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Physical ageing of wheat flour-based confectionery wafers

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The effect of ageing wheat flour-based wafers below their glass transition temperature has been studied by DSC, X-ray and infrared methods. It is found that a new peak develops well below T_g during the ageing process, and the temperature of this peak depends on the storage conditions. Analysis of the time development of the enthalpy change and peak position associated with this new peak support the view that its origin lies in enthalpy relaxation. However, it appears from the X-ray and IR observations that a specific molecular arrangement is associated with the relaxation process; within the starch component the formation of small ordered single helical domains occurs. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

There has recently been a growth of interest in the behaviour of foods in the glassy state (see for instance Blanshard & Lillford, 1993). For many low moisture products it is now recognized that the glassy state plays a crucial role, e.g. Levine & Slade, 1988; Slade & Levine, 1993. However, it is not sufficient to assume that below the glass transition temperature, T_g, all molecular motions cease (Scandola *et al.*, 1991)

Studies into low (< 30%) moisture starch systems are not only applicable to the food industry with food systems such as confectionery wafers and pasta, but also to the pharmaceutical industry where starch may be used to enrobe pharmaceutical tablets. At present, there is also much interest in the use of starch as a biodegradable plastic (Shogren *et al.*, 1993). For both wheat starch and amylopectin, containing 25–30% moisture, a glass transition (T_g) at about 0°C has been observed by differential scanning calorimetry (DSC) (Zeleznak & Hoseney, 1987; Orford *et al.*, 1989; Kalichevsky *et al.*, 1992). The T_g for both materials was found to increase in temperature with decreasing water content, as would be expected for solvent plasticization, but at low moisture contents the T_g is rarely observed since the polysaccharide decomposes before the glass transition. Using a double reciprocal plot of T_g versus the degree of polymerization of D-glucose, and malto-oligomers from dimer to hexamer, an extrapolated T_g of 230±10°C was obtained for "dry" amylose and amylopectin (Orford *et al.*, 1989).

Of the studies carried out on low moisture starch systems, most, if not all, have involved a pre-treatment of heating in excess of 100°C (Gidley *et al.*, 1993). It is only recently that investigations into the thermal events that occur during the first heating process for a wide range of polysaccharides at low moisture levels have been undertaken (Kalichevsky *et al.*, 1992; Shogren, 1992; Appelqvist *et al.*, 1993; Gidley *et al.*, 1993; Lawton and Wu, 1993; Livings *et al.*, 1993; Yuan and Thompson, 1994). Common to these articles is the observation of the appearance of a peak at a temperature well below the glass transition temperature. This peak appears at around 60°C (the precise temperature depending on the ageing conditions), which is similar to that usually reported for amylopectin crystals during retrogradation in high moisture systems (Longton & Le Grys, 1981; Ring *et al.*, 1987; Kalichevsky *et al.*, 1990). However, the fact that this peak is below T_g for these low moisture systems rules out this explanation for the peak. Gidley

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et al. (Appelqvist *et al.*, 1993; Gidley *et al.*, 1993), who observed this peak in a range of carbohydrate systems, interpreted it as due to enthalpic associations between water and the carbohydrate. However, Shogren (Shogren, 1992) suggested the peak's origin lay in enthalpy relaxation, although without testing this hypothesis very far. Moreover, Lawton and Wu (Lawton and Wu, 1993) suggested that the enthalpic peak observed in wheat gluten glasses was due to enthalpy relaxation. An additional suggestion is due to Yuan and Thompson (Yuan and Thompson, 1994), who postulated that for waxy starch and β-limit dextrins the sub-T_g peak (at around 50°C) was due to water–hydroxyl group interactions, whereas the enthalpic peak superimposed on the glass transition in acetylated starch was due to enthalpy relaxation.

In an attempt to clarify the situation, in this paper we present data on the changes that occur during controlled ageing of wheat flour-based wafers below T_g, and relate these to the phenomena of enthalpy relaxation to test the validity of this suggestion for the origin of the peak. Most of the data reported here have been obtained by DSC, with an analysis of the shift in peak position and change in the enthalpy of the sub-T_g peak with annealing time being presented. Some results from infrared and X-ray diffraction are also presented, to focus in on any specific ordering that may be occurring in the glass. In a subsequent paper an extended DSC analysis of the ageing of amylopectin wafers in terms of different enthalpy relaxation models will be presented (Livings *et al.*, 1998).

EXPERIMENTAL

All experiments were carried out on a standard yeasted wafer formulation (see for example Almond, 1991). Most of the wafers selected for DSC analysis were initially aged for 18 months at room temperature in sealed polythene bags, followed by controlled annealing, within the DSC, either at 51°C, or at higher temperatures for 70 min. Additionally some wafers were aged for shorter periods of 4–6 weeks, under controlled conditions of humidity (40%, 70% or 75% R.H.). Differential scanning calorimetry was carried out on a Perkin Elmer DSC7 at a heating rate of 10°C min^{-1}, using indium as the calibrant.

X-ray diffraction experiments were carried out using both a CPS-120 Spectrolab Series 3000 and a Philips PW 1730 vertical diffractometer. For the X-ray experiments, 12 month-old and fresh yeasted wafers were compared, together with a 12 month-old wafer which had been heated up to 80°C and quickly cooled immediately prior to examination.

Fourier transform infrared (FTIR) spectroscopy was carried out using a 10 reflection zinc selenide Perkin Elmer ATR cell. The FTIR spectrometer was a Mattson 40-20 spectrometer with a DGTS detector. An aged wafer (aged for 1 year in a sealed polythene bag) was crushed using a pestle and mortar and tightly packed against the ATR cell. To remove water vapour from the sample chamber the spectrometer was well purged using dry nitrogen gas at a flow rate of 50 l min^{-1}. The ATR cell was fitted with a lid to prevent water evaporation during the heating cycle (heating up to 100°C, and subsequent cooling back to 30°C). Heat was supplied to the ATR cell via a base plate heating element connected to a temperature controller. Deconvolution of spectra was carried out using the Mattson deconvolution software program supplied with the spectrometer.

RESULTS

DSC data

Figure 1 shows how the DSC traces of the original wafer (aged for 18 months at room temperature within a sealed polythene bag) change with annealing time at 51°C. The peak present at ~56°C following the room temperature ageing treatment essentially disappears after a short time at 51°C, and then grows in magnitude and shifts to higher temperatures as annealing at 51°C continues. Using the Perkin-Elmer software the enthalpy of the peak in the vicinity of 60°C, and the precise temperature of this peak were calculated as a function of annealing time. These two quantities are plotted as a function of annealing time in Figs 2 and 3 respectively. It can be seen that both are linearly dependent on log time. It should be noted that the enthalpy was calculated by extrapolating a line from below to above the transition. For sub-T_g peaks – as is the case here – this method probably gives enthalpy values similar to the well described method of Richardson & Savill, 1977.

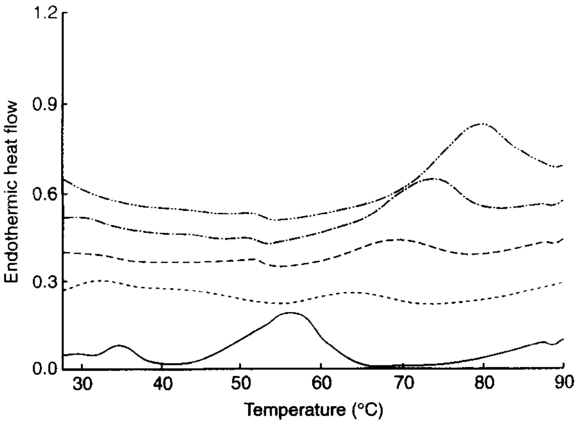

Fig. 1. DSC traces showing the effect of time of annealing at 51°C on a wafer originally stored for 18 months at room temperature (— 0 min, - - - 14 mins, – – – 70 mins, –·–·– 320 mins, ·········· 3900 mins).

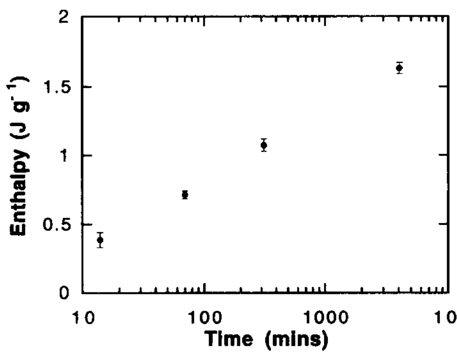

Fig. 2. The development of enthalpy associated with the sub-T_g peak with annealing time at 51°C for a wafer originally stored for 18 months at room temperature.

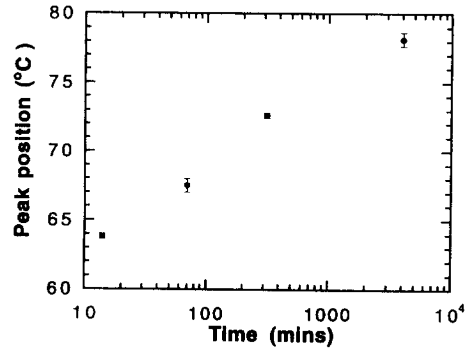

Fig. 3. Change in the peak position associated with the sub-T_g peak with annealing time at 51°C for a wafer originally stored for 18 months at room temperature.

It is also possible to age the wafers for a constant time but at varying temperature. Data on the peak position as a function of annealing temperature is shown in Fig. 4. There is a linear relationship between peak temperature and annealing temperature. Finally, the effect of scan rate on the transition enthalpy was explored, and the results for a wafer aged at 25°C for one month at 70% relative humidity are shown in Fig. 5.

FTIR and WAXS data

Only limited FTIR and WAXS data have been obtained, to help elucidate the underlying molecular ordering changes that are associated with the growth of the sub T_g peak measured by DSC. Figure 6 shows diffractometer traces of a wafer aged for one year before and immediately after heating to 80°C and cooling (Livings *et al.*, 1993). For the well-aged sample, superimposed on the broad amorphous halo typical of gelatinized starch products is a small but well-defined peak at a 2Θ angle of about 20° in the aged wafer. This peak disappears after heating through the temperature of the endotherm. As a comparison, data from a freshly prepared wafer is also shown. This only shows the amorphous halo.

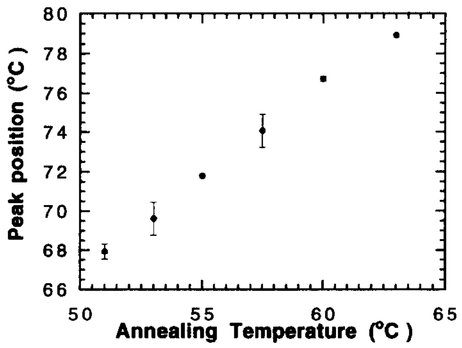

Fig. 4. Peak position as a function of annealing temperature for a set time of 70 minutes for a wafer originally stored for 18 months at room temperature.

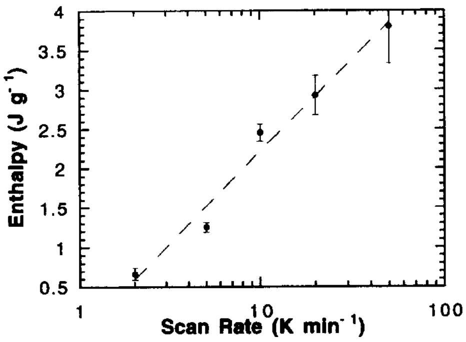

Fig. 5. Effect of scan rate on the enthalpy of transition for a wafer aged at 70% relative humidity at room temperature for one month.

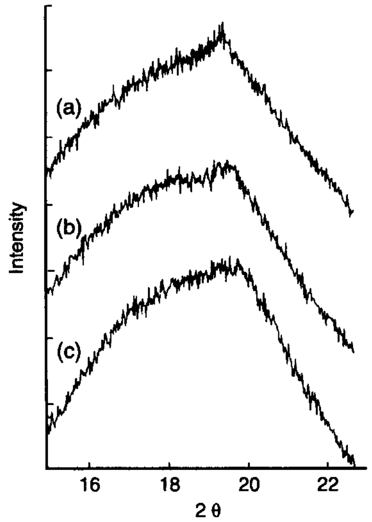

Fig. 6. X-ray diffractograms of wafers: (a) aged for 1 year at room temperature; (b) wafer as in (a) but then heated to 80°C and then cooled; (c) a freshly prepared wafer.

The position of this 20° peak is characteristic of a six-fold single helix structure (V_6) (Gidley & Bociek, 1988). Figure 7 shows the X-ray spectra of wafers conditioned at 75% and 40% relative humidity at 25°C for 6 weeks. Again a peak at 20° is visible on the sample with the

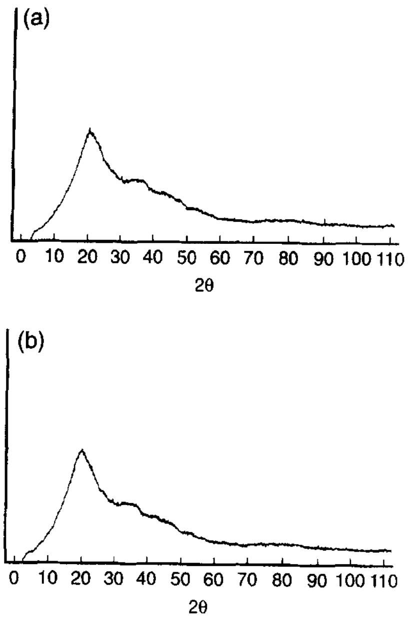

Fig. 7. X-ray diffractograms of wafers conditioned at (a) 75% and (b) 40% relative humidity at 25°C for 6 weeks.

higher moisture level; for the 40% sample the peak is hardly discernible. DSC traces of the same wafers are shown in Fig. 8, from which it can be seen that the wafer stored at the higher moisture levels shows a greater enthalpy (2.22 as compared with 0.10 $J g^{-1}$) although the peak position remains approximately the same.

FTIR has also been used to investigate chain conformation in the wafers. Figure 9 shows how the deconvoluted spectrum of an aged wafer changes as the temperature is increased. From the deconvoluted spectra the intensities of the peaks at 992 and 1018 cm^{-1} as well as the trough at 1006 cm^{-1} were recorded. The peak intensity was then taken as the intensity of the peak minus the intensity of the trough. This procedure was carried out to minimize the effect of baseline movement during the course of the experiment. The results were then plotted to show up any phase transition undergone during heating. Figure 10 shows how the ratio of peak intensities of the two peaks at 992 and 1018 cm^{-1} in the spectrum of a room-temperature aged wafer change with temperature (Livings *et al.*, 1993). These peaks are due to the starch entity within the wafer and have been attributed to highly coupled C–O and C–C stretching modes of the polysaccharide backbone (Wilson & Belton, 1988). Although no obvious discontinuities are apparent on heating the sample to 100°C, on cooling the sample to 30°C and rescanning, the peak intensity ratio remains approximately at the level of the sample at 100°C. A possible reason for the rather broad transition observed in the infrared is that the sample is being annealed as it is run. During an infrared run the sample is held at a particular temperature for approximately 15 minutes between successive scans.

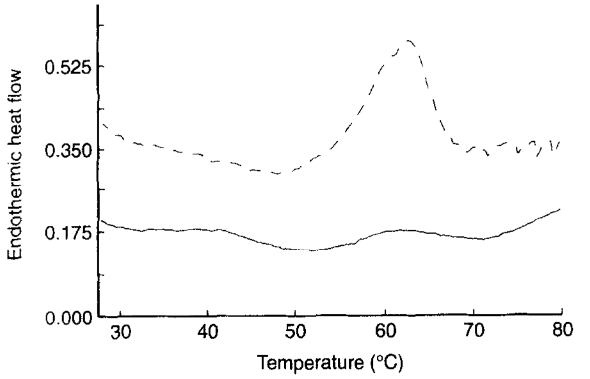

Fig. 8. DSC traces for the same samples as in Figure 6 (—— 40% RH, – – – 75% RH).

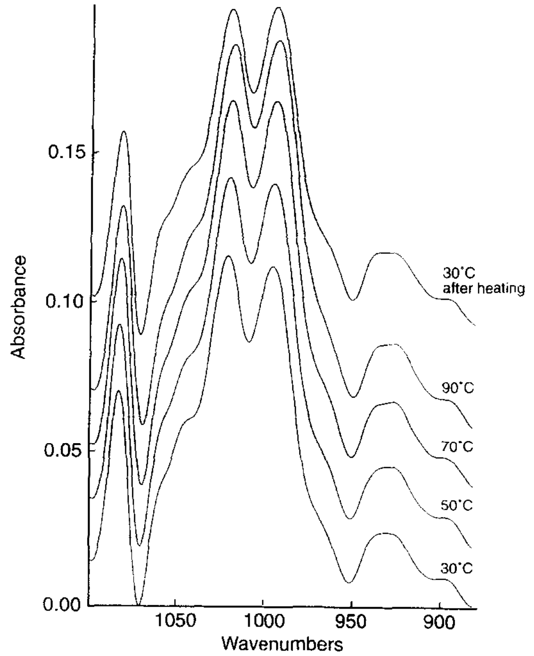

Fig. 9. Deconvoluted FTIR-ATR spectra of a wafer originally stored for 1 year at room temperature and then heated to 100°C. The spectra were taken at different temperatures during the heating programme. The top spectrum is of the wafer immediately after recooling to 30°C.

DISCUSSION

Before discussing the results presented here in detail, it is necessary to make some comments about the nature of the glassy state and the phenomenon of enthalpy relaxation. When a glass forming liquid is cooled, one of two events may occur: either the liquid crystallizes or it supercools to form a glass. As the liquid is supercooled, the volume of the sample decreases and the time for molecular relaxation increases. Eventually

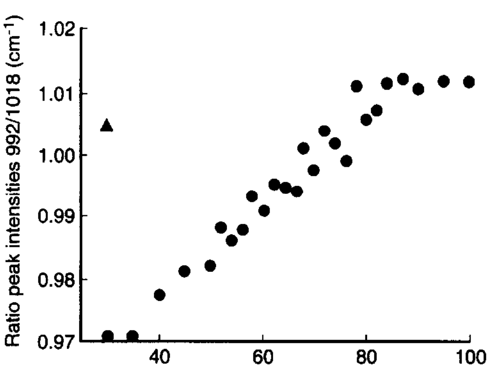

Fig. 10. The ratio of the MR peak intensity at 992 to 1018 cm^{-1} as a function of temperature (●), derived from Figure 8. (▲) indicates the equivalent data point for the sample recooled to 30°C.

the molecules within the sample cannot respond within the timeframe of the cooling, and the structure is essentially frozen in; the temperature at which this happens is known as the glass transition temperature. If the sample is cooled at a slow rate, since the molecules have more time to relax, a lower T_g is recorded. In practice, a glass can never achieve an equilibrium state; it is inherently a non-equilibrium structure. Consequently, when a glass is stored below its T_g it will relax, through local motions, towards a more nearly equilibrium form. This is what underlies the process of volume and enthalpy relaxation, and will occur for all glasses (Struik, 1980).

Through volume relaxation on ageing the glass densifies. As a consequence, on reheating the sample responds sluggishly to the externally applied heating rate. Eventually there is sufficient thermal energy for the volume to relax back to the equilibrium rubbery line, and a fairly abrupt rise in volume results. Using DSC this jump is seen to correspond to an endothermic peak. For short annealing times in the glass, this peak appears at a lower temperature than the T_g. At long annealing times, this sub-T_g peak eventually becomes the characteristic enthalpy relaxation overshoot. Hodge and co-workers, detailed the changes that took place in DSC traces (Hodge & Berens, 1981; Hodge & Berens, 1982; Hodge & Huvard, 1983) for various synthetic polymers. They state the following features for the sub-T_g peak as being indicative of enthalpy relaxation: peak position increases approximately linearly with log annealing time; the enthalpy increases approximately linearly with log annealing time; at annealing temperatures well below T_g (by 30°C or more) the peak temperature is calculated to increase almost linearly with annealing time.

These features can now be compared with the data presented in the results section. Figure 1 shows qualitatively that the sub-T_g peak grows with annealing time when the wafer is held at 51°C (after the structure present in the wafer due to storage at room temperature, and which gives rise to a transition peak at ~56°C has been lost due to raising the temperature to 51°C). Quantitatively Figs 2 and 3 show that there is a logarithmic time dependence of both the peak position and enthalpy change. Figure 4 demonstrates that the peak temperature is linearly dependent on the temperature of annealing.

It is interesting to note that in Fig. 3 the sample appears to de-age, i.e. there is a decrease in the enthalpy loss as the sample changes from its well-aged state (18 months at room temperature) due to annealing for 14 min at 51°C. This type of behavior is well known in the enthalpy relaxation literature, and is termed either the memory or cross-over effect. The memory effect is best described in terms of volume relaxation, which is equivalent to enthalpy relaxation, and necessitates the assumption of a distribution of relaxation times (Adachi & Kotaka, 1982; Hofer *et al.*, 1991). At the annealing temperature T_l the sample's short relaxation time components relax and the sample's volume decreases from its initial value *A*, towards its equilibrium metastable position *B*. If the sample temperature is then rapidly increased to a temperature $T_l(2)$ the sample volume is found to be below its metastable equilibrium position (*E*) for that annealing temperature. To approach the equilibrium position (*E*) at temperature $T_l(2)$ the sample's short relaxation time components relax and the sample's volume increases. Once these short relaxation processes have occurred the components with longer relaxation times will start to relax toward the equilibrium position. In the aged wafer, on raising the temperature to 51°C all the previous ageing is eradicated, since the sample's volume is below that of the equilibrium position. After 14 minutes at 51°C it is clear from the peak in the DSC that the longer relaxation components have started to relax. It is noteworthy that a very similar trend has been observed for PVC(Struik, 1980). As far as the authors are aware this is the first time that the memory effect has been observed for a biopolymer based system. Its observation gives further credence to the fact that the sub-T_g peak is due to enthalpy relaxation. Furthermore, this result shows that the annealing temperature does not have to be higher than the T_g to eradicate ageing.

Figure 5 shows the effect of scan rate on the enthalpy of the transition. Extrapolation of the least squares fit to the data intersects the *x* axis at ~0, i.e. at zero scan rate no endothermic transition is observed. Qualitatively, Lawton and Wu (1993) have shown the same phenomenon for wheat gluten, and Appelqvist *et al.*, 1993 have suggested that at decreasing thermal scan rates a point would be reached where endotherm annealing occurs continuously during a scan so that it cannot seen. This is consistent with arguments based on enthalpy relaxation: as the sample is scanned at a very slow rate, it has sufficient time to relax to its equilibrium position and hence no transition is observed. A short précis and critique is now given on these earlier works.

These results are, therefore, all in accord with the known manifestations of enthalpy relaxation, and represent an extension of the previous work on acetic acid soluble wheat gluten(Lawton and Wu, 1993) and corn starch (Shogren, 1992). However, other work on low moisture biopolymers have turned to mechanisms, other than enthalpy relaxation, to explain the growth of the sub-T_g peak.

Gidley and coworkers (Appelqvist *et al.*, 1993; Gidley *et al.*, 1993) also carried out DSC studies on a range of range of polysaccharides at moisture levels between 5–25%. For all the samples studied, when the sample was stored at room temperature, an endothermic peak in the range 50–70°C was observed. The DSC peaks were relatively narrow, which the authors suggested indicated a high degree of cooperativity. The transition enthalpy increased with increasing moisture content, although the melting temperature did not vary systematically with water content. On immediate thermal rescanning, the endotherm disappeared; however the peak did gradually reappear following storage. For maltoheptose following its reappearance, the peak was shown to exhibit increasing enthalpy and melting temperature with increasing storage time. The T_g observed for maltoheptose was always higher than the endothermic event and was independent of storage time (Gidley *et al.*, 1993).

It was concluded from the above observations that the exothermic event was due to enthalpic associations between water and carbohydrates. It has been seen that such associations are not permanent for solutions, where lifetimes are of the order of picoseconds (Brady & Ha, 1991). However, Gidley *et al.*, 1993 suggest that in high concentrations of carbohydrates there will be an increase in the local microviscosity. This leads to the lifetimes of the associations being sufficiently long to be detected by physical techniques, e.g. DSC.

Gidley *et al.* (1993) also showed that the observed endotherm occurred irrespective of whether a glass transition is observed for the system: agar, kappa carrageenan and native (granular) starches do not show glass transition behavior, whereas pullulan and dextran do. This difference in behavior of the polysaccharides was attributed to the geometry of the glycosidic bond. This leads to a diversity of "immobilization" mechanisms available to polysaccharides, of which glass formation is only one.

Gidley *et al.* (1993) seem to have excluded enthalpy relaxation as the mechanism underlying the growth of the sub-T_g peak because the peak position aged at different moisture contents did not appear to follow any systematic trends. However, as mentioned by Hutchinson and Ruddy (1988) and will be documented more fully in a subsequent paper Livings *et al.* (1998), the peak position is dependent on both the thermal history of the sample and the sample composition. Appelqvist *et al.* (1993) state that the thermal history of the samples (both temperature and time) were not well controlled, and thus enthalpy relaxation remains a possible mechanism based on their data.

Appelqvist *et al.* (1993) also showed that for a range of starches, dextrans and pullulan at the same moisture contents (and similar T_g values) endotherm enthalpy values differed significantly. They commented that one would expect that these would have the same value if enthalpy relaxation were invoked. However. this is a misconception of glass transition theory. The theory states that a glass cannot be fully characterized by only one parameter, i.e. T_g, and other parameters in the Tool–Narayanaswamy theory – a theory that describes the rate of enthalpy relaxation – (Tool, 1946; Gardon & Narayanaswamy, 1970; Narayanaswamy, 1971) need not be the same for all the polysaccharides. Thus, it is an incorrect supposition that the rate of evolution of the enthalpy for various polysaccharides, having the same T_g, need be the same.

As an extension of the Gidley model, Yuan and Thompson (1994) suggest that the sub-T_g peak is due to water–hydroxyl group interactions whereas enthalpic overshoot was due to enthalpy relaxation. As with the Gidley model there is no reason advocated why enthalpy relaxation does not occur in all systems. As discussed previously, the thermodynamic instability of a glass is an inherent feature. In neither model is a reason given why biopolymer glasses should be treated differently to synthetic polymers. If we attempt to interpret the Yuan and Thompson data all within the framework of enthalpy relaxation, there is no fundamental inconsistency. The role of water, which turns out to be so crucial when aged samples are subsequently dried out over P_2O_5, can be considered solely as a kinetic factor without needing to invoke any specific interactions.

The peak due to enthalpy relaxation will manifest itself when there is sufficient mobility for the chains to move towards the equilibrium state. These relaxation processes will be impeded by drying. Thus in effect the spectrum of relaxation times will be altered after the initial ageing treatment due to the P_2O_5 drying, and it is not surprising there is a change in the response seen in the DSC. Further evidence to support this picture comes from examining both the original model calculations of Berens and Hodge (1982) and the data on cornstarch of Shogren (1992). Hodge and Berens showed that for a fixed T_e, the T_{max} increased as T_g was raised – in this case corresponding to an decrease in moisture content. The data of Shogren confirms this, with T_{max} being higher at low moisture contents (although the range of moistures examined was quite modest). Since T_g rises dramatically with a reduction in moisture, it is possible that the temperatures accessed in the DSC experiments of Yuan and Thompson did not reach the appropriate T_{max}.

A similar transition in DSC has also been observed in breakfast cereals (Sauvegot & Blond, 1991). In this work it was speculated that the transition could be due to either starch retrogradation or protein denaturation. Protein denaturation as a cause of the transition can be dismissed since, as has been observed by Kalichevsky *et al.* (1992) and ourselves, amylopectin which contains no protein also shows the same transition. The appearance of an endotherm at ~65°C is normally indicative of starch retrogradation in high moisture systems, but the melting point would be expected to be much higher at the low moisture content of the wafers. This is because of the plasticizing effect of the water (Whittam *et al.*, 1990). The WAXS data on the aged wafers presented here also show no evidence of the B-type structure which is associated with retrograded starch.

The DSC data presented in this paper strongly suggest that enthalpy relaxation is taking place during sub-T_g storage, and it is this process which is giving rise to the growth of the peak at around 60°C. The WAXS and IR evidence suggest that for this system the rearrangements that are occurring during the relaxation may be rather specific, but there is no need for this to be a general phenomenon. WAXS diffractometer traces of a wafer aged for one year at room temperature show superimposed on the broad amorphous halo typical of gelatinized starch products a small but well-defined peak at a 2Θ angle of about 20° in the aged wafer (Fig. 6). This peak disappears after heating through the temperature of the endotherm, when the trace resembles that of a wafer freshly prepared which only shows the amorphous halo. The peak seen in the WAXS trace can therefore be linked to the effects of ageing studied via DSC.

The position of this small peak at 20° is characteristic of a six-fold single helix structure (V_6) (Gidley & Bociek, 1988). Using the Bragg formula, this 2Θ angle corresponds to a d-spacing of 4.43Å. This corresponds to the 310 reflection for V-type amylose (Rappenecker & Zugenmaier, 1981). This evidence suggests that on ageing below the glass transition, small domains with helical ordering form. These domains must have little crystallographic register since only one WAXS peak is observed. Usually the 20° peak is seen when amylose complexes with straight-chain aliphatic lipids or when there are organic inclusion complexes i.e. when aqueous solution of amylose is precipitated out of solution by ethanol (Gidley & Bociek, 1988). In this case the helix formation could possibly involve the amylopectin chains rather than the amylose, and be of only short length (for amylopectin determined by the branch length). Whether the helices formed on ageing contain lipid or not is unclear. In an attempt to clarify this point, WAXS was attempted on amylopectin films obtained from a 5% amylopectin/DMSO solution (in which no lipid was present). However, this proved unsuccessful because of the difficulty of placing sufficient film in the X-ray beam. The standard type of V-type amylose–lipid complexes, with substantial long range order, can be eliminated as the source of the DSC peak at around 60°C, since these are known to melt at the much higher temperature of 95–130°C when in an excess of water, i.e. 50% w/w (Biliaderis, 1992). Shogren (1992) has shown that for corn starch, the V-type amylose–lipid complex is plasticized by water and at 10% moisture it melts at ~230°C. Thus, the melting of these amylose–lipid complexes is not a viable proposition for the transition observed in the DSC, as it would occur at a far higher temperature.

In a recent review Slade and Levine (Slade & Levine, 1994) suggest that although the overall moisture level gives a suspected T_g which is well above room temperature, there may be certain domains where the moisture level is sufficient to plasticize the starch to below room temperature. Indeed, Larsson and Eliasson (1991) have shown that at intermediate water levels annealing of starch can occur at temperatures below T_g. They indicated that this may be due to uneven distribution of the water. However, if Slade and Levine's (Slade & Levine, 1994) suggestion is correct it would be thought that any reordering process above T_g would result in the formation of the B-type structure. Zeleznak and Hoseney (Zeleznak & Hoseney, 1987) have shown that at 22% moisture the T_g of starch is at room temperature. Longton and LeGrys (Longton & Le Grys, 1981) showed that at moisture levels between 20–80% starch retrograded into the B-type structure. Since there is no B-type structure evident from the X-ray spectra of aged wafers Slade and Levine's suggestion seems not to be valid in this case.

Figures 7 and 8 compare the X-ray spectra of wafers conditioned at 75% and 40% (R.H.) at 25°C for 6 weeks with their DSC traces. The wafer stored at the higher moisture levels shows both a greater enthalpy (2.22 $J g^{-1}$ compared with 0.10) and a much clearer signature of the X-ray peak associated with the V_h structure. This corroborates the view that the ordering associated with the growth of the sub-T_g DSC peak is associated with the formation of regions with helical packing. In a related vein, Nicholls *et al.*, 1995 note that their wheat starch sheets showed very little crystallinity when freshly prepared, whereas after one month's storage at 20°C crystallinity was more evident. Furthermore, the extent of crystallinity increased with increasing relative humidity at which the sample had been stored. Although, Nicholls *et al.*, 1995 do not comment on the nature of the crystallinity the similarities between the two observations are apparent.

Turning now to the FTIR evidence, Figs 9 and 10 show that changes to the spectrum have occurred during storage. Heating above the temperature of the sub-T_g transition causes a gradual change in the spectrum and the subsequent room temperature spectrum is now comparable with the high temperature spectrum. Although this spectral change cannot be immediately identified with any specific type of ordering, it does suggest that specific conformational changes are occurring, at least to some limited extent, during storage and these changes are eradicated by heating up beyond the temperature at which DSC shows the new peak appearing. It is interesting to note that Gidley *et al.*, 1993 failed to detect changes in FTIR spectra of various polysaccharides during heating, but their experimental set-up was unable to prevent moisture loss during the heating run, whereas the cell in the experiments described here was fitted with a lid to minimize evaporation.

Based on the WAXS and DSC behavior, the mechanism that we are hypothesizing is that below T_g starch undergoes an ordering process to form (single) helical structures, but without the aggregation of these to give crystals. The mechanism by which the chain segments move is that of enthalpy (volume) relaxation. This requires molecular movement below the glass transition but not diffusion. Beta transition chain motions have been observed for polysaccharides below T_g using techniques such as DETA and DMTA (Scandola *et al.*, 1991). Whether in wafers the helices formed do, or do not, contain lipid is uncertain.

In addition to the WAXS data for aged wafer, further support for the formation of helical domains in aged starch samples has been supplied by Gidley *et al.* (1993). These workers, using high-resolution solid state ^{13}C NMR (cross-polarization magic-angle spinning), have noted the appearance of the V-type structure in starch systems conditioned to various moisture levels in the region of the ^{13}C spectrum at a chemical shift value of approximately 100 ppm; this is due to the carbon at the 1 position for starch. The major determinant in its structural features is due to the geometry of the glycosidic linkage (Gidley & Bociek, 1988). By comparing the spectra of amorphous and V-type starch with spectra from starch conditioned at various moisture levels, it was seen that as the moisture level is increased the preponderance of the V-type conformation also increases. However, it should be noted that Shogren (1992) did not see differences in NMR data for aged and unaged starch samples.

Gidley et al. (Gidley & Bociek, 1988) concluded from the NMR data that $\alpha(1\text{–}4)$ glucans have greater linkage flexibility than that of β-(1–4) glucans. However, what seems to have been neglected was the time the samples were left to age. Appelqvist et al. (Appelqvist *et al.*, 1993) state that moisture contents for DMTA bars were manipulated by storing over supersaturated salt solutions for at least one month. Gidley et al. (Gidley & Bociek, 1988) do not mention how long the NMR samples were allowed to equilibrate over the salt solutions before the experiment was run. However it seems reasonable to assume a similar timescale. For this period of time even storing at 20°C will result in significant ageing at the higher moisture levels.

The NMR evidence from Gidley's group is therefore in agreement with our conclusions that specific rearrangements occur to form single helices of V_h type starch. Our premise is that these rearrangements occur as a result of enthalpy relaxation, and the results from DSC are all in agreement with this picture.

CONCLUSION

When wheat flour wafers are annealed at temperatures below T_g, so that conventional retrogradation and crystal formation cannot occur, a new peak appears below T_g. DSC evidence suggests that this peak is associated with enthalpy relaxation. It grows in magnitude with the logarithm of the annealing time, as does the peak position. Annealing at different temperatures shows a linear dependence of the peak position with the annealing temperature. These observations are all in accord with the known manifestations of enthalpy relaxation in synthetic polymers. For this particular system, X-ray and FTIR evidence suggests that the rearrangements that are occurring during this enthalpy relaxation are rather specific, leading to the formation of single helices in the V_h configuration.

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