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Oxygen-17 NMR relaxation studies on gelatinization temperature and water mobility in maize starches

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The effects of starch/water ratio, amylose content, degree of phosphorylation, and added KI on water mobility in maize starch–water dispersions were studied by oxygen-17 spin–spin relaxation time measurements over a range of temperatures. The results demonstrate that: (i) the changes in spin–spin relaxation time (ΔT_2) reflect the degree of starch–water interaction at different stages of the heating process; (ii) the amount of added water affects the initial T_2 and ΔT_2 during gelatinization; (iii) higher amylose contents result in lower water mobility in starch–water systems; (iv) higher degrees of phosphorylation lead to a decrease in water mobility, accompanied by a decrease in gelatinization temperature; and (v) added KI effectively decreases water mobility and gelatinization temperature in the starch–water systems studied. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Polysaccharide–water interactions are important determinants of polysaccharide functionality in foods. The gelatinization temperature of starch, the apparent gelatinization enthalpy, and many other changes are influenced by water. The effects of the amount of water on starch gelatinisation have been studied by differential scanning calorimetry (DSC) measurements (Donovan, 1979; Eliasson, 1980; Biliaderis et al., 1980), and recently by ^{17}O-NMR (Lim et al., 1992; Chinachoti et al., 1991).

Because ^{17}O-NMR relaxation is free from complications of cross-relaxation and chemical exchange, it has been found to be the most direct and effective NMR method for studying the dynamics of water–starch interactions (Richardson, 1989; Richardson et al., 1987a) as decreasing T_2 values indicate a reduction in water mobility.

Gelatinization is an irreversible process of swelling and hydration of the starch granule, with accompanying order/disorder transition of the polymers. The hydrogen-bonded network of the native granule structure may be partially or completely disrupted by heat or appropriate chemicals. Furthermore, gelatinization will cause the loss of granule birefringence, consistency/viscosity changes, and affect granule swelling and solubility behaviour (Zobel, 1992). Thus, water mobility is likely to strongly reflect the degree of gelatinization and other properties.

The gelatinization temperature range of starch granules is affected by many factors; for instance, by reducing the amount of water available to the granules, or by adding high levels of hydrophilic solutes such as sugars or other polyhydroxy compounds, or even by the addition of simple salts (Evans and Haisman, 1982). Heating facilitates the hydration of starch granules, and results in a more open structure of the swollen granule that allows fast exchange between the bound and free (or bulk) water. The beginning of gelatinization is characterised by an increased hydration and fluidity of polymer chains (Mora-Gutierrez and Baianu, 1989).

Amylose content, KI concentration and the degree of starch phosphorylation directly affect water-binding ability and swelling properties during gelatinization (Wootton and Bamunuarachchi, 1978; Jane, 1993). Although starch–water interactions have been studied extensively, ^{17}O studies on the effects of the above factors on starch–water interactions have not been reported. Because gelatinization is a continuous process involving the melting of crystallites and the swelling of granules in the presence of water as the temperature is increased, it is a precondition of gelatinization that the starch granules need to absorb water and swell to some extent. Therefore, observation of changes in water mobility with increasing temperature is likely to provide a better understanding of the starch gelatinization process and of the underlying molecular mechanisms.

MATERIALS AND METHODS

Maize starches (MS) with amylose content 28% (MSA), 40% (MSB), 56% (MSC), and 65% (MSD) obtained from Starch Australasia Ltd were used for all experimental work. Deuterium oxide instead of pure water was used to study starch–water interactions to avoid proton-exchange broadening of the O-17 peak (Richardson et al., 1987b). Maize starch was phosphorylated as described previously (Paschall, 1964). Different degrees of substitution of starch phosphate esters were obtained by increasing the phosphate salt buffer concentration and extending the heating time. Moisture contents of the samples were determined gravimetrically, and expressed on the basis of weight loss of samples dried at 130°. For each sample, the average of three determinations was taken as the moisture content. Maize starch solutions with the ratio of maize starch to deuterium oxide 1:5, 1:2, 1:1.5 and 1:1 w/w were prepared and left to equilibrate overnight before the gelatinization experiment. The total water in the sample was taken as the sum of the original moisture content and the amount of water added in the preparation of the starch–D_2O mixture. For KI–maize starch and starch phosphate investigations, starch dispersions were prepared in the ratio of starch–D_2O (1:2, w/w) and mixed mechanically (KI concentration from 0.5 M to 2 M). The gelatinization temperature of starches was determined by polarizing microscopy with a Kofler electrically-heated microscopic hot stage, at a heating rate of 2°C min^{-1} according to the method of Watson (1964). Approximately 2%, 50% and 100% loss of polarization in these granules were taken as the onset temperature, peak temperature and completion temperature of gelatinization. Triplicate runs were averaged.

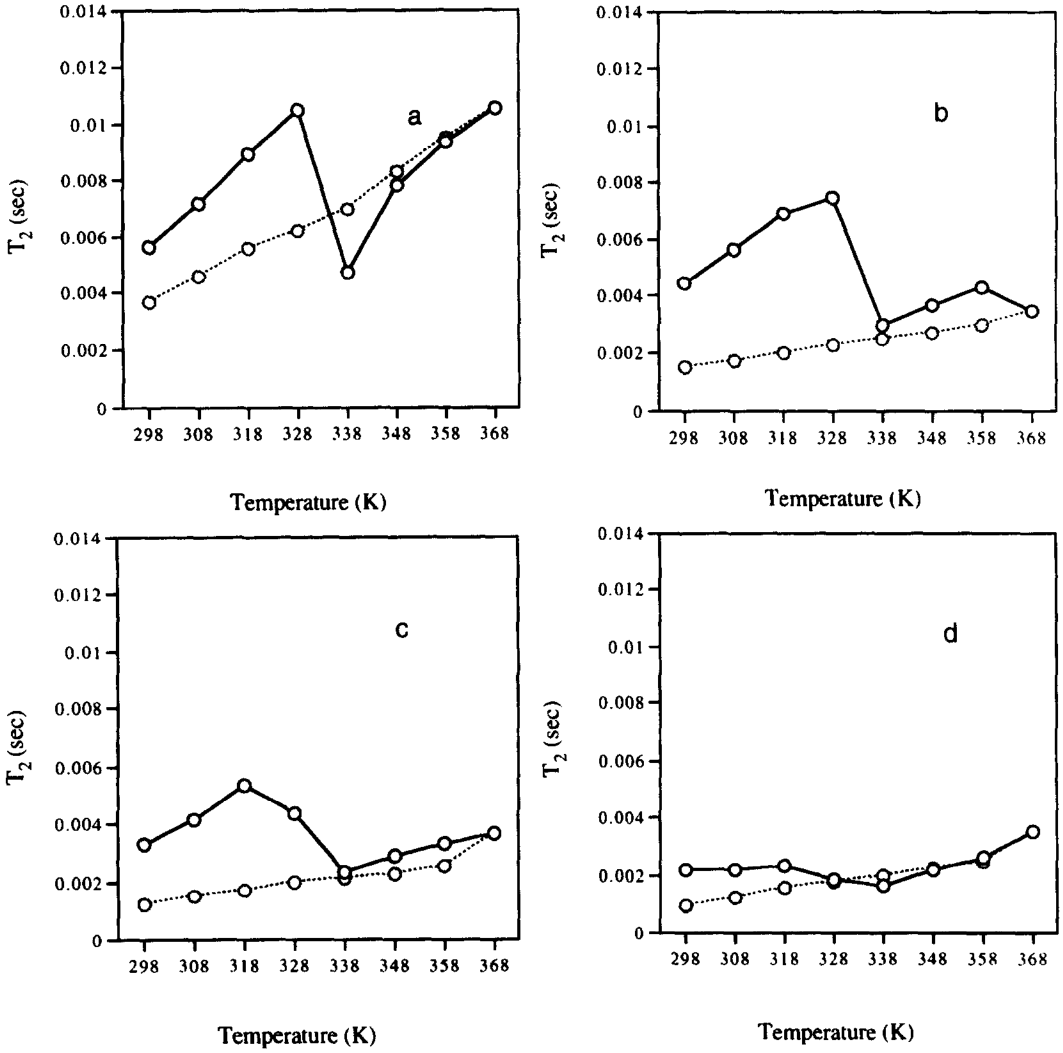

Fig. 1. Oxygen-17 spin–spin relaxation times vs. temperature profiles. (a) starch:D_2O = 1:5; (b) starch:D_2O = 1:2; (c) starch:D_2O = 1:1.5; (d) starch:D_2O = 1:1. The sample is the maize starch with 28% amylose (MSA). ——, heating curve; - - - -, cooling curve.

Oxygen-17 NMR

A Brüker ACF-300 NMR spectrometer (Bruker instruments) was used for the O-17 NMR spectra. A 10 mm diameter NMR sample tube was used in all experiments. The probe was equipped with a programmable heating and cooling control. The program V.TEXP.AU was used to give heating and cooling control under the following conditions: Temperature was raised from 298 to 368 K at 2 K min^{-1}, and cooled back from 368 to 298 K at 1 K min^{-1}, with 5 min delays at each 10 K step. The spectra were obtained at 75.44 MHz; sweep width, 5000 Hz; pulse width, 15 μs; number of scans, 2000. Line shapes were evaluated by deconvolution in terms of fit of least-squares of the peaks to a Lorentzian equation. Line widths ($\Delta\nu_{obs}$) were the values at half-height for the water peak at each temperature. The spin–spin relaxation time (T_2) was calculated from $T_2 = 1/\pi(\Delta\nu_{obs})$.

RESULTS AND DISCUSSION

Effect of the ratio of starch–water ratio on T_2

Oxygen-17 spin–spin relaxation time vs. temperature plots are shown in Fig. 1 for different ratios of maize starch and water. Table 1 lists some of the data for each ratio. These include initial T_2 value, T_2 at onset and final gelatinization temperature, ΔT_2 during gelatinization, and gelatinization temperature range. In order to avoid difficulties in obtaining homogeneous samples and incomplete gelatinization (Bamunuarachchi, 1978), water levels above 90% and below 50% were avoided.

For maize starch concentrations between 20% and 50%, a single Lorentzian peak is observed, indicating a rapid exchange between free water and bound water. As the ratio of starch to D_2O was increased (Fig. 1(a)–(d)), the following trends were noted.

(i) Initial T_2 values fell.
(ii) T_2 values at gelatinization onset decreased and the initial slope of the T_2 vs. temperature curves decreased.
(iii) ΔT_2, i.e. change in T_2 during gelatinization, decreased with increasing starch concentration.
(iv) On cooling the samples, T_2 values decreased in an essentially linear fashion.We interpret these results as follows:

(i) The overall result shows that the T_2 vs. temperature profile is dependent on the starch/water ratio. At low ratios of starch/ water (Fig. 1(a)) the 'free water'/ 'bound water' ratio is high, and T_2 is dominated by the former. At high ratios of starch/water (Fig. 1(d)) a greater proportion of the water is 'bound' and lower initial (and overall) T_2 values result.
(ii) The same reasoning can be applied to the lower T_2 values observed at gelatinization onset. High initial slopes in T_2 vs. temperature curves occur as the large percentage of 'free water' molecules become highly mobile on heating (Fig. 1(a)). The low percentage of 'free water' at all temperatures in Fig. 1(d) is reflected in low T_2 values.
(iii) The large ΔT_2 at high water levels occurs only because the excess free water is 'allowed' to reach such high levels of mobility. At lower water levels most water molecules are bound even at the initial temperature. Immediately after gelatinization, T_2 falls as much more water becomes bound in the starch paste and its mobility decreases (c.f. the well-known initial viscosity increase during gelatinization). Note that the initial T_2 value at high water levels is still quite high (Fig. 1(a)) compared with the minimum T_2 values at higher starch levels (Fig. 1(b)–(d)).
(iv) The linear nature of the cooling curve probably indicates that there is no significant gelation during cooling. If gelation had occurred, we would expect a sharp drop in T_2 spanning the gelation temperature range. An alternative explanation could be that the extra water one would expect to be bound during gelation is insufficient to be detected by the present method. We plan to investigate this possibility.

Overall, the data in Fig. 1 and Table 1 demonstrate the potential of T_2 measurements for the study of water mobility in starch granule gelatinization, provided that an appropriate ratio of starch to water is selected and used in all comparative studies. We have used a starch to water ratio of 1:2 in subsequent sections. Table 2 shows that the gelatin-

Table 1. Spin–spin relaxation time (T_2) parameters from O-17 NMR measurements of maize starch/water mixtures

Starch:D_2O	Initial T_2 $\times 10^{-3}$ (s)	T_{2o} [a] $\times 10^{-3}$ (s)	T_{2f} [b] $\times 10^{-3}$ (s)	ΔT_2 $\times 10^{-3}$ (s)	Gelatinization temperature range (approximate) (K) [c]
1.5	5.65	10.47	4.75	5.72	328–338
1:2	4.42	7.48	2.94	4.54	328–338
1:1.5	3.31	5.33	2.37	2.96	318–338
1:1	2.24	2.36	1.64	1.68	318–338

The sample is normal maize starch with 28% amylose (MSA).
[a] $T_{2o} = T_2$ at onset gelatinization temperature.
[b] $T_{2f} = T_2$ at final gelatinization temperature.
[c] Gelatinization temperature range estimated according to T_2 change.

Table 2. Spin–spin relaxation time (T_2) parameters from O-17 NMR measurements of maize starches with different amylose contents

Sample	Amylose content (%)	Initial T_2 $\times 10^{-3}$ (s)	T_{2_0} $\times 10^{-3}$ (s)	T_{2f} $\times 10^{-3}$ (s)	ΔT_2 $\times 10^{-3}$ (s)	Gelatinization temperature range (approximately) (K)
MSA	28	4.42	7.48	2.94	4.54	328–338 [a] (330–338–345) [b]
MSB	40	3.74	6.46	3.03	3.43	328–338 (329–335–342)
MSC	56	3.29	5.04	3.17	1.87	338–348 (337–341–346)
MSD	65	2.86	3.81	2.46	1.35	328–348 (333–339–352)

[a] Gelatinization temperature range estimated according to T_2 change. The ratio of starch to water is 1:2.

[b] Gelatinization temperature range determined by polarizing microscopy. The data shown in parentheses are onset temperature, peak temperature and completion temperature respectively, which occur at approximately 2%, 50% and 100% loss of polarization in the granules.

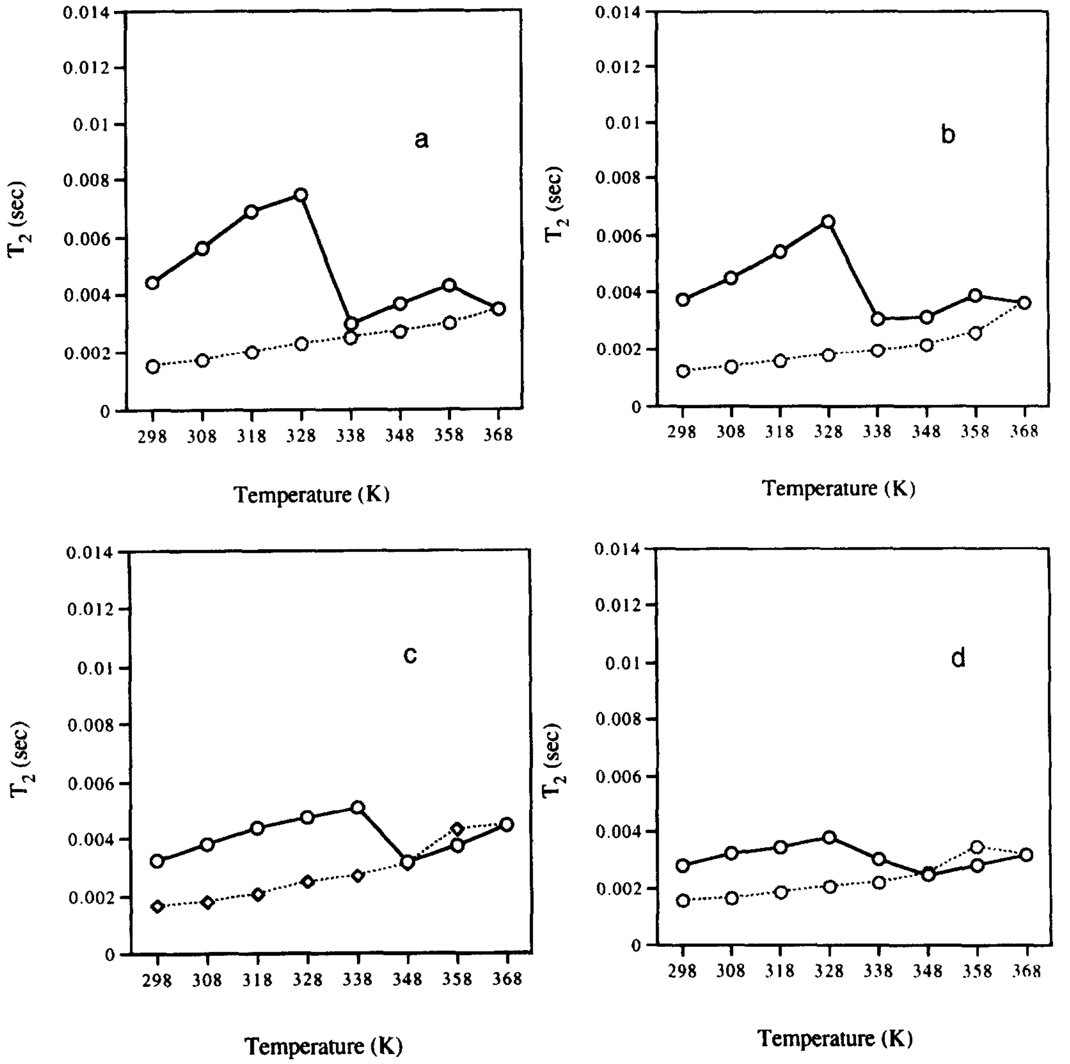

Fig. 2. Oxygen-17 spin–spin relaxation times of water in a mixture of starch and D_2O (1:2) for different maize starches. (a) MSA, (b) MSB, (c) MSC, (d) MSD. ——, heating curve; - - - -, cooling curve.

ization temperature ranges determined by T_2 measurements compared well to those found by polarizing microscopes considering measurements of T_2 were taken at 10° intervals.

The effect of amylose content on starch hydration

Samples of maize starches with amylose content from 28% to 65% (MSA, MSB, MSC and MSD) were chosen for these studies. Fig. 2 shows the T_2 vs. temperature profiles at a starch/water ratio of 1:2. Broadly speaking, these follow the shapes of the respective curves in Fig. 1. i.e. flatter slopes up to gelatinization onset, smaller ΔT_2 values with increasing amylose content, and progressively lower initial T_2 values (Table 2). This latter observation apparently indicates that with increasing amylose content, the granule interior becomes more accessible to the D_2O. We have found (unpublished results) that the degree of crystallinity of maize starches decreases with an increase in amylose content. Thus, the amylopectin in high amylose starches is likely to be less crystalline, and more solvent-accessible than that in low-amylose starches. The combination of amylopectin accessibility even at 298 K, combined with its water-binding capacity, is presumably responsible for reduced water mobility, resulting in lowering of T_2 values as the amylose content is increased. As observed previously, the gelatinization temperature increases with amylose content.

Variation of T_2 at different concentrations of KI

The effects of KI concentrations (0–2 M) on the water oxygen-17 T_2 in a range MSA starch/KI dispersions are shown in Fig. 3. Initial T_2 and ΔT_2 values decrease as the concentration of KI is increased, until at 2 M KI there is no observable phase transition (Table 3). At 2 M KI levels, polarizing microscopy showed that most of the granules had lost birefringence.

The effects of KI have been explained in terms of the Donnan potential in the system (Oösten, 1982; Jane, 1993). As KI solution viscosity is low, and the large iodide ion has a low charge density, diffusion into the granule is relatively unrestricted.

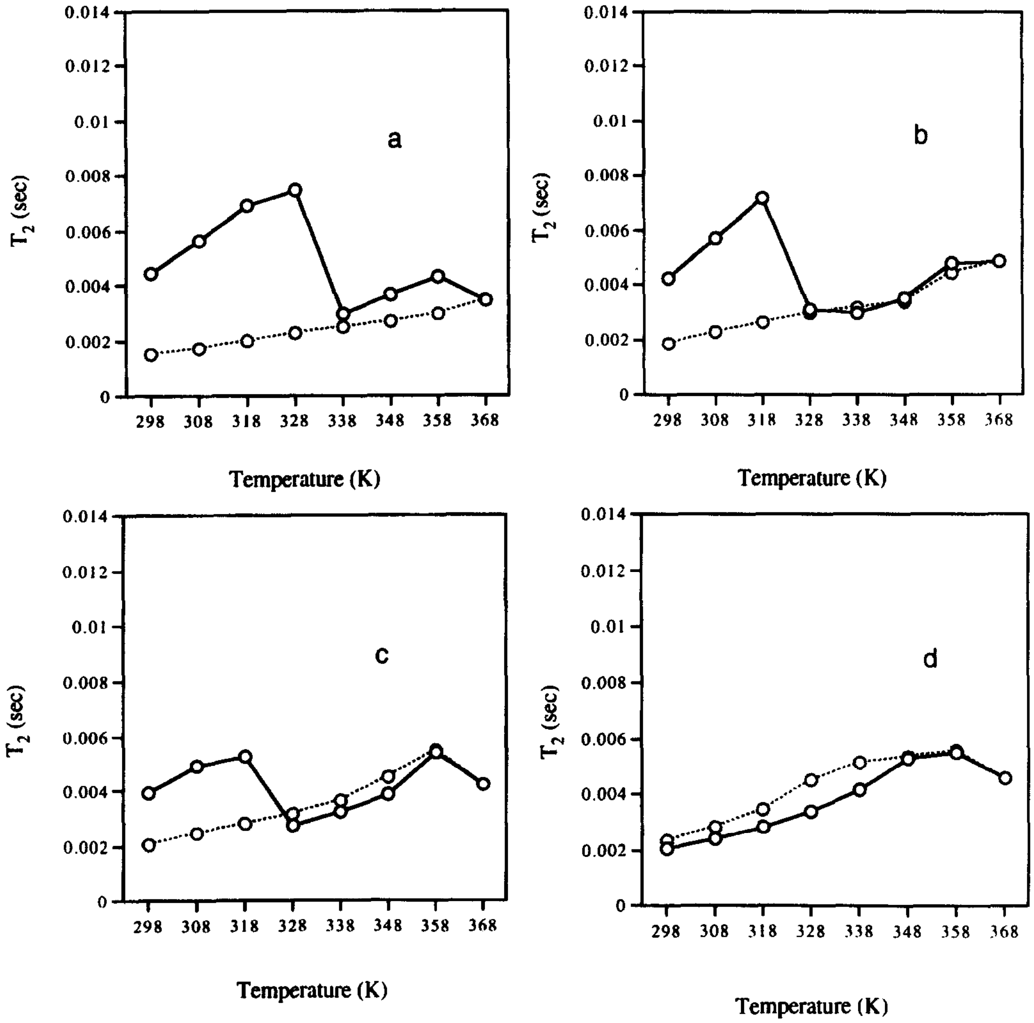

Fig. 3. The effects of KI concentration on the T_2 vs. temperature profiles of starch/water mixtures. (a) control, (b) 0.5 M KI, (c) 1 M KI, (d) 2 M KI. The sample is MSA. ——, heating curve; - - - -, cooling curve.

Table 3. Spin–spin relaxation time (T_2) parameters from O-17 NMR measurements of maize starch (MSA) at different concentrations of KI

KI conc. (M)	Initial T_2 $\times 10^{-3}$ (s)	T_{2o} $\times 10^{-3}$ (s)	T_{2f} $\times 10^{-3}$ (s)	ΔT_2 $\times 10^{-3}$ (s)	Gelatinization temperature range (approximate) (K)
0	4.42	7.48	2.94	4.54	328–338 (330–338–345)
0.5	4.25	7.17	3.14	4.03	318–328 (324–328–333)
1	3.93	5.23	2.75	2.47	318–328 (322–324–327)
2	2.04	–	–	–	– (305–307–309)

At 0.5 M net KI concentration (Fig. 3(b)), the major change is the shift in gelatinization range from 328–338 K in water to 318–328 K. This range is maintained at 1 M KI, but ΔT_2 decreases markedly. At 2 M KI no gelatinization transition is observed, and the initial T_2 is essentially the same as T_2 after the heating and cooling cycle. This implies that gelatinization has occurred at 298 K. Jane (1993) considers the KI effect on gelatinization to involve the electrostatic interactions between starch and ions, and damage to water structure by the low charge-density iodine ions. These two effects facilitate the absorption of water and destabilize starch granules at temperatures far below the 'normal' gelatinization temperature. The cooling curves in 1 M and 2 M KI (Fig. 3(c) and (d)) lie slightly

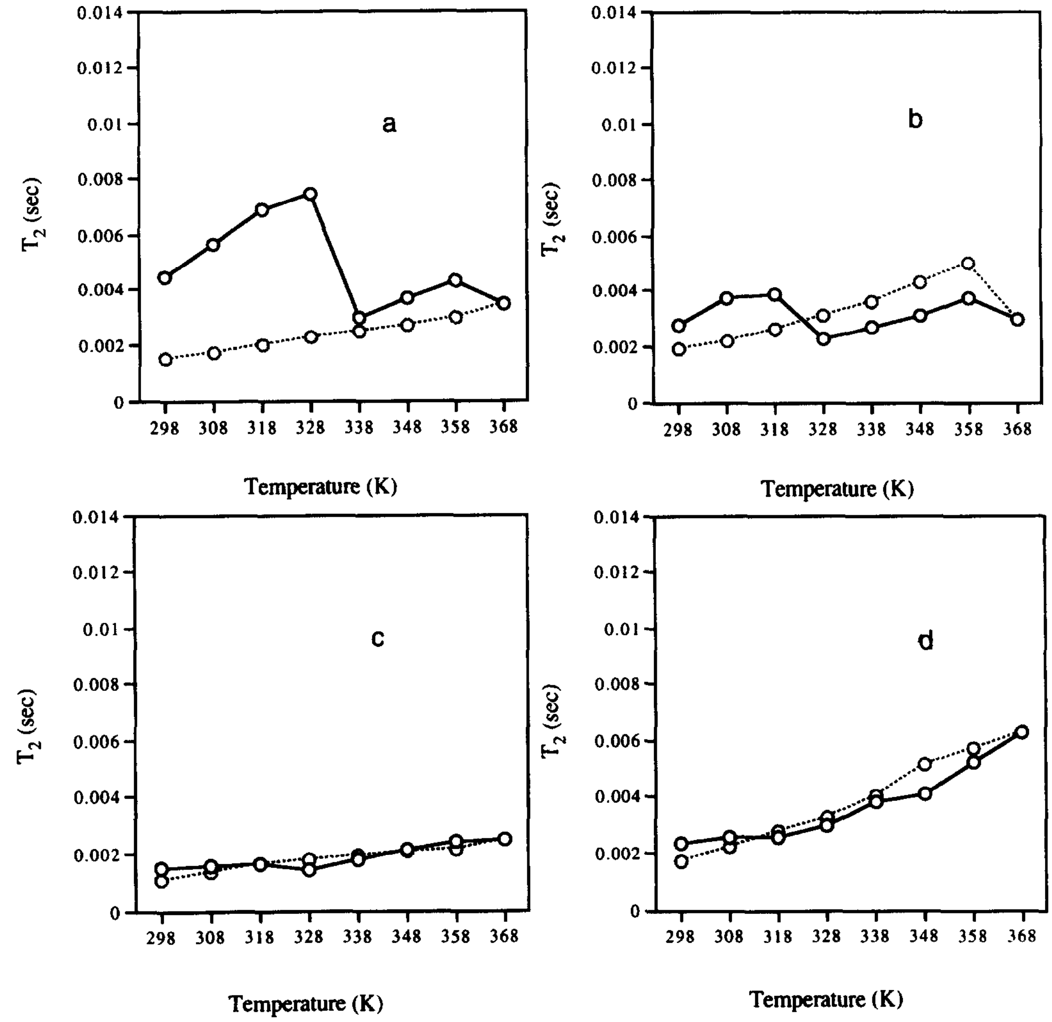

Fig. 4. Profiles of oxygen-17 spin–spin relaxation times of water vs. temperature in a mixture of starch and D_2O (1:2) for MSA maize starch and its phosphates. (a) Maize starch (MSA), (b) maize starch phosphate (DS = 0.024), (c) maize starch phosphate (DS = 0.072). ——, heating curve; - - - -, cooling curve.

above the heating curve. This probably reflects the destructive effect of KI on the structure of water after complete destruction of the granule by heating, leading to an increase in T_2. The fact that the T_2 values in 1 and 2 M KI reach the highest post-heating levels achieved in any experiment in this study, supports such an interpretation.

Starch phosphates: variation of T_2 with degree of substitution

Starch phosphate, a cross-linked mixture of mono-, di-, and trisubstituted starch molecules, is an important industrial derivative. The influence of degree of substitution (DS) on the T_2 of water ^{17}O is shown in Fig. 4. With the increase in DS, the gelatinisation temperature range and ΔT_2 decrease. The DS-0.024 (Fig. 4(b)) sample shows a reduction of T_2 compared with the control at the same temperature, a lowering of the gelatinisation range to 318–328 K, and a reduced ΔT_2. When the DS is increased to 0.031 (Fig. 4(c)) initial T_2 is further reduced, indicating that a higher degree of phosphate substitution can effectively weaken internal bonding within the granules and lead to an increase in water-binding ability and a corresponding decrease in water mobility. At DS = 0.072 (Fig. 4(d)), the gelatinisation temperature range decreases to 308–318 K, and ΔT_2 decreases further as the high degree of substitution causes more and more hydrogen bonding to be broken, and the energy required for breaking the residual hydrogen-bonding decreases. Judging by the almost horizontal T_2 curve, the water binding of starch phosphate at DS = 0.031 is 'strongest' of all the samples in this study. Perhaps at this 1:2 ratio of starch phosphate to water, essentially all the water available is bound. The greater mobility of water molecules at higher temperatures in Fig. 4(d) compared to Fig. 4(c) is probably due to a combination of (a) too high a degree of crosslinking, and (b) the greater overall mobility associated with smaller molecular weight chains. Polarized light microscopy showed that some granules had lost birefringence, and the molecular weight of the starch as determined by GPC was significantly lower than in the control.

CONCLUSIONS

(i) During gelatinisation, the amorphous parts (mainly amylose) of starch undergo irreversible swelling and increased water binding which leads to a rapid decrease in water mobility. Above the gelatinisation temperature, the solubility and swelling properties of the starch determine the water mobility. Cooling curves reflect the expected decrease in water mobility. If gelation occurs during cooling, it is not detected by T_2 measurements.

(ii) The effect of the amount of water on starch–water interaction is prominent. When the ratio of starch to water is low (1:5), T_2 change mainly results from the effect of temperature on pure water (D_2O). As the ratio is raised to 1:1, T_2 at onset and final gelatinisation temperature, and ΔT_2 show a significant decrease.

(iii) The ratios of free water to bound water in starch–D_2O mixtures are correlated with amylose content. Initial T_2 and ΔT_2 is progressively reduced with the increase in amylose content. The ΔT_2 of the phase transition reflects volume expansion, as ΔT_2 is dependent on the degree of swelling and solution viscosity. As with volume expansion values, the ΔT_2 value of the phase transition has a good correlation with amylose content. Therefore, the T_2 vs. temperature profile may provide important information about maize starch quality, because it reflects the combined effects of amylose content, water-binding ability, swelling ability, and gelatinisation temperature range.

(iv) KI decreases water mobility and gelatinisation temperature in starch–water systems by improving starch–water interaction. The effect of KI on the gelatinisation temperature of maize starch is the result of a rapid increase in water absorbing ability and volume expansion.

(v) The alteration of the granular structure of maize starch by phosphorylation leads to water-binding and swelling properties which are highly dependent upon the degree of substitution. Moderate degrees of substitution cause sharp increases in water absorption capacity, even at low temperatures, indicated by a decrease in oxygen T_2. Phosphorylation of starch changes the water absorption behaviour, and leads to a decrease in the gelatinisation temperature.

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