The influence of glycerol on structural changes in waxy maize starch as studied by Fourier transform infra-red spectroscopy

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The influence of glycerol on the retrogradation kinetics for waxy maize starch–water systems was monitored by Fourier transform infra-red spectroscopy. The spectra showed the C–C and C–O stretching region (1300–800 cm^{-1}) to be sensitive to the retrogradation process. A multistage kinetic process in terms of structural changes on a molecular level was observed during the retrogradation of waxy maize in a 10% (w/w) gel. For the first two stages (formation of helices and induction time for helix aggregation) no significant kinetic differences were observed in the gels with various glycerol contents. Stage three is described as the primary aggregation and crystallization step. In the final stage, syneresis of water occurs. The calculated rate constants clearly show decreasing retrogradation of waxy maize starch with increasing glycerol content. The effects are explained in terms of starch–glycerol interactions and stabilization of water resulting in a decreased mobility of the starch chains.

(Keywords: waxy maize starch; *FT*i.r. spectroscopy; glycerol)

INTRODUCTION

Application of thermoplastic starch as a biodegradable plastic is a subject of growing interest. A drawback of these materials is the loss of mechanical properties with time. This is caused by structural changes due to water loss, crystallization and retrogradation. Therefore, it is of importance to investigate the possible effects of additives used in thermoplastic starch materials on structural changes. The retrogradation of starch and starch-containing materials has traditionally been studied on time-scales up to several weeks by X-ray diffraction, d.s.c. rheological methods and light scattering[1–10]. However, none give direct information on the processes involved at the molecular level. Even the X-ray diffraction technique is limited to the long-term appearance of the final, crystalline form of the retrograded material. More recently, spectroscopic methods, such as Raman[11–16], n.m.r.[17–21] and Fourier transform infra-red (*FT*i.r.) spectroscopy[22–27] have been used. Spectroscopy is non-invasive and allows direct observation of structural changes during retrogradation over a long time-scale.

The influence of glycerol as the most commonly used plasticizer in thermoplastic starch has been studied. The starch gel system has been used as a model for the complex thermoplastic starch materials. Research has been focused on the role of amylopectin by the use of waxy maize starch. *FT*i.r. spectroscopy coupled with sampling methods such as attenuated total reflection (ATR) appears to be a powerful method. The region of the i.r. spectrum between 1300 cm^{-1} and 800 cm^{-1} is sensitive to the conformation of polysaccharides in aqueous solution[28,29], even though most bands arise from highly coupled and difficult to assign C–O and C–C stretching vibrations[30]. Molecular conformational changes due to retrogradation can be observed by analysis of the band-narrowing process in the 1300–800 cm^{-1} region during storage. The kinetics of this process is measured by quantification of the extent of the line narrowing as a function of time.

EXPERIMENTAL

Preparation of gels

The amylopectin gels were prepared by gelatinization of waxy maize starch (var. Amioca), 99% amylopectin on the basis of dry material and 12% water on the basis of total mass, dispersed in deionized water, with or without glycerol added in various concentrations. Both gels contained 10% (w/w) starch. The gelatinization was performed in a Contraves Rheomat 115 viscometer at standard conditions (temperature profile: logarithmic incline from 40 to 95° over 30 min). The hot solutions were poured into a preheated (65°C) ATR cell and sealed to prevent water loss. The preservative used was 0.01% (w/w) thimerosal (Sigma Chemical Co.). The samples were stored at 5°C.

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X-ray diffractometry

Wide-angle X-ray diffraction patterns were measured by using a Philips powder diffractometer (model PW 3710) operated at the CuKα wavelength of 1.542 Å. Measurements of diffracted intensities were made over the angular range of 2.5–20° at ambient temperature.

F*Ti.r. measurements*

All *FT*i.r. spectra were obtained on a Digilab FTS-60A spectrometer (BIO-RAD) at 4 cm^{-1} resolution. Measurements were carried out using a deuterated triglycine sulfate detector (DTGS) at a mirror velocity of 0.3 $cm\ s^{-1}$. The number of interferograms being co-added for each Fourier transformation was 64 for the initial measurements and 256 for the measurements from 5 h onwards. An ATR cell (BIO-RAD) with a ZnSe crystal was used. Spectra were run at irregular intervals up to 500 h. The ATR cell was allowed to equilibrate at room temperature before each measurement. A spectrum of the empty cell was used as the background. The spectrum of water with glycerol added in the proper amount was subtracted from all starch gel spectra in order to eliminate the distorting effect of water in the region 1300–800 cm^{-1} and to correct for the spectrum of glycerol.

Resolution enhancement was applied to all resulting spectra by using the deconvolution technique described by Cameron and Moffat[31] and modified for use on the Digilab FTS-60A. The assumed line shape was Lorentzian with a half-width of 15 cm^{-1}. The resolution enhancement factor (K) was optimized at 1.5. Absorbance ratio measurements were made only on the enhanced spectra.

Data treatment

The *FT*i.r. data from each gel were analysed separately by fitting a linear zero-order equation or by fitting an Avrami equation to each data set[32]:

$$\phi = (A_\infty - A_t)/(A_\infty - A_0) = \exp(-kt^n)$$

where ϕ is the fraction of the total change in measured property still to occur; A_0, A_t and A_∞ are experimental values of the absorbance ratio at time zero, t and infinity, respectively, k is the rate constant and n is the Avrami exponent. The Avrami exponent was assumed to be equal to one. The fits were obtained by means of an iterative, non-linear least squares procedure.

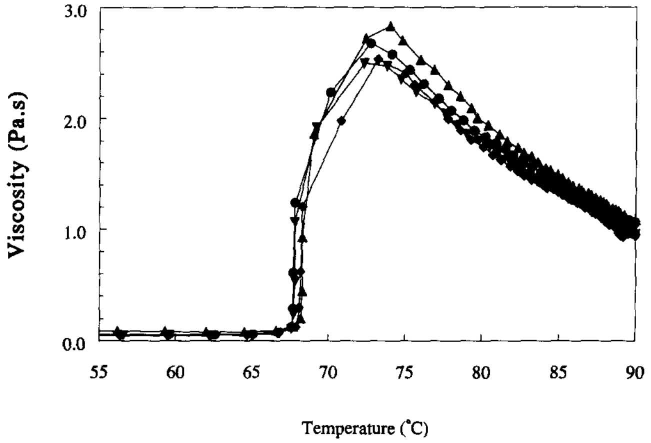

Figure 1 Viscograms of the four gels with 0% (◆), 1% (▼), 5% (●) and 20% (▲) glycerol

RESULTS AND DISCUSSION

The viscograms of the waxy maize gels with different amounts of glycerol added are shown in *Figure 1*. The onset gelatinization temperature for all the gels is 67°C and the peak gelatinization temperature is 74°C. The added amounts of glycerol have no significant influence on the gelatinization behaviour of the waxy maize starch. Apparently, there are no differences in the preparation of the waxy maize gels. Possible differences in retrogradation kinetics cannot therefore be ascribed to variations in gel preparation.

The retrogradation of waxy maize starch has been measured in relation to glycerol content in a 10% (w/w) gel with the aid of *FT*i.r. spectroscopy. The spectra at selected time points (0, 10 and 300 h) are shown for the waxy maize gels in *Figure 2*. All spectra show eight major distinct bands in the region of 1300–800 cm^{-1} at similar frequencies. In *Table 1* the band frequencies are summarized for two samples (0% and 20% glycerol) at time zero and at the last time point for each experiment. The two other gels (1% and 5% glycerol) give similar frequencies and shifts in time. The spectrum of freshly gelatinized starch shows an intense broad band with a maximum intensity at 1022 cm^{-1} and a noticeable shoulder on the high frequency side. During retrogradation for several weeks, this broad line becomes resolved into three bands around 1053, 1022 and 1000 cm^{-1} (with a minimum of the intervening valley at 1035 cm^{-1}). The most pronounced changes in the spectra occur at 1000 (peak), 1035 (valley) and 1053 (peak) cm^{-1}. These observations represent the process of a range of amylopectin polymer conformations in the gelatinized disordered state to a more ordered system with a reduced spread of conformations. The distribution of bond energies will become smaller during retrogradation. These changes are measured with *FT*i.r. spectroscopy as variation in the intensity instead of line narrowing because of the deconvolution process applied to enhance

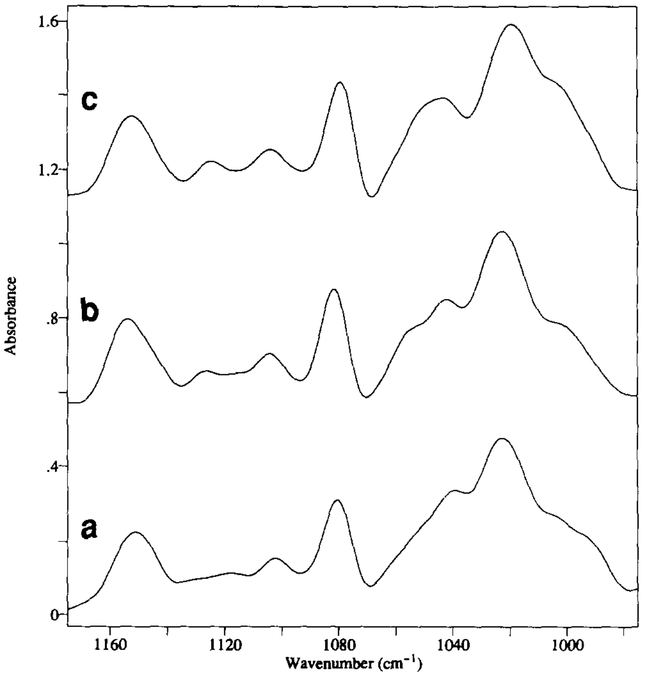

Figure 2 *FT*i.r. spectra of a waxy maize gel (10% w/w) at selected time points during retrogradation: (a) 0 h; (b) 10 h; (c) 300 h

Table 1 *FT*i.r. band frequencies (in the region 1300–800 cm^{-1}) of the waxy maize starch gels at various time points during retrogradation at 5°C

Sample	Time (h)	Band (cm^{-1}) 1	2	3	4	5	6	7	8
0% glycerol	0	1151	1125	1102	1080	1053 sh^a	1039 sh	1022	1003 sh
	288	1153	1126	1105	1080	1053 sh	1045	1020	1004 sh
20% glycerol	0	1152	1125	1105	1080	1053 sh	1041	1022	999 sh
	502	1154	1122 sh	1107	1081	1053 sh	1042	1024	1001 sh

^{a} sh = shoulder

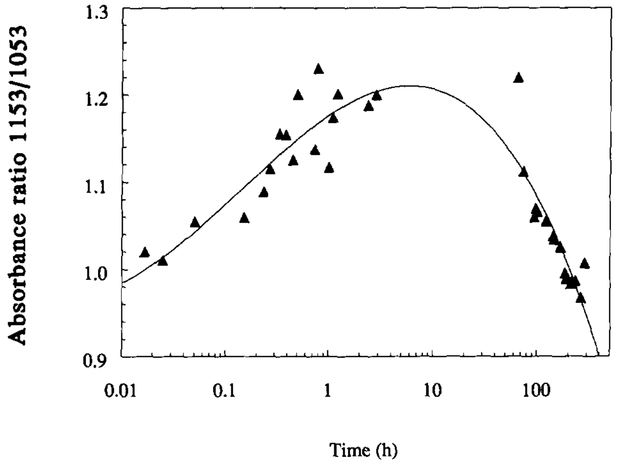

Figure 3 Spectral changes over time for the waxy maize gel (10% w/w) for the absorbance ratio 1153/1053

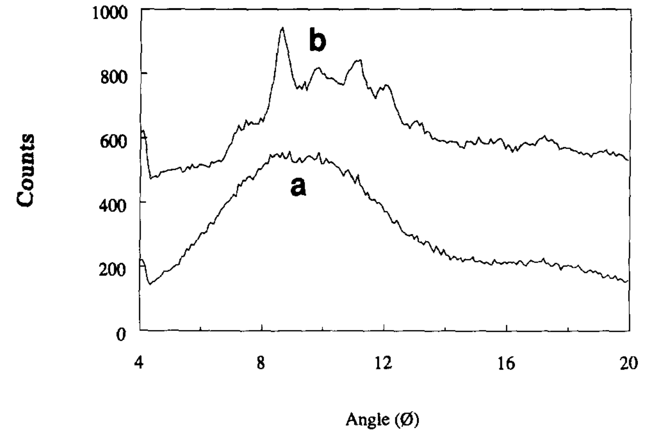

Figure 4 X-ray diffractograms of waxy maize after (a) 5 h and (b) 3 weeks of retrogradation

resolution. The absorbance ratios of band intensities between selected peaks were measured at different time points. The use of ratios allows the compensation of path-length differences that might arise from incomplete coverage of the ATR crystal between different samples.

The spectral changes in the waxy maize gel (10% w/w without glycerol) in time are shown in *Figure 3* on a logarithmic time-scale for the absorbance ratio 1153/1053. Three different steps are clearly visible: from 0 to 1 h the ratio increases, from 1 to 10 h there is a plateau region, and beyond 10 h the ratio declines. It has to be concluded that a multistage kinetic process occurs during retrogradation. Stage 1 has been described[16,25] as a purely conformational ordering, possibly involving the formation of double helices in the amylopectin branches within a single polymer molecule. Stage 2 is the induction time for onset of helix aggregation and crystal growth. Stage 3 is described as the primary aggregation and crystallization step. This crystallization has been confirmed by the appearance of diffraction in X-ray diffractometry measurements for gels without glycerol and with 20% glycerol after 3 weeks of retrogradation (see *Figure 4*). The crystal structure is B-type for both gels. The addition of glycerol does not influence the type of crystal structure. The fourth step, during which the absorbance at 1000 cm^{-1} increases, involves phase separation of water (syneresis) (*Table 2*).

Table 2 Syneresis onset times^a

Sample	Time (h)
0% glycerol	288
1% glycerol	400
5% glycerol	500
20% glycerol	610

^{a} Defined as the onset time of increase in intensity of the absorbance at 1000 cm^{-1}

Kinetics

The rate of structural changes (kinetics) of the separate steps have been determined by analysis of the ratios 1153/1035, 1153/1053 and 1053/1035. In *Figure 5* the ratios 1153/1035 and 1053/1035 are plotted against time over the periods 0–5 h and 0–500 h for the waxy maize starch gel without glycerol.

The data for the multistage structural changes are treated as individual steps. Stage 1 can be described by first-order Avrami kinetics. The ratio 1153/1035 is the best representation of the fast changes occurring in this step (*Figure 5*). The ratio 1153/1053 gives similar results, but the deviation in the calculated kinetic parameters is higher. The calculated k values are summarized in *Table 3*. The plateau region in stage 2 can be characterized as the delay time between the end of stage 1 and the beginning of stage 3. For the first two stages no significant differences can be seen between the various waxy maize

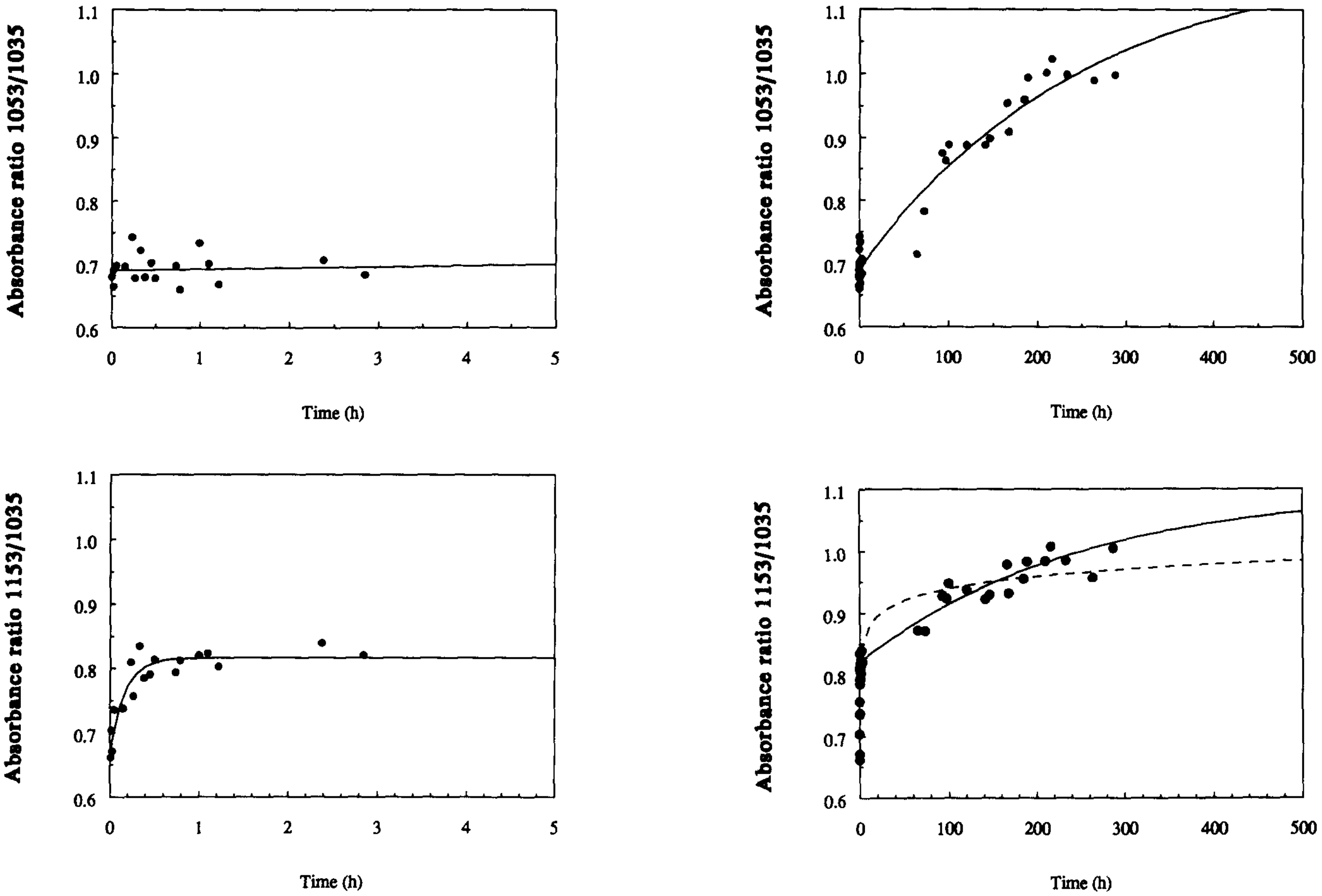

Figure 5 Spectral changes of waxy maize starch gels for the absorbance ratios 1053/1035 (top) and 1153/1035 (bottom) for the first stage (left) and for the overall process (right). (Bottom right) Avrami fit: (– – –) 0–500 h; (—) 5–500 h

Table 3 Kinetic parameters of waxy maize starch gels with various glycerol contents[a]

Absorbance ratio	Glycerol (%) (w/w)	A_∞	A_0	k (h^{-1})
1153/1053	0	1.18±0.01	0.99±0.02	4±1
	1	1.040±0.002	0.98±0.01	8±2
	5	1.055±0.004	1.08±0.02	4±8
	20	1.096±0.003	1.04±0.02	7±3
1153/1035	0	0.816±0.008	0.67±0.02	6±2
	1	0.780±0.002	0.61±0.02	7±1
	5	0.815±0.002	0.71±0.01	8±2
	20	0.876±0.005	0.66±0.04	10±3

[a] The parameters were calculated with an Avrami fit (0–5 h). The data were fitted with variables k, A_∞ and A_0

Table 4 Kinetic parameters of stage 3 depending on glycerol content[a]

Absorbance ratio	Glycerol (%) (w/w)	a_1 (slope) × 10^4 (h^{-1})	a_0 (intercept)
1153/1053	0	−7.0±0.5	1.137±0.009
	1	−3.8±0.2	1.094±0.001
	5	−1.6±0.3	1.072±0.009
	20	−1.4±0.5	1.22±0.01
1153/1035	0	5.22±0.03	0.864±0.001
	1	2.4±0.2	0.81±0.01
	5	2.0±0.2	0.78±0.01
	20	0.06±0.04	0.87±0.01
1053/1035	0	13.3±0.2	0.70±0.01
	1	6.3±0.2	0.72±0.01
	5	3.4±0.2	0.72±0.01
	20	1.3±0.5	0.72±0.05

[a] The parameters are calculated by a linear fit of the data

gels, probably due to the low time resolution which is obtained with a DTGS detector. In stage 3 the absorbance ratios 1153/1035, 1153/1053 and 1053/1035 change almost linearly over time and are characterized by zero-order kinetics. The slopes of the straight line regions yield the rate constants (*Table 4*). The final stage can be characterized by the onset time of syneresis, which becomes visible by a growth of the absorbance at 1000 cm^{-1}. These data show a retardation of the retrogradation rate by addition of glycerol.

The overall retrogradation kinetics of the first three stages, i.e. the total process of forming of helices, aggregation and crystallization, can be accurately described by a first-order Avrami fit for the ratio 1053/1035 (*Figure 5*), which is more or less a combination of the ratios 1153/1035 and 1153/1053. An apparent smooth change over time in the spectroscopic parameters is observed. The overall reaction rate constants k (Avrami fit) for the absorbance ratio 1053/1035 with fixed A_∞ and A_0 are given in *Table 5*. A_∞ and A_0 are derived for the 0% glycerol gel with an Avrami fit with free k, A_∞ and A_0.

The overall (first-order Avrami) reaction rate constant k as a function of the amount of glycerol clearly shows a reduction of the retrogradation rate of waxy maize starch in terms of short-range structural changes by the addition of glycerol (*Figure 6*). The inhibition of retro-

Table 5 Kinetic parameters of waxy maize starch gels with different glycerol contents[a]

Absorbance ratio	Glycerol (%) (w/w)	A_∞ (fixed)	A_0 (fixed)	$k \times 10^3$ (h^{-1})
1053/1035	0	1.18	0.69	4.1±0.1
	1	1.18	0.69	2.3±0.2
	5	1.18	0.69	1.1±0.1
	20	1.18	0.69	0.5±0.05

[a] The parameters are calculated with an Avrami fit over the period 0 h until syneresis of water occurred. The A values were taken from an Avrami fit with variables k, A_∞ and A_0 for the gel without glycerol

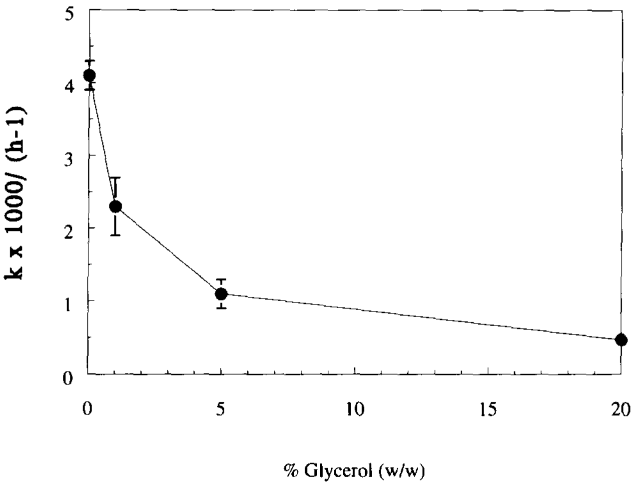

Figure 6 Dependence of the overall (first-order Avrami) reaction rate constant, k, as a function of glycerol content

gradation by polyhydroxy compounds can be explained in terms of stabilization of the amorphous and entangled starch gel matrix[33–39]. The structure of water is stabilized and the thermal motion of water is controlled by saccharides, resulting in a reduced flexibility of starch chains in the water–starch–sugar system. Hydrogen bonding plays an important role in this stabilization.

The antiplasticizing effect of glycerol relative to water alone is shown in raising the gelatinization temperature (T_{gel}) of native starches[40,41]. Analogous to the elevation of the T_{gel}, glycerol is presumed to raise the glass transition temperature (T_g) of the amylopectin gel matrix in a freshly made gel. This elevated T_g controls subsequent recrystallization of B-type starch in the undercooled rubbery gel[42,43]. For storage at $T > T_g$ there is sufficient mobility for devitrification and subsequent formation of crystalline regions, resulting in a partially crystalline polymer system which constitutes the retrograded starch gel. A higher T_g translates to a smaller ΔT above T_g which lowers the rate of propagation of starch retrogradation for storage at T. The antiplasticizing effect of glycerol relative to water can be explained by the Williams–Landel–Ferry free volume concept as used for intermediate moisture food materials[43]. Because of the higher molar mass of glycerol, less free volume is available to the starch polymer resulting in a higher T_g. The question can be posed as to whether this theoretical polymer approach can be applied to describe high moisture starch gel systems accurately. The antiplasticizing effect could be more influenced by the hydrogen-bonding effects which occur between the water, starch and glycerol molecules. However, it has been claimed for polymers capable of hydrogen bonding, that the free volume contribution to the crystallization mechanism and the extent of polymer–solute hydrogen bonding can be co-operative[43,44].

CONCLUSIONS

*FT*i.r. spectroscopy can be used to monitor the influence of glycerol on starch retrogradation for short-range ordering in the starch gel system, which is directly related to conformational changes at a molecular level. The retrogradation of waxy maize starch in a 10% (w/w) gel appears to be a multistage kinetic process. The calculated rate constants clearly show a decreasing rate of the retrogradation of waxy maize starch with increasing glycerol content.

The effects can be explained in terms of stabilization of water and a decreasing mobility of the starch chains in which hydrogen bonding plays an important role. Further studies on the relation between thermal and physicochemical properties will be necessary to give new information about starch mobility in relation to the T_g and, as a result, retrogradation.

REFERENCES

1 Miles, M. J., Morris, V. J., Orford, V. J. and Ring, S. G. *Carbohydrate Res.* 1985, **135**, 271
2 Miles, M. J., Morris, V. J. and Ring, S. G. *Carbohydrate Res.* 1985, **135**, 257
3 Ring, S. G., Colonna, P., I'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J. and Orford, P. D. *Carbohydrate Res.* 1987, **162**, 277
4 Zeleznak, K. J. and Hoseney, R. C. *Cereal Chem.* 1986, **63**, 407
5 Del Rosario, R. R. and Pontiveros, C. R. *Starch* 1983, **35**, 86
6 Kim, S. K. and D'Appolonia, B. L. *Cereal Chem.* 1977, **54**, 150
7 Ciacco, C. F. and Fernandes, J. L. A. *Starch* 1979, **31**, 51
8 Germani, R., Ciacco, C. F. and Rodriguez-Amaya, D. B. *Starch* 1983, **35**, 377
9 Doublier, J.-L. and Choplin, L. *Carbohydrate Res.* 1989, **193**, 215
10 Orford, P. D., Ring, S. G., Carroll, V., Miles, M. J. and Morris, V. J. *J. Sci. Food Agric.* 1987, **39**, 169
11 Cael, J. J., Koenig, J. L. and Blackwell, J. *Carbohydrate Res.* 1973, **29**, 123
12 Cael, J. J., Koenig, J. L. and Blackwell, J. *Biopolymers* 1975, **14**, 1885
13 Galat, A. *Acta Biochim. Polym.* 1980, **27**, 135
14 Wang, I. C. and Ting, C. H. *J. Chin. Chem. Soc. (Peking)* 1972, **19**, 63
15 Barrett, T. W. *Spectrochim. Acta A* 1981, **37**, 233
16 Bulkin, B. J., Kwak, Y. and Dea, I. C. M. *Carbohydrate Res.* 1987, **160**, 95
17 Gidley, M. J. and Bulpin, P. V. *Macromolecules* 1989, **22**, 341
18 Clark, A. H., Gidley, M. J., Richardson, R. K. and Ross-Murphy, S. B. *Macromolecules* 1989, **22**, 346
19 Gidley, M. J. *Macromolecules* 1989, **22**, 351
20 Wu, J. Y. and Eads, T. M. *Carbohydrate Polym.* 1993, **20**, 51
21 Teo, C. H. and Seow, C. C. *Starch* 1992, **44**, 288
22 Wilson, R. H., Kalichevski, M. T., Ring, S. G. and Belton, P. S. *Carbohydrate Res.* 1987, **166**, 162
23 Wilson, R. H., Goodfellow, B. J., Belton, P. S., Osborne, B. G., Oliver, G. and Russell, P. L. *J. Sci. Food Agric.* 1991, **54**, 471
24 Wilson, R. H. and Belton, P. S. *Carbohydrate Res.* 1988, **180**, 339
25 Goodfellow, B. J. and Wilson, R. H. *Biopolymers* 1990, **30**, 1183
26 van Soest, J. J. G. Report IOP-k: period 01-09-91/01-03-92, ATO-DLO, 1992, p. 15
27 van Soest, J. J. G. Report IOP-k: period 01-03-92/01-09-92, ATO-DLO, 1992, p. 1
28 Belton, P. S., Wilson, R. H. and Chenery, C. H. *Int. J. Biol. Macromol.* 1986, **8**, 247

29 Belton, P. S., Goodfellow, B. J. and Wilson, R. H. *Macromolecules* 1989, **22**, 1636

30 Tipson, R. S. and Parker, F. S. in 'The Carbohydrates' (Eds W. Pigman and D. Horton), Vol. IB, Academic Press, London, 1980, p. 87

31 Cameron, D. G. and Moffat, D. J. *J. Test. Eval.* 1984, **12**, 78

32 Avrami, M. *J. Chem. Phys.* 1939, **7**, 1603

33 Krüsi, H. and Neukom, H. *Starch* 1984, **36**, 300

34 Cairns, P., Miles, M. J. and Morris, V. J. *Carbohydrate Polym.* 1991, **16**, 355

35 I'Anson, K. J., Miles, M. J., Morris, V. J., Besford, L. S., Jarvis, D. A. and Marsh, R. A. *J. Cereal Sci.* 1990, **11**, 243

36 Cairns, P., I'Anson, K. J. and Morris, V. J. *Food Hydrocolloids* 1991, **5**, 151

37 Kohyama, K. and Nishinari, K. *J. Agric. Food Chem.* 1991, **39**, 1406

38 Katsuta, K., Nishimura, A. and Miura, M. *Food Hydrocolloids* 1992, **6**, 387

39 Katsuta, K., Nishimura, A. and Miura, M. *Food Hydrocolloids* 1992, **6**, 399

40 Evans, I. D. and Haisman, D. R. *Starch* 1982, **34**, 224

41 Slade, L. and Levine, H. in 'Industrial Polysaccharides' (Eds S. S. Stivala, V. Crezenzi and I. C. M. Dea), 1987, p. 387

42 Levine, H. and Slade, L. in 'Water Science Reviews', Vol. 3, 1988, p. 79

43 Moy, P. and Karasz, F. E. *Am. Chem. Soc Symp. Ser.* 1980, **127**, 505

44 Carfagna, C., Apicella, A. and Nicolais, L. *J. Appl. Polym. Sci.* 1982, **27**, 105