Gelation Mechanism of Curdlan by Dynamic Viscoelasticity Measurements

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ABSTRACT

Gelation of curdlan was classified into two forms: thermo-reversible and thermo-irreversible gelation. The temperature dependence of the rheological properties indicated that the temperature at which the transition to thermo-irreversible gel system occurred was concentration-dependent, while thermo-reversible gel formation temperature was independent of concentration. An increase in concentration lowered the irreversible gel formation temperature from the generally reported 80°C and decreased the amount of thermo-reversible component in the final gel. This indicates that an increase in the curdlan concentration promoted hydrophobic interactions between micelles at junction zones at a lower temperature than 80°C.

Key Words: curdlan, gel, thermo-reversible, thermo-irreversible, dynamic viscoelasticity

INTRODUCTION

CURDLAN IS A POLYSACCHARIDE PRODUCED BY *ALCALIGENES faecalis* var. *myxogenes* (Harada et al., 1966). Curdlan has been approved by the U. S. Food and Drug Administration as a direct food additive (Anonymous, 1996), and has been widely studied and used in Japan as a key ingredient in various types of processed foods for many years (Harada et al., 1993; Miwa et al., 1994). Curdlan is a linear homopolymer of D-glucose with β-1,3-glucosidic linkages, and its aqueous suspension is capable of forming a gel by heating. Curdlan forms two types of heat-induced gels depending on the temperature termed low-set gel and high-set gel. The low-set gel is thermo-reversible, similar to behaviors of agar-agar and gelatin, whereas the high-set gel is irreversible and very stable at temperatures ranging from freezing to those used in retorting (Nakao et al., 1991). The low-set gel is formed when the aqueous suspension is heated to 50 to 60°C and then cooled to below 40°C, whereas the high-set gel is typically formed when the aqueous suspension is heated above 80°C (Harada et al., 1987, Konno and Harada, 1991).

The molecular mechanism of high-set gel formation is different from that of low-set gel formation (Konno et al., 1994). In high-set gel, there is cross-linking between curdlan micelles (Fulton and Atkins, 1980), which are occupied by molecules of multiple-chain helix (Marchessault and Deslandes, 1979) or triple-stranded helix (Kasai and Harada, 1980) forming a three-dimensional network caused through hydrophobic interactions. In the low-set gel, cross-linking between curdlan micelles, which are occupied by molecules of single-helix (Saito et al., 1989), is caused through hydrogen bonding.

There is need for further investigation of the temperature range in which the transition from thermo-reversible to irreversible gels takes place. The reported transition temperature of 80°C has been based on the relationship between gel strength and heating temperatures at a relatively low curdlan concentration level. The gel strength of curdlan at 3% remained unchanged between 60 and 80°C, but increased linearly with increasing heating temperature above 80°C (Maeda et al., 1967). Another result has been reported which supports that the transition temperature is around 80°C. When heated at higher than 85°C, the gel strength of curdlan at 2% increased as heating time increased, whereas when heated at 65°C or 75°C, the gel strength was almost independent of heating time (Kimura et al., 1973). Although the transition temperature from thermo-reversible to irreversible gel can be dependent on curdlan concentration, there have been limited reports regarding the influence of curdlan concentration on the gelation behavior or gel properties. Our main objective was to clarify the gelation mechanism of curdlan by analyzing the temperature dependence of dynamic viscoelasticity of curdlan aqueous suspensions as well as the physical properties of gels at various concentrations.

MATERIALS & METHODS

Materials

Curdlan was provided by Takeda Chemical Industries, Ltd. (Osaka, Japan). The initial moisture content of curdlan is <5% (w/w) (Nakao, 1991).

Preparation of curdlan aqueous suspensions

All curdlan suspensions were prepared on a % w/w basis. Five aqueous suspensions with concentrations of curdlan from 2 to 10% were prepared in 2% increments. Curdlan powder was dispersed in water at 25°C with an AM-8 homogenizer (Nihon Seiki Co., Ltd., Tokyo, Japan) and deaerated under vacuum. The dispersing rate was fixed at 13,000 rpm and agitation time was varied from 2.5 to 15 min depending on concentration. Aqueous suspensions of curdlan after deaeration were subjected to dynamic viscoelasticity measurements.

Dynamic viscoelasticity

Aqueous suspensions of curdlan of various concentrations were placed on the bottom plate of an Ares strain-controlled rheometer (Rheometric Scientific, Piscataway, NJ, USA). Dynamic strain sweep, dynamic frequency sweep, and dynamic temperature ramp measurements were conducted. Dynamic strain sweep measurement from 10^{-1} to 10^2% was performed at 30°C at a constant frequency of 6.28 rad/s using a parallel plate geometry (gap 1.3 mm, plate dia 25 mm). Dynamic frequency sweep measurement from 10^0 to 10^2 rad/s was performed at 30°C at a constant strain of 1%, which was previously determined by a strain sweep test within the linear viscoelastic region of curdlan aqueous system using the same geometry described. Dynamic temperature ramp measurements from 30 to 90°C at a heating rate of 1°C/min and then again to 30°C at a cooling rate of 1°C/min were performed at a constant strain of 1% and at a constant frequency of 6.28 rad/s using the same geometry. For the aqueous suspensions at concentrations of 2 and 10%, the temperature ramp measurements from 30°C to various upper limit temperatures (55, 65, 70, 80, and 90°C) at the same heating rate

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and then again to 30°C at the same cooling rate were also performed. Heating and cooling were done by circulating temperature-controlled water through a water jacket. Samples of curdlan aqueous suspension were covered with a layer of silicon oil to avoid dehydration during measurements. Each datum was presented as the mean value of duplicate measurements.

Preparation of curdlan gel

Aqueous suspensions of curdlan after de-aeration were poured into glass test tubes with inner diameter of 15 mm (10g/tube). Each test tube was plugged with a rubber stopper before heating to prevent evaporation of moisture. Curdlan aqueous suspensions were heated in a temperature-controlled water-bath at 60, 70, 80, or 90°C for 15 min and then cooled with running water to 20°C for 30 min.

Puncture tests

Curdlan gels at 2 and 10% concentration were cut into 10 mm sections and subjected to puncture tests to analyze their physical properties. Tests were carried out using a Rheoner RE2-33005 (Yamaden Co. Ltd., Tokyo, Japan) with a 2 Kgf load cell at 20°C. Gels were compressed with a cylindrical plunger (5 mm dia) at 1 mm/sec. From the stress vs strain curve, apparent breaking stress was determined. Data were presented as means±SD of duplicate measurements.

RESULTS & DISCUSSION

Relationship between curdlan concentration and dynamic viscoelasticity

Temperature-dependence curves of storage modulus (G′) (Fig. 1) during heating were compared for dynamic viscoelasticity of curdlan aqueous suspensions at various concentrations. G′ of each suspension started to increase at around 45°C, which indicated that swelling of the molecule began at that temperature regardless of concentrations. This confirmed findings that the incipient swelling temperatures of curdlan aqueous suspensions determined from differential scanning calorimetry were independent of concentration (Konno and Harada, 1991). All suspensions except 2% peaked at around 55°C, indicating the maximum water uptake occurred there. This peak temperature was independent of curdlan concentration between 4 and 10%.

At temperatures above 60°C, the G′ increased with rising temperature, suggesting the formation of a thermo-irreversible gel. The curdlan suspension at 2%, however, did not show such peak and the G′ remained unchanged between 60 and 80°C. Above 80°C, G′ increased with temperature. These results suggested that the incipient temperature for thermo-irreversible gel formation was dependent on curdlan concentration: the higher the concentration, the lower the incipient temperature. Temperature-

dependence curves of both mechanical loss tangent (tan δ) (Fig. 2) and G′ (Fig. 3) during cooling were also examined. The tan δ curve of each suspension peaked at around 40°C. Since tan δ is a measure of the dynamic character of inter- or intra-molecular bonds in a gel network (Boye et al., 1997), the peak in tan δ curve is closely related to the transition of molecular conformation.

The formation of thermo-reversible gel occurred at 40°C and this temperature was independent of curdlan concentration. As the peak height in tan δ curve became smaller with increasing curdlan concentration, the degree of the transition became smaller with curdlan concentration. That is, the mass of thermo-reversible portions in the final gel decreased with increasing curdlan concentration. Tan δ became less temperature dependent as concentration increased. For the 2% gel, tan δ at 90°C and 30°C were 0.13 and 0.06, respectively, while for the 10% gel, tan δ values were 0.06 and 0.04, respectively. The measured values of G′ at 90, 40, and 30°C (Fig. 3) were plotted against the curdlan concentration on a double-logarithmic scale to obtain a regression line (Fig. 4). The slopes of the regression line (r^2>0.98) for G′ at 90°C and 40°C were about 2.4, while that for G′ at 30°C was about 1.7. This indicated the difference of gel structure, especially cross-linking or junction zone occurring between curdlan micelles. The cross-linking within the gel formed at 90°C and 40°C was furnished by hydro-

phobic interactions which contributed to thermo-irreversibility of the gel. The cross-linking within the gel formed at 30°C was furnished by not only hydrophobic interactions but also hydrogen bonds which contributed to the thermo-reversibility. G′ of each suspension started to increase markedly at around 40°C as well as tan δ during cooling. The increase in G′ at 30 to 40°C during cooling was hypothesized to be attributed to the increase in thermo-reversible components in the gel and calculated as follows:

$$\text{Thermo-reversible component (\%)} = 100 \times [(\text{G}' \text{ at } 30°\text{C} - \text{G}' \text{ at } 40°\text{C})/\text{G}' \text{ at } 30°\text{C}]$$

Increasing curdlan concentration decreased this indicator logarithmically (Fig. 5), which supported our hypothesis quantitatively that thermo-reversible structure in the final gel decreased with increasing curdlan concentration. These dynamic viscoelasticity measurements suggested that increasing curdlan concentration shifted the transition temperature where thermo-reversible gels changed to irreversible gels, to lower temperatures. This was probably because the likelihood of forming cross-linking between curdlan micelles increased with concentration, which led to an increase in the number of junction zones. Also, it became evident that with increasing concentration, hydrophobic interactions between micelles at junction zones, which formed inter-molecular bonds and consequently a three-
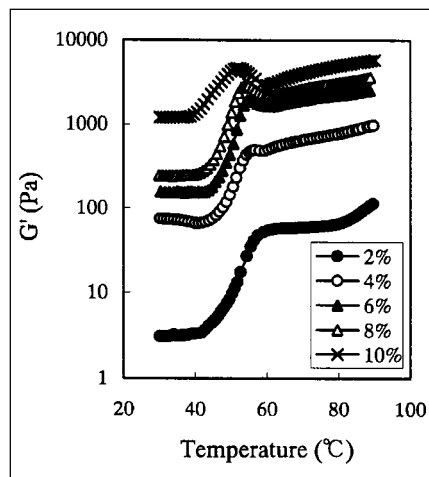
Fig. 1—Temperature dependence curve of dynamic storage modulus (G′) of curdlan aqueous suspensions at various concentrations during heating. The measurement was performed from 30°C to 90°C at a heating rate of 1°C/min at a constant strain of 1% and at a constant frequency of 6.28 rad/s using a 25 mm dia parallel plate geometry. Data are presented as means of duplicate measurements.
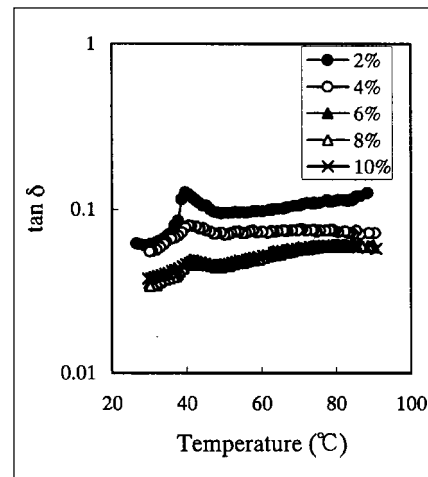
Fig. 2—Temperature dependence curve of mechanical loss tangent (tan δ) of curdlan aqueous suspensions at various concentrations during cooling. The measurement was performed from 90°C to 30°C at a cooling rate of 1°C/min at a constant strain of 1% and at a constant frequency of 6.28 rad/s using the same geometry as Fig. 1. Data are presented as means of duplicate measurements.

dimensional network, could be formed at temperatures <80°C.

Relationship between heating temperature and dynamic viscoelasticity

Regardless of the higher end of heating temperatures, G' at either 2% or 10% concentration, started to increase markedly at 40°C

during cooling (Fig. 6, (A): 2%, (B): 10%). From each G' curve, the percentage of thermo-reversible component in the final gel was calculated as before and plotted against heating temperature (Fig. 7). The portion of thermo-reversible structure in the 2% gel was higher than that in the 10% gel at each heating temperature. For the 2% gel, the thermo-reversible gel fraction reached a maximum

(84%) between 70 and 80°C and decreased (74%) at 90°C, indicating that the thermo-irreversible gel formation occurred above 80°C. For the 10% gel, however, it decreased slightly from 55 to 65°C, but decreased markedly above 65°C, indicating that the thermo-irreversible gel formation occurred above 65°C, which was much lower than that for 2% gel. These results correlated well with the temper-
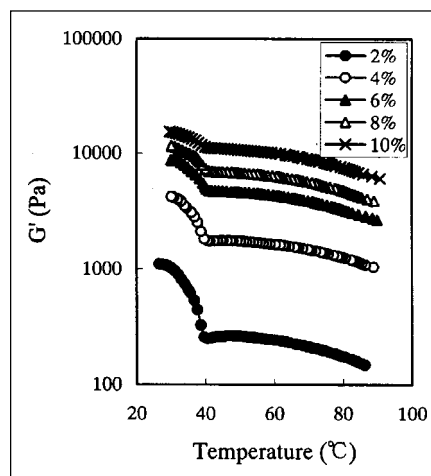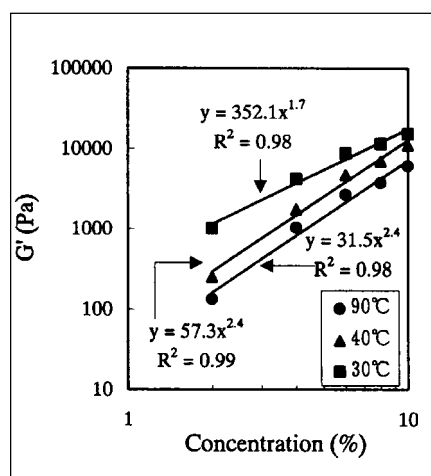

Fig. 3—Temperature dependence curve of dynamic storage modulus (G') of curdlan aqueous suspensions at various concentrations during cooling. The measurement was performed in the same manner as Fig. 2. Data are presented as means of duplicate measurements.
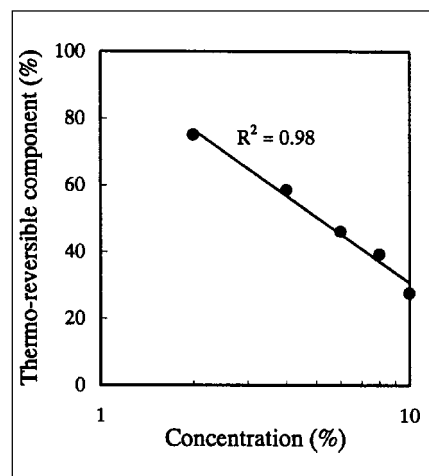

Fig. 5—Relationship between curdlan concentration and thermo-reversibility of the gel. Percentage of thermo-reversible component in gel was calculated using changes in G' during cooling (Fig. 3) as follows: Thermo-reversible component (%)=100 × [(G' at 30°C − G' at 40°C)/G' at 30°C]
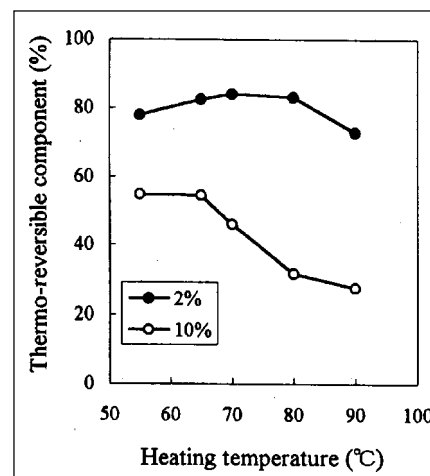

Fig. 7—Relationship between thermo-reversibility of curdlan gel and heating temperature. Percentage of thermo-reversible component in gel was calculated in the same manner as Fig. 5.

Fig. 4—Relationship between curdlan concentration and dynamic storage modulus (G') at various temperatures during cooling. G' figures at 90, 40, and 30°C were determined from the cooling curve (Fig. 3) and plotted against the curdlan concentration. The relationship between G' and curdlan concentration was determined by power law regression.
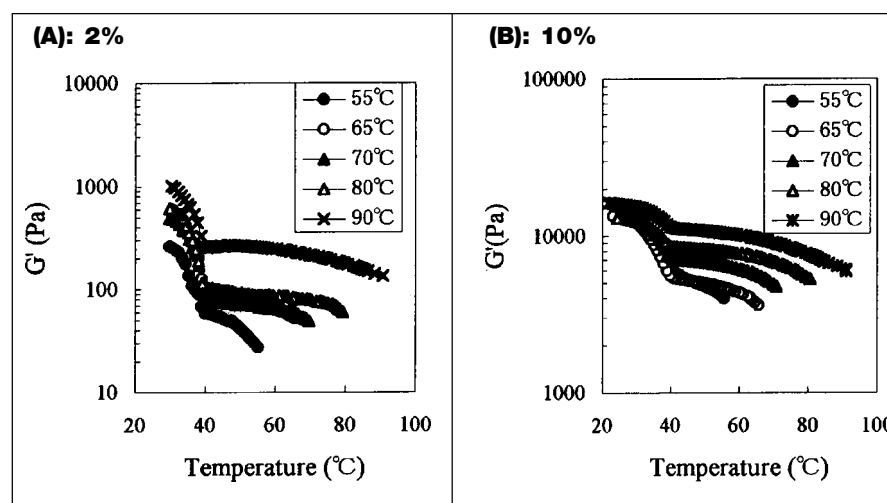
Fig. 6—Temperature dependence curve of dynamic storage modulus (G') of curdlan aqueous suspension during cooling. Temperature was raised from 30°C to various upper limit temperatures (55, 65, 70, 80, and 90°C) at a heating rate of 1°C/min and then cooled to 30°C again. The measurement was performed at a constant strain of 1% and at a constant frequency of 6.28 rad/s using the same geometry as Fig. 1. Data are presented as means of duplicate measurements. (A): 2%, (B): 10%.
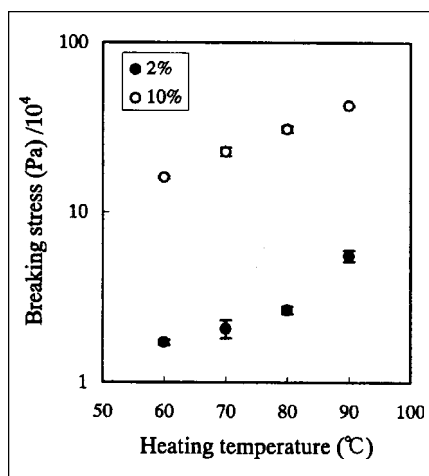
Fig. 8—Dependence of apparent breaking stress of curdlan gels on heating temperature. Curdlan gels at 2 and 10% concentration were cut into 10 mm height 15 mm in diameter and subjected to puncture test. The gels were punctured with a cylindrical plunger (5 mm dia) at 1mm/sec. The measurement was performed at 20°C. Data are presented as means±SD of duplicate measurements.

ature dependence of G′ for the aqueous suspension of various concentrations and also suggested that increasing curdlan concentrations shifted the transition temperature from thermo-reversible gel to irreversible gel to a temperature lower than 80°C.

Physical properties of curdlan gel

The apparent breaking stress was dependent on heating temperature at each curdlan concentration (Fig. 8), and increased with heating temperature. For the 2% gel, a marked increase in the apparent breaking stress occurred at 90°C. For the 10% gel, however, such marked increase in breaking stress was not observed and the stress increased at a constant rate. Results along with the thermo-reversible component calculations suggested that the increase in thermo-irreversible structure was responsible for the increase in breaking stress or gel firmness.

CONCLUSIONS

PREVIOUS STUDIES SHOWED THAT AN AQUEOUS SUSPENSION OF curdlan formed a thermo-irreversible gel at 80°C or higher. Those results were based on the result of a relationship between gel strength and heating temperature at relatively low curdlan concentrations (2 to 3%). The temperature-dependence of dynamic viscoelasticity of curdlan aqueous systems at various concentrations indicates that the thermo-irreversible gelation transition temperature was concentration-dependent. That is, increases in concentration shifted the irreversible gel formation transition zone to a temperature lower than 80°C and decreased the thermo-reversible component in the final gel.

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