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Some Recent Observations on the Retrogradation of Amylose

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SUMMARY

Starch is the major storage polysaccharide of higher plants where it occurs as water insoluble granules. Two component polymers may be extracted from the starch granules, namely amylose and amylopectin. Both polymers are based on chains on α-1,4-linked D*-glucose. However, whereas amylose is an essentially linear polymer, amylopectin is highly branched with branches linked at the 6-position.*

INTRODUCTION

As well as providing a source of carbohydrate, starch may also be used to manipulate the texture of many food products. When an aqueous suspension of starch granules is heated above a certain temperature, referred to as the gelatinisation temperature, irreversible swelling of the granules occurs resulting predominantly in the release of amylose into solution. If the starch concentration is high enough this polymer solution containing swollen granules behaves as a viscoelastic paste. On cooling the paste thickens and becomes opaque and an elastic gel is formed. The pastes and gels may be regarded as composites consisting of polymer solutions or gels 'filled' with swollen granules (Ring & Stainsby, 1982). The changes on cooling have been attributed to the association of the amylose and the process is termed retrogradation. This paper is concerned with studies of the form of association respon-

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sible for retrogradation and hence will be concerned with studies of pure amylose solutions and gels.

In dilute aqueous solution at 20°C and neutral pH high molecular weight ($>100\,000\ \mathrm{g\ mol^{-1}}$) amylose behaves as a flexible polymer (Banks & Greenwood, 1975). These solutions are unstable and retrogradation results in an increase in turbidity and eventually precipitation of the amylose. Above a certain critical concentration the amylose solution forms an opaque elastic gel on cooling. X-ray diffraction studies of such gels yield weak diffraction patterns of the so-called B type (Katz, 1930). By analogy with X-ray diffraction studies on oriented fibres of amylose (Wu & Sarko, 1978), it has been suggested that the X-ray data provide evidence for the presence of crystallites consisting of side-by-side stacked amylose helices as junction zones of the gel (Morris, 1979). Retrogradation and gelation of amylose is normally attributed to a crystallisation process (Collison, 1968). The opacity of the gels and the turbidity of dilute solutions is normally taken to imply the presence of large semi-crystalline regions comparable in size to the wavelength of light. We have here compared the onset of turbidity, as a monitor of aggregation, with the onset of crystallinity as monitored by X-ray studies on amylose gels.

METHOD AND RESULTS

Starch was prepared from dried, smooth, seeded peas, variety Filby, by the aqueous extraction method of Adkins & Greenwood (1966). Samples of amylose were prepared by leaching the amylose from a 2% aqueous suspension of granules over a temperature range 62–70°C. The granules were removed by centrifugation and the amylose purified by *n*-butanol precipitation. Amylose solutions were regenerated by heating suspensions of the complex to 90°C and removing the butanol by passing a heated stream of nitrogen through the suspension. The amylose fraction prepared in this way had an iodine binding capacity, determined using a semi-micro differential potentiometric method (Banks *et al.*, 1971), of 19·5 ± 0·5 mg iodine per 100 mg polymer. The intrinsic viscosity, measured using an Ostwald capillary viscometer at 20°C, was $80\ \mathrm{ml\ g^{-1}}$. Using the relation

$$[\eta] = KM^{\alpha}$$

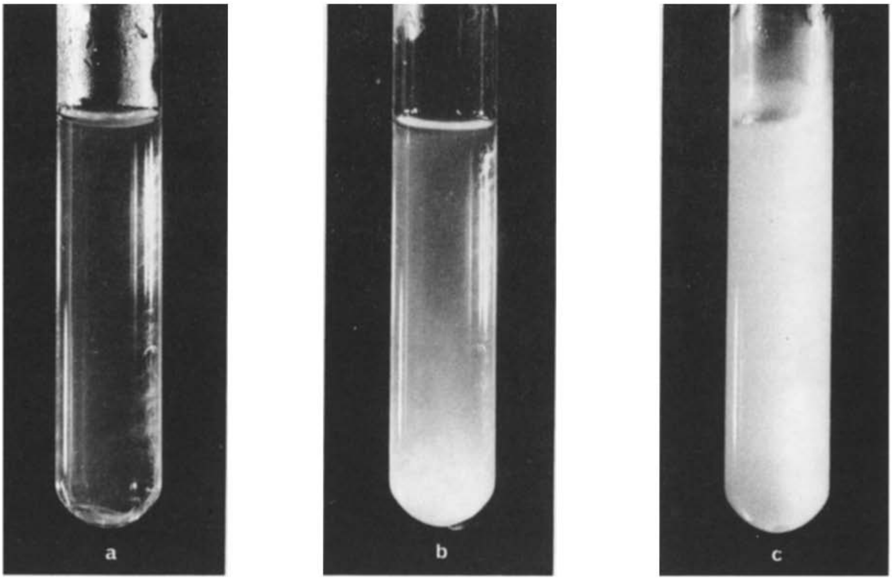

Fig. 1. Development of turbidity as a function of time for a 7% w/w amylose solution quenched from 90 to 32°C at zero time (a) 1·5 min (b) and 3 min (c).

with $\alpha = 0{\cdot}5$ and $K = 0{\cdot}113$ (Banks & Greenwood, 1975), this corresponds to a weight-average molecular weight of 500 000 g mol^{-1}.

Gels were prepared by quenching amylose solutions from 90 to 32°C. Typically slowest cooling rates were observed for samples used in the rheological studies. Gels cooled to 36°C within ~2 min and 33°C within ~3 min. Cooling rates were sensitive to sample size, shape and polymer concentration.

The development of turbidity was monitored at 600 nm using a Pye Unicam SP 800.B spectrophotometer (0·1 cm pathlength). Typical changes are illustrated in Fig. 1 for the gelation of a 7% w/w solution of amylose. The samples became opaque within 3 min and no further detectable change in turbidity was observed on standing. The development of the gel structure, as monitored by the storage modulus, G', measured at a frequency of 200 Hz under low deformation (10^{-4}) using a Rank Brothers pulse shearometer was found to follow closely the turbidity change (Fig. 2). The modulus reached ~98% of its 'final value' (measured after 8 h) after a period of 40 min. Crystallinity was

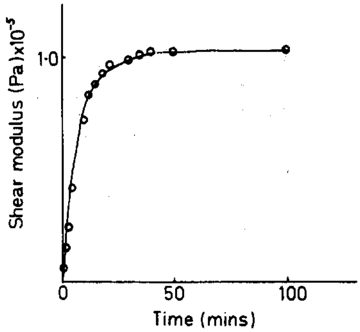

Fig. 2. Change in shear modulus as a function of time for a 7% w/w amylose gel quenched from 90 to 32°C.

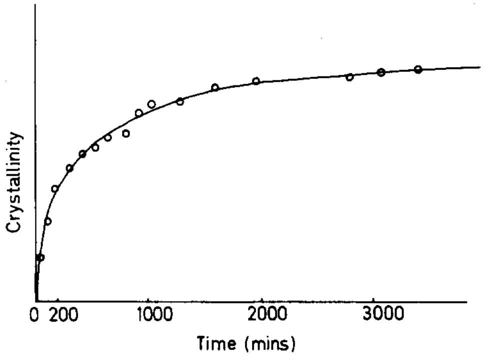

Fig. 3. Development of crystallinity as a function of time for a 7% w/w amylose gel quenched from 90 to 32°C.

monitored by following the development of the 100 reflection (*d* spacing 1·6 nm) characteristic of the B type crystal structure using a Kratky small angle X-ray scattering system ($\lambda = 0{\cdot}154$ nm). Crystallinity was measured in terms of the integrated intensity of the diffraction peak above the background intensity of the non-crystalline polymer and solvent. From Fig. 3 it can be seen that the crystallinity developed over a period of several hours and approached a constant value after approximately 50 h. After 40 min, at a time when the gels are opaque and G' has reached 98% of its final value, the detectable crystallinity is only ~12% of its 'final value'.

CONCLUSION

The conclusion to be drawn from these preliminary studies is that there are two main processes involved in the retrogradation of amylose, and hence presumably of starch. The first initial increase in turbidity and the development of gel structure appear to result from a phase separation (aggregation) of polymer molecules. The development of a gel structure rather than a precipitate is favoured by the high molecular weight (high viscosity) and high rate of cooling both of which hinder polymer diffusion. The development of crystallinity in these polymer rich regions is a secondary process in the creation of the network structure.

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