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Carbohydrate Research 292 (1996) 143–151

CARBOHYDRATE
RESEARCH

Evidence for sulfite induced oxidative reductive depolymerisation of starch polysaccharides

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Received 25 March 1996; accepted 12 July 1996

Abstract

The influence of low levels of sodium sulfite on the viscosity of 0.90 g/100 mL dispersions of wheat and potato starch pasted at 95 °C has been studied. It is shown that, if pasting is carried out in 0.01 g/100 mL sulfite, the viscosity of the starch following solubilisation in 0.5 M KOH is lower than a control pasted in the absence of sulfite. The starch intrinsic viscosity is also reduced after pasting in sulfite, indicating that some polysaccharide degradation has occurred. It is suggested that this is the reason for the increased release of polysaccharide from the starch granule when pasted in the presence of sulfite. For both starches the intrinsic viscosities show that there is a reduction in degradation when the sulfite level is increased or when the polar antioxidant propyl gallate is included in addition to sulfite indicating that the mechanism is oxidative reductive depolymerisation of the starch polysaccharides. It is also suggested that oxidative degradation may have an important influence on the integrity of the starch granule following pasting and may be a factor in the functional behaviour of oxidising agents in baked products. Low levels of sodium sulfite and sodium chloride both dramatically reduce the viscosity of potato starch as a result of a non-specific ionic effect. © 1996 Elsevier Science Ltd.

Keywords: Starch; Sulfite; Oxidative reductive depolymerisation

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PII S0008-6215(96)00186-3

1. Introduction

The main constituents of starch are the two polysaccharides amylose and amylopectin. The former is a predominantly linear polymer of α-(1 → 4)-linked glucose whereas amylopectin is a highly branched polysaccharide consisting of α-(1 → 4)-linked glucose with α-(1 → 6) linkages at the branch points. Typically starches contain about 70–80% amylopectin and 20–30% amylose. The starch granule is built up of a series of concentric growth rings. These have alternating amorphous and crystalline characteristics. In the crystalline growth ring, the short amylopectin chains, which have degrees of polymerisation of 15–20 glucose units, are associated into double helices. These double helical regions show a periodicity in the radial direction and this ordering results in the granule displaying crystallinity when examined by X-ray diffraction, and birefringence when viewed under a polarising microscope [1,2].

When the starch granule is heated in the presence of water, the double helical and hence the crystalline order is lost and the granule will swell. This swelling is accompanied by a loss of polysaccharide, particularly amylose, from the granule structure. At high temperatures and under conditions of high shear, the granule integrity is lost [3–5]. The extent of swelling and the amount of polysaccharide released by the granule following the initial loss of order is of substantial significance. It will govern the rheology of starch pastes and thus determines the performance of starch when it is used as a thickener [6]. It will also influence the structure of baked products.

Recently, we reported that the addition of low levels of sodium sulfite can influence the degree of swelling of the starch granule pasted at high temperatures. The most dramatic effects were seen with cassava, sago, and potato starch [7,8]. If these starches were pasted at 95 °C in the presence of 0.01 g/100 mL sulfite, the swollen volume as determined by the volume occupied by the pellet following centrifugation decreased to less than 25% of the value found in the absence of sulfite. For both tuber and cereal (wheat, maize, and rice) starches the presence of these low levels of sulfite substantially increased the amount of material found in the supernatant. These observations would suggest that sulfite promotes granule disintegration. Two aspects of these results were of particular interest. Firstly, with the exception of potato starch, it was observed that there was a concentration at which the influence of sulfite was a maximum. Secondly it was found, again with the exception of potato starch, that the sulfite effect was substantially reduced when low levels of the polar antioxidant, propyl gallate, were included in the system [7,8]. This latter observation resulted in the suggestion that sulfite acted to promote a free radical attack associated with the formation of oxygen and hydroxyl radicals and resulted in the breakage of bonds within the polymer chain. This degradation mechanism is known as oxidative reductive depolymerisation (ORD). If starch granule integrity following pasting is the result of interactions, presumably predominantly entanglements, between the high molecular weight amylopectin molecules it is easy to understand why a molecular weight reduction should result in a greater tendency for granule disintegration.

The objective of the work described in this paper was to determine if the postulated molecular weight reduction of starch granule polysaccharides in the presence of sulfite does indeed occur. To obtain information on molecular weight changes we have measured the viscosity of gelatinised starches after solubilisation in KOH.

2. Materials and methods

Materials.—Wheat and potato starches were purchased from Sigma. Sodium sulfite, sodium chloride, and propyl gallate were of analytical grade. Double distilled water was used throughout.

Methods.—1% (w/v) starch suspensions were prepared by dispersing 1.0 g of starch in 100 mL of distilled water containing various levels of sodium sulfite with or without propyl gallate. To ensure that homogeneous dispersions were obtained, the samples were agitated with a magnetic stirrer in a water bath at 95 °C until gelatinization occurred which took 2 and 3 min.

Completion of gelatinization was indicated by an increase in clarity of the starch pastes. The samples were then further heated in the water bath at 95 °C for 60 min without any agitation. After pasting, the samples were cooled to room temperature and divided into two. 50 mL was used to obtain the solubilized samples. Viscosity measurements were made on a sample of the remainder within two hours of ambient temperature being attained. To allow direct comparison with the solubilised starch, suspensions were diluted to a concentration of 0.9 g starch/100 mL (0.9%) with distilled water.

Solubilisation of starch suspensions.—The 1% pasted starch suspensions were solubilised by the addition of 5 M KOH to achieve a 0.9% solution in 0.5 M KOH. The samples were mechanically stirred overnight and a clear solution was then visible. Tests indicated that sample viscosity did not alter when left in 0.5 M KOH for between 8 and 24 h.

Rheological measurements.—The viscosity of the starch suspension was measured on a Bohlin Controlled Stress Rheometer (Model CS 10) using a double gap concentric cylinder measuring geometry (DG 40/50) over the increasing shear rate range 1 to 100 s^{-1}. Owing to its large surface area, this particular measuring geometry is most suitable for measuring low viscosity fluids. The samples were equilibrated at 25.0 ± 0.1 °C for 10 min on the rheometer before the first measurement was taken.

Where non-Newtonian behaviour was observed the flow curve data obtained was fitted to the power law equation:

$$\eta = k\dot{\gamma}^{n-1}, \tag{1}$$

where η is the viscosity, $\dot{\gamma}$ the shear rate, and k and n are constants: termed the consistency index and flow behaviour index respectively.

Intrinsic viscosities were obtained from measurements made at a range of concentrations (1–5 mg/mL) on the solubilised starch in 0.5 M KOH. Measurements were made at a temperature of 25.0 ± 0.05 °C using a 2 mL capacity Ostwald type capillary viscometer in a Schött–Geräte automated measuring system. For each concentration 10 flow times were recorded and the results are the mean of measurements made using two stock solutions prepared on different days. The intrinsic viscosity was obtained using the Huggin's extrapolation.

3. Results and discussion

Wheat starch.—Table 1 displays the intrinsic viscosity for the starches measured after gelatinisation and solubilisation in 0.5 M KOH. A significant reduction in viscosity is seen when pasting was carried out in 0.01 g/100 mL sulfite, as compared to a control in the absence of sulfite. The inclusion of 0.005 g/100 mL propyl gallate in the system containing 0.01 g/100 mL sulfite prevented the apparent degradation which occurred when sulfite alone was present. When the sulfite level increased to 0.1 g/100 mL, the intrinsic viscosity was higher than that observed for the system containing 0.01 g/100 mL sulfite.

Fig. 1A displays the viscosity (measured at a shear rate of 20 s^{-1}) of the solubilised wheat starch in 0.5 M KOH and the starch suspension in water as a function of sulfite concentration. In water the suspension showed Newtonian behaviour and gave a viscosity of 0.0017 Pa, which is about twice the value expected for water alone. There was no evidence for any dependence on the level of added sulfite. When the starch was solubilised in KOH the viscosity was significantly higher and slight non-Newtonian behaviour was observed. A significant decrease in viscosity was observed with increasing sulfite content, with evidence for a shallow minimum at a concentration of 0.01 g/100 mL. Inclusion of 0.005 g/100 mL propyl gallate substantially prevented the viscosity reduction caused by sulfite addition. When the data was fitted to eq (1), the exponent n varied in the range 0.89 to 0.94 (Table 2). The conclusions regarding the effect of sulfite would be independent of the shear rate at which the data is presented.

Potato starch.—The intrinsic viscosities of solubilised potato starch after pasting in sodium sulfite, sulfite plus propyl gallate, and sodium chloride are shown in Table 1.

Sodium chloride has no measurable effect on the intrinsic viscosity, but as with wheat starch this is reduced when low levels of sodium sulfite are included and recovers on

Table 1
Intrinsic viscosity (mL/g) of wheat and potato starch pasted in the absence and presence of additives at 95 °C for 60 min. Subsequently the samples were dissolved overnight in 0.5 M KOH. Additional dilutions were made in KOH and intrinsic viscosities determined

Additive conc. (g/100 mL)	Starch		
	Wheat	Potato	
	sodium sulfite	sodium sulfite	sodium chloride
0.00	137±5	224±4	224±4
0.01	94±4	182±4	226±4
0.10	113±5	193±4	223±4
0.01 + 0.005 propyl gallate	132±5	220±4	n.d.

n.d. = not determined.

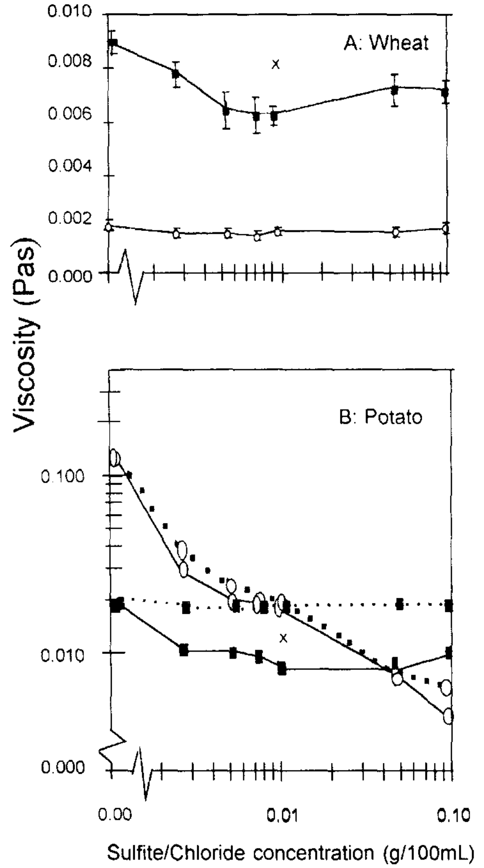

Fig. 1. Viscosity of starch samples ((A) wheat, (B) potato) at concentrations of 0.9 g starch/100 mL solvent at a temperature of 25.0 ± 0.2 °C and a shear rate of 20 s^{-1}. Starch suspensions (○) and starch solubilised in 0.5 M KOH (■) after pasting in sodium sulfite (————) or in sodium chloride (- - -). Starch in KOH and originally pasted in 0.01 g/100 mL sodium sulfite and 0.005 g/100 mL propyl gallate is denoted by X. Standard deviation is noted in (A).

addition of propyl gallate or higher levels of sulfite. This behaviour is qualitatively similar to that observed with wheat starch.

Fig. 1B displays the viscosity of potato starch suspensions in water at 20 s^{-1} as a function of the level of added sulfite or chloride. The viscosity is substantially higher than that observed for wheat starch and in contrast to wheat starch the addition of sulfite has a large influence on the viscosity. A similar dramatic reduction in viscosity is also shown on chloride addition. These potato starch suspensions show pronounced non-Newtonian behaviour in the absence of electrolyte when the exponent n in eq (1) was 0.50 (Table 2). This parameter increases when low levels of either sulfite or chloride are added. For all the systems, the flow behaviour indices show the expected negative correlation with the measured viscosities. The higher the viscosity, the greater the departure from Newtonian behaviour. It has previously been reported that chloride

Table 2
Exponent n in power law equation describing flow behaviour of starch systems prepared by pasting at 90 °C for 60 min. The concentration of starch in all cases was 0.9 g starch/100 mL solution (the estimated error for n is ± 0.02)

Conc. (g/100 mL)	Starch				
	Wheat	Potato			
	sodium sulfite	sodium sulfite		sodium chloride	
	0.5 M KOH	water	0.5 M KOH	water	0.5 M KOH
0.00	0.91	0.50	0.77	0.50	0.77
0.025	0.90	0.71	0.85	0.75	0.78
0.050	0.90	0.77	0.87	0.82	0.78
0.075	0.94	0.80	0.87	0.85	0.78
0.100	0.90	0.84	0.89	0.82	0.78
0.500	0.91	0.87	0.83	0.88	0.77
1.000	0.89	0.93	0.82	0.87	0.77

addition results in a large reduction in the viscoelastic parameters of potato starch suspensions. In contrast, cassava starch was unaffected [9].

The influence of sodium sulfite and sodium chloride on the viscosity of potato starch solubilised in 0.5 M KOH and measured at 20 s^{-1} is shown in Fig. 1B. In contrast to the behaviour of the suspension in water, it can be seen that sodium sulfite has a significant viscosity reducing effect whereas sodium chloride does not. The flow behaviour index is unaffected by chloride addition whereas it is increased by the addition of sulfite. Since the viscosity of the solubilised starch is related to the molecular weight, this supports the conclusion that can be drawn from the intrinsic viscosity data: that sulfite degrades the polysaccharide whereas sodium chloride does not. In contrast however to what might be inferred from the intrinsic viscosity data, propyl gallate inclusion does not prevent the viscosity reduction induced by the inclusion of 0.01% sulfite nor is there a substantial recovery in viscosity seen when the sulfite level rises to 0.1%. This may be because the viscosity of the 0.9% solutions is influenced by intermolecular interactions as well as the molecular weight.

Starch pastes in water.—The dilute starch pastes in water can be regarded as suspensions of swollen granules [10,11]. Their viscosity can then be described by an equation of the form

$$\eta = \eta_0 f(\phi) \tag{2}$$

where η is the viscosity of the solution, η_0 the viscosity of the continuous phase, and ϕ the volume fraction occupied by the swollen granules. ϕ is equal to cS, where S is the volume occupied by the swollen starch granule, and c is the starch concentration [12]. The viscosity of the potato starch suspensions is higher than the solubilised systems whereas the reverse is found for wheat starches. This, and the far higher viscosities found for potato starch compared with wheat starch, can be interpreted in terms of the greater volume fraction occupied by the swollen potato starch granules. In the absence of electrolytes, S has been determined to be 127 mL/g when pasted under these conditions, whereas in the presence of 0.01 g/100 mL sulfite it had decreased to 33 mL/g [8]. This is still substantially higher than the value of 13 mL/g found for wheat starch, which, in contrast to potato starch, was unchanged by the inclusion of low concentrations of electrolyte [8]. The value of ϕ when starch granules close pack has been reported [10] to be in the range 0.35–0.55. As this value is approached the viscosity will be a very strong function of ϕ. Thus, for potato starch, the volume fraction of the granules will dominate the viscosity, whereas for wheat starch the polysaccharide in solution outside the granular phase will be important, since for wheat starch ϕ is much lower than the close packing concentration. The large decrease in viscosity on addition of sulfite to the potato starch suspensions can easily be interpreted in terms of the decrease in the volume occupied by the swollen granule previously reported. The fact that similar results are found for sodium chloride suggests that this is a manifestation of a general ionic effect and not primarily related to molecular degradation. The unique behaviour of potato starch is almost certainly due to the presence of a significant level of phosphate groups on the potato amylopectin [13]. The potato starch granule behaves like a swollen polyelectrolyte gel presumably maintained primarily by entanglements between the highly branched amylopectin.

Solubilised systems.—The rheology of the systems solubilised in KOH should be interpreted in terms of the properties of polymer solutions rather than suspensions. If the starch polysaccharides are completely solubilised, then the viscosity would be expected to be a function of the coil overlap parameter $[\eta]c$ where $[\eta]$ is the intrinsic viscosity and c the concentration [14]. The data shown in Table 1 shows that there has been a significant reduction in intrinsic viscosity when sulfite is included in the solution which is nullified by the inclusion of the polar antioxidant propyl gallate. This data would thus support our previous hypothesis that the reason why low levels of sulfite increase the amount of polysaccharide released from the granule on pasting is related to a decrease in polysaccharide molecular weight due to oxidative reductive depolymerisation reactions. It should be appreciated that although the changes in intrinsic viscosity are fairly modest this could reflect a substantial amount of damage to the polysaccharide since the exponent in the Mark–Houwink equation for a highly branched polymer such as amylopectin would be expected to be low [3].

Factors determining the swelling and solubilisation of starches.—If the swollen starch granule is considered as a polymer gel, its integrity will be determined by two types of interaction between the polymer chains: non-specific entanglements, and microcrystalline regions involving interactions between ordered parts of the polysaccharide chain. During the gelatinization event amylopectin double helices will be melted out although amylose lipid complexes will not melt until temperatures in excess of 100 °C are reached. It is therefore not surprising that a negative correlation has been reported between the amount of bound lipid and the extent to which the starch granule swells [5,15]. Where the starch polysaccharides are charged (which will be the case for potato starch where the amylopectin is phosphorylated), polyelectrolyte effects will also be important in determining the gel volume. This is seen clearly in the dramatic effects of electrolytes on the swollen volume and hence the rheology of potato starch. Amylopectin is a polymer with a massive molecular weight (estimates as high as 6×10^7 Da have been given [16]) and is highly branched. Both these factors will result in entanglements between amylopectin molecules with very long life times. It would therefore be expected that the predominantly linear, lower molecular weight amylose will find it far easier to reptate out of the swollen granule as is observed.

Movement of molecules within polymer melts have been studied and the self diffusion coefficient of a bulk polymer reptating through an entangled melt was found to be proportional to $1/M^2$ where M is the molecular weight [17,18]. If these ideas can be applied to the starch granule it is not surprising that a reduction in molecular weight, which appears to be promoted by sulfite addition, results in more material leaving the granule. For low lipid starches such as cassava and sago starch this results in a complete loss of granule integrity even when pasted at 95 °C. For cereal starches at these pasting temperatures, the molecular weight reduction is evidenced by a substantial increase in the amount of material detected outside the granule [8]. For these starches a change in swollen volume as a result of sulfite addition is only seen at autoclaving temperatures where the polysaccharide lipid complexes will be melted.

This leaves the question as to why sulfite addition should cause a molecular weight reduction. We have previously postulated that interactions between the sulfite and dissolved oxygen results in the formation of superoxide radicals [7]. At higher concentrations sulfite will scavenge oxygen and this effect will be reduced, explaining the recovery in the intrinsic viscosity. Some support for this comes from the observation that in the case of cassava starch, loss of the sulfite induced granule disruption is not seen when the oxygen level is kept high by bubbling oxygen gas through the solution [8]. A question that we cannot answer at present is why starches appear to be degraded by sulfite whereas sulfite is generally recognised to protect other polysaccharides. This type of radical induced degradation is complex and can be strongly influenced by the presence of very small amounts of material. Oxidising agents are extensively used as bread improvers and sulfite in particular is used at low levels to reduce the elastic properties of biscuit doughs [19]. The conventional understanding is that they act entirely on the protein component [19,20]. The work reported here suggests that the integrity of the wheat starch granule may be influenced by the oxidative environment, so it seems possible that the oxidising agents could also have an effect on the starch component.

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