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Studies of the Effect of the Sugars Ribose, Xylose and Fructose on the Retrogradation of Wheat Starch Gels by X-ray Diffraction

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ABSTRACT

The effects of the sugars ribose, xylose and fructose on the retrogradation of wheat starch gels were investigated by measuring the area under the strong 0·516 nm diffraction peak (characteristic of B-type crystalline retrograded starch) as a function of storage time for a series of gels containing different amounts of added sugars. Retrogradation was monitored as the increase in peak area with storage time. The results obtained suggested that all three sugars altered crystallisation and hence retrogradation of the gels. For the concentration regimes studied, xylose and ribose acted by progressively reducing crystallisation with increasing sugar concentration. In the case of fructose two effects were noted. The fructose led to an increase in both thermally reversible and thermally irreversible crystallisation upon storage. For xylose and ribose the increase in crystallisation upon storage was almost totally thermoreversible suggesting that the retrogradation upon storage was dominated by amylopectin crystallisation.

INTRODUCTION

Foods such as bread, cakes and other baked products, which contain gelatinised starch, are prone to undesirable increases in elastic shear

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modulus, whilst stored over a period of weeks, due to a process commonly referred to as 'staling'. This problem is of commercial significance and has been the subject of extensive investigation. Some of these studies (Hellman *et al.*, 1954; Zobel & Senti, 1959; Dragsdorf & Varriano-Marston, 1980; Russell, 1983; Ghiasi *et al.*, 1984; Miles *et al.*, 1985*a*, *b*) have shown that the increase in firmness due to staling can be linked to a thermoreversible starch crystallisation process.

When an aqueous dispersion of starch granules is heated to a characteristic 'gelatinisation' temperature (usually in the range 60–70°C), the granules swell irreversibly releasing the linear molecule amylose into solution. Provided the starch concentration is sufficiently high then, upon cooling, the mixture forms an opaque elastic gel. Upon storage the increase in elastic modulus is accompanied by an increase in the development of B-type crystalline X-ray diffraction patterns (Katz, 1930; Cluskey *et al.*, 1959; McIver *et al.*, 1968). This return to a crystalline structure has been dubbed retrogradation. Recent experimental studies have provided a molecular description of starch gelation and retrogradation (Ring & Stainsby, 1982; Miles *et al.*, 1984, 1985*a*, *b*; Orford *et al.*, 1987). Development of this model resulted from the recognition (Ring & Stainsby, 1982) that, provided the gelatinised starch granules remained intact, starch gels can be modelled as a composite structure. From comparative investigations (Miles *et al.*, 1984, 1985*a*, *b*) of the amylose matrix, the intact starch gel and the gelatinised granules, the starch gel may be pictured as gelatinised granules, composed of an amylopectin skeleton, suspended in an interpenetrating amylose matrix. Gelation is dominated by that of the amylose matrix and this step determines the opacity of amylose and starch gels (Miles *et al.*, 1984, 1985*a*, *b*). Gelation of the amylose involves a phase separation into an amylose-rich amorphous network within which limited amylose crystallisation occurs. The amylose crystals can only be melted at high temperatures above 100°C (Mestres, 1986) and this step is essentially thermally irreversible. The long-term increases in stiffness upon storage are thermoreversible and are related to crystallisation of the branched amylopectin (Miles *et al.*, 1985*b*). The simplest picture is to consider the crystallisation of the amylopectin skeletons of the gelatinised granules resulting in a stiffening of the granular structure and enhancement reinforcement of the amylose matrix (Miles *et al.*, 1985*b*). Amylose–amylopectin binding would improve the granule matrix interaction, and cannot be ruled out, as discussed by Mestres (1986). Orford *et al.* (1987) have extended the earlier studies of Miles *et al.* (1984, 1985*a*, *b*) to include higher starch concentrations and the effects of the botanical

source of the starch. At high starch–water ratios, such as those found in bakery products, the role of amylopectin crystallisation in the retrogradation stage becomes increasingly important as the starch–water ratio is increased. This arises due to the restricted swelling of granules upon gelatinisation and the resultant reduced solubilisation of the amylose.

Traditionally, starch recrystallisation processes and their role in retrogradation have been treated as equilibrium processes and examined by the classical Avrami equation. Recently, Slade and Levine (1987, 1988) have emphasised the importance of treating such crystallisation as a non-equilibrium process. The role of water and cosolutes is explained in terms of their influence on the glass transition temperature of the amorphous amylose network (Tg) and the melting temperatures of amylose and amylopectin crystals. The primary factors which influence crystallisation during gelation and storage are the relative values of the storage temperatures after cooling of the starch gel, or the starch-based food product, and the glass transition and melting temperatures, since these parameters primarily control the nucleation and growth of crystals.

Starch-based food products, such as cakes, not only contain relatively low amounts of water but also contain other components such as proteins, lipids and sugars. Little research has been done into the effects of these other components upon starch retrogradation. Sugars are known to affect the rates of retrogradation. Maxwell and Zobel (1978) have investigated the effects of fructose, glucose (dextrose) and sucrose on the rate of stiffening of wheat starch gels, and Germani *et al.* (1983) have compared the effects of glucose and sucrose on the firming of maize starch gels. Recently, I'Anson *et al.* (1990) have carried out a limited examination of the relative effects of ribose, glucose and sucrose at a single sugar concentration on the increase in shear modulus of wheat gels upon storage and compared such changes with the influence of these sugars on the development of crystallinity within the gels. This latter study demonstrated that the sugars reduced the rate of stiffening of the gels by reducing the rate of crystallisation upon storage (I'Anson *et al.*, 1990). These results are consistent with the suggested antiplasticising effect of sugars on the amorphous amylose matrix (Slade & Levine, 1987, 1988) thus raising Tg and reducing the growth of crystallites.

This article describes a more detailed study of the effect of three different sugars and the influence of sugar concentration on the crystallisation of amylopectin in stored wheat starch gels. X-ray diffraction has been used to provide a direct measure of crystallisation in a time-course study of stored wheat gels containing different concentrations of ribose, xylose or fructose.

EXPERIMENTAL

Wheat starch was a gift from RHM Research Ltd. The wheat starch was prepared from Challenge breadmaking flour using a pilot scale (Martin Process) dough washer. The pH of the starch milk was reduced to 2·5 (by addition of HCl) for 1 h in order to denature indigenous amylopectic activity, carefully neutralised (using Na_2CO_3), and spray dried. The resulting starch had a protein content of 0·34% ($N \times 5{\cdot}7$), starch damage of 2% and a moisture content of 13%.

Ribose, xylose, fructose and sodium azide were purchased from Sigma Chemicals Ltd. Colourless paraffin was purchased from BDH Chemicals Ltd.

Wheat starch gels were prepared in the following way: the wheat starch, sodium azide as a preservative and sugar (as required) were stirred into water according to the recipes given in Table 1. After dispersion the slurry was carefully poured into cylindrical aluminium moulds with removable tops and bases. The moulds containing the slurry were heated to 80°C in a water bath for 5 min in order to initiate thickening and thus lessen settling of the starch, and then immediately transferred to an oven (preheated to 105°C) for 30 min. The aluminium moulds containing the samples were removed from the oven and allowed to cool to room temperature.

The gel discs were removed from the aluminium moulds. Immediately upon removal a slice (12 mm × 3 mm × 2 mm) was cut from each disc. These slices were stored by immersion in liquid paraffin in order to prevent water loss by evaporation. The same slice from each gel disc was re-used throughout the course of the experiment. Each slice was weighed before immersion in order to check for adsorption of paraffin or water loss during the experiment.

TABLE 1
Composition by Weight (g) of Wheat Starch Gels

Starch	*Water*	*Sugar*	*Sodium azide*	*% Sugar*
10·0	10·0	0·0	0·015	0·0
10·0	10·0	2·0	0·015	9·0
10·0	10·0	4·0	0·015	16·7
10·0	10·0	6·0	0·015	23·0
10·0	10·0	8·0	0·015	28·6
10·0	10·0	10·0	0·015	33·3

X-ray diffraction measurements were carried out using X-ray radiation of wavelength 0·154 nm. The diffractometer was a Phillips Scientific PW1820 vertical goniometer with attached camera. Data was collected using a proportional detector and stored in a BBC microcomputer. The strong 0·516 nm diffraction peak for B-type crystalline starch was scanned over the range 0·591 nm to 0·467 nm. The angular region 15–19° (2θ) was scanned at a rate of 4×10^{-3} (2θ) per second, with a step size of 0·15. Scanning and data collection were controlled by a BBC microcomputer. The slit setting of the camera was slightly different for each of the three sugars studied. All data were normalised to unity with respect to a pure starch gel to offset any effects from this variation.

All measurements were carried out at ambient temperature and pressure. Before measurement each gel slice was carefully blotted in order to remove excess paraffin, with care being taken to avoid drying out the surface of the gel. The slice was then placed in an upright position on the sample holder of the camera, with its long axis perpendicular to the X-ray beam. In order to minimise moisture loss during measurement, each gel slice was enclosed together with two thin strips of wetted blotting paper, under a tent of thin transparent plastic film secured to the edges of the sample container with double-sided tape. The plastic film had been previously examined in order to ensure that it did not produce a diffraction peak, or significant amounts of background scatter in the angular range of interest. The strips of blotting paper were rewetted before each measurement and care was taken to avoid the sample touching them, and thus adsorbing moisture and altering the level of crystallinity. Helium gas was passed through the camera during each measurement in order to reduce air-scatter.

After each measurement the sample was reweighed in order to monitor any significant water loss during measurement.

At the end of each investigation the gel slices were placed in sealed glass vials, which were then immersed in a water bath for 20 min at 80°C. These samples were cooled to room temperature, removed from the glass vials and rescanned in the diffractometer. This procedure was used to distinguish between thermally reversible and thermally irreversible crystallisation upon storage.

RESULTS AND DISCUSSION

The experimental data collected for wheat starch gels containing ribose, xylose and fructose is shown in Figs 1, 2 and 3, respectively.

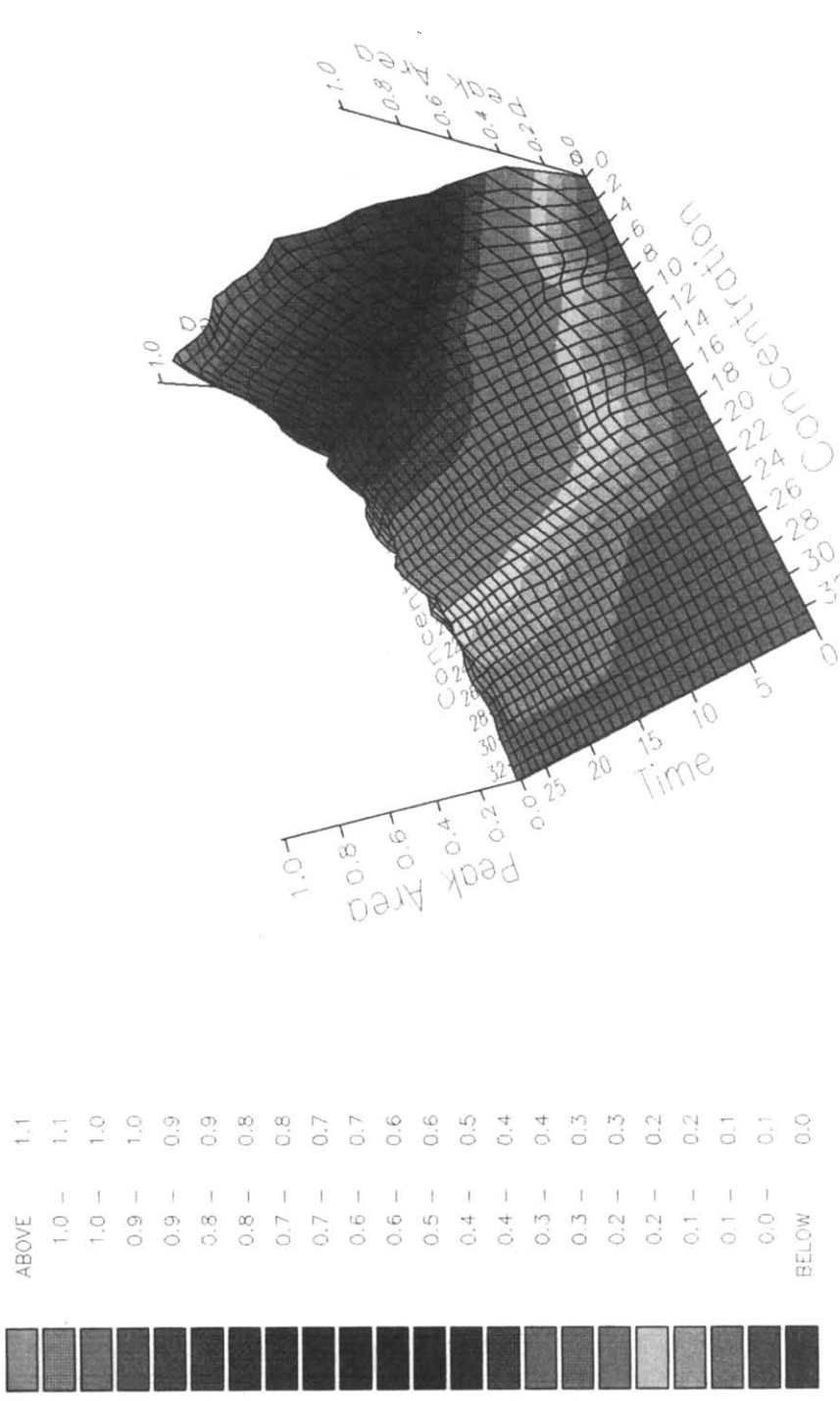

Fig. 1. Three-dimensional representation of the effect of added ribose on the retrogradation of wheat starch. Crystallisation is expressed (arbitrary units) in terms of the area under the 0·516 nm X-ray peak for B-type crystalline starch normalised to unity for the pure starch gel at 23 days. Variations of crystallinity with storage time (days) and sugar concentration (wt%) are displayed on the graph.

TABLE 2
Crystallisation in Starch–Sugar–Water Gels[a]

Sugar content	*Ribose*			*Xylose*			*Fructose*		
	Day 1	*Day 25*	*Heated*	*Day 1*	*Day 24*	*Heated*	*Day 1*	*Day 23*	*Heated*
0	0·34	1·00	0·29	0·35	1·00	0·13	0·33	1·00	0·24
9·0	0·12	0·50	0·15	0·16	0·64	0·07	0·38	0·82	0·31
16·7	0·06	0·35	0·08	0·07	0·29	0·05	0·61	0·95	0·33
23·0	0·04	0·19	0·05	0·05	0·20	0·03	0·62	0·98	0·37
28·6	0·02	0·07	0·02	0·03	0·05	0·03	—	—	—
33·3	0·02	0·03	0·01	0·03	0·05	0·03	0·62	0·74	0·32

[a]Crystallisation is measured as 0·516 nm peak areas normalised to unity at 23 days for pure starch gels to correct for different camera apertures used to collect data for different sugar systems.

Results of the ribose study are shown in Fig. 1. It can be seen that on the first day after preparation the gels showed a descending level of crystallisation with ascending ribose concentration. This trend remained constant throughout storage. The crystallinity of the pure starch gel increased rapidly during the first few days and then slowed down tending to a plateau value. Increasing ribose concentration reduced the rate of crystallisation such that, at and above ~ 33·3% ribose concentration, the level of crystallinity remained essentially unchanged throughout the storage period. These results are consistent with the earlier limited data published by I'Anson *et al.* (1990). Towards the end of the study all the gels showed a small increase in crystallinity possibly due to the accumulative effects of small losses of water during measurement.

Heating the samples to 80°C at the end of the experiment (28 days) reduced their crystallinity to values equal to, or slightly greater than, the level of crystallinity observed at day 1 (Table 2). Since it is known that the amylose crystals can only be melted at temperatures in excess of 100°C (Mestres, 1986), these observations support the hypothesis that the retrogradative changes in starch during storage involve crystallisation of amylopectin.

Results obtained for the sugar xylose (Fig. 2) are very similar to those observed with ribose. Heating the samples to 80°C at the end of the experiment (25 days) reduced the levels of crystallinity to approximately those values observed at day 1 (Table 2). Thus both xylose and ribose appear to function in a similar manner in suppressing the rate of amylopectin crystallisation.

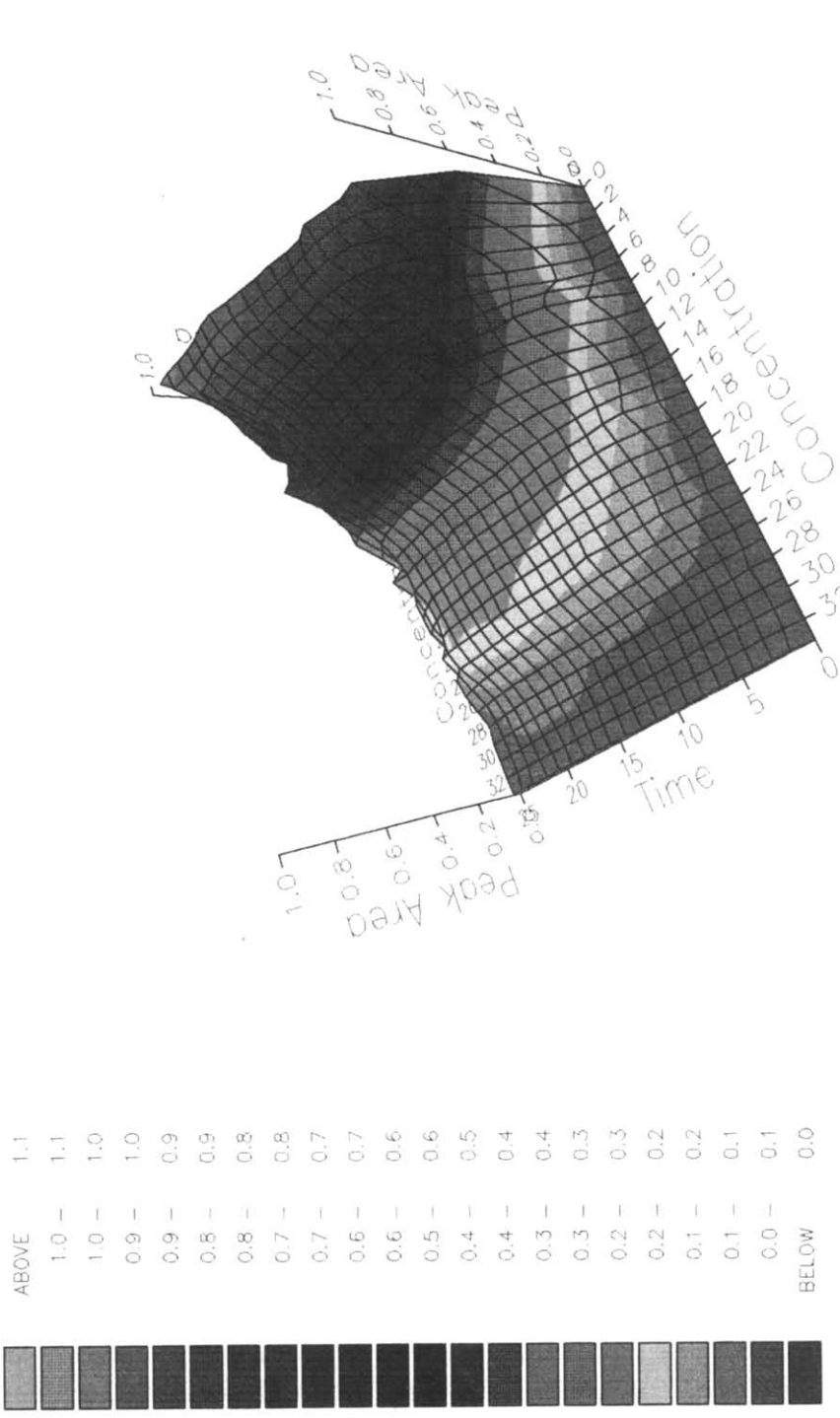

Fig. 2. Three-dimensional representation of the effect of added xylose on the retrogradation of wheat starch. Changes in crystallinity (peak area) normalised to unity for the pure starch gel at 23 days are displayed as a function of storage time and sugar concentration.

The results obtained for fructose are shown in Fig. 3. These results are strikingly different to the previous two studies involving xylose and ribose. On day 1 after preparation of the gels, all samples containing fructose exhibited a higher crystallinity than the pure starch gel. The gels show a time dependent increase in crystallinity superimposed on an increasing level of crystallinity at day 1 with increased fructose concentration. Heating at 80°C at the end of the experiment (26 days) resulted in partial removal of crystallinity (Table 2). Unlike the gels containing xylose and ribose the residual crystallinity increased with increasing sugar concentration. These results suggest that the crystallinity of starch gels containing fructose consists of a non-reversible component which increases with sugar concentration and thermally reversible component which also increases with increasing sugar concentration.

CONCLUSIONS

Ribose and xylose have been found to suppress the growth rate of amylopectin crystals although the mechanism of suppression remains unclear. The X-ray diffraction methods used in this study are insufficient in themselves to shed any light upon the relative merits of the model proposed by Slade and Levine (1987, 1988). Further studies combining X-ray and physical measurements, such as thermal transitions, will be required.

The results for fructose are unusual and are less easily interpretable than those for ribose and xylose. Addition of fructose appears to increase both the thermally reversible and irreversible crystalline components of starch. The mechanisms responsible for these increases are obscure and, as with ribose and xylose, more extensive studies involving the measurement of thermal parameters are required.

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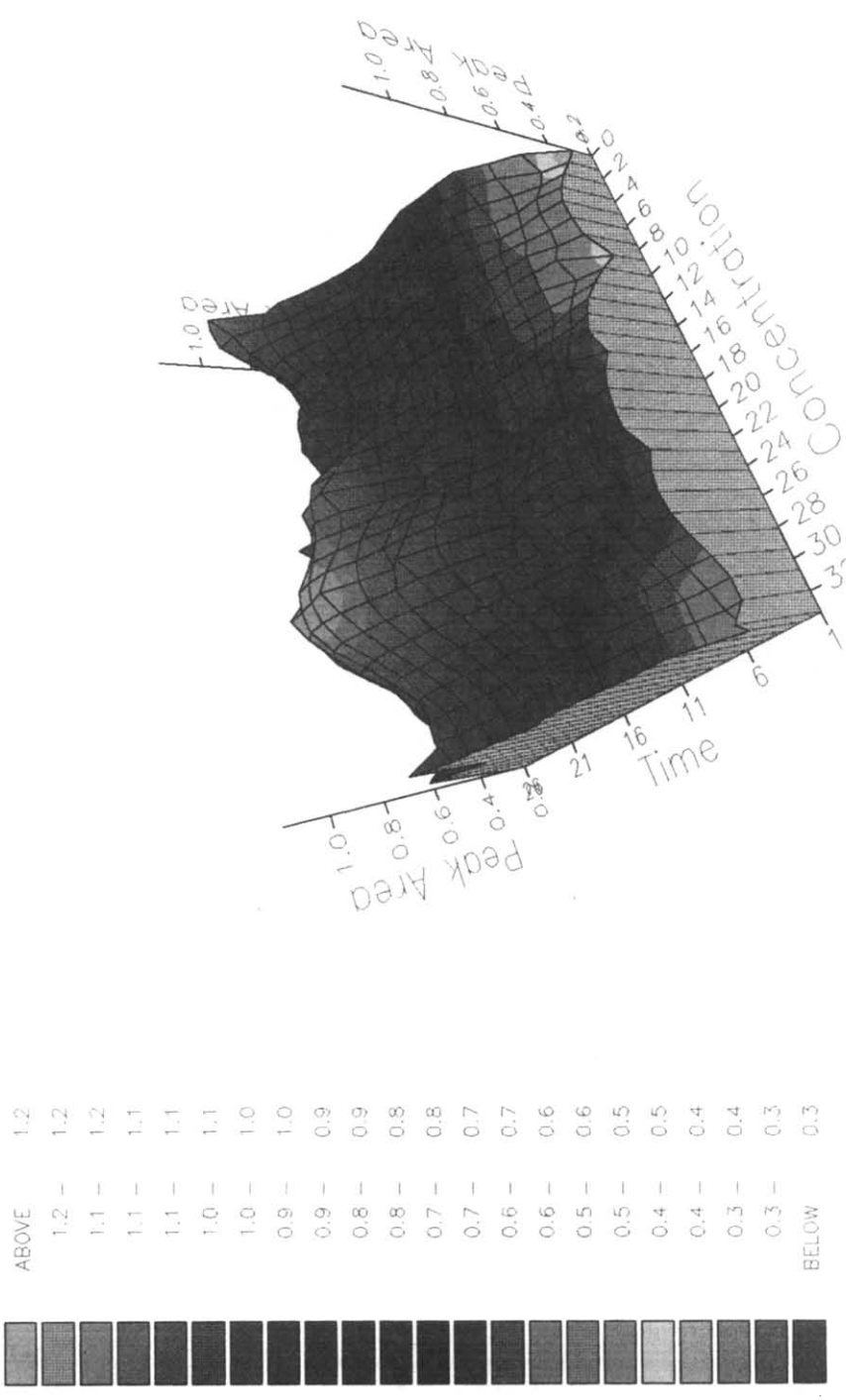

Fig. 3. Three-dimensional representation of the effect of added fructose on wheat starch retrogradation. Changes in crystallinity (peak area) normalised to unity for the pure starch gel at 23 days with storage time and sugar concentration are displayed on the plot.

EDITOR'S NOTE

The authors have asked us to point out that the delay between the received and revised dates was mainly due to a delay in the refereeing procedure.

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