swollen KM were also

investigated.

2. EXPERIMENTAL

2.1 Material

Commercial konjac flour (Akagi Ohodama species) was supplied by Ogino Shoten Co. Ltd. The flour was washed with methanol aqueous solution several times before air-drying. The flour was dissolved in distilled water and centrifuged to remove cell wall debris and afterwards freeze-dried.

2.2 Measurements

2.2.1 DSC measurements: The freeze-dried KM (0.5 - 2 mg) was placed in aluminum pans, and excess water added. In order to adjust the water content of the samples, surplus water was removed at room temperature. The pans were subsequently sealed hermetically and kept at room temperature overnight. A DSC 6200 (EXSTAR6000 series, Seiko Instruments, Inc.) equipped with an automatic nitrogen gas cooling unit was used to measure the phase transition of sorbed water in the samples. The measurements were carried out from room temperature to -140 °C and thereafter up to 50 °C at 10 °C /min. The transition temperature was defined as the extrapolated onset temperature as given by the DSC curves. The temperature scale and heat of transition were calibrated using distilled water and indium as standards.

2.2.2 Water content: After DSC measurements, the sample pans were pricked and dried under reduced pressure at 110 °C for 30 min and then left overnight at room temperature. After weighing, the water content (Wc)

was determined:

$$W_c \text{ (g/g)} = \frac{\text{Weight of water (g) in KM}}{\text{Weight of dry KM (g)}} \quad (1)$$

The following designations are used for the weights of the various types of water: W_{nf} , non-freezing, W_{fb} , freezing-bound, and W_f , free water. W_{fb} and W_f were calculated from the endothermic transitions. W_{nf} was then obtained from the expression:

$$W_c = W_{nf} + W_{fb} + W_f \quad (2)$$

2.2.3 Polarized optical microscopy: A very small quantity of freeze dried KM was placed on a slide glass, with a small amount of distilled water alongside and covered with glass. Water gradually seeped into the KM, giving water swollen KM material with a concentration gradient. An Olympus BX51-P polarized optical microscope equipped with a temperature control unit TH-99 was used to measure the higher order structure of the swollen KM. The sample was observed under the crossed Nicols condition using a color sensitive plate. The observations were taken at temperatures from -5 to 50 °C.

3. RESULTS

DSC cooling and heating curves for KM-water systems with various W_c are shown in Figures 1 and 2. The W_c values are given on the curves. When W_c is less than 0.40, no marked change due to a phase transition of the sorbed water is observed, but the base line shows some change near -40 °C on the cooling curves and a small endothermic peak appears near 5 °C on heating curves. All of such water is categorized as non-freezing water (W_{nf}), and is water which attaches closely to the polymer matrix. The base line change can be attributed to a glass transition of the KM molecule. At W_c 0.42, no exothermic peak is detected on the cooling curve, but a broad exothermic transition and a small endothermic peak are observed on the heating curve. The exothermic transition is due to cold crystallization (T_{cc}) of glassy water [11, 12]. The endothermic transition is due to melting of crystallized glassy water.

Figure 1 (W_c 0.54) shows a broad transition on the cooling curve. When W_c is increased, the intensity and temperature of the transition is enhanced to W_c 0.87. When W_c increased further, the transition moves to higher temperature and changes to what appears to be a single peak at W_c 1.59.

For the heating process (Fig.2), the system at W_c 0.54 shows a broad exothermic peak at T_{cc} and an endothermic peak due to the melting of freezing-bound water 1 (W_{fb1}). The melting of glassy water is incorporated in this transition. The intensity of the exothermic peak increases with W_c up to 0.87 and then decreases. A new endothermic peak due to freezing-bound water 2 (W_{fb2})

appears at W_c 0.71 and the intensity increases with increasing W_c . Since the peak which appears at higher temperature shows a small bulge on higher temperature side, a peak due to free water (W_f) is thought to overlap this envelope (see W_c 0.87). When W_c reaches 1.33, the exothermic peak of T_{cc} becomes indistinct and the transitions due to W_{fb1} , W_{fb2} and W_f can no longer be distinguished. At W_c 1.59, the endothermic peak changes to what appears to be a single peak and a small endothermic peak appears anew at -6.2 °C. This was associated with the melting of a new type of freezing-bound water, namely freezing-bound water 3 (W_{fb3}).

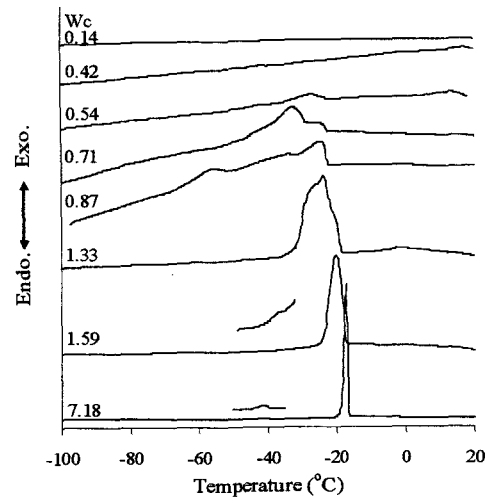


Fig. 1 DSC cooling curves for KM-water system with various water content.

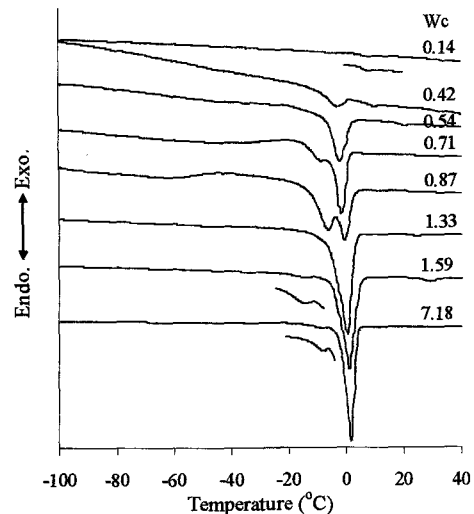


Fig. 2 DSC heating curves for KM-water system with various water content.

The transition temperatures determined from the DSC heating curves plotted against W_c are shown in Figure 3. The onset temperature of T_{cc} shifts to the low temperature side with increasing W_c and has a minimum near 1.0 of W_c . T_{cc} rises and disappears at W_c 1.6. The melting

temperature (T_m) of glassy water occurs at about $-8\text{ }^\circ\text{C}$ at W_c 0.42 but overlaps with T_m for W_{fb1} . The overlapping T_m shifts gradually to higher temperature with increasing W_c . T_m of W_{fb2} at W_c 0.71 is ca. $-12.5\text{ }^\circ\text{C}$ and becomes higher with increasing W_c . When W_c exceeds 1.33, both T_m overlap with T_m increased gradually with W_c to approach $0\text{ }^\circ\text{C}$. T_m of W_{fb3} is ca. $-22\text{ }^\circ\text{C}$ at W_c 1.59 and increases gradually with W_c . At temperatures over $0\text{ }^\circ\text{C}$ the transition temperature for the endothermic peak changed from 5 to $25\text{ }^\circ\text{C}$ with W_c up to 1.5 and afterwards decreased. The peak became indistinct in the high W_c region.

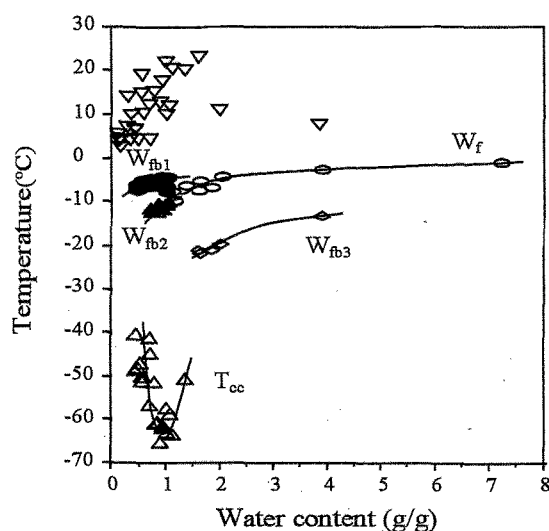


Fig. 3 Transition temperature for KM-water system with various water content.

It is assumed that the heat of fusion (ΔH) of all freezing-bound water is 334 J/g and on this basis the weight of various type of W_{fb} can be determined from the DSC heating curves. Figure 4 shows variation of weight of various types of water with W_c for the KM-water system. W_{nf} was calculated by eq. (2). W_{nf} increases with W_c up to 1.2 and then reaches a constant value of $0.73\text{ (g water/g KM)}$ and number of W_{nf} per pyranose ring is ca. 6.6 (mol/mol) . Fig. 2 demonstrates that it is difficult to separate glassy water, W_{fb1} and W_f on DSC curves, so their weights are given as a sum of both, which is plotted against W_c (open circle). After an initial slow increase there is a linear relationship in the high W_c regions. W_{fb2} increases with W_c and overlaps W_{fb1} . W_{fb3} is of constant value regardless of W_c . The average value of W_{fb3} was $0.08\text{ (g water/g KM)}$ and number of W_{fb3} per pyranose ring was ca. 0.7 (mol/mol) .

Polarization optical micrographs of water swollen KM with concentration gradient are shown in Fig. 5. At $-5\text{ }^\circ\text{C}$ (see Fig. 5-a), swollen KM shows polarizability in the high concentration region and the polarized light is quenched at low concentrations. The liquid crystalline (LC) pattern hardly changes with increasing temperature.

Dave et al. [13] reported that KM exhibited LC behavior in aqueous solutions above 7% concentration and the anisotropy of KM solution was retained when heated up to $80\text{--}90\text{ }^\circ\text{C}$. KM-water systems with lower W_c exhibited lyotropic LC behavior, but did not have thermotropic LC character in the temperature range studied.

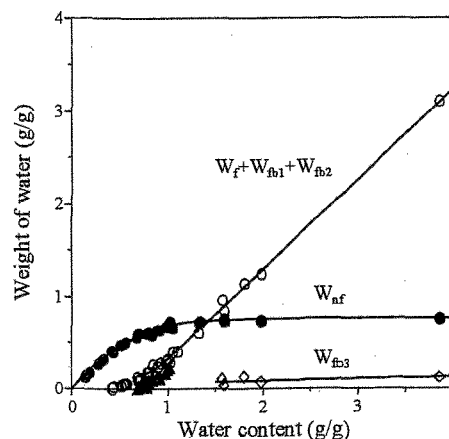


Fig. 4 Amount of various types of water as a function of water content for KM-water system.

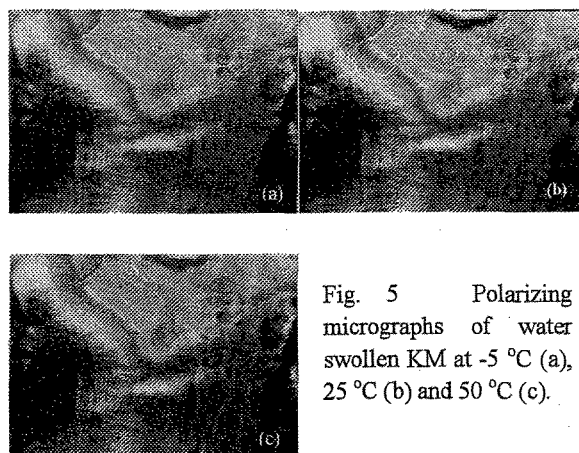


Fig. 5 Polarizing micrographs of water swollen KM at $-5\text{ }^\circ\text{C}$ (a), $25\text{ }^\circ\text{C}$ (b) and $50\text{ }^\circ\text{C}$ (c).

4. DISCUSSION

All water in the KM-water system when W_c is less than 0.42 exists as W_{nf} . Then glassy water appeared with increasing W_c , and on its disappearance W_{nf} reached a constant value. Glassy water is probably closely related to W_{nf} and located in the vicinity of W_{nf} . T_g could be seen on the cooling curves when W_c is lower than 0.42, but could not be detected in other systems. Hatakeyama et al. observed T_g for polysaccharides-water systems [14] and they attributed this behaviour to W_{nf} being held tightly by the hydrophilic groups. T_g shows a minimum with increasing W_c , and this behaviour can be attributed to a plasticizing effect of the W_{nf} in association with the increasing mobility of the polymer chains. Though we could not detect T_g over a wide range of W_c values, T_{cc}

does shift to lower temperatures with increasing Wc and shows a minimum near Wc 1.0. The shift of T_{cc} is considered to be due to the plasticizing effect of KM chains by W_{nf} . The molecular motion of glassy water trapped in KM-water systems could be activated by the chain movement of KM with T_{cc} decreasing with increasing Wc. The rise of T_{cc} over the minimum is attributed that the molecular motion of KM chains which are disturbed by the freezing W_{fb} ice.

Freezing water appeared with increasing Wc in order: W_{fb1} , W_{fb2} , W_f and W_{fb3} . KM is a branched polysaccharide, which without annealing at high temperature shows amorphous X-ray patterns [15]. Low solubility of KM in water was considered due to strong hydrogen bonding in its structure [16]. It can be concluded that water is first sorbed in the amorphous region where there is less hydrogen bonding in the form of W_{nf} and thereafter as glassy water. KM chains expand with increasing Wc, so water can be sorbed as W_{fb1} . As Wc increases further, more W_{nf} and glassy water can penetrate into the regions with dense hydrogen bonding where the KM chains coagulate tightly and allow space where water can exist as W_{fb2} . W_{fb2} is the water which is strongly affected by the interactions within the KM matrix. The coagulated parts disaggregate with increasing Wc and the regions where water can stay as W_{fb2} are expanded. Consequently, W_{fb2} changes to W_{fb1} with less interaction among KM chains. When Wc increased even more, W_{fb1} overlaps with W_f and it is at this point that W_{fb3} appears. The number of W_{fb3} per pyranose ring was ca. 0.7 (mol/mol) and the value is about one tenth of W_{nf} . KM is a neutral polysaccharide with side chains and weight average molar mass and the α value of Mark-Houwink-Sakurada equation is 1.17×10^6 and 0.78, respectively [17]. It is considered that W_{fb3} is true freezing bound water, maintaining interactions between KM molecules. The amount of W_{fb3} is small because hydroxyl groups are the only hydrophilic groups exist in KM. W_{fb1} and W_{fb2} are bound water that strongly receive interaction from tough polymer networks at lower Wc region and change to W_f with increasing Wc. It was found that most of sorbed water in KM-water system with high Wc is held as W_f .

KM-water systems with lower Wc exhibit lyotropic LC behavior, with the LC pattern the same, regardless of temperature. We consider, therefore, that the endothermic peak which appears at temperatures over 0 °C is not the transition from the mesophase to the isotropic liquid state. The transition temperature increases with Wc up to 1.5 and then decreases and could be related to the molecular motions of KM. It is our view, therefore, that the transitions reflect conformation change of KM induced by the breaking of hydrogen bonds [18].

5. CONCLUSION

There are at least six kinds of sorbed water found in the

KM-water system: 1) non-freezing water, 2) three types of freezing bound water, i.e., W_{fb1} , W_{fb2} and W_{fb3} , 3) free water, and glassy water. The proportion of each type of water changes with water content. The equivalent value of non-freezing water per pyranose ring is ca. 6.6 (mol/mol). W_{fb1} and W_{fb2} are categorized as freezing bound water, which strongly interact with polymer networks in the smaller Wc region and change to W_f with increasing Wc. W_{fb3} is true bound water which maintains the interaction between the KM chains and the equivalent value of W_{fb3} per pyranose ring is ca. 0.7 (mol/mol). Most of the sorbed water in KM-water system is held as W_f in the high Wc region. The system with lower Wc exhibits lyotropic LC behavior, but without thermotropic LC property. The endothermic transition observed over 0 °C could reflect conformation changes of KM induced by the breaking hydrogen bond.

6 REFERENCES

- [1] H. Shimahara, H. Suzuki, N. Sugiyama and K. Nisizawa, *Agric. Biol. Chem.*, **39**, 293-99 (1975).
- [2] M. Maeda, H. Shimahara and N. Sugiyama, *Agric. Biol. Chem.*, **44**, 245-52 (1980).
- [3] K. Kato and K. Matsuda, *Agric. Biol. Chem.*, **33**, 1446-53 (1969).
- [4] K. Katsuraya, K. Okuyama, K. Hatanaka, R. Oshima, T. Sato and K. Matsuzaki, *Carbohydr. Polym.*, **53**, 183-89 (2003).
- [5] K. Maekaji, *Agric. Biol. Chem.*, **38**, 315-21 (1974).
- [6] K. Nishinari, P.A. Williams and G.O. Phillips, *Food Hydrocolloids*, **6**, 199-222 (1992).
- [7] S. Takigami, "Handbook of Hydrocolloids", Ed. by G.O. Phillips and P.A. Williams, Woodhead Publishing, Cambridge (2000) pp. 413-424.
- [8] S. Takigami, M. Takigami and G.O. Phillips, *Carbohydr. Polym.*, **26**, 11-18 (1995).
- [9] T. Hatakeyama, F. X. Quinn and H. Hatakeyama, *Carbohydr. Polym.*, **30**, 155-60 (1996).
- [10] S. Takigami, Y. Etoh and G.O. Phillips, *Food Hydrocolloids*, **14**, 609-13 (2000).
- [11] H. Yoshida, T. Hatakeyama and H. Hatakeyama, *Polymer*, **31**, 693-98 (1990).
- [12] S. Takigami, M. Takigami and G.O. Phillips, *Carbohydr. Polym.*, **22**, 153-60 (1993).
- [13] V. Dave, M. Sheth, S.P. McCarthy, J.A. Ratto and D.L. Kaplan, *Polymer*, **39**, 1139-48 (1998).
- [14] H. Yoshida, T. Hatakeyama, K. Nakamura and T. Hatakeyama, *Kobunshi Ronbunshu*, **46**, 597-602 (1989).
- [15] K. Ogawa, T. Yui and T. Mizuno, *Agric. Biol. Chem.*, **55**, 2105-11 (1991).
- [16] K. Kohyama, H. Iida and K. Nishinari, *Food Hydrocolloids*, **7**, 213-26 (1993).
- [17] P. Prawitwong, S. Takigami, R. Takahashi and G.O. Phillips, *Proceedings of MRS*, 2005, Japan.
- [18] Y. Jin, H. Zhang, Y. Yin and K. Nishinari, *Carbohydrate Research*, **341**, 90-99 (2006).