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A study of the effect of water on the glass transition of 1:1 mixtures of amylopectin, casein and gluten using DSC and DMTA

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The glass transition temperature (T_g) and mechanical properties of binary mixtures of the food biopolymers amylopectin, casein and gluten have been studied in the ratio 1:1 in the presence of water. In general these polymers appear to be immiscible, showing two glass transitions due to the two polymers when there is sufficient difference between the T_gs of the two components. Increasing the water content reduces the T_g of both components.

INTRODUCTION

In amorphous polymers the glass transition temperature (T_g) is the single most important parameter in understanding mechanical properties. Recently the importance of the glass transition in understanding the mechanical and storage properties of foods has begun to be appreciated (e.g. Levine & Slade, 1988). The glass transition properties of various food polymers have been studied as a function of water content (Hoseney *et al.*, 1986; Zeleznak & Hoseney, 1987; Cocero & Kokini, 1991; Kalichevsky *et al.*, 1992*a*). The effects of low molecular weight additives such as sugars, emulsifiers and fats (Kalichevsky & Blanshard, 1992; Kalichevsky *et al.*, 1992*b, c, d*) on this transition have also been investigated. However real food systems also often consist of polymer mixtures (such as gluten and starch in baked products). Thus it is of interest to study the T_g of biopolymer mixtures.

Studies of various synthetic polymer mixtures have shown that miscibility is the exception rather than the rule (Olabisi *et al.*, 1979). The generally quoted reason for this is that for compatibility the Gibbs free energy of mixing (ΔG_m) must be negative; this implies that there must be a negative enthalpy of mixing (ΔH_m) or an increase in entropy (ΔS_m) on mixing (since $\Delta G_m = H_m - T\,\Delta S_m$). In the case of polymers, due to their high molecular weight there is very little change in entropy on mixing, so that any unfavourable interactions between the polymers readily result in phase separation.

For glassy polymers, mixing must occur above T_g and the resulting cooled glass is a non-equilibrium system, the phase state of which will depend on the sample history and will not reflect the miscibility at the measurement temperature (Wetton *et al.*, 1978; Olabisi *et al.*, 1979). In the case of mixing biopolymers an exothermic heat of mixing may be obtained if hydrogen bonds are formed between the different polymers, but if self associations are reduced on mixing, this may result in a positive ΔH_m (Painter *et al.*, 1991). In this study mixtures of two biopolymers were studied in the presence of water. This further complicates the system. In dilute solutions, maximum miscibility is obtained when both polymers have a similar affinity for the solvent. If polymer self-associations are not too strong, however, the presence of a solvent can enhance miscibility by diluting unfavourable polymer interactions (Olabisi *et al.*, 1979). In this case the water contents are relatively low and the water is not expected to have much effect on polymer miscibility.

Incompatibility of food biopolymers has been observed at higher water contents (at about 90% water) in food gels including phase separation of dextran with amylose (Kalichevsky *et al.*, 1986), gelatin with agar (Clark *et al.*, 1983) and even the very similar starch polymers amylose and amylopectin (Kalichevsky & Ring, 1987). It is quite likely, therefore, that at low water contents, biopolymer mixtures will be incompatible. In general, miscible polymer mixtures have a single T_g between the T_gs of the two components and broadening of the transition is observed as the components become more immiscible. In contrast, an incompatible system

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will have two T_gs corresponding to the two components, but if the component T_gs are less than 20°C apart, it is not possible to distinguish a phase separated system from a miscible one by this method (Olabisi *et al.*, 1979). In the case of strong interactions between the polymers, there may be an increase in T_g above that of either component, reaching a maximum at a particular composition (Olabisi *et al.*, 1979; Fahrenholtz & Kwei, 1981; de Meftahi & Frechet, 1988). Miscible systems should appear clear (by eye), although where phase separation does occur, if phase sizes are small, it may only be detected by light or X-ray scattering (Wetton *et al.*, 1978). (Visual clarity implies a phase size smaller than *c.* 100 nm, whereas a domain size of more than *c.* 15 nm is required for two transitions to be observed mechanically (Kaplan, 1976). At smaller domain sizes (*c.* 5 nm) the properties would be dominated by interfacial material (Wetton *et al.*, 1978)).

The behaviour of amylopectin-gluten, gluten-casein and amylopectin-casein two component mixtures (1:1) in the presence of water, are studied in this paper, using DSC (differential scanning calorimetry) and DMTA (dynamic mechanical thermal analysis). The behaviour of each of these components (in the presence of water) has been studied separately previously (Kalichevsky & Blanshard, 1992; Kalichevsky *et al.*, 1992*a*, *c*).

EXPERIMENTAL

Sample preparation

Pregelatinised waxy maize starch was supplied by National Starch and Chemical Corporation, Manchester; rennet casein (30 mesh) was supplied by Kerry Milk Products Ltd, (Uxbridge, Middlesex) and gluten (Stadis brand) was supplied by RHM. Samples were prepared by pressing to a thickness of 1-2 mm in a mould under pressure of *c.* 3·1 × 10^3 Pa. Water was added by grinding the polymers with ice in liquid nitrogen, as described previously (Kalichevsky *et al.*, 1992*a*). The water content was adjusted, by this method, to allow the samples to be pressed well above the T_g of both components, without heating above 60-70°C. Samples were then cut into bars (*c.* 8 × 20 mm) and stored over salt solutions of various relative humidities to obtain a variety of water contents. Samples were stored for at least a week before measurements were made. Water contents were obtained by drying to constant weight at 105°C.

Instrumentation

Differential Scanning Calorimetry (DSC)

Calorimetric measurements were carried out using a Perkin-Elmer DSC-2B calorimeter. The glass transition was determined from the midpoint of the heat capacity change observed at a heating rate of 10°C/min. If possible, the T_g was determined from the second heating scan after cooling at 10°C/min, in order to eliminate previous differences in the thermal treatment of samples.

DMTA measurements

The Polymer Laboratories dynamic mechanical thermal analyser (DMTA) Mark I was used in the single cantilever bending mode at a frequency of 1 Hz and strain × 1 (corresponding to a nominal peak to peak displacement of 16 μm). Samples were heated at 2°C/min. Sample dimensions were typically 1·5 × 8 × 16 mm. As described previously (Kalichevsky *et al.*, 1992*a*), the glass transition was defined as that region between the onset of the fall in elastic modulus ($\Delta E'$ slope) and the peak in tan δ ($=E''/E'$), which occurs 20-50°C higher for the biopolymers used in this study. The midpoint of the DSC transition generally falls between these two temperatures. The drop in $\log E'$ was obtained from the intersection of the extrapolated glassy E' and an extrapolation of the inflection point in the modulus drop.

RESULTS AND DISCUSSION

Gluten + amylopectin

Previous studies have shown that the glass transition temperature (T_g) of gluten (Kalichevsky *et al.*, 1992*c*) is about 30-60°C lower than that of amylopectin (Kalichevsky *et al.*, 1992*a*). This difference increases with decreasing water content. This is thus a good system for investigating miscibility, as immiscibility will result in two clearly resolved peaks.

DSC clearly showed two transitions for most of the samples on the first scan. On rescanning, the two wettest samples showed only one transition, whereas the others showed two. On the first run large relaxation peaks were generally observed, whereas on the second run the transitions were less clear and broader. This can be seen in Fig. 1, where the first and second DSC runs for a sample stored at 75% RH (13·3% aq.) are plotted. The DSC T_gs obtained from the second run are plotted against water content in Fig. 2. The additional curves represent the T_g (by DSC) for amylopectin and gluten as determined in the previous papers (Kalichevsky *et al.*, 1992*a*, *c*). It appears that the upper DSC transition arises from amylopectin or an amylopectin-rich phase, whereas the lower transition arises from the gluten-rich phase. The upper transition is quite close to, or a little lower than, the transition for amylopectin alone, whereas the lower transition is close to that for gluten alone at low water contents, becoming higher as the water content is increased. This suggests greater miscibility at higher water contents, although it may also be partly due to the T_gs of the two components

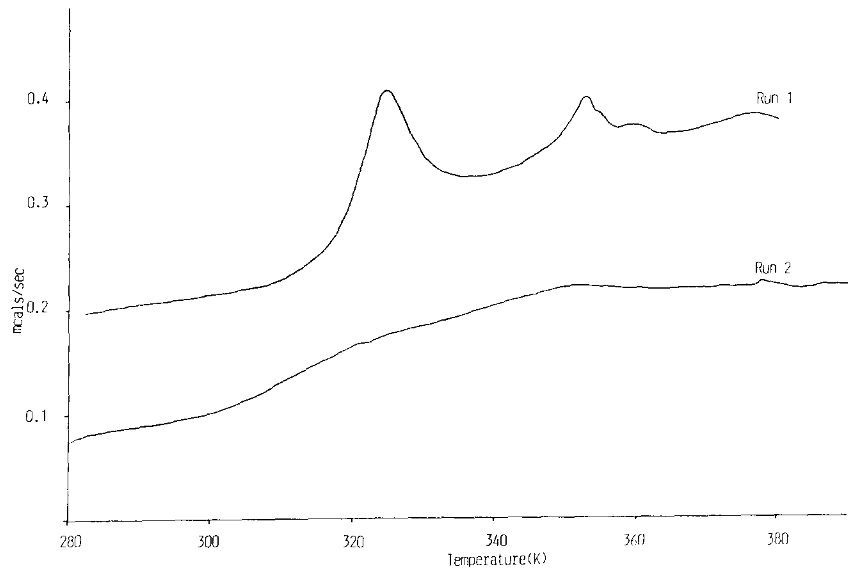

Fig. 1. DSC scans for gluten + amylopectin 1:1 stored at 75% RH (13·3% w/w aq.). (Run 2 after cooling at 10 K/min.)

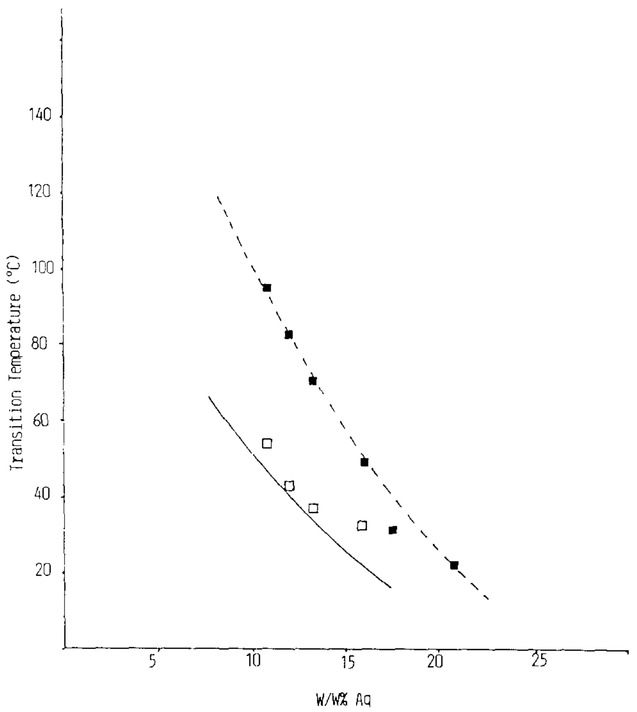

Fig. 2. Summary of T_g by DSC (midpoint, run 2) of gluten + amylopectin (1:1) as a function of water content. — — —, position of DSC T_g for amylopectin alone; ———, position of DSC T_g for gluten alone.

coming closer together. These results suggest that these biopolymers may be miscible under certain conditions.

Tan δ as a function of temperature for gluten + amylopectin (1:1) is plotted in Fig. 3 at various water contents. A lower temperature shoulder is clearly observed at intermediate water contents, but not at the highest water contents, which is in agreement with the DSC results. It is also interesting to note that the tan δ peak height increases with increasing water content, which was also observed for gluten and amylopectin alone. This behaviour is reflected in the elastic moduli, plotted as a function of temperature in Fig. 4, for the same water contents. The magnitude of the modulus drop at T_g increases with increasing water content, as the rubbery modulus decreases.

The height of the tan δ peak has been found to be related to the volume of the relaxing phase (Wetton, 1986) and the magnitude of the rubbery modulus is related to the crosslink density. It appears that water increases the mobility of these biopolymers, but may also in some way reduce self-association (by hydrogen bonding), thus reducing crosslinking and increasing the volume of the relaxing phase. This type of mechanical behaviour has also been observed in plasticised ionic polymers (Nomura *et al.*, 1977; Yeo & Eisenberg, 1977).

The tan δ peak temperatures and drop in modulus temperatures are plotted against water content in Figs 5 and 6 respectively. The lower temperature tan δ peaks fall slightly above those for gluten alone at the same water content, whereas the upper transition is slightly below that for amylopectin, indicating some effect of the two components on each other, or possibly a redistribution of water in favour of the amylopectin phase. The elastic modulus results show similar trends, although the upper transition temperatures are very similar to those for amylopectin alone.

Recent DMTA studies (Davies & Nicholls, 1992) carried out elsewhere on similar samples have not shown phase separation. These samples were prepared by pressing at 90°C, whereas the samples studied above were pressed at 60°C to avoid any heat-setting of the gluten. Thus the difference in behaviour appears to arise from the different sample history. This may be

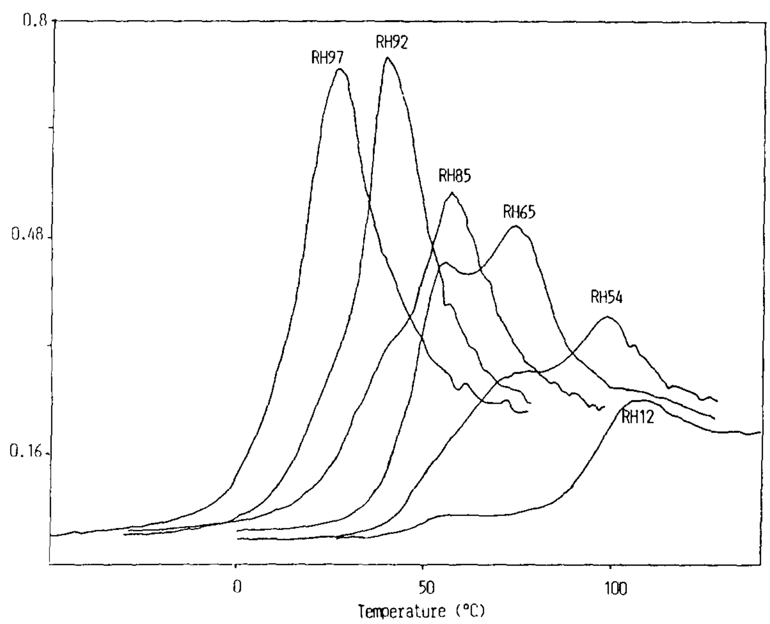

Fig. 3. DMTA tan δ as a function of temperature for gluten + amylopectin (1:1) stored at various RHs.

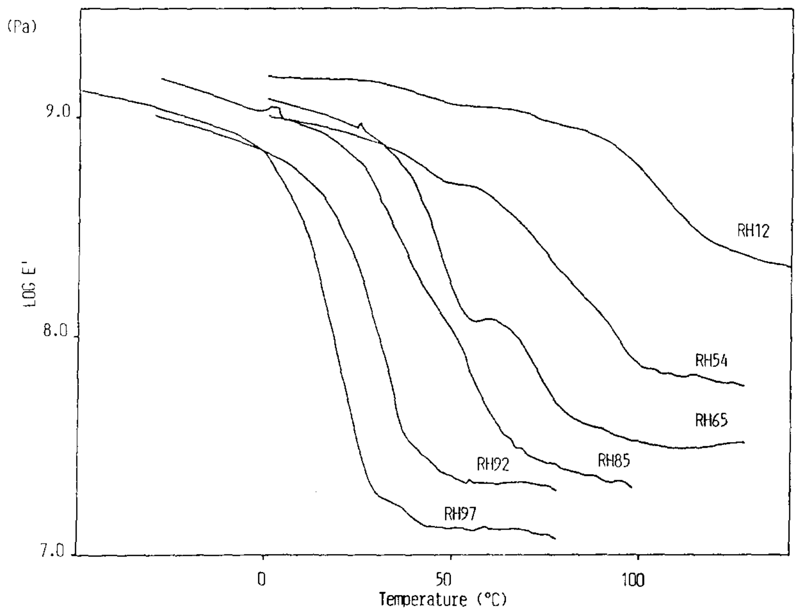

Fig. 4. DMTA log E' plotted as a function of temperature for gluten + amylopectin stored at various RHs.

due to increased miscibility of amylopectin and gluten or due to other interactions becoming possible at the higher pressing temperature. This result illustrates the importance of sample history in these systems.

Casein + gluten (1:1)

These samples again showed evidence of phase separation by DMTA, with broad tan δ peaks and frequently double peaks. Some of the tan δ results are plotted in Fig. 7. These results are summarised in Fig. 8 and compared with values previously obtained for the two components individually (Kalichevsky & Blanshard, 1992; Kalichevsky *et al.*, 1992*b*). There is a great similarity between the two peaks and those obtained for the separate components as a function of water content, although the lower transition may be somewhat depressed when compared with gluten. The onset temperatures of the drop in modulus as plotted in Fig. 9 show a greater scatter. This is largely due to the fact that the onset of the second drop in modulus is very difficult to define. As in the case of the tan δ peaks, the lower temperature transition occurs at a similar or slightly higher temperature than that for gluten alone.

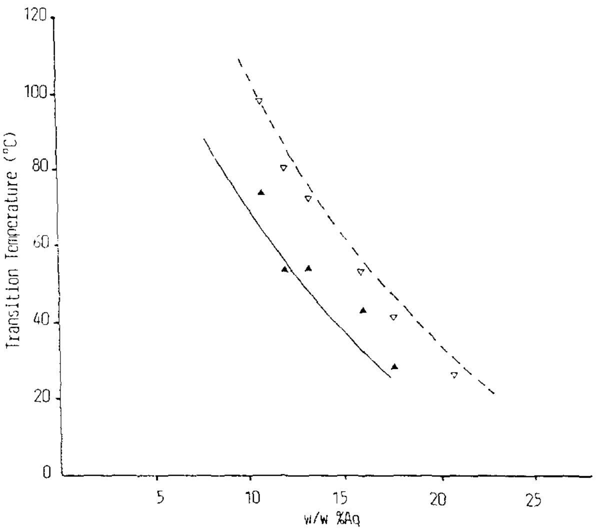

Fig. 5. T_g of gluten + amylopectin (1:1), as defined by DMTA tan δ peak temperatures (▽, ▲), plotted as a function of water content. — — —, position of tan δ peak for amylopectin alone; ———, position of tan δ peak for gluten alone.

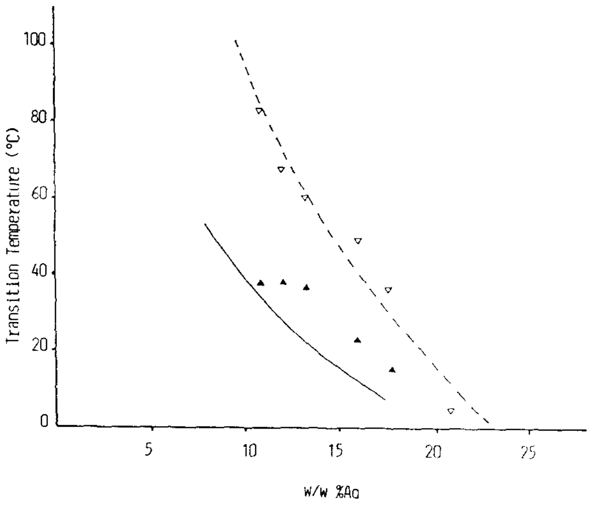

Fig. 6. T_g of gluten + amylopectin (1:1) as defined by the change in slope of log E' ($\Delta E'$ temperature) as a function of water content (▽, ▲). The curves correspond to the $\Delta E'$ transition temperatures in gluten and amylopectin alone (———, — — —).

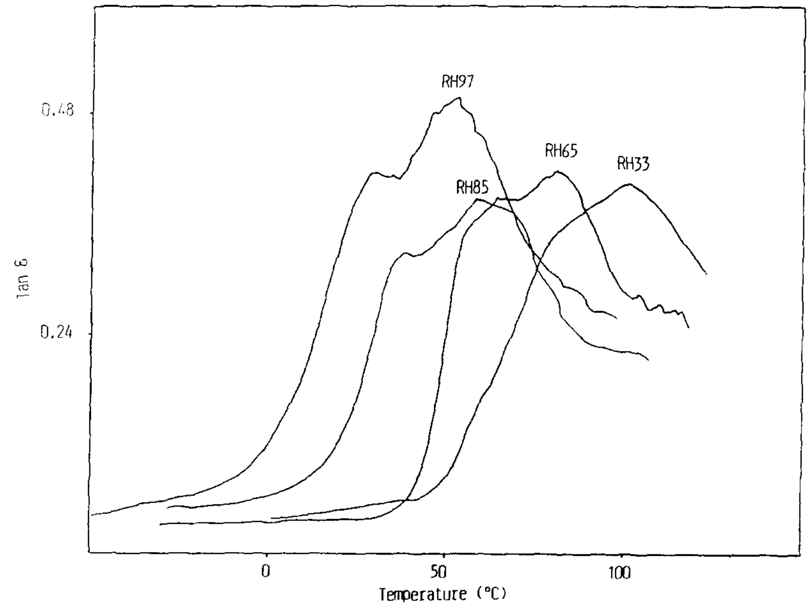

Fig. 7. DMTA tan δ plotted as a function of temperature for casein + gluten 1:1 stored at various RHs.

The DSC data were less clear for this mixture, but two transitions could be distinguished for some samples. The data obtained by averaging several runs are summarized in Fig. 10. The two transitions approached each other at higher water contents as was observed for amylopectin and gluten, which may be due to redistribution of water between the phases.

Amylopectin + casein (1:1)

In previous studies the Couchman-Karasz equation has been used to fit the data (Kalichevsky & Blanshard, 1992; Kalichevsky *et al.*, 1992*a*, *b*, *c*, *d*). This has been used in the following form:

$$T_g\,(\text{mixture}) = \frac{W_1\,\Delta C_{p_1}\,T_{g_1} + W_2\,\Delta C_{p_2}\,T_{g_2}}{W_1\,\Delta C_{p_1} + W_2\,\Delta C_{p_2}}$$

where W_1 is the weight fraction of component 1, ΔC_{p_1} is the change in heat capacity at T_g for component 1 and T_{g_1} is the glass transition of pure component 1. The subscript '1' refers to water, and '2' to the polymer. For a three component system another component may be added to the equation (Kalichevsky *et al.*, 1992*b*). In

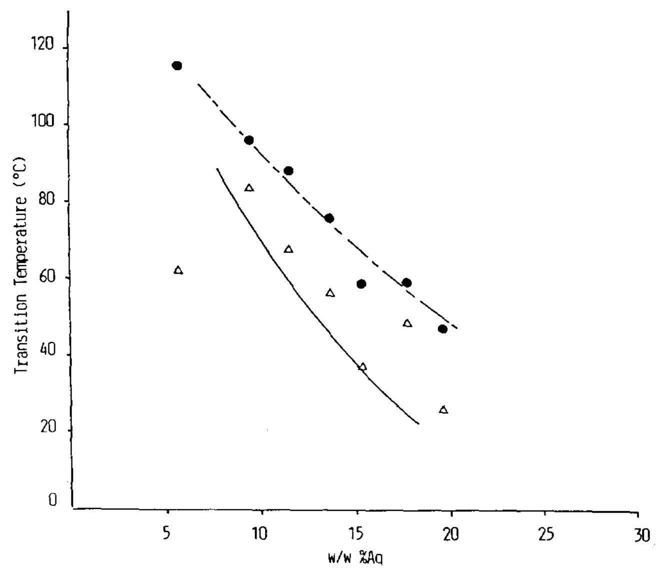

Fig. 8. DMTA tan δ peak temperatures (●, △) plotted as a function of water content for casein + gluten 1:1. — — —, position of tan δ peak temperature for casein alone; ———, for gluten alone.

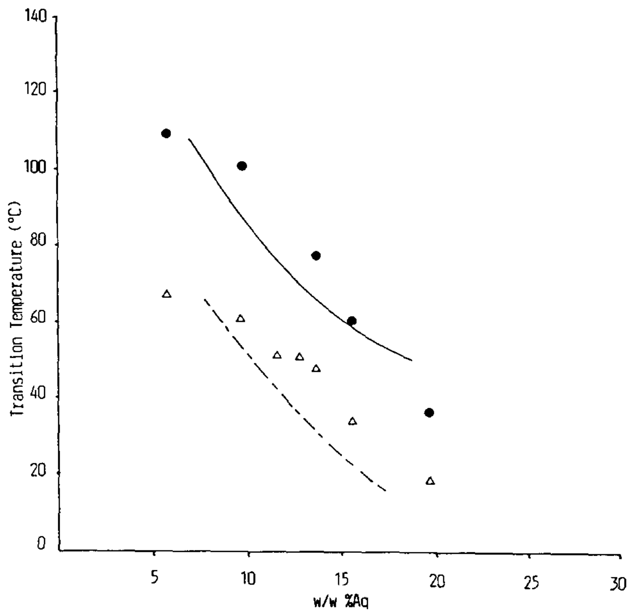

Fig. 10. T_g by DSC for casein + gluten 1:1 as a function of water content. ———, — — — represent $T_{g_{DSC020}}$ previously obtained for casein and gluten alone respectively.

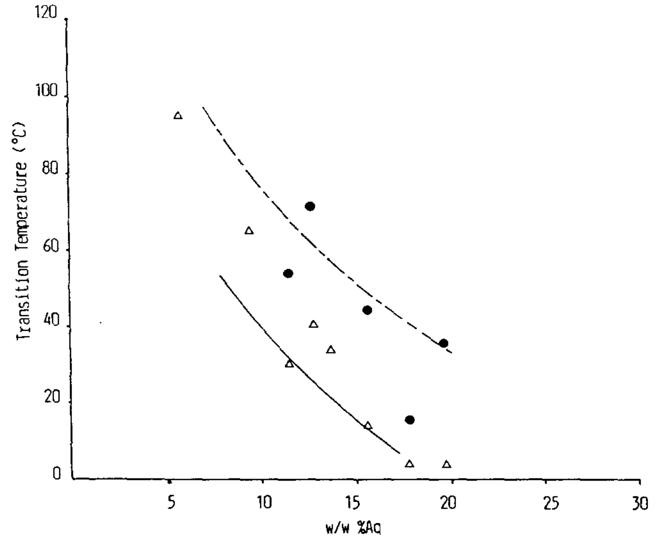

Fig. 9. DMTA ΔE' temperatures for casein + gluten (1:1) (●, △) plotted against water content. — — —, ——— represent the position of the corresponding transitions in casein and gluten alone respectively.

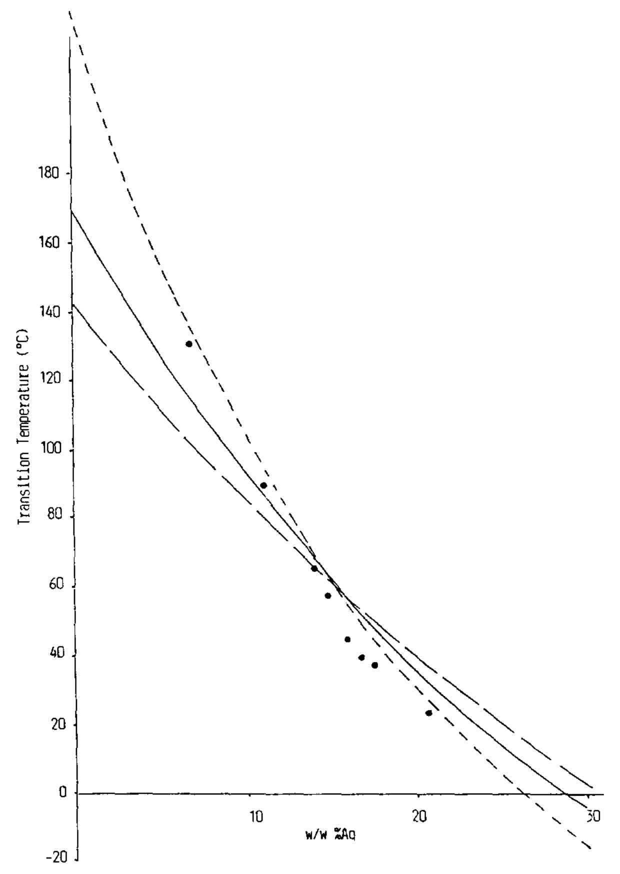

Fig. 11. Best fit (Couchman-Karasz) of T_g (DSC midpoint) for casein (— —), amylopectin (----) and expected position of $T_{g_{DSC}}$ for a 1:1 mixture (———) as a function of water content. $T_{g_{DSC}}$ observed is plotted for comparison (●).

Fig. 11 the best fits to the Couchman-Karasz equation previously obtained for amylopectin and casein (Kalichevsky *et al.*, 1992*b* and Kalichevsky & Blanshard, 1992, respectively) are plotted with the predicted (DSC) values for a miscible 1:1 mixture, as a function of water content. The T_g values used in this calculation were 500 K, 417 K and 134 K for amylopectin, casein and water respectively. The corresponding ΔC_p values used were 0·425, 0·84 and 1·94 J g^{-1} K^{-1} respectively. This shows that, particularly at intermediate water contents, the transition temperatures of the two components are very close. Any evidence of immisci-

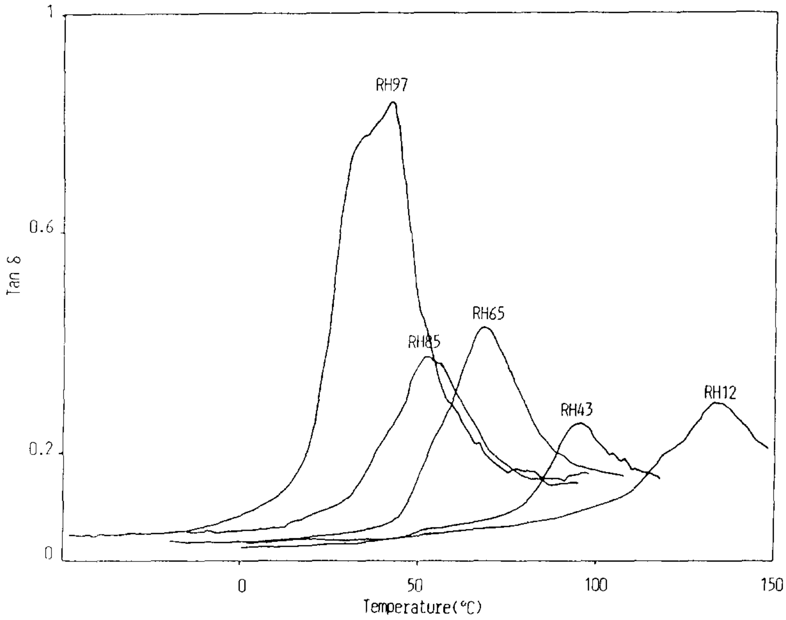

Fig. 12. DMTA tan δ for casein + amylopectin (1:1) stored at various RHs.

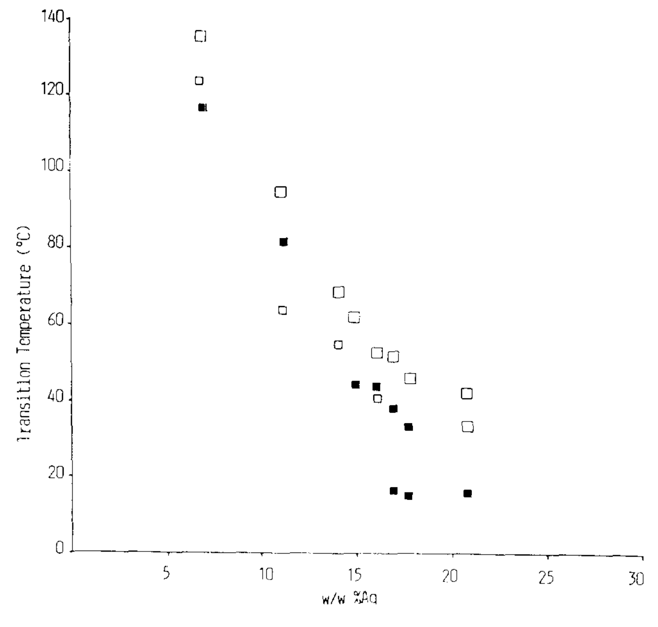

Fig. 13. Summary of DMTA results for casein + amylopectin (1:1): tan δ, peak temperatures (□, ▫) and $\Delta E'$ temperature (■).

bility from T_g would thus only be expected to be observable at high or low water contents. The DSC T_g values obtained on the second run are also plotted in Fig. 11. Only one transition was observed in all cases. The transition temperature appears to correspond most closely to the values previously obtained for amylopectin alone, rather than being in between the two samples as might have been expected.

It should be pointed out, in view of the debate about the value of the ΔC_p of water (very low values in the region of 0·1 J g^{-1} K^{-1} have recently been obtained by Hallbrucker *et al.*, 1989), that the use of a smaller value of ΔC_p of water would result in similar predictions for the three component system, if the values of ΔC_p for amylopectin and casein are also adjusted to fit the data for the separate components.

The DMTA tan δ peaks show some broadening, particularly at high and low RH, as shown in Fig. 12. The samples containing casein appear to show less effect of water content on the magnitude of the drop in modulus at T_g. It seems that casein is less affected by water than gluten or amylopectin, which may be due to its greater hydrophobicity (Adrian & Frangne, 1991). The DMTA results for casein + amylopectin (1:1) in the presence of water are summarised in Fig. 13. As in the case of the DSC results, the transitions appear to follow the corresponding amylopectin transitions quite closely. There are secondary transitions, probably due to casein, at high and low water contents.

CONCLUSION

The glass transitions of binary (1:1) mixtures of casein, amylopectin and gluten have been studied as a function of water content. All of these biopolymers appear to be immiscible, although this is not so clear cut in the case of casein + amylopectin, as at most water contents studied their T_gs are within 10°C of each other. Miscibility may be very dependent on sample history. Immiscibility results in each system having two glass transitions or considerable broadening. These observations may be important in the control of food mechanical properties and stability.

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