

Glass Transition Properties as a Function of Water Content for Various Low-Moisture Galactans

Masata Mitsuiki,* Youko Yamamoto, Akinori Mizuno, and Masao Motoki

Food Research and Development Laboratories, Ajinomoto Company, Inc.,
1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan

The glass transition temperatures (T_g) of various low-moisture galactans, such as agars and carrageenans, were measured by differential scanning calorimetry (DSC) and occasionally by dynamic mechanical analysis (DMA). The shift in heat flow on the DSC thermogram was detected for all samples. The typical thermally activated relaxation process was observed in the DMA thermogram for an agar and a carrageenan that were measured as the representatives of galactans, and it was confirmed that the shift on the DSC thermogram was caused by the glass transition. The T_g values of agars leveled off at higher water contents. Thus, the minimum T_g values of agars were much higher than those of the carrageenans and a sample of wheat starch. The tightness of binding of the water, which was analyzed by proton nuclear magnetic resonance and water sorption isotherm data, was somewhat weaker to an agar than to a carrageenan even for the same water content, suggesting that most of the water molecules sorbed by the agar were free water and could not plasticize it.

Keywords: *Glass transition; agar; carrageenan; water binding; plasticizability*

INTRODUCTION

Galactans such as agars and carrageenans are cell-wall polysaccharides extracted with water from certain members of marine red algae. Agars are mainly composed of alternating $\beta(1-4)$ -D-galactose and $\alpha(1-3)$ -3,6-anhydro-L-galactose repeating units (Araki and Hirase, 1953). On the other hand, carrageenans are divided into three main types: κ , ι , and λ . They are basically composed of alternating $\beta(1-4)$ -D-galactose and $\alpha(1-3)$ -3,6-anhydro-D-galactose or $\alpha(1-3)$ -D-galactose repeating units, containing a variety of sulfate group contents (O'Neill, 1955). There are similarities in the structures of the agar and carrageenan, both of them being widely used as gel-forming agents, thickeners, water-holding agents, and stabilizers in the food industry.

In recent years, a growing number of food scientists have increasingly recognized the practical significance of the glass transition as a physicochemical event that can govern food processing, product properties, quality, safety, and stability (Slade and Levine, 1991a). Thus, the characteristic temperature at which the glass transition occurs, called the glass transition temperature (T_g), has been studied as a function of water content for various food polymers. A number of food scientists have particularly investigated the T_g of the major components of cereal-based foods, starch (Slade and Levine, 1984; Zeleznak and Hosney, 1987; Mizuno et al., 1998), gluten (Slade, 1984; Hosney et al., 1986), and their component polymers (Cocero and Kokini, 1991; Scandola et al., 1991; Slade and Levine, 1991a; Kalichevsky et al., 1992a,b). As a matter of fact, there are many reports of the T_g values of such foods themselves (Hallberg and Chinachoti, 1992; Levine and

Slade, 1993; Wollny and Peleg, 1994; Martinez and Chiralt, 1995).

Because it has recently become evident that the T_g of a food influences its shelf life, increasing the T_g leads to an important technology for extending this period (Slade and Levine, 1991a). According to Couchman and Karasz (1978), the T_g of a compatible polymer mixture shows a dependence on its composition and increases with an increase in the molar fraction of the polymer with the higher T_g . Thus, the need for a food polymer with a significantly higher T_g than those of the major components of cereal-based foods has become evident.

There have been many studies on the T_g values of food polysaccharides other than starch and its component polymers. These have included gellan gum (Papageorgiou et al., 1994), xanthan gum (Yoshida et al., 1990), sodium alginate (Hatakeyama et al., 1996), cellulose (Salmen and Back, 1977), carboxymethylcellulose (Hatakeyama et al., 1996), pullulan (Scandola et al., 1991), dextran (Scandola et al., 1991), and chitosan (Pizzoli et al., 1991). However, in those studies, no food polymer with a T_g higher than that of starch, under low-moisture conditions (<40%), was reported.

There have also been a few papers on the glass transition properties of galactans (Nishinari et al., 1991; Gidley et al., 1993). However, because galactans are typically used for the previously stated purposes, no report of their T_g values at low moisture contents has been found, except for a study by Gidley et al. (1993). They reported that galactans did not show glass transition behavior in their study. Thus, there seems to have been no studies on the T_g values of galactans at low moisture conditions.

Therefore, the first objective of this study was to search for food polymers with T_g values higher than those of cereal polymers, under low moisture conditions, by characterizing the T_g values of various agar and

* Author to whom correspondence should be addressed [fax +81-44-211-8096; e-mail arsoumu3@sunfield.or.jp].

Table 1. Agar and Carrageenan Samples Studied

	sample name	origin	co.
agar	S6	<i>Gracilarria verrucosa</i>	I ^a
	UP16	<i>Gracilarria verrucosa</i>	I ^a
	AX30	<i>Gracilarria verrucosa</i>	I ^a
carrageenan	type III (κ)	<i>Euचेuma cottoni</i>	S ^b
	type IV (λ)	<i>Gigartina aciculairae</i> and <i>pistillata</i>	S ^b
	type V (ι)	<i>Euचेuma spinosa</i>	S ^b
	X4945 (κ)	<i>Euचेuma cottoni</i>	C ^c
	X2949 (κ)	<i>Euचेuma cottoni</i>	C ^c
	X0910 (λ)	<i>Gigartina</i> var.	C ^c

^a Ina Food Corp., Nagano, Japan. ^b Sigma Chemical Co., St. Louis, MO. ^c Copenhagen Pectin Japan Co., Tokyo, Japan.

carrageenan samples, using differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). As a result, because there was a significant difference in the dependence of T_g on water content among these samples, the second objective was to investigate the cause of this difference, on the basis of water binding data obtained by nuclear magnetic resonance (NMR) and water sorption isotherms.

MATERIALS AND METHODS

Materials. The agar and carrageenan samples studied were obtained from various companies. Table 1 lists the samples used. A sample of wheat starch was purchased from Wako Pure Chemical Ind. (Osaka, Japan).

Preparation of Samples for Measurement. Each agar and carrageenan sample was first dissolved in boiling distilled water, at ~10% (w/w). The solution was lyophilized after rapid freezing with liquid nitrogen. The wheat starch sample was suspended at a ~10% concentration and then heated and gelatinized at 100 °C for 15 min. The suspension was then lyophilized after rapid freezing with liquid nitrogen. Each lyophilized sample was humidified at various water contents according to the method of Mizuno et al. (1998), by which the samples were exposed to supersaturated water vapor for an appropriate time and equilibrated in a hermetically sealed vessel for >48 h at ambient temperature (25 °C). The water content of each sample was determined by measurement of the weight loss after heating for 24 h at 105 °C.

Measurement of T_g by DSC. Heat-flux DSC (DSC120, Seiko Instruments, Chiba, Japan) was used for measurement of the T_g values of the various samples. The DSC was calibrated with Ga, In, and Sn. Each sample (~45 mg), prepared with a different water content, was placed into a silver pan (70 μ L). The pan was then hermetically sealed and accurately weighed. An empty silver pan was used as the reference. The pans were heated in the DSC at 10 °C/min to detect the incremental change in heat flow associated with the glass transition. It is well-known that the glass transition does not occur at a single temperature but over a whole range of temperatures. There are a lot of papers showing T_g as temperature range [e.g., Cherian and Chinachoti (1997)]. However, it is thought that a maximum change in heat flow or rheological property occurs at the midpoint of T_g and that the midpoint is approximately the mean value of the distribution. One objective in this study is to search for polymers with T_g values higher than those of cereal polymers. We believe that the midpoint T_g data suffice for such comparison of the height of T_g . Hence, we defined T_g as the peak in the derivative of the heat flow curve accompanying the shift in the heat flow, which is considered by us provide the most unequivocal T_g (Mizuno et al., 1998). Samples that did not clearly reveal such a change during the first heating were rapidly cooled and then reheated at 10 °C/min to more clearly reveal the change in heat flow.

Measurement of T_g by DMA. A lyophilized disk-shape galactan sample (20 mm diameter and ~2.5 mm thickness) was used for DMA measurement. The disk sample was

humidified according to the previously mentioned method. A Stresstech rotary rheometer CE95 (Seiko Instruments) with a parallel plate (20 mm diameter) was used for the measurement. First, the galactan sample behavior was analyzed in the oscillation stress sweep mode to find a stress range (and corresponding strain range) inside which the measurement results are linear and independent of applied stress. The analysis was performed in a stepwise manner, changing stress amplitude at 1 Hz and 25 °C. The disk sample was then subjected to an applied shear stress in the oscillation strain control mode at frequencies of 0.05, 0.1, 0.5, 1, or 5 Hz, and the strain at which the stress was measured was 0.02%, within which the oscillation stress sweep mode measurement results are linear and independent of applied stress. The temperature was raised from 0 to 120 °C at 2 °C/min. We observed the changes in the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta = G''/G'$) with heating for detecting the glass transition.

NMR Analysis. The tightness of binding of the water to galactan was studied by using proton (¹H) NMR (α -400, JEOL, Tokyo, Japan), at a frequency of 400 MHz at 25 °C. Each sample, with a water content ranging from 5 to 25%, was placed in an NMR tube (5 mm o.d.) and then analyzed. The line-width at half-height ($\delta_{1/2}$) of the water ¹H signal was determined from the NMR spectra. We estimated the tightness of binding of water to galactan from the line-width.

Measurement of Water Sorption Isotherm. We investigated the tightness of binding of the water to galactan also by its water sorption isotherm analysis. The analysis of water sorption isotherm was performed with an automatic water vapor sorption apparatus (Belsorp 18, Bel Japan, Osaka, Japan). This unit was designed for obtaining sorption/desorption isotherms of solid samples with water vapor. Samples (~100 mg) were held at 25 °C and sequentially exposed to increasing water vapor, of which the weight was accurately known, until the water vapor pressure was at equilibrium.

The water activity (a_w) versus water content data were fit to the Brunauer–Emmett–Teller (BET) sorption isotherm model (Brunauer et al., 1938). The BET model is described by

$$p/v(p_0 - p) = 1/v_m c + (c - 1)p/v_m c p_0 \quad (1)$$

where v is the volume of water sorbed at the vapor pressure p , v_m is the volume of water sorbed in a complete unimolecular layer of water molecules, p_0 is the vapor pressure of water at saturation, and c is a constant. Equation 1 is a linear equation, and a plot of $p/v(p_0 - p)$ versus $p/p_0 (= a_w)$ gives a straight line, if the theory is obeyed. Using the y -intercept of the straight line, $1/v_m c$, and the slope, $(c - 1)/v_m c$, the constants v_m and c can thus be obtained from the experimental data. The constant c is described by

$$c = \exp(E_1 - E_L)/RT \quad (2)$$

where E_1 is the average heat of sorption of water in the first sorbed layer, E_L is the heat of liquefaction of water, R is the gas constant, and T is the absolute temperature. This equation means that the tightness of binding of the water to a material with a higher c value, that is, $E_1 > E_L$, is stronger (Jendrsiak et al., 1996).

RESULTS AND DISCUSSION

T_g Measurement for an Agar and a Carrageenan by DSC. We first tried to determine, by DSC, the T_g and its dependence on water content for agar AX30 and carrageenan X0910 (see Table 1). Figure 1 shows the DSC thermogram for agar AX30 with a water content of 22.8% (w/w). A clear shift in heat flow and a peak in the derivative of the heat flow curve (dDSC), which were considered to be associated with the glass transition, were observed at ~90 °C. Thus, it was estimated that

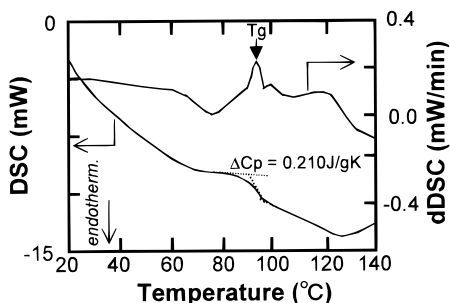


Figure 1. DSC thermogram for agar AX30 (water content = 22.8%).

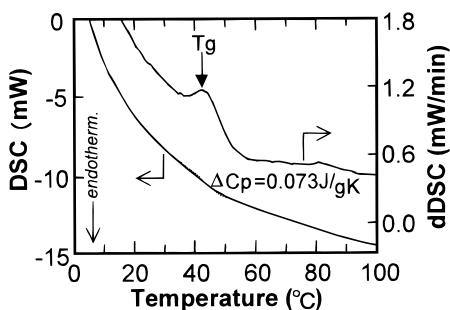


Figure 2. DSC thermogram for λ-carrageenan X0910 (water content = 25.1%).

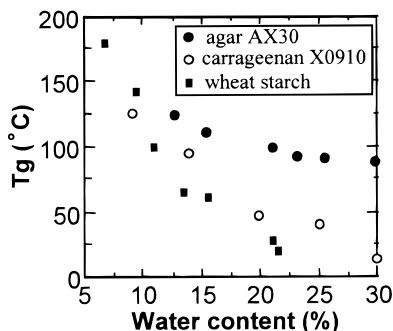


Figure 3. Variation of T_g with water content for agar AX30, carrageenan X0910, and a sample of wheat starch.

the T_g of agar AX30 containing 22.8% water was 93.8 °C, from the temperature of the maximum in the dDSC.

On the other hand, it turned out to be more difficult to detect the T_g values of the carrageenan than it was for the agar because of their smaller heat capacity change (ΔC_p) at the glass transition. Figure 2 shows a typical example of a DSC thermogram for carrageenan X0910 with a water content of 25.1%. As is evident from Figures 1 and 2, the ΔC_p at the glass transition for this carrageenan sample (0.073 J/g·K) was much smaller than that for agar (0.210 J/g·K). As already mentioned, Gidley et al. (1993) reported that galactans did not show glass transition behavior in their study. We could detect a T_g for carrageenan with the DSC used in this study, which had a high sensitivity, but not with other DSCs, suggesting that the ΔC_p at the glass transition for galactans, especially carrageenans, was too small to be detected by a DSC with an ordinary sensitivity.

The T_g values of agar AX30, carrageenan X0910, and a sample of wheat starch with water contents ranging from 10 to 40% were measured in a similar manner and are plotted as a function of water content in Figure 3. It was found that their T_g values decreased with increasing water content, indicating that water acts as a plasticizer for these samples, as it does for other

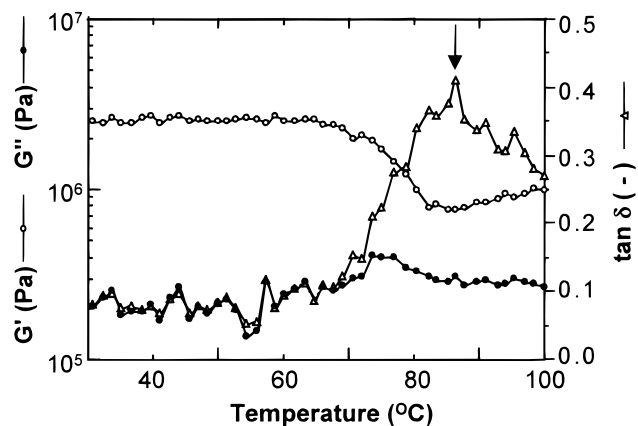


Figure 4. Plots of G' , G'' , and $\tan \delta$ against temperature at 1 Hz for agar AX30 (water content = 24.5%).

polysaccharides reported before (Zeleznaek and Hosoney, 1987; Scandola et al., 1991; Slade and Levine, 1991a; Kalichevsky et al., 1992a; Hatakeyama et al., 1996). However, it was clear that T_g values of agar leveled off at higher water contents (>20%) and that the minimum value (~ 90 °C at 30% water content) was well above ambient temperature. The equilibrium of T_g values at ~ 90 °C was distinctly different from that for wheat starch, carrageenan, and other food polysaccharides (Scandola et al., 1991; Slade and Levine, 1991a; Kalichevsky et al., 1992a; Hatakeyama et al., 1996), where the T_g values decreased to below ambient temperature (25 °C) with increasing water content. This behavior of the agar was rather similar to that of lignin, which is not a food polysaccharide but rather a major component of wood (Kelly et al., 1987).

In any event, we could measure the temperature at which the thermal change, considered to be associated with the glass transition, took place (Figures 1–3). It was thus believed that the T_g of this agar sample was much higher than those of samples of wheat starch and carrageenan, especially at higher water contents.

Our measurements on the water content dependence of T_g for wheat starch was quite similar to those of previous studies [e.g., Zeleznaek and Hosoney (1987) and Mizuno et al. (1998)] (Figure 3). Therefore, we were confident that the accuracy of the obtained T_g data was high, not only for starch but also for agar and carrageenan.

To confirm that the shift in heat flow and the peak in the dDSC curve (Figures 1 and 2) were associated with the glass transition, measurements other than DSC have often been employed in the past (Cocero and Kokini, 1991; Scandola et al., 1991; Kalichevsky et al., 1992a; Shogren, 1992). We tried to measure the T_g of these agar and carrageenan samples by DMA. Figure 4 shows plots of G' , G'' , and $\tan \delta$ against temperature, at 1 Hz, for agar AX30 with a water content of 24.5%. The resulting G' started from relatively high values and dropped sharply to lower values at ~ 80 °C. At that temperature, the $\tan \delta$ curve rose to a peak and then dropped, thus indicating a glass transition. We defined the temperature of the maximum in $\tan \delta$ as the DMA T_g (Kalichevsky et al., 1992a; Mizuno et al., 1998). Thus, it was estimated by DMA, at 1 Hz, that the DMA T_g of agar AX30 containing 24.5% water was 88 °C. Figure 5 shows plots of G' , G'' , and $\tan \delta$ against temperature, at 1 Hz, for carrageenan X0910 with a water content of 24.5%. The resulting G' started from

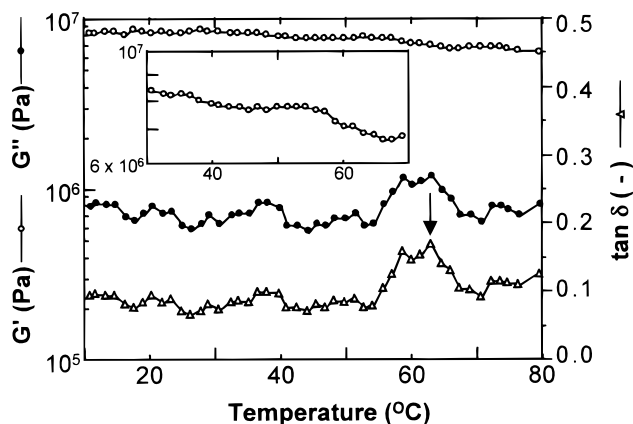


Figure 5. Plots of G' , G'' , and $\tan \delta$ against temperature at 1 Hz for carrageenan X0910 (water content = 24.5%). Enlarged plot of G' is shown in the inset.

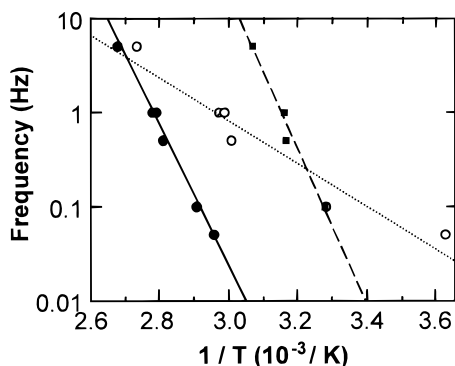


Figure 6. Plot of log frequency versus the reciprocal temperature of the maximum in $\tan \delta$ for agar AX30 (●), carrageenan X0910 (○), and a sample of potato starch (■), with water contents of 24.5, 24.5, and 23.2%, respectively. The plot for potato starch was performed by Mizuno et al. (1998).

relatively high values and dropped to lower values at ~ 60 °C. At that temperature, the $\tan \delta$ curve rose to a peak and then dropped, thus indicating a glass transition as agar. Thus, it was estimated by DMA, at 1 Hz, that the DMA T_g of carrageenan X0910 containing 24.5% water was 62 °C. However, as is evident from Figures 4 and 5, the extent of the drop in G' at the glass transition for this carrageenan sample was much smaller than that for agar, as was the ΔC_p at the glass transition for these samples on DSC thermogram.

The DMA T_g values of the agar and the carrageenan samples at a water content of 24.5% were measured at different frequencies, and the log frequencies were plotted versus the reciprocal DMA T_g (Figure 6). The reciprocal DMA T_g versus log frequency (Arrhenius plot) for a sample of potato starch, which was measured by Mizuno et al. (1998), is also plotted in Figure 6. The figure clearly shows that the DMA T_g for both agar and carrageenan increased with increasing frequency, the same as for the potato starch sample. This Arrhenius plot for these samples gave a single straight line with a good correlation coefficient ($R^2 > 0.93$), as would be expected for any thermally activated relaxation process (Kaličevsky et al., 1992a). From these results, we concluded that the sharp drops in modulus and the peaks in $\tan \delta$ on DMA plots for agar and carrageenan (Figures 4 and 5) were attributable to the glass transition. The value of the apparent activation energy, E_{act} , was estimated from the slope of the straight line, using

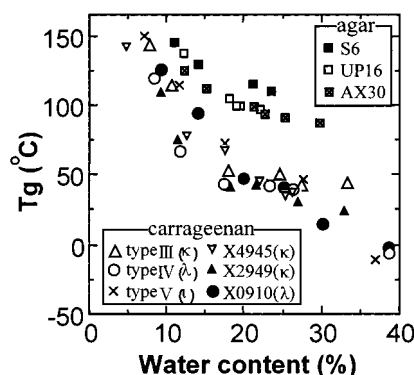


Figure 7. Variation of T_g with water content for various agars and carrageenans. The origin and manufacturer of each sample are listed in Table 1.

the Arrhenius equation

$$\nu = A \exp(E_{act}/RT) \quad (3)$$

where ν is the DMA measurement frequency, A is a constant, and T is the absolute temperature of the DMA T_g . As the result, the E_{act} values of the glass transition in agar AX30 and carrageenan X0910 were 142.1 and 43.3 kJ/mol, respectively. This E_{act} value for the glass transition of agar agrees with that of a typical biopolymer, that is, 200–400 kJ/mol (Kaličevsky et al., 1993). On the other hand, the E_{act} value for the glass transition of carrageenan is lower than those of these polymers.

From Figure 3, we could estimate the DSC T_g of these agar and carrageenan samples at the same water content (24.5%). The estimated T_g values of agar AX30 and carrageenan X0910 containing 24.5% water were 91 and 40 °C, respectively. The estimated T_g of a sample of starch containing 23.2% water from previously measured T_g data (Mizuno et al., 1998) was 20 °C. Therefore, the estimated T_g decreased in the order agar, carrageenan, and potato starch. On the other hand, the DMA T_g values of the agar, carrageenan, and potato starch for measurement frequencies of 0.05–5 Hz were in the ranges 50–100, 30–70, and 30–50 °C, respectively (Figure 6). Thus, their DMA T_g values agree, in their value and order, with their DSC T_g . Therefore, we concluded that the thermal event associated with the shift on the DSC thermogram for agar and carrageenan was the same as the thermally activated relaxation process seen in the DMA thermogram, which we assumed to be a glass transition.

In any event, on the basis of this assumed confirmation of the validity of the T_g values, we hereafter assumed the DSC T_g to be the T_g for each sample.

Water Content Dependence of the T_g Values for Various Agars and Carrageenans. The variation of T_g with water content for various agars and carrageenans was measured by DSC. The water content dependence of T_g for various agars and carrageenans is summarized in Figure 7. It was found that the T_g values for all samples decreased with increasing water content, indicating that water acts as a plasticizer for all of these galactans. However, there was a significant difference in the water content dependence of T_g for the agars and carrageenans, as mentioned previously (Figure 3). The T_g values for all agar samples leveled off at higher water contents, and their minimum values were all well above ambient temperature, as already described (Figure 3). In contrast, the T_g values of all

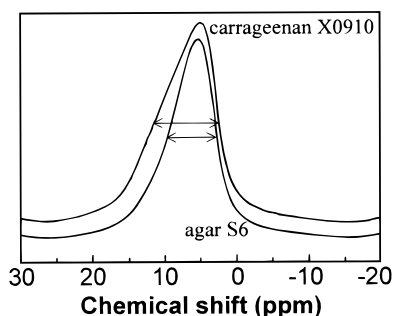


Figure 8. NMR spectra for agar S6 and carrageenan X0910, with water contents of 15.1 and 16.2%, respectively.

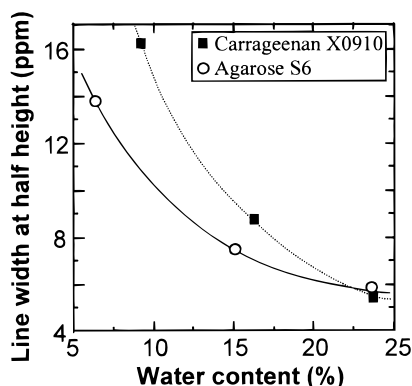


Figure 9. Variation of the line-width at half-height ($\delta_{1/2}$) of the water ^1H signal with water content for agar S6 and carrageenan X0910. Each point is the average of three measurements.

the carrageenans did not level off up to the higher water contents, and the minimum values were in the range of -10 to 40 °C. The T_g values of carrageenan samples were rather similar in water content dependence to that for starch (Figure 3) and other food polysaccharides (Scandola et al., 1991; Kalichevsky et al., 1992a; Hatakeyama et al., 1996). Among the carrageenans, the minimum T_g values for λ -carrageenan seemed to be lower than those for the others. It is noteworthy that there was a significant difference in the moisture dependence of T_g for the agars and carrageenans, despite the similarity in their structures.

As previously mentioned, polymers with high T_g values can be very important in extending the shelf life of foods. Therefore, we believe that agars might be very useful food polymers for this purpose. We decided that it was necessary to study the cause of the higher T_g value for agar, by analyzing the state of sorbed water. Hence, the tightness of binding of the water to galactan was analyzed by studying ^1H NMR and water sorption isotherms.

Tightness of Binding of Water to Galactan Samples. Agar S6, which showed the highest T_g value (Figure 7), and carrageenan X0910, a λ -carrageenan that showed the lowest T_g value (Figure 7), were used for comparing tightness of binding water to the samples. Typical NMR spectra for the agar (water content = 15.1%) and carrageenan (water content = 16.2%) are shown in Figure 8. As can be seen from the figure, the water ^1H NMR resonance for the carrageenan sample was broadened toward low field, and its $\delta_{1/2}$ (8.73 ppm) was wider than that for the agar sample (7.55 ppm). Figure 9 shows the variation of $\delta_{1/2}$ with water content for the agar and carrageenan samples. The $\delta_{1/2}$ values for both samples decreased with increasing water

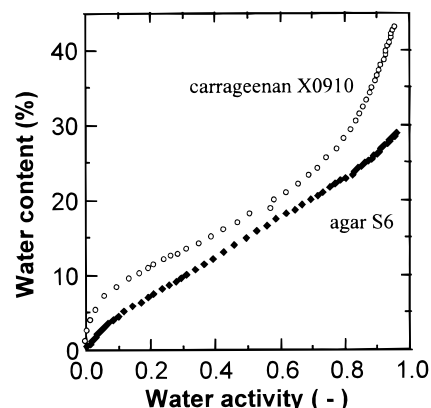


Figure 10. Sorption isotherm data for agar S6 and carrageenan X0910 at 25 °C. The water content is wet-base w/w %.

content, indicating that the molecular motion of sorbed water increased with increasing water content for both samples. For the lower water contents (5–16%), the $\delta_{1/2}$ for the agar sample was lower than that for the carrageenan sample (Figure 9). From these results, we believe that the water molecules sorbed by carrageenan sample were more motionally restricted than those sorbed by agar at the same water contents and that the hydrogen bonding by the water sorbed in the sample was stronger (Rouse et al., 1982; Mizuno et al., 1995). It is, however, thought that $\delta_{1/2}$ of proton is not influenced only by water protons. Many factors such as solid protons, diffusional exchange, cross-relaxation, and chemical exchange also affect slightly the $\delta_{1/2}$. Thus, water sorption isotherm data were employed as a method to back up the discussion for the NMR data.

Figure 10 presents water sorption isotherms for agar S6 and carrageenan X0910 at 25 °C. According to the BET isotherm type that describes the shape of the isotherm type over the entire range of vapor sorption, from zero to the saturation vapor pressure, it seems that the isotherm for the carrageenan is a typical type II and that for the agar is a type V (Jendrasiak et al., 1996). Isotherm type II is thought to indicate strong water binding, whereas type V indicates weak binding. As can be seen from Figure 10, over the entire range of water content, from 0 to 30%, the a_w for agar was much higher than that for carrageenan. This result suggests that the water molecules sorbed by the carrageenan sample were more motionally restricted than those sorbed by agar at the same water contents.

On the basis of eq 1, $p/v(p_0 - p)$ was plotted as a function of a_w . As the results, the plots gave a single straight line with an excellent correlation coefficient ($R^2 > 0.997$) for both agar and carrageenan. Thus, their sorption behaviors seem to obey good BET theory. Hence, we calculated the constants v_m and c from their correlation equation. There was not so much difference in the constant v_m between agar (134.4 mL/g) and carrageenan (141.3 mL/g), whereas the constant c for carrageenan (32.02) was much higher than that for agar (5.34). As mentioned previously, the level of the constant c value indicates the tightness of binding of the water to a material. This result, therefore, suggests that the water molecules sorbed by the carrageenan sample were more tightly bound than those sorbed by agar. These results in water sorption isotherms fairly agree with the result obtained by ^1H NMR (Figure 9). It was, therefore, thought that the discussion for the NMR data was backed up by these data. That is to say,

the influence of previously mentioned factors other than water proton on $\delta_{1/2}$ seems to be little in these low-moisture polysaccharide systems.

According to these results, we believe that the tightness of binding of the water was somewhat weaker to an agar than to a carrageenan even for the same water content and that most of the water molecules sorbed by the agar were free water.

As shown in Figures 3 and 7, the T_g values for agars and carrageenans decreased with increasing water content; in other words, water acts as a plasticizer for them. However, the T_g values for agars leveled off at higher water contents and their minimum values were well above ambient temperature. Consequently, there was a significant difference in the water content dependence of T_g values for these two galactans.

On the basis of the results shown in Figures 9 and 10, the water molecules sorbed by carrageenan showed highly restricted motions. It could be considered that most of them are "bound" water and act as plasticizer. In contrast, the molecular motion of water molecules in the agar sample was relatively rapid, even for the lower water contents. This result suggests that the practical limit to the extent of plasticization by water for agar was considerably lower. The excess water, therefore, would have been separated from the polymer phase. Thus, the T_g for agar appeared to level off at higher water contents.

According to the classification of T_g curves (water content dependence of T_g) proposed by Slade and Levine (1991a,b), agars and carrageenans appear to be classifiable as "water-sensitive" and "water-compatible" polymers, respectively. Slade and Levine also suggested that there is a practical limit to the extent of plasticization by water for water-sensitive polymers and that the excess water would separate from the polymer phase. Hatakeyama et al. (1996) suggested that T_g values for polysaccharides decreased, due to the severing of inter- and intramolecular interactions, when water molecules are bound to hydrophilic groups and counterions as nonfreezing water.

In this study, we tried to clarify the cause of the significant difference in the T_g curves for agars and carrageenans by employing ^1H NMR and water sorption isotherm data. These results showed that the tightness of binding of the water was somewhat weaker to an agar than to a carrageenan, even for the same water content, and that most of the water molecules sorbed by the agar were free water. It might be concluded from these findings that the difference could be explained by a difference in the plasticizability of the two polysaccharides by water, which could be estimated from the data of tightness of binding of water to the polysaccharides. It is noteworthy that there was a significant difference in the tightness of binding of water, even at the lower water contents, and this difference can be related to the difference in the T_g curves.

As previously mentioned, agars are similar to carrageenans in main-chain structure. We speculated, therefore, that the significant difference between them, in the tightness of binding of water, could originate from the structures of the side chains. It is well-known that these two galactans have two major characteristic functional groups, the anhydro and sulfate groups, in different contents. According to previous papers, the anhydro group content decreases in the order agar, κ -carrageenan, and λ -carrageenan (Yaphe, 1960), and

the sulfate group content decreases in the order λ -carrageenan, κ -carrageenan, and agar (Nishinari et al., 1991; Maitani et al., 1994). The hydrophile-lipophile balance (HLB) number developed by Davies (Sherman, 1968) is known as the index of hydrophilicity of functional groups. The HLB number for the sodium salt of the sulfate group (38.7) is the highest of all functional groups. On the other hand, the number of the anhydro group (1.3) is lower than that for the hydroxyl group (1.9). Thus, it can be estimated that the hydrophilicities of these polysaccharides increase in the order agar, κ -carrageenan, and λ -carrageenan. That is to say, the water-binding capacities of agars seem to be lower than those of carrageenans. This estimate agrees fairly well with the ^1H NMR and water sorption isotherm data (Figures 9 and 10).

From the above discussion, we conclude that the T_g values of agars and carrageenans would be reduced by the severing of inter- and intramolecular interactions, according to the quantity of water molecules interacting with their functional groups, and that the water content dependence of their T_g values would, therefore, be different.

Functional groups, such as anhydro and sulfate groups, seem to be necessary to explain the difference in the water content dependence of the T_g values for these galactans. Certainly, it is well-known that the content of such groups, in addition to molecular weight, shows a good correlation with gel strength of agars and agar sulfates (Fuse and Suzuki, 1975). We believe that an investigation of the correlation between the T_g curves and functional group contents for various galactans is necessary.

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LITERATURE CITED

- Araki, C.; Hirase, S. Studies on chemical constitution of agar-agar. XV: Exhaustive mercaptolyses of agar-agar. *Bull. Chem. Soc. Jpn.* **1953**, *26*, 463–467.
- Brunauer, S.; Emmett, P. H.; Teller, E. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **1938**, *60*, 309–319.
- Cherian, G.; Chinachoti, P. Action of oxidants on water sorption, ^2H nuclear magnetic resonance mobility, and glass transition behavior of gluten. *Cereal Chem.* **1997**, *74*, 312–317.
- Cocero, A. M.; Kokini, J. L. The study of the glass transition of glutenin using small amplitude oscillatory rheological measurements and differential scanning calorimetry. *J. Rheol.* **1991**, *35*, 257–270.
- Couchman, P. R.; Karasz, F. E. A classical thermodynamic discussion of the effect of composition on glass-transition temperatures. *Macromolecules* **1978**, *11*, 117–119.
- Fuse, T.; Suzuki, T. Preparation and properties of agar sulfates. *Agric. Biol. Chem.* **1975**, *39*, 119–126.
- Gidley, M. J.; Cooke, D.; Ward-Smith, S. Low moisture polysaccharide systems: thermal and spectroscopic aspects. In *The Glassy State in Foods*; Blanshard, J. M. V., Lillford,

- P. J., Eds.; Nottingham University Press: Nottingham, England, 1993; pp 303–316.
- Hallberg, L. M.; Chinachoti, P. Dynamic mechanical analysis for glass transition in long shelf-life bread. *J. Food Sci.* **1992**, *57*, 1201–1204.
- Hatakeyama, T.; Nakamura, K.; Hatakeyama, H. Glass transition of polysaccharide electrolyte-water systems. *Kobunshi Ronbunshu (Jpn. J. Polym. Sci. Technol.)* **1996**, *53*, 795–802.
- Hoseney, R. C.; Zeleznak, K.; Lai, C. S. Wheat gluten: a glassy polymer. *Cereal Chem.* **1986**, *63*, 285–286.
- Jendrsiak, G. L.; Smith, R. L.; Shaw, W. The water adsorption characteristics of charged phospholipids. *Biochim. Biophys. Acta* **1996**, *1279*, 63–69.
- Kalichevsky, M. T.; Jaroszkiewicz, E. M.; Ablett, S.; Blanshard, J. M. V.; Lillford, P. J. The glass transition of amylopectin measured by DSC, DMTA and NMR. *Carbohydr. Polym.* **1992a**, *18*, 77–88.
- Kalichevsky, M. T.; Jaroszkiewicz, E. M.; Blanshard, J. M. V. The glass transition of gluten. I. Gluten-sugar mixtures. *Int. J. Biol. Macromol.* **1992b**, *14*, 257–266.
- Kalichevsky, M. T.; Blanshard, J. M. V.; Marsh, R. D. L. Applications of mechanical spectroscopy to the study of glassy biopolymers and related systems. In *The Glassy State in Foods*; Blanshard, J. M. V., Lillford, P. J., Eds.; Nottingham University Press: Nottingham, England, 1993; pp 133–156.
- Kelly, S. S.; Rials, T. G.; Glasser, W. G. Relaxation behavior of the amorphous components of wood. *J. Mater. Sci.* **1987**, *22*, 617–624.
- Levine, H.; Slade, L. The glassy state in applications for the food industry, with an emphasis on cookie and cracker production. In *The Glassy State in Foods*; Blanshard, J. M. V., Lillford, P. J., Eds.; Nottingham University Press: Nottingham, England, 1993; pp 333–373.
- Maitani, T.; King, D. R.; Terai, R.; Yoshihara, K. Application of vacuum-ultraviolet ICP atomic emission spectrometry for tests of carrageenan. *Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jpn.)* **1994**, *35*, 631–635.
- Martinez, N.; Chiralt, A. Glass transition and texture in a typical Spanish confectionery product: Xixona turrón. *J. Texture Stud.* **1995**, *26*, 653–663.
- Mizuno, A.; Mitsuiki, M.; Motoki, M. Effect of crystallinity on the glass transition temperature of starch. *J. Agric. Food Chem.* **1998**, *46*, 98–103.
- Mizuno, K.; Miyashita, Y.; Shindo, Y.; Ogawa, H. NMR and FT-IR studies of hydrogen bonds in ethanol–water mixtures. *J. Phys. Chem.* **1995**, *99*, 3225–3228.
- Nishinari, K.; Watase, M.; Williams, P. A.; Phillips, G. O. Effect of sugars and polyols on water in agarose gels. In *Water Relationships in Foods*; Levine, H., Slade, L., Eds.; Plenum Press: New York, 1991; pp 235–249.
- O'Neill, A. N. 3,6-Anhydro-D-galactose as a constituent of κ -carrageenan. *J. Am. Chem. Soc.* **1955**, *77*, 2837–2839.
- Papageorgiou, M.; Kasapis, S.; Richardson, R. K. Glassy-state phenomena in gellan-sucrose-corn syrup mixtures. *Carbohydr. Polym.* **1994**, *25*, 101–109.
- Pizzoli, M.; Ceccorulli, G.; Scandola, M. Molecular motions of chitosan in solid state. *Carbohydr. Res.* **1991**, *222*, 205–213.
- Rouse, R. E.; Wiltbank, W. J.; Brey, W. S., Jr. Nuclear magnetic resonance of water in deuterated citrus leaf tissue. *J. Am. Soc. Hort. Sci.* **1982**, *107*, 1124–1128.
- Salmen, N. L.; Back, E. L. Influence of water on T_g of cellulose. *Tappi* **1977**, *60*, 137–140.
- Scandola, M.; Ceccorulli, G.; Pizzoli, M. Molecular motion of polysaccharides in the solid state: dextran, pullulan, and amylose. *Int. J. Biol. Macromol.* **1991**, *13*, 254–260.
- Sherman, P. General properties of emulsions and their constituents. In *Emulsion Science*; Sherman, P., Ed.; Academic Press: London, England, 1968; pp 131–216.
- Shogren, R. L. Effect of moisture content on melting and subsequent physical aging of corn starch. *Carbohydr. Polym.* **1992**, *19*, 83–90.
- Slade, L. Starch properties in processed foods: staling of starch-based products. Presented at the American Association of Cereal Chemists Annual Meeting, Minneapolis, MN, 1984; Abstr. 112.
- Slade, L.; Levine, H. Thermal analysis of starch and gelatin. In *Proceedings of the 13th Annual Conference, North American Thermal Analysis Society*, Sept 23–26, 1984; McGhie, A. R., Ed.; Philadelphia, PA, 1984; p 94.
- Slade, L.; Levine, H. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *CRC Crit. Rev. Food Sci. Nutr.* **1991a**, *30*, 115–360.
- Slade, L.; Levine, H. A food polymer science approach to structure–property relationships in aqueous food systems: non-equilibrium behavior of carbohydrate-water systems. In *Water Relationships in Foods*; Levine, H., Slade, L., Eds.; Plenum Press: New York, 1991b; pp 29–101.
- Wollny, M.; Peleg, M. A model of moisture-induced plasticization of crunchy snacks based on Fermi's distribution function. *J. Sci. Food Agric.* **1994**, *64*, 467–473.
- Yaphe, W. Colorimetric determination of 3,6-anhydrogalactose and galactose in marine algal polysaccharides. *Anal. Chem.* **1960**, *32*, 1327–1330.
- Yoshida, H.; Hatakeyama, T.; Hatakeyama, H. Phase transitions of the water-xanthan system. *Polymer* **1990**, *31*, 693–698.
- Zeleznak, K. J.; Hoseney, R. C. The glass transition in starch. *Cereal Chem.* **1987**, *64*, 121–124.

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