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The effects of low molecular weight additives on the viscosities of cassava starch

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The influence of a range of additives on the change in the viscosity of cassava starch pastes on ageing following gelatinisation has been investigated. The inclusion of sulfite with pasted starch markedly decreases the viscosity of the paste with time and this is prevented by the addition of propyl gallate. Intrinsic viscosity measurements strongly support the view that sulfite addition promotes polysaccharide degradation. Ascorbate has a greater degradative effect than sulfite, whereas only limited degradation is observed with glutathione. Salts such as sodium chloride and sodium sulfate show no effect on viscosity. If the chelating agent EDTA is added as well as sulfite, the reduction in viscosity is smaller, although the effect is not cancelled out completely. Interestingly, the degradative effect of sulfite and ascorbate can be shown at 30, 60 and 80°C. The results presented uphold the previous suggestion that sulfite addition promotes oxidative reductive depolymerisation (ORD) and largely rule out any residual enzyme activity as the cause. It is suggested that the strong degradative action of ascorbate on starch could be one of the explanations for its action in baked products. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Previous work by this group (Mat Hashim *et al.*, 1992; Paterson *et al.*, 1994; Mitchell *et al.*, 1997) demonstrated that gelatinisation of starches in the presence of low levels of sulfite altered the swelling volume of the starch and the amount of carbohydrate released from the granule. The influence on swelling volume was most marked for cassava and sago starches, although all starches studied showed changes in the amount of polysaccharide released from the granule. We also reported that the effect of sulfite could be largely cancelled out by the inclusion of the polar antioxidant propyl gallate, which led us to believe that a free radical mechanism was responsible. The hypothesis was, therefore, that in the presence of sulfite energy put into the system as heat and mechanical energy can trigger the formation of radical species (perhaps via a metal ion initiator) and that these radicals can attack the starch polysaccharides, lowering their molecular weight. Evidence to support this hypothesis came from intrinsic viscosity data from potato and wheat starch samples. Samples of both these starches showed a decrease in molecular weight (as assessed by intrinsic viscosity) when 1% starch was gelatinised in the presence of 0.01 g/100 ml sodium sulfite (Paterson *et al.*, 1996) when compared with samples gelatinised in water alone.

In this paper we describe the influence of sulfite and other oxidant systems on the viscosity of cassava starch pasted at concentrations of 5%. Although this concentration is pertinent to the use of this starch as a thickener, the main reason for its choice was that the viscosity at this concentration would be expected to be a sensitive indicator of changes in swollen volume and/or degradation of polysaccharide in solution outside the granular environment. Swollen granules at this concentration, if intact, will close pack (Evans & Haisman, 1979; Steeneken, 1989) and polysaccharide in solution at this concentration will be in the semi-dilute domain. This domain will be achieved at concentrations (g/dl) higher than $\sim 200/[\eta]$ (Lapasin &

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Pricl, 1995) where $[\eta]$ is the intrinsic viscosity in ml/g (approximately 180 for cassava starch). A reduction in the volume occupied by swollen granules or in molecular weight in this region will cause a large viscosity decrease.

In the previous studies the sulfite was added before gelatinisation of the starch, which was then pasted at temperatures of 95°C or 120°C. It was therefore not known at what stage the sulfite induced the changes observed for the starch. To determine if it could act on the starch subsequent to gelatinisation, sulfite was added after pasting. In the absence of other salts the pH of an aqueous suspension of starch is dependent on the sulfite level. To avoid this complication all measurements were carried out in a pH 7.0 phosphate buffer.

Other materials with a potential oxidising action that were investigated were ascorbic acid and glutathione. These were chosen because, like sulfite (Wade, 1972), they are known to have a significant influence on aspects of the baking process (Fichett & Frazier, 1986; Grosch, 1986). A possible hypothesis is that in addition to influencing disulfide crosslinking reactions in proteins, these materials also function because they exert some degradative effect on starch polysaccharides.

MATERIALS AND METHODS

The experiments were designed to give maximum reproducibility and ease of handling, as well as to facilitate the throughput of a large number of experiments utilising different additives. The reagents were added after pasting and then the solutions were normally held at 60°C to prevent retrogradation until viscosity measurement, as detailed in the protocol below.

Materials

Cassava starch was obtained from the Central Tuber Crop Research Institute, Trivandrum, India. Sodium sulfite, silver nitrate, *n*-propyl gallate, EDTA, ascorbic acid and sodium sulfate were purchased from Sigma Chemical Company. Sodium chloride was purchased from British Drug Houses Ltd and the buffer salts were obtained from Fisons Ltd. All reagents were of analytical grade.

Intrinsic viscosity

The intrinsic viscosity of starch was measured following pasting of a 1% dispersion at 95°C and then solublisation in 0.5 M KOH as described by Paterson *et al.* (1996). Pasting was carried out in water and in dilute solutions of sodium sulfite (0.01 and 0.1%) with and without *n*-propyl gallate (0.05%).

Viscosity of 5% starch suspensions

200 ml of 5% starch suspensions in 0.1 M sodium phosphate buffer at pH 7.0 were prepared in 250 ml sample bottles and gelatinised for 30 min at 90°C with gentle stirring. Shear was applied using a Silverson mixer set at maximum speed for 2 min and the samples were split into 50 ml aliquots. Where appropriate, additives were incorporated at this stage. In these buffered systems, the pH was found to vary by no more than ±3%. The samples were held at constant temperature in a water bath without agitation before analysis.

Initially viscosity measurements were made after holding the sample at 60°C for up to 12 h. To determine the effect of holding temperature, some experiments were repeated with holding at 30 and 80°C after inclusion of additives. Immediately prior to analysis a 5 ml aliquot was transferred to a new universal sample bottle and cooled to 25°C. Rheological measurements were carried out at this temperature using a Bohlin CS10 rheometer and 4° cone and plate (CP 4/40) geometry. Constant rate measurements were made using shear rates in the range 1.35 to 70 s^{-1} (shear rate increasing).

RESULTS AND DISCUSSION

Intrinsic viscosity

The intrinsic viscosities of cassava starch solution (solubilised in 0.5 M KOH) was found to be 182 ml/g. The inclusion of sulfite reduced the viscosity to 118 and 158 ml/g for 0.01 and 0.1% sulfite, respectively. The reduction in intrinsic viscosity expected by the presence of 0.01% sulfite is almost entirely prevented if the antioxidant propyl gallate is incorporated at a level of 0.05%. These results are consistent with previous measurements of the swelling volume of cassava starch (Mat Hashim *et al.*, 1992) if the decrease in swelling volume measured is attributed to depolymerisation of the starch polysaccharides. Rather similar changes in intrinsic viscosity have been measured for wheat and potato starches (Paterson *et al.*, 1996).

Viscosity of 5% pastes

The effect of sodium sulfite with and without propyl gallate

Figure 1 displays the shear rate dependence of the viscosity as a function of holding time at 60°C in the presence of 0.1% sodium sulfite. It can be seen that there is a large and continuous reduction in viscosity with time. Initially the pastes show substantial shear thinning but with ageing the behaviour becomes more Newtonian. In subsequent figures measurements are reported at a constant shear rate of 27 s^{-1}.

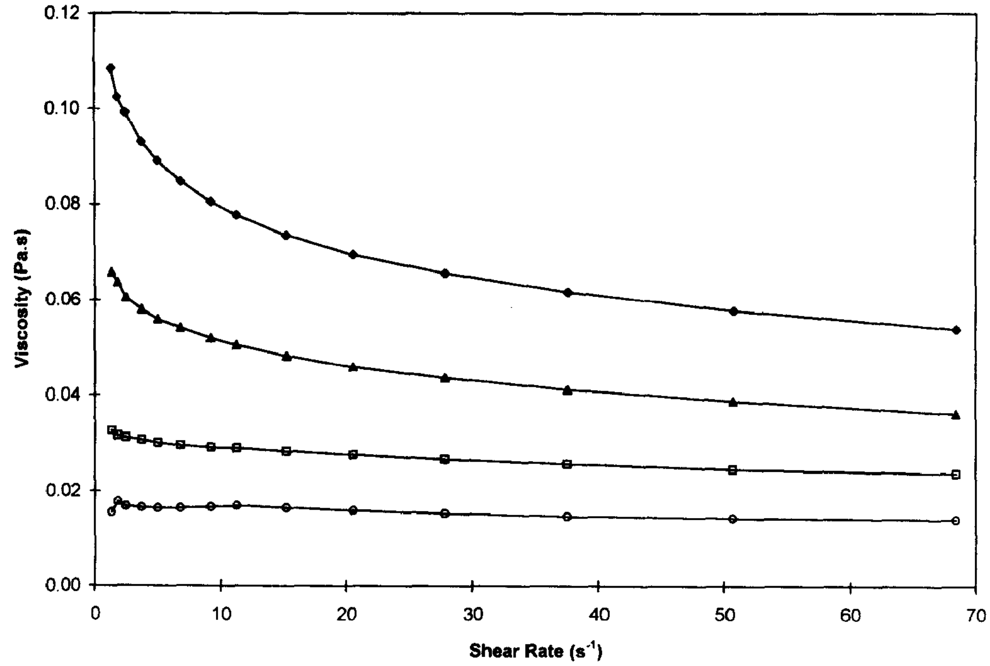

Fig. 1. The evolution of flow curves with time at 60°C in the presence of 0.1% sulfite: ◆, 0 h; ▲, 1.5 h; □, 4 h; ○, 7.5 h. Measurements were made on 5% cassava starch at pH 7.0.

Figure 2 shows the effect of sulfite inclusion with and without propyl gallate on the viscosity of the starch paste following ageing at 60°C. On inclusion of 0.1% sulfite the solution approaches a minimum viscosity of 0.013 Pa s. At 1% sulfite the decrease in viscosity is slower but more linear, reaching the same low viscosity after 10 h. The lower concentration of 0.01% sulfite induces a sharp initial fall in the viscosity, tailing off quickly to a final viscosity of around 0.035 Pa s. When 0.01% propyl gallate is incorporated with 0.1% sulfite, the viscosity drop exactly parallels the control with no additives. At the higher propyl gallate concentration of

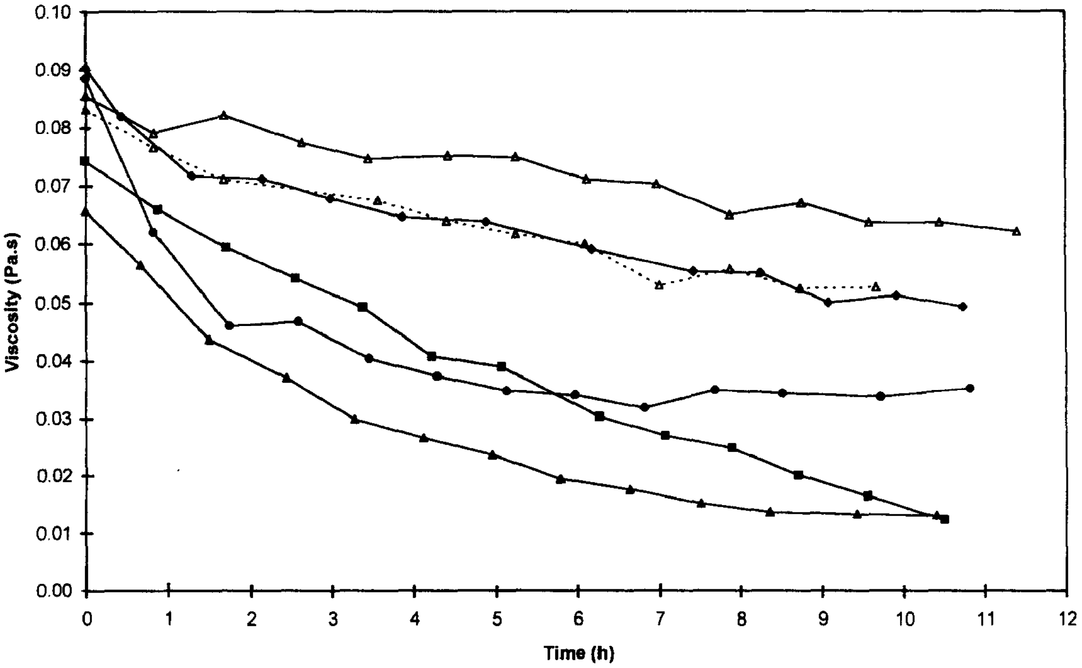

Fig. 2. The effect of added sodium sulfite with and without propyl gallate on the viscosity of cassava starch: ◆, no additives; •, 0.01% sulfite; ▲, 0.1% sulfite; ■, 1% sulfite; △ (solid line), 0.1% sulfite + 0.05% propyl gallate; △ (dashed line), 0.1% sulfite + 0.01% propyl gallate. Measurements made on 5% cassava starch at pH 7.0. Temperature is 60°C and shear rate 27 s^{-1}.

0.05% the viscosity of the starch sample in the presence of 0.1% sodium sulfite remains even higher than that of the control.

The effect of other salts

To show that the influence of sulfite was specific rather than a general ionic effect we repeated the experiment using sodium sulfate and sodium chloride (Fig. 3). Clearly, these salts have no effect on the viscosity of the pasted cassava starch. Another possible interpretation would be that sulfite activated an enzyme system, which caused polysaccharide degradation. For these purified starches it could be expected that the only enzymes which might radically degrade the starch would be amylases. To try and eliminate this possibility we added silver nitrate, which would be expected to interfere with the action of amylase (Burnett *et al.*, 1995). This had no influence on the viscosity change with time observed in the presence or absence of 0.1% sulfite (Fig. 4). Also shown in Fig. 4 is the influence of the chelating agent EDTA. EDTA incorporation substantially reduced the viscosity drop observed with sulfite alone.

The effect of temperature

In an effort to elucidate the effect of temperature, some of the sulfite experiments were repeated with holding at temperatures of 30 and 80°C (Fig. 5). The 30°C control shows almost no change in viscosity after holding for over 11 h. In contrast, on holding at 30°C with sulfite included there was a relatively large drop in viscosity, analogous to the drop observed at 60°C without sulfite. Remarkably, holding the pastes at 80°C degrades the polysaccharide almost as much as at 60°C with sulfite. Furthermore, with sulfite at 80°C the viscosity decrease is not much greater than observed at 60°C with sulfite.

The effect of other oxidants

The results displayed in Fig. 6 show that ascorbic acid is more effective than sulfite in lowering the viscosity of the cassava starch. When 0.01% ascorbate is incorporated the viscosity, reduction is not prevented by the addition of 0.05% *n*-propyl gallate. Little difference in the viscosity behaviour is observed for ascorbate additions in the range 0.01 to 1%. The temperature dependence (Fig. 7) is similar to that observed with sulfite. In contrast to ascorbate the influence of glutathione (Fig. 8) is relatively small although on storage at 60°C for long periods there is a significant effect on the starch viscosity.

DISCUSSION

Our previous studies have been confined to low concentrations of starch pasted at high temperatures. In our view the current work significantly advances our knowledge of the oxidative reductive depolymerisation of starch. In the first place the data in this paper clearly confirm that the previous observations could not be explained by a pH change on sulfite addition. Second, we had assumed that the sulfite-induced disruption of the starch granule was confined to high temperatures. This assumption should now be questioned since there is now

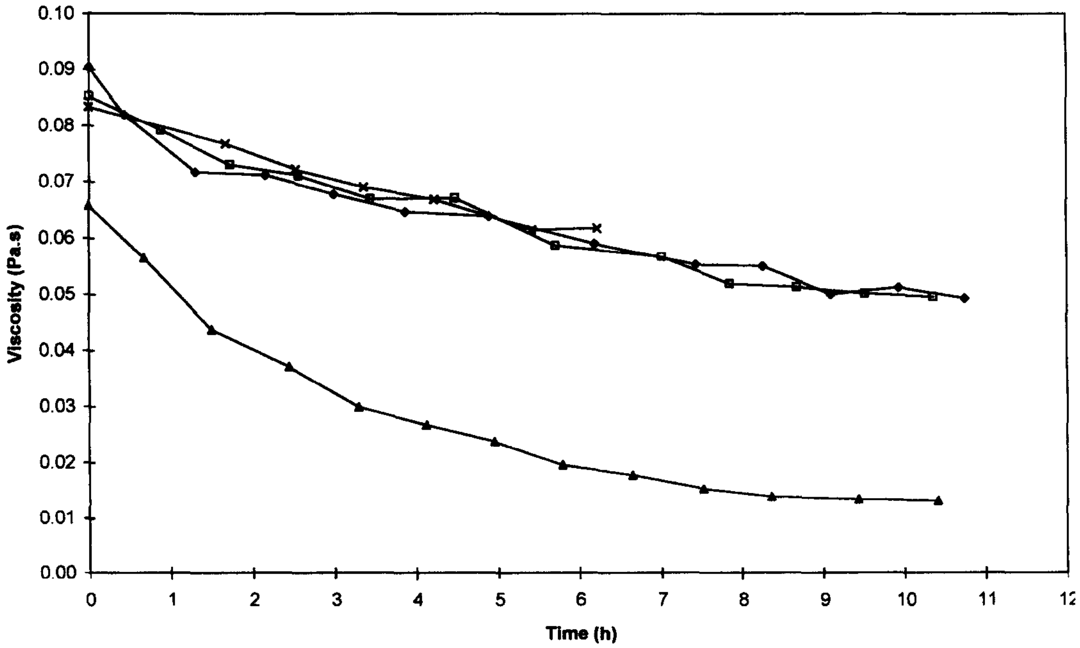

Fig. 3. The effect of salts on the viscosity of cassava starch: ◆, no additives; □, 0.1% sodium chloride; x, 0.1% sodium sulfate; ▲, 0.1% sulfite. Other details as for Fig. 2.

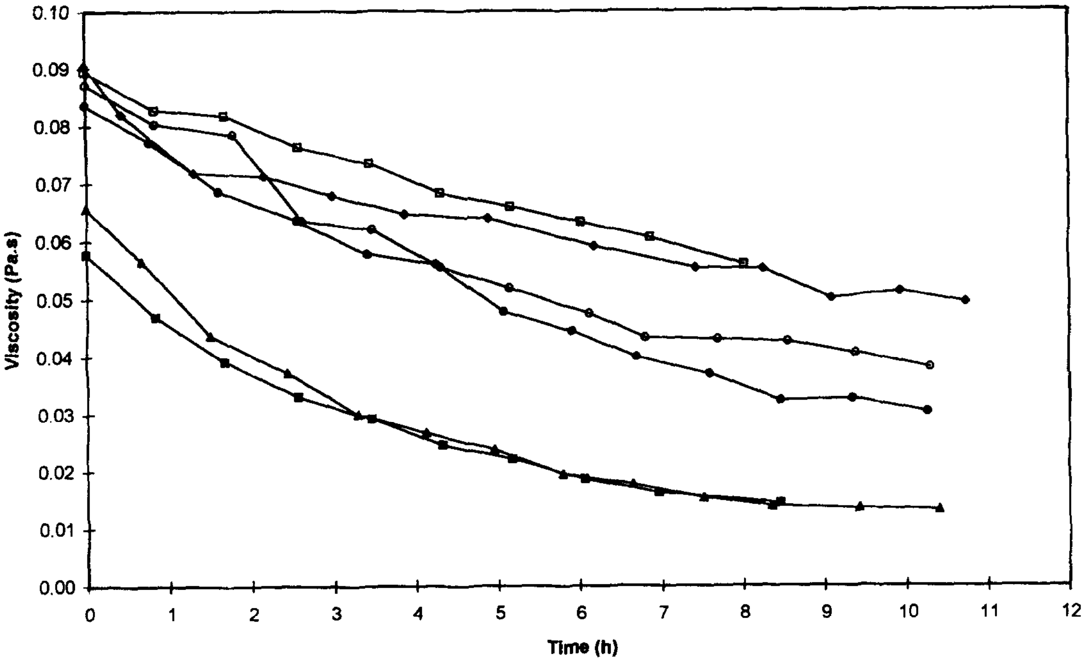

Fig. 4. The influence of EDTA and $AgNO_3$ on the sulfite-induced viscosity reduction: ◆, no additives; ▲, 0.1% sulfite; •, 0.01% EDTA + 0.1% sulfite; o, 0.05% EDTA + 0.1% sulfite; ■, 0.01% silver nitrate + 0.1% sulfite; □, 0.01% silver nitrate. Other details as for Fig. 2.

clear evidence that sulfite produces viscosity changes at temperatures as low as 30°C. Indeed it seems easier to observe the phenomena at 60°C than at 80°C since the viscosity results suggest that degradation occurs rapidly even in the absence of an oxidant at the higher temperatures. Third, the intrinsic viscosity data provide further evidence to support the view that it is the degradation of the polysaccharides which causes the changes in swelling volume previously described and an increase in the amount of polysaccharide detected outside the granular environment. Fourth, this work, when taken in conjunction with the intrinsic viscosity data, shows

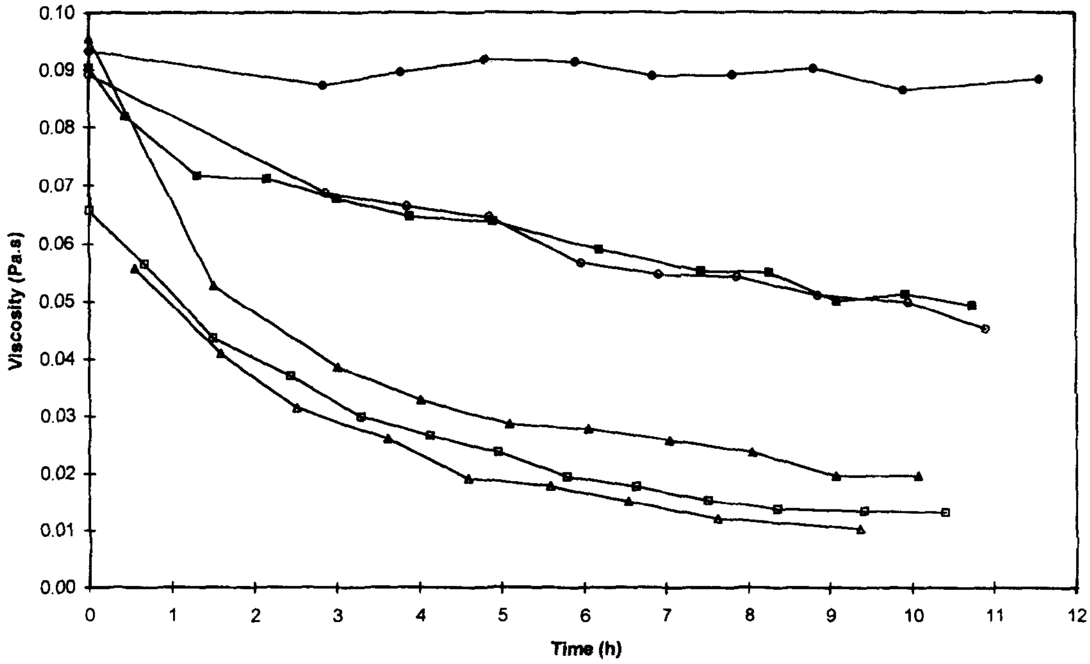

Fig. 5. The effect of holding temperature on the viscosity of cassava starch: •, no additives 30°C; o, 0.1% sulfite 30°C; ■, no additives 60°C; □, 0.1% sulfite 60°C; ▲, no additives 80°C; △, 0.1% sulfite 80°C. Other details as for Fig. 2.

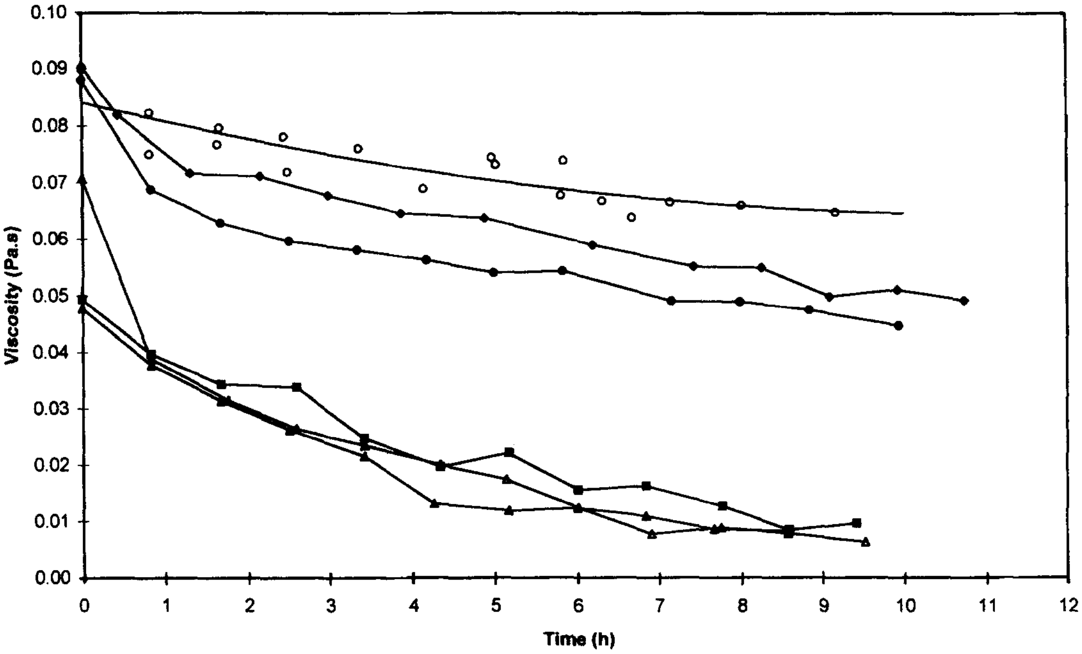

Fig. 6. The influence of added ascorbate with and without propyl gallate on the viscosity of cassava starch pastes: ◆, no additives; •, 0.001% ascorbate; ▲, 0.01% ascorbate; ■, 1% ascorbate; ○, 0.001% ascorbate + 0.05% propyl gallate; △, 0.01% ascorbate + 0.05% propyl gallate. Other details as for Fig. 2.

that the oxidants can degrade the starch polysaccharides whether or not they are within the granular environment since accompanying microscopic studies showed little evidence of intact structures. The shear treatment prior to additive addition apparently disrupted the swollen granular structure. Fifth, it is now clear that this is not an effect specific to sulfite but is observed with other oxidants, most dramatically with ascorbic acid although it can also be seen with glutathione addition.

It is well recognised that sulfite can exert a protective influence on the heat degradation of polysaccharides at higher temperatures through its oxygen scavenging role (Wellington, 1983). In our view the current work is of interest because it clearly reveals that at low levels

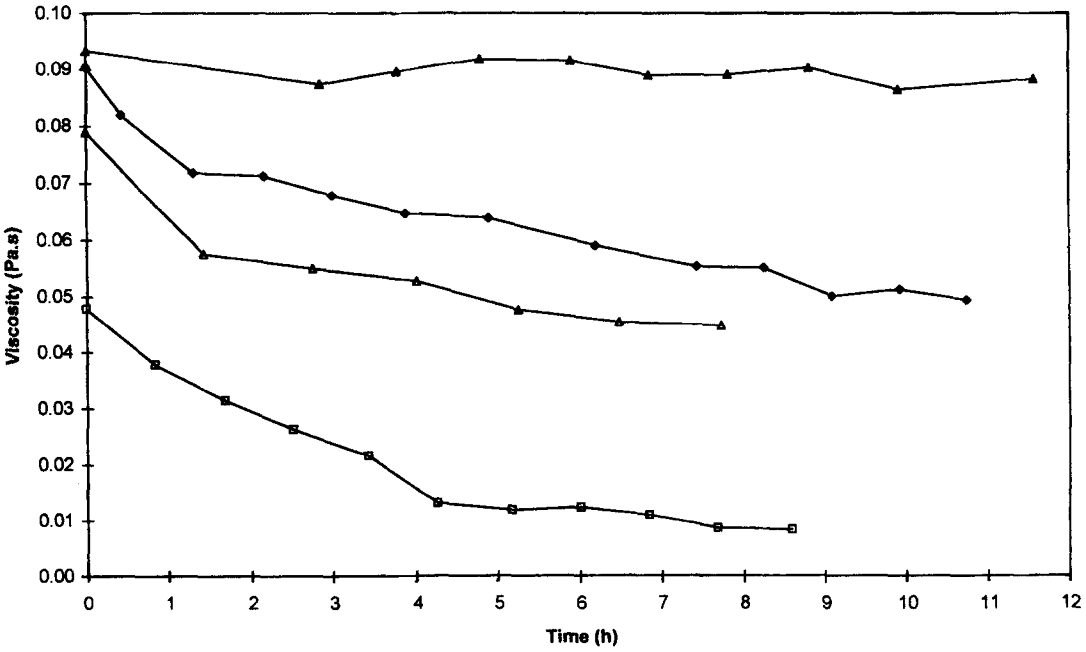

Fig. 7. The effect of holding temperature on cassava starch in the presence and absence of ascorbate: ▲, no additives 30°C; ◆, no additives 60°C; △, 0.01% ascorbate 30°C; □, 0.01% ascorbate 60°C. Other details as for Fig.2.

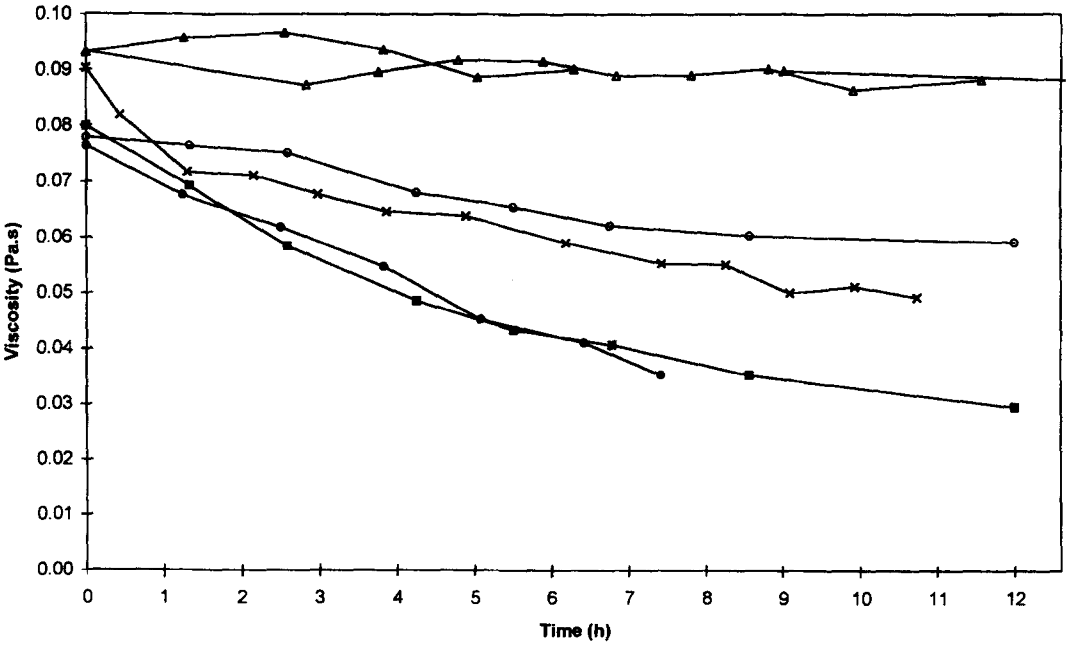

Fig. 8. The influence of added glutathione with and without propyl gallate on the viscosity of cassava starch at 60°C unless otherwise stated: x, no additives; •, 0.1% glutathione; ■, 1% glutathione; o, 0.1% glutathione + 0.05% propyl gallate; △, no additives at 30°C; ▲, 0.1% glutathione 30°C. Other details as for Fig. 2.

sulfite can act in an oxidative role rather than showing the generally accepted oxygen scavenging and reducing behaviour. A possible mechanism for its oxidative role through free radical products is shown in Scheme 1.

At higher sulfite levels there will not be sufficient free oxygen for the initiation stage to occur, which explains why the degradation rate decreases when the sulfite level is increased to 1% (Fig. 2). In the absence of oxidants there is a significant viscosity decrease on ageing at 60°C. This is reduced on addition of 0.1% sulfite plus 0.05% propyl gallate (Fig. 2). For this higher (5%) level of cassava starch, the level of sulfite addition giving maximum degradation appears to be greater than the 0.01% reported for the 1% starch pastes previously studied. This and the critical rapid drop in viscosity to a constant level observed with 0.01% sulfite in this work suggests that the radicals produced at this concentration are exhausted at higher levels of starch.

Initiation:

$$M^{n+} + O_2 \rightarrow M^{n+1+} + {}^{\bullet}O_2^-$$

Propagation:

$$SO_3^{2-} + {}^{\bullet}O_2^- + 3H^+ \rightarrow HSO_3^{\bullet} + 2\,{}^{\bullet}OH$$

$$SO_3^{2-} + {}^{\bullet}OH + 2H^+ \rightarrow HSO_3^{\bullet} + H_2O$$

$$HSO_3^{\bullet} + O_2 \rightarrow SO_3 + {}^{\bullet}O_2^- + H^+$$

Termination:

$$HSO_3^{\bullet} + {}^{\bullet}OH \rightarrow SO_3 + H_2O$$

$$HSO_3^{\bullet} + {}^{\bullet}OH \rightarrow SO_3 + H_2O$$

$$SO_3 + H_2O \rightarrow SO_4^{2-} + 2\,H^+$$

Scheme 1. A possible mechanism for the formation, propagation and termination of radicals with the potential to depolymerise starch.

Propyl gallate inhibits sulfite-induced degradation by forming a stable free radical, thereby blocking the propagation of the chain reaction. It would be expected that EDTA would chelate out transition metal ions and interfere with the initiation phase. Propyl gallate may also have a chelating role. Although ascorbate is known to act in both an oxygen scavenging role and as a prooxidant, in these experiments there is only evidence for the latter. The viscosity of the starch is almost independent of the ascorbate concentration in the range 0.01% to 1%. Why ascorbate should be more effective than sulfite in degrading starch is unclear. One can imagine, however, that this may be due to the more easily reducible hydroxyl groups of ascorbate. Electron spin resonance studies must be carried out to shed more light on this interesting problem.

Ascorbate is used extensively as an additive in baking where it has a role in increasing loaf volume. Its mechanism of action is not clearly understood but it is generally considered that it is involved in a redox reaction with wheat protein sulfhydryl groups (Fichett & Frazier, 1986; Grosch, 1986). In extrusion processing, increased starch conversion results in a greater degree of expansion and one interpretation is that extragranular polysaccharide is more effective at entangling and maintaining a coherent film at the surface of the expanding water vapour bubbles (Fan *et al.*, 1996). If oxidants can promote starch conversion a similar explanation might be used to partly explain the role of oxidants as bread improvers. Glutathione occurs naturally in wheat flours and is sometimes also added to the baking process and, although this does appear to have some oxidative effect on starch, we would not dispute the conventional view that its primary influence is on SH/SS interchange reactions with flour proteins (Grosch, 1986; Chen & Schofield, 1995). Of course, in order to relate these observations to what could happen during the bread baking process it is important to extend this work to wheat starch and this is currently being undertaken.

Although this study is concerned with the effect of additives it is noteworthy that starch alone at 60°C shows a viscosity decrease and this can be reduced by the addition of a mixture of sulfite and propyl gallate at appropriate levels. It is possible that over long storage times degradation of starch can occur by these mechanisms in the absence of added sulfite. This degradation rate could be strongly influenced by trace metal levels, dissolved oxygen, UV light and possibly other naturally occurring materials such as lipids. If proven this mechanism may explain variation in starch functionality from apparently similar materials.

Although the intrinsic viscosity data provide evidence that polysaccharide degradation is promoted, it is not clear to what extent this occurs and whether it is the amylopectin, the amylose component or both which is attacked. Chromatographic studies on whole starch and viscosity measurements on amylose and amylopectin are being undertaken to determine this and characterise the degradation process further.

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