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Reactivity of sulphite during the dehydration of a glucose–glycine–sulphite–agar-microcrystalline cellulose gel

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This paper considers the extent to which sulphite species, S(IV), react with intermediates in the glucose–glycine reaction, when a gel matrix consisting initially (on a wet weight basis) of cellulose (22% w/w), agar (1% w/w), glycine (0.19% w/w), and variable amounts of glucose (0.14–9.0% w/w) and sodium disulphite $Na_2S_2O_5$ (0.012–0.24% w/w) is dehydrated in air at 80°C. The amount of S(IV) which undergoes reaction increases with S(IV) and glucose concentration when (on a dry weight basis) S(IV) is 2 g SO_2/kg and glucose is $<10\%$ (w/w). At higher concentrations of S(IV) and glucose, the amount of S(IV) which reacts is independent of the concentration of these reactants. It is suggested that high concentrations of glucose give rise to an increase in the viscosity of the medium thereby causing the extent of reaction to be limited by the rate of molecular diffusion. On the other hand, specific interactions involving S(IV) may be responsible for its concentration behaviour.

INTRODUCTION

The inhibition of Maillard browning in mixtures of glucose and glycine by sulphite species, S(IV), is accompanied by the irreversible binding of S(IV), i.e. it becomes unavailable when analysed by the conventional Monier-Williams distillation procedure (Monier-Williams, 1927; McWeeny *et al.*, 1974). The kinetics of the loss of S(IV) in homogeneous aqueous systems reveal that two consecutive reactions are involved, as illustrated in Fig. 1 (Wedzicha & Vakalis, 1988). The first step consists of two parallel reactions, one of which is of first order with respect to S(IV) and is presumed to be catalysed by sulphite ion at the Amadori rearrangement. This step gives rise (Wedzicha & Garner, 1991) to 3-deoxyhexosulose, DH, which is subsequently converted to another intermediate (**I**). The possible identity of this intermediate is 3,4-dideoxyhexosulos-3-ene, DDH, formed by loss of a water molecule from DH. The final product, 3,4-dideoxy-4-sulphohexosulose, DSH, is the result of a nucleophilic reaction of sulphite ion with DDH (McWeeny *et al.*, 1974).

In homogeneous aqueous systems, the rate of conversion of S(IV) to DSH is controlled by the reactant concentration (Wedzicha & Vakalis, 1988; Bellion & Wedzicha, 1993). However, when S(IV)-treated vegetables are dehydrated in air at 80°C, the fraction of S(IV) lost is independent of the type of vegetable used or the amount of S(IV) in the final product when present in the following amounts (mg SO_2/kg dry wt): Brussels sprouts, 800–2000; cabbage, 260–1900; carrot, 270–1300; peas, 250; potato, 32–220 (Wedzicha & Adamu, 1987). In the case of potato dehydration, measurement of the yield of sulphonate product suggests that the major reaction of S(IV) in this food is the result of its inhibition of Maillard and ascorbic acid browning (Wedzicha, 1987). Similarly, chromatographic investigations of the sulphonates present in dehydrated S(IV)-treated cabbage show that the products are the same as those formed in the glucose–glycine–S(IV) reaction (Wedzicha *et al.*, 1984). One might expect the reactivity of S(IV) in all the vegetable dehydrations to be the result of the inhibition of nonenzymic browning reactions. However, the compositions of these vegetables with respect to reducing sugars and amino compounds differ markedly (Wedzicha & Adamu, 1987) and it is possible that the reactivity of S(IV) during food dehydration is not controlled by such composition but by physical factors such as reactant or water transport behaviour (Wedzicha, 1987).

The purpose of this investigation is to consider the contribution of the solid matrix to the extent of reaction of S(IV) in a model dehydration involving a cellulose support in an agar gel containing glucose and glycine. This model is based on one used by Schrader and Litchfield (1992) to study moisture transport in such systems and is currently being evaluated (Gogus *et*

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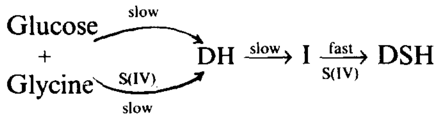

Fig. 1. Kinetic model of the reaction of S(IV) in mixtures of glucose, glycine and S(IV). DH is 3-deoxyhexosulose, DSH is 3,4-dideoxy-4-sulphohexosulose and I is an unspecified intermediate.

al., unpublished) for its solute transport properties during dehydration.

MATERIALS AND METHODS

Agar, microcrystalline cellulose (MCC) and water were heated in a boiling water bath for 1 h, and an aliquot of the reaction mixture containing glucose, glycine and S(IV) was added. The mixture was made up to a known weight so that it contained (on a wet weight basis) 22% (w/w) MCC and 1% (w/w) agar, rapidly poured into a mould and allowed to set for 15 h. Pieces of gel (2 cm × 2 cm × 8 mm) were cut, placed in a fluidised bed dryer (80°C, 0.7–1.1 m/s) and dried to constant weight. The amount of S(IV) present in the gels before and after dehydration was measured by the method of Wedzicha and Bindra (1980). Amounts were expressed in terms of the weight of SO_2 per kg (dry weight) of the material. The dry matter content was obtained from the initial composition of the gels. The S(IV) content of dehydrated gels was also measured by dispersing the sample in water, adjusting to pH > 10 (1 M NaOH), adding acid (2.5 M H_2SO_4) to give pH 2, and titrating with iodine (McWeeny *et al.*, 1969).

RESULTS AND DISCUSSION

In all experiments reported, the dehydrated sulphited gels did not undergo significant browning and maintained a constant geometry during the dehydration process. The maintenance of shape is largely a property of the cellulose component which behaved as a rigid filler for the gel; similar gels prepared using agar alone (5% w/w, wet weight basis) shrank anisotropically.

The measurement of the extent to which S(IV) undergoes reaction during the model dehydration experiment requires that the total amount of S(IV) be measured. In a mixture of glucose, glycine and S(IV) a significant proportion of the S(IV) is present in a reversibly bound form as hydroxysulphonate ($C(OH)SO_3^-$) adducts, a result of the addition of HSO_3^- to carbonyl groups. To obtain the total S(IV) content it is necessary to decompose any such adducts. This is accomplished by raising the pH (>10) of aqueous dispersions of the solid sample to be analysed prior to iodimetric titration of the S(IV) content (McWeeny *et al.*, 1969), or during the prolonged boiling in acid recommended in the Monier-Williams distillation procedure (Monier-Williams, 1927) and still regarded as the reference method for S(IV) determination in food. The method of analysis used in the present investigation was that of Wedzicha and Bindra (1980), which has the advantage of high sensitivity because it couples the effectiveness of a distillation procedure for separating SO_2 from other components, with a spectrophotometric measurement of SO_2 in the distillate; good results have been reported for the analysis of dehydrated vegetables in comparison with the conventional Monier-Williams method. However, the procedure uses a relatively short distillation time of 5 to 10 min and it is important to show that this is sufficient to allow decomposition of any hydroxysulphonates present. In the case of agar–MCC gels containing initially 0.5 M glucose and 11 mM S(IV), the S(IV) contents of individual gel pieces after dehydration were 1165 ± 23 mg SO_2/kg (n=4) in comparison with 1134 and 1116 mg SO_2/kg obtained when duplicate measurements, each using five gel pieces, were made by the iodimetric procedure. These samples represent the highest glucose concentrations used in the experiments reported here, and the agreement between the two procedures is good. The relatively small standard deviation (*c.* 2% of the mean value) of the data obtained by the distillation procedure indicates that the dehydration operation leads to reproducible levels of S(IV) in the final product.

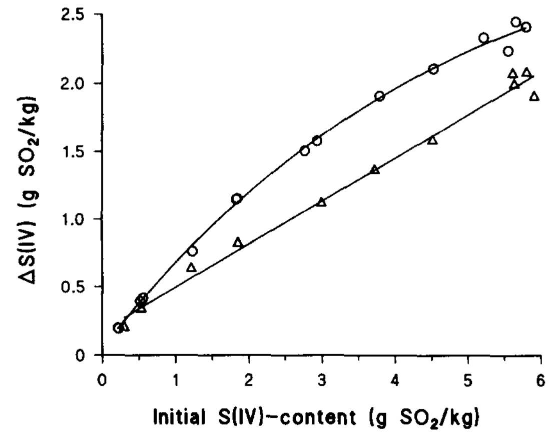

Fig. 2. Effect of initial S(IV) content of agar–cellulose gels on the change in S(IV) content (ΔS(IV)) when the gels are dehydrated in air at 80°C. All amounts are expressed per dry weight of gel. Initial gel composition (wet weight basis): agar (1%, w/w); MCC (22%, w/w); glucose (0.9%, w/w); $Na_2S_2O_5$ (0.012–0.24%, w/w); glycine (zero △; 0.19%, w/w ○).

In all experiments except those with the highest glucose content (*c.* 290 g/kg glucose) the drying time was 3 h; gels with the highest glucose contents required 4 h to reach constant weight.

Figure 2 shows the relationship between the amount of S(IV) lost during dehydration and the amount present initially in the gel. The linear relationship observed in the absence of glycine is surprising and suggests that there is either significant irreversible binding of S(IV) to glucose in the absence of glycine in such a dehydration, or the loss of S(IV) is the result of autoxidation or some physical process. However, the reactivity is increased on the addition of glycine and it is assumed here that the difference between the extent

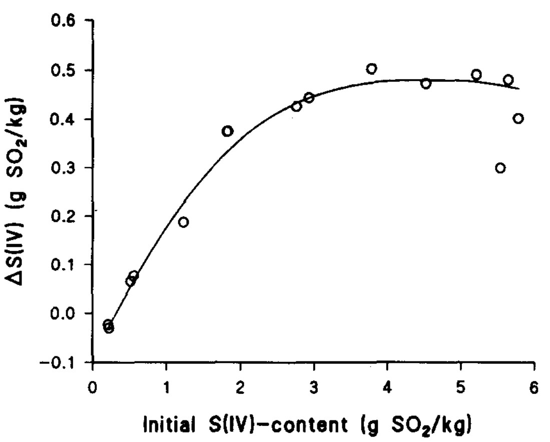

Fig. 3. Effect of initial S(IV) content of agar–cellulose gels on the difference in the amount of S(IV) (ΔS(IV)) which has undergone reaction when the gels are dehydrated in air at 80°C with and without glycine. All amounts are expressed per dry weight of gel. Initial gel composition (wet weight basis): agar (1%, w/w); MCC (22%, w/w); glucose (0.9%, w/w); $Na_2S_2O_5$ (0.012–0.24%, w/w); glycine (zero or 0.19%, w/w). The difference in S(IV) content is obtained by subtracting the change in S(IV) content, measured when gels containing glycine were dehydrated, from that observed when gels contained no glycine.

of reaction in the absence and presence of glycine represents the contribution of the Maillard reaction to the reactivity of S(IV) in the system. This difference is plotted in Fig. 3.

At low concentrations (< 1 g SO_2/kg) the slope of the graph of the amount of S(IV) reacted versus the initial amount present (Fig. 3) is *c.* 0.3, suggesting that the conversion of S(IV) to reaction products arising from the inhibition of Maillard browning is *c.* 30%. This may be compared with the 50% conversion of S(IV) to sulphonates during the dehydration of potato (Hererra-Viloria, 1984; Wedzicha, 1987). The observation that the graph shown in Fig. 3 passes close to the origin suggests that the rates of the major reactions of S(IV) with glucose–glycine during dehydration probably depend on the concentration of S(IV) and the extent of the S(IV)-independent reaction is insignificant in comparison with that of the S(IV)-dependent reaction. This is in contrast to the behaviour of the glucose–glycine–S(IV) reaction (e.g. at concentrations [glucose] = 0.2–2.0 M, [glycine] = 0.1–1.0 M and [S(IV)] = 0.01–0.1 M (Bellion, 1992)) in homogeneous solutions where S(IV)-dependent *and* S(IV)-independent mechanisms are observed. The initial concentrations of glucose and glycine (based on the water content of the gels) were 52 and 26 mM, respectively, and S(IV) concentrations were in the range 0.8–22 mM. The final concentrations are expected to be those at saturation, and the concentrations of the reaction mixtures within the gels therefore pass through the range previously investigated in homogeneous model systems.

Measurements of the rate of reaction of S(IV) in glucose–glycine–water–glycerol mixtures indicates that the effect of water content or water activity, a_w, on the S(IV)-independent and S(IV)-dependent reactions is different; the rate of the S(IV)-dependent reaction increases much more rapidly as the water content is initially decreased from 100 to 40% (a_w = 1.0–0.7), whilst the rate of the S(IV)-independent reaction is much more sensitive to water contents below 40%. Whereas experiments in glycerol cannot be directly related to the effects of a_w in solid matrices, because glycerol also shows solvent behaviour (Eichner & Karel, 1972), the results indicate a complex interplay between the compositional variables and the overall rate of reaction of S(IV). In the case of the dehydration of the gel, the S(IV)-dependent reactions could include the known catalysis of the formation of DH by S(IV).

The tendency for the graph shown in Fig. 3 to level off at high S(IV) contents is indicative of a possible change in mechanism. The molar ratio of glucose to S(IV) varies from 64:1 to 2:1 as the amount of S(IV) is increased; the extent of reaction is independent of concentration when the ratio is in the range 5:1 to 2:1. If, in the first instance, we assume that the transport behaviour of reactants is relatively unaffected by S(IV) concentration in the range studied, then the effect of S(IV) concentration on its reactivity could be the result of specific interactions involving S(IV) species.

The simplest discussion considers an application of the law of mass action to equilibria such as those for the formation of hydroxysulphonates or ion-pair interactions involving S(IV) oxoanions with their counterions (Wedzicha & Goddard, 1991). In all such reactions the position of the equilibrium is displaced to the side of products with concentration. These reactions will serve to oppose the enhancing effect of S(IV) content on reactivity, observed at low S(IV) contents, by reducing the effective concentrations of glucose, compounds containing the carbonyl group generated in the Maillard reaction, free S(IV) and the activities of the S(IV) oxoanions. The formation of disulphite ion:

$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O$$

is similarly enhanced as concentration is increased and the position of equilibrium should be further displaced to the right as the water activity is reduced on dehydration. It has been suggested that $S_2O_5^{2-}$ could be a good catalyst for the formation of DH in the Maillard reaction (Wedzicha, 1987), but FTIR data indicate (Wedzicha *et al.*, 1992) that less, rather than more, $S_2O_5^{2-}$ is formed as the concentration of water in water–glycerol mixtures is reduced. The formation of $NaS_2O_5^-$ ion pairs is a possible reason for this observation. In any case, the tendency is for a reduction of the activity of S(IV) species as a_w is reduced.

The observation that the reaction at low concentration of S(IV) is completely dependent on S(IV) is particularly interesting. Not only has this behaviour been reported for the dehydration of potato (Wedzicha, 1987), but it implies that the rate of formation of intermediates in the Maillard reaction is very considerably increased by the presence of S(IV); the additive markedly changes the behaviour of the glucose–glycine system, i.e. it does not merely inhibit a browning reac-

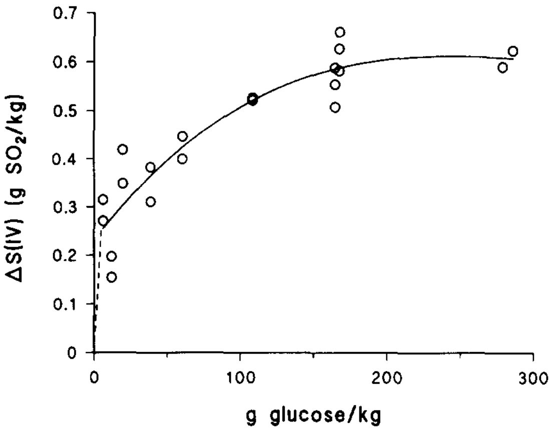

Fig. 4. Effect of initial glucose content of agar–cellulose gels on the difference in the amount of S(IV) (ΔS(IV)) which has undergone reaction when the gels are dehydrated in air at 80°C with and without glycine. All amounts are expressed per dry weight of gel. Initial gel composition (wet weight basis): agar (1%, w/w); MCC (22%, w/w); glucose (0.14–9.0%, w/w); glycine (zero or 0.19%, w/w); $Na_2S_2O_5$ (0.12%, w/w). The difference in S(IV) content is obtained by subtracting the change in S(IV) content, observed when gels containing glycine were dehydrated, from that observed when gels contained no glycine.

tion which otherwise takes place at the same rate in its absence.

Figure 4 shows the effect of glucose content on the difference in the amount of S(IV) which reacts when gels containing glucose and S(IV) are dehydrated with and without glycine. The graph has been extrapolated to pass through the origin because there is no reaction possible in the absence of glucose. Thus, the reactivity of S(IV) in this situation increases steeply with glucose concentration at low concentration, but reaches a constant value at high glucose concentration. The rate of reaction of S(IV) in mixtures of glucose and glycine is of first order with respect to glucose in dilute aqueous solution (Wedzicha & Vakalis, 1988) and there is no change in the kinetics when the solvent is replaced by water–glycerol mixtures containing up to 82% (w/w) glycerol (Bellion & Wedzicha, 1993). No glucose-independent reaction has yet been reported in homogeneous systems. It is suggested here that the reaction rate could be limited at high glucose concentration by the high viscosity of the partially dehydrated gels, leading to a diffusion-controlled reaction, i.e. the effect of glucose is to reduce the rate of diffusion of reactant molecules to balance the normal kinetic effect of glucose concentration. Thus, there is no change in the extent of reaction as the glucose content of the gels is approximately doubled. It is now plausible to suggest that the extent of reaction of S(IV) during vegetable dehydration could also be limited by the rate of reactant transport through a viscous partially dehydrated food matrix, giving the overall observation that the extent of S(IV) loss is independent of glucose content.

Preliminary work on solute transport behaviour in this model material (Gogus *et al.*, unpublished) suggests that it behaves as a capillary porous material, with macroscopic movement of solutes towards the surface, as dehydration progresses. The increase in solute concentration close to the surface is probably responsible for the observed reactivity of S(IV) during dehydration, whilst the diffusion of solutes on a molecular scale of distances, within the aqueous phase, is responsible for encounters which give rise to reaction. The latter may be controlled by high viscosity due to glucose at high concentration.

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