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Phase transition of pectin with sorbed water

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Abstract

The phase-transition behaviour of pectin having various degrees of methyl esterification (DE), in the presence of sorbed water, was investigated by differential scanning calorimetry (DSC). In the first run DSC curves of pectin in the dry state, a melting endothermic peak (T_m) was observed at about 152°C. In the second run DSC curves, glass transition (T_g) was observed at about 35°C. The above facts indicate that the original pectin formed a regular molecular arrangement. However, amorphous glass is formed when the crystalline pectin is melted once. In pectin–water systems, the T_m of pectin decreases with increasing water content ($W_c =$ mass of sorbed water/mass of dry pectin (g/g)). When W_c is greater than 0.4 g/g, melting of free water and the glass transition of the pectin–water system were observed. The T_g of the pectin–water system decreased with increasing W_c in the bound water region. After reaching a minimum value, T_g slightly increased and approached a constant value. Water content at the minimum T_g ($W_{c_{min}}$) decreased with increasing DE. This suggests that the number of water molecules required to break the hydrogen bonds between –OH and –COOH groups decreases with increasing DE. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Pectin; Sorbed water; Phase transition

1. Introduction

Pectin is a complex polysaccharide found in many plant tissues, such as fruits and vegetables (Van Buren, 1991). Pectin consists of 1,4-linked α-D-galacturonic acid having a part of the carboxyl group esterified by a methyl group. The chemical structure of pectin is shown in Fig. 1. The gelation mechanism of pectin varies according to the degree of methyl esterification (DE) (Pedersen, 1980a). When DE is greater than 42.9%, gel is formed in the presence of acids and saccharides. When DE is lesser than 42.9%, gel is formed in the presence of divalent cations. It is known that these two groups of pectins gelatinize by different gelation mechanisms (Thakur, Singh & Handa, 1997). The junction zones of high-methoxyl pectins are stabilized by a combination of hydrogen bonds and hydrophobic interactions between molecules. In contrast, gelation in low-methoxyl pectins occurs by ionic linkages via calcium bridges between two carboxyl groups belonging to two different chains in close contact.

Much research (Al-Ruqaie, Kasapis, Richardson & Mitchell, 1997; Antonov, Lashko & Glotova, 1996; Clark & Farrer, 1996; Coffin & Fishman, 1994; Garnier, Axelos & Thibault, 1993; Grosso & Rao, 1998; Hoagland & Parris, 1996; Kawabata, 1985; Kawabata & Sawayama, 1975; 1977; Kawabata, Sawayama & Kamata, 1979; Kawabata, Sawayama & Nayoga, 1977; Rao & Cooley, 1994; Willians, Keenan & Halstead, 1998; Yao, Liu, Xiang, Lu & Tu, 1996) on pectins has been carried out, particularly on constituent sugars Kawabata and Sawayama, 1975, solution properties Kawabata and Sawayama, 1977 and mechanism of gelation (Kawabata et al., 1979; Thakur et al., 1997), in the field of food science (Kawabata, 1985), since pectins are used in many foods. Although a large number of studies on pectins have been reported, the physical properties has received little attention (Coffin & Fishman, 1994; Hoagland & Parris, 1996).

Pectins are water-soluble polymers and on this account, it is important to establish the phase-diagram of pectin related to the water content. In our previous studies, we reported that the glass-transition temperature (T_g) is markedly affected in the presence of bound-water, namely non-freezing water (W_{nf}), which is strongly restrained by the hydroxyl group and the ionic group of polysaccharides. In many water–polysaccharide–electrolyte systems, such as water–carboxymethylcellulose, water–cellulose sulfate and water–xanthan gum, T_g remarkably decreases by the absorption of non-freezing water. When free water was

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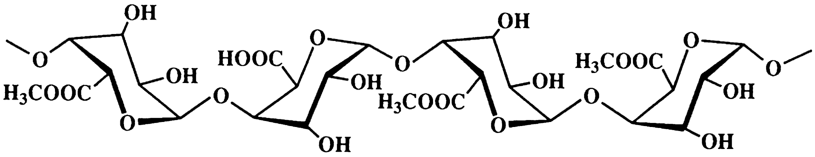

Fig. 1. Chemical structure of pectin. Segment of high-methoxyl pectin, degree of methyl esterification (DE) of 75%.

observed in the system, T_g increases, because the molecular motion of polysaccharides associated with bound-water is restricted by ice grown in the intermolecular spaces (Hatakeyama, Nakamura & Hatakeyama, 1996; Nakamura, Hatkeyama & Hatakeyama, 1996).

In this study, the phase-transition behavior of pectins having various DEs, in the presence of non-freezing water and free-water, is investigated by differential scanning calorimetry (DSC).

2. Experimental

2.1. Sample preparation

Pectin in powder-form was obtained from the Taiyo Kagaku Co. DE and viscosity (η) are listed in Table 1. The samples with low DE (P1, P2 and P3) were prepared by de-esterification of the high-methoxyl pectin (P4) (Pedersen, 1980b). It may be noted that high-methoxyl pectin is an intermediate product in the low-methoxyl pectin process. Two methods for de-esterification of acid-demethylated and ammonia-demethylated low-methoxyl pectins are followed in industrial processes. In this experiment, high-methoxyl (P3, P4) and low-methoxyl (P1, P2) pectins were prepared by acid-demethylation and ammonia-demethylation, respectively.

2.2. Measurements

A Seiko Instruments Inc. differential scanning calorimeter EXSTAR 6000, equipped with a cooling apparatus, was used for all the thermal analysis experiments. Temperature and enthalpy calibrations were carried out using indium. When the dry sample was measured, the sample mass was about 10 mg and an aluminium open-pan was used. Dry nitrogen was used as a purge gas and the flow rate was 30 ml/min. The scanning rate was 10°C/min. The samples were dried at 120°C for 10 min in a DSC sample-holder. It was confirmed that no endothermic deviation in the sample baseline due to the vaporization of water was observed after heat treatment. The sample was heated to 180°C (first-run measurement), and then rapidly cooled to −150°C. Then the second-run was carried out from −150 to 180°C. Liquid nitrogen was used a coolant.

Table 1
Characteristics of pectin samples

Sample		DE (%)	η (m Pa s)[a]
Pectic acid	(P0)	0	
Pectinic acid	(P1)	18–30	20.6
	(P2)	26–36	21.9
	(P3)	64–68	35.9
	(P4)	70–74	166.0

[a] $\eta = 2\%$ solution, 25°C.

When the wet sample was measured, the sample weight was 3–5 mg and an aluminium sealed-type sample-pan was used. The sample-pan was hermetically sealed and the total weight of the pectin and water recorded. A Sartorius ultramicro-balance ($\pm 0.1 \times 10^{-6}$ g) was used for sample weight measurements. The accurate water contents (W_c) of the samples were calculated according to the following equation:

$$W_c = \text{mass of sorbed water/mass of dry pectin (g/g)}. \tag{1}$$

The sample pan was pierced with a pin after DSC measurements, in order to remove water from the measured sample. The pan was then dried at 120°C for 90 min. The dried sample was then quickly re-weighed and the intrinsic water content determined.

The melting temperatures (T_m) of pectin and water were defined as DSC endothermic peaks. T_g was defined as the intersection of the extrapolations of the baseline and slope, and the heat capacity difference (ΔC_p) was defined between the glassy and the rubbery states at T_g.

3. Results and discussion

Fig. 2 shows the representative DSC curves of pectin (P4) in the dry state. A melting endothermic peak (T_m) was observed at 154°C in the first-run. When the sample was cooled from 180 to 0°C and heated from 0 to 180°C (second-run), T_g was observed at 37°C. The T_g value was constant even if the cooling rate was changed. The crystalline structure of pectin has been investigated by the X-ray diffraction method (Oakenfull, 1991). As shown in Fig. 2, it is clear that the original pectin is a crystalline polymer, but once the crystalline sample is melted, amorphous glass is formed. The amorphous structure of pectin was stable, i.e. the sample was not crystallized by slow cooling or by annealing. It is known that amorphous polysaccharides are crystallized in the presence of water (Hatakeyama, Hatakeyama & Nakamura, 1983). In order to examine the possibility of water-induced crystallization, amorphous pectin

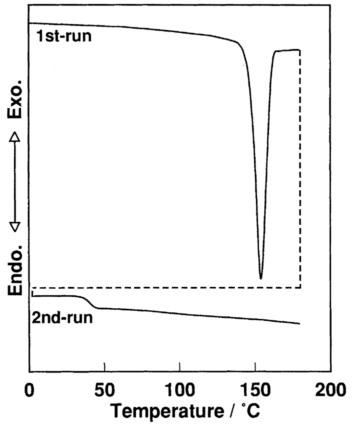

Fig. 2. DSC curves of pectin (P4) powder in the dry state.

was immersed in water at 20°C for 1 month. However, crystallization was not observed.

Fig. 3 shows the relationships between T_{m}, melting enthalpy (ΔH_{m}) and DE of pectins in the dry state. T_{m} and ΔH_{m} increased with increasing DE, indicating that the crystallinity increases with increasing DE. As stated in Section 2, the low-methoxylated pectins were prepared by de-esterification of high-methoxylated pectin. It is thought that the crystalline region of a pectin is destroyed during the chemical reaction.

Fig. 4 shows the relationships between T_{g}, ΔC_{p}, estimated from the second-run, and DE of pectins in the dry state. T_{g} maintains a constant value regardless of DE. It is known that bulky side-chains increase intermolecular distance, and main-chain molecular motion is readily enhanced (Nakamura, Hatakeyama & Hatakeyama, 1981). For the samples used in this study, DE values vary, and at the same time, the molecular mass of the pectin decreases with decreasing DE, as shown in Table 1. The high molecular weight introduces chain entanglement that disturbs free molecular rotation. It is thought that T_{g} maintains an almost constant value due to the variate of molecular mass and DE, i.e. molecular mass and bulky side-chain. The slight increase of ΔC_{p} suggests that the effect of bulky side-chains is more prominent than the effect of molecular mass.

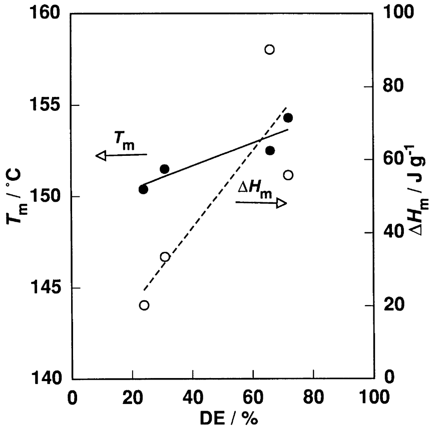

Fig. 3. Relationships between melting temperature (T_{m}), melting enthalpy (ΔH_{m}) and degree of methyl esterification (DE) of pectins in the dry state.

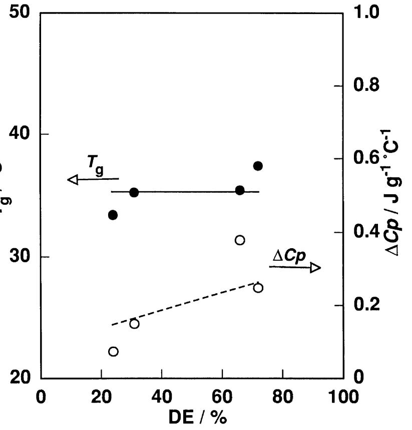

Fig. 4. Relationships between glass transition temperature (T_{g}), ΔC_{p} and degree of methyl esterification (DE) of pectins in the dry state.

Fig. 5 shows representative DSC curves of pectin (P4)–water systems having various water contents. Fig. 5(a) shows the heating curves of the first-run and Fig. 5(b) shows those of second-run. Similar DSC curves were obtained in the cases of the other samples (P1, P2 and P3). In the first-run DSC curves, the T_{m} of pectin decreased with increasing W_{c}. Glass transition and cold crystallization are clearly observed at $W_{\mathrm{c}} = 0.437$. At the same time, a small melting peak of ice is observed at a temperature lower than 0°C. Melting enthalpy (ΔH_{m}) of water in the system increases with increasing W_{c}. In the second run DSC curves, when W_{c} is small, T_{g} is observed at -50 to 50°C. T_{g} decreased with increasing W_{c}. When W_{c} exceeds about 0.5 g/g, the T_{m} of water was observed and T_{g} was constant at about -90°C.

Fig. 6 shows representative phase diagrams of the pectin (P4)–water systems. Similar diagrams were obtained in the other samples. Fig. 6(a) shows the results of the first-run and Part (b), those of the second-run. In the first-run, T_{m} of pectin decreased with increasing W_{c}. When W_{c} is greater than 0.4 g/g, T_{m} of sorbed water was observed. When T_{m} of pectin disappeared, T_{g} of pectin was clearly observed. It was found that pectin crystals melt in the presence of more than 0.45 g/g of water.

In the second-run, T_{g} of the pectin decreased remarkably with increasing W_{c} (ranging from 0 to 0.4 g/g). After reaching a minimum value (for example, -92°C for P4), T_{g} slightly increased and approached a constant value. The minimum value of T_{g} corresponded to the W_{c} where a melting peak of free-water appeared. ΔC_{p} remarkably increased with increasing W_{c} (ranging from 0 to ca. 1.0 g/g), and ΔC_{p} decreased when W_{c} increased beyond 1.0 g/g, though the ΔC_{p} data are not shown in Fig. 6.

Fig. 7(a) shows the variation of T_{g} of pectin–water systems having various DEs and W_{c}s. The variation of T_{g} was magnified in a W_{c} range from 0.4 to 1.0 g/g as shown in Fig. 7(b). T_{g} remarkably decreases in the low W_{c} range, since

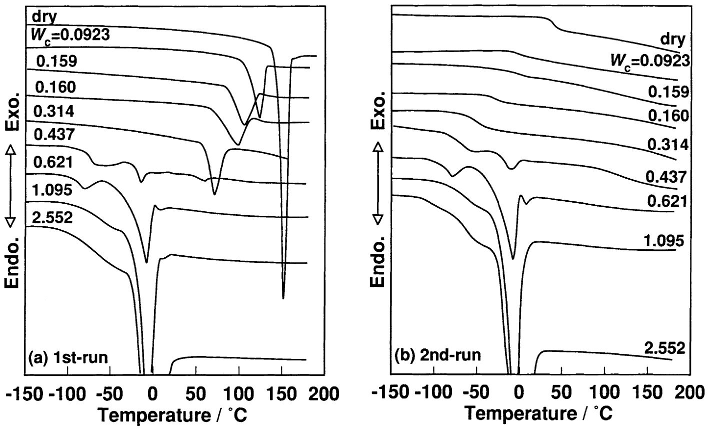

Fig. 5. DSC curves of pectin–water systems containing various water contents (P4): (a) first-run; (b) second-run.

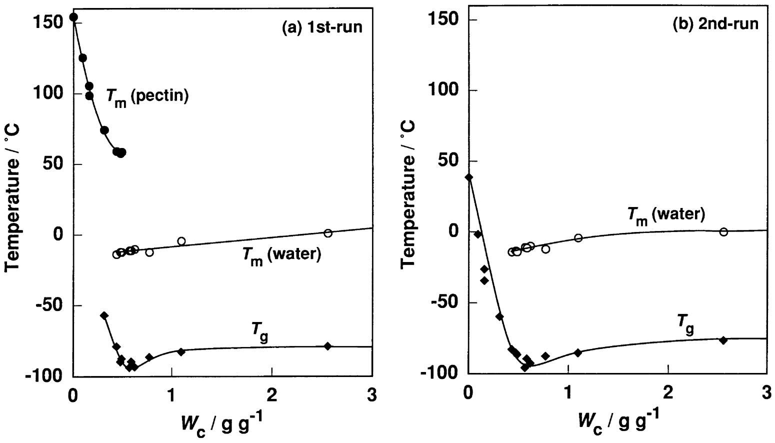

Fig. 6. Phase diagram of the pectin–water system (P4): (a) first-run; (b) second-run.

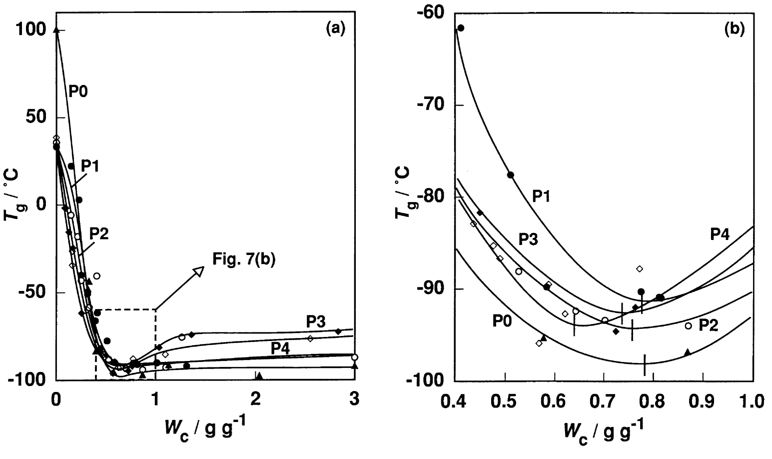

Fig. 7. Relationship between glass transition temperature (T_g) of pectin having various DEs and water content (W_c): (a) W_c ranging from 0 to 3.0 (g/g); (b) W_c ranging from 0.4 to 1.0 (g/g).

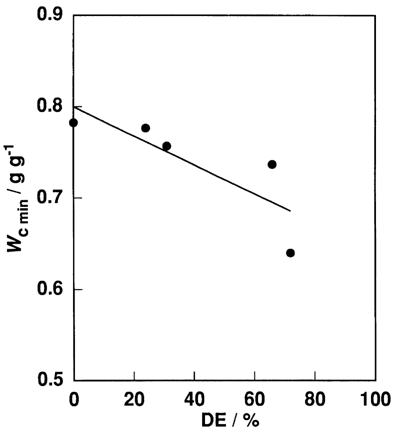

Fig. 8. Relationship between water content at the minimum T_g value ($W_{c_{min}}$) and degree of esterification (DE).

intermolecular hydrogen bonding is broken by water molecules. T_g values in the initial stage were compared with calculated values derived from Couchman's formula (Couchman, 1978). In the calculation, T_g and ΔC_p of dry pectin and those of amorphous ice were used (Sugisaki, Suga & Seki, 1968). The calculated values did not agree well with obtained values. This suggests that bound-water does not exist homogeneously. As shown in Fig. 7(b), T_g of pectins shows a minimum value ($T_{g\,min}$) at -91 to -97°C in the W_c ranging from 0.6 to 0.8 g/g. $T_{g\,min}$ shifted to the low W_c side with increasing DE.

Fig. 8 shows the relationship between water content at the minimum T_g value ($W_{c\,min}$) and DE. $W_{c\,min}$ decreased with increasing DE. When the hydrophilic group (–COOH) was replaced by the hydrophobic group (–$COCH_3$), the extent of intermolecular hydrogen bonding decreases. This causes the number of water molecules necessary to cut the hydrogen bonds between the –OH and –COOH groups to decrease.

4. Conclusion

From the above, the following are concluded: (1) phase diagrams of pectins with various DEs were established in a W_c range from 0 to 4.0 g/g; (2) glass-transition temperature of pectin–water systems markedly decreased in a W_c range from 0 to 0.4 g/g suggesting that water molecules break intermolecular hydrogen bonding; (3) when ice is formed in the system, T_g is constant or slightly increases due to the restriction of free molecular motion by the presence of ice; and (4) the W_c showing the lowest T_g decreases with increasing DE corresponding to the decrease of the hydroxyl group in the system.

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