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Effect of glyceraldehyde on the kinetics of Maillard browning and inhibition by sulphite species

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Abstract

The kinetics of browning (A_{470}) are reported for glyceraldehyde + glycine and glucose + glycine + glyceraldehyde mixtures ([glucose] = 0–1 mol l^{-1}; [glycine] = 0–1 mol l^{-1}; [glyceraldehyde] = 0–10 mmol l^{-1}; pH 5.5, [acetate buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C). In the absence of glucose the rate of reaction depends only on glyceraldehyde concentration, whereas glycine is required for colour development. The rate of browning when all 3 reactants are present is much greater than calculated as the sum of the individual glyceraldehyde + glycine and glucose + glycine reactions. This synergistic behaviour is accompanied by the removal of the induction phase normally seen for the Maillard reaction, and a browning reaction whose kinetics depend on the concentrations of all 3 reactants. The apparent dissociation constant of the hydroxysulphonate of glyceraldehyde is 6.76×10^{-5} mol l^{-1} (pH 5.5, 55°C, ionic strength≈0.2 mol l^{-1}). Theoretical calculations suggest that the conversion of glyceraldehyde to its hydroxysulphonate could contribute to the mechanism of the inhibition of Maillard browning by sulphite species.

1. Introduction

An overall scheme for the two major pathways in the Maillard reaction of glucose is given in Fig. 1 (Tressl, Nittka, Kersten, & Rewicki, 1995). At pH < 5, the predominant reaction proceeds by way of 3-deoxyhexosulose, 3DH, or the enol form of this intermediate, to give high molecular weight products (polymers) known as melanoidins. At pH > 7, the intermediates are the 1- and 4-deoxyhexosuloses, 1DH and 4DH, respectively, which undergo facile degradation (retro-aldol reactions) to produce fragments of all possible sizes, C_1-C_5. These include precursors of the important flavour volatiles.

Sulphite species, S(IV), inhibit browning by the reaction of sulphite ion with the α,β-unsaturated carbonyl moiety of 3,4-dideoxyhexosulos-3-ene, DDH, which is formed from 3DH, to give 3,4-dideoxy-4-sulphohexosulose (Wedzicha, 1984). This product cannot be converted back to an α,β-unsaturated carbonyl compound and so is relatively unreactive towards browning. Davies, Wedzicha, and Gillard (1997) developed an approach which uses this reaction of S(IV) to measure the rates of formation of 3DH and a subsequent intermediate inferred to be DDH, and hence to determine the kinetics of the individual rate-limiting steps in the browning reaction. The kinetics of colour formation can be modelled as a 3-stage reaction, illustrated in Fig. 2.

This approach has been used (Molero-Vilchez & Wedzicha, 1997) to confirm that the route to melanoidins at pH 5.5 does, indeed, depart from the route to the Amadori product (as given by Tressl et al., 1995). Thus, the early stages in the browning reaction are relatively simple and now well characterised. On the other hand, the process described by k_3 (Fig. 2) is likely to be very complex, involving the formation of polymer sub-units and their assembly into melanoidins.

Some insight into the involvement of low molecular weight aldehydes in this process can be gained from the fact (Vasiliauskaite & Wedzicha, 1997) that the Strecker aldehyde of glycine (formaldehyde) is incorporated stoichiometrically into high molecular weight (M_r > 12,000) melanoidins from glucose and glycine (pH 5.5, 55°C). It is possible that such substances are essential ingredients in melanoidin formation, but their role has not yet been identified. Equally, C_3 sugar fragments such as glyceraldehyde, ga, or pyruvaldehyde, pa, are known to be very reactive towards browning (Hayashi & Namiki, 1986), but their participation in Maillard browning, when they are formed in situ, has not yet been discussed comprehensively in the literature.

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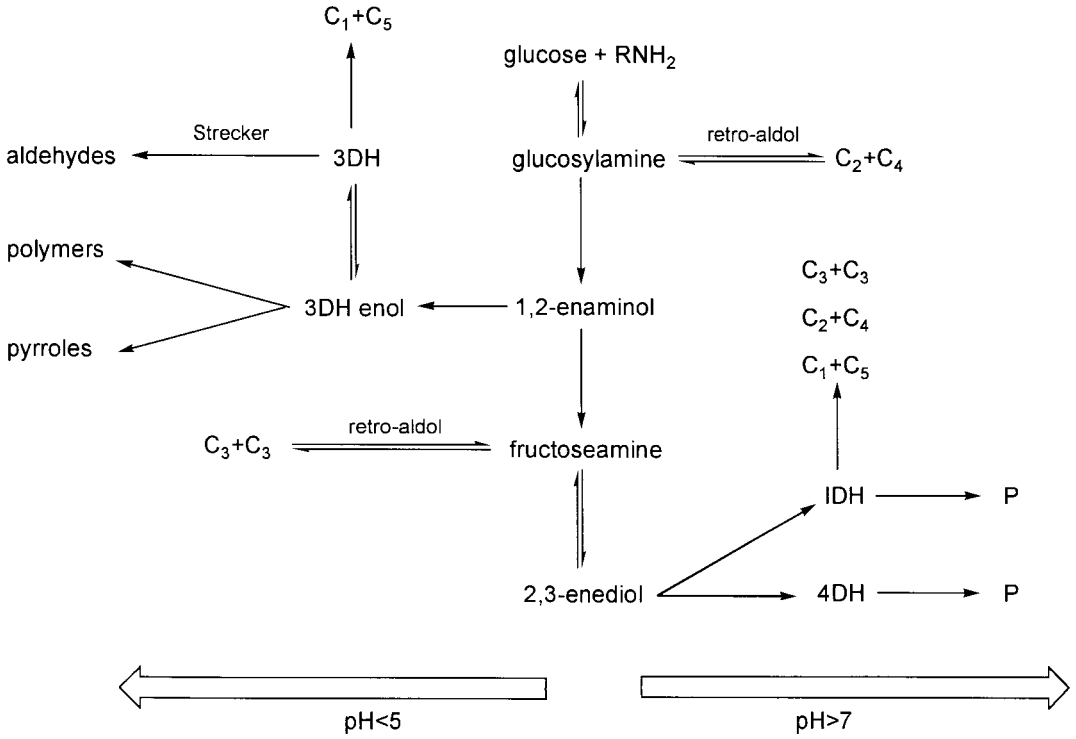


Fig. 1. Outline of the mechanism of the Maillard reaction of glucose based on an illustration by Tressl et al. (1995). 1DH, 3DH and 4DH refer to 1-, 3- and 4-deoxyhexosuloses which are key intermediates in the mechanism. Low molecular weight fragments are shown as C_1, C_2, etc. to indicate the lengths of the carbon chains.

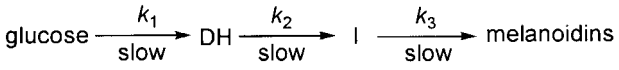


Fig. 2. Simple kinetic model of Maillard browning. I is an unspecified intermediate, DH is 3-deoxyhexosulose and k_1, k_2 and k_3 are rate constants.

Here we aim to provide experimental evidence for an extraordinary involvement of ga, in the glucose–glycine reaction, to suggest that this compound plays an important role in melanoidin formation. The reactivity of aldehydes in the Maillard reaction depends on the availability of the carbonyl group and these moieties are made unreactive by conversion to hydroxysulphonates. Whereas glucose forms a relatively unstable hydroxysulphonate, that of the important intermediate in browning, 3-deoxyhexosulose, is relatively stable (dissociation constant 1.64×10^{-2} mol l^{-1}) (Wedzicha & Kaban, 1986) but not sufficiently stable to inhibit the progress of the browning reaction in the presence of S(IV). On the other hand, aldehydes such as ga are expected to form much more stable adducts with S(IV). Here we will also measure the dissociation constant of the hydroxysulphonate of ga to deduce whether hydroxysulphonate formation is likely to be significant in relation to the anti-browning behaviour of S(IV). Model Maillard systems investigated by our group are studied at pH 5.5 and 55°C. The pH value is in the middle of the range of many plant and animal foods. The temperature represents conditions suitable for "accelerated storage" trials and these model systems are, therefore, appropriate to the study of the Maillard reaction in stored sulphited foods.

2. Materials and methods

Where possible analytical grade reagents were used and were obtained either from Sigma or Aldrich. DL-Glyceraldehyde was obtained from Fluka and radiochemicals from Amersham International.

2.1. Kinetics of browning

Stock acetate buffer contained sodium acetate (2 mol l^{-1}) and sufficient acetic acid to give pH 5.5. Reaction mixtures were prepared by dissolving the solid reactants in water, adding the stock buffer and re-adjusting to pH 5.5 (NaOH/acetic acid) before making up to the mark. The final concentrations were [glucose] = 0–1.0 mol l^{-1}, [glycine] = 0.2–1.0 mol l^{-1}, [ga] = 0-10 mmol l^{-1}, [buffer] = 0.2 mol l^{-1} (with respect to sodium acetate). Solutions were heated at 55°C and aliquots withdrawn at timed intervals. Absorbance measurements were made at 470 nm in 1 cm cells.

2.2. Hydroxysulphonate dissociation constant

To measure the dissociation constant of the ga-S(IV) adduct, a series of mixtures containing DL-glyceraldehyde (10 mmol l^{-1} by weight) and variable concentrations of S(IV) (6–13 mmol l^{-1}) prepared from sodium metabisulphite, buffered at pH 5.5 with acetate buffer (0.2 mol l^{-1} with respect to sodium acetate), were heated at 55°C for 24 h to attain equilibrium. To aliquots (20 ml) of the mixtures was added hydrochloric acid (25 vol%, 2 ml) and starch solution (1 ml). The solutions were titrated rapidly with iodine solution (5 mmol l^{-1}) to give a measure of the concentration of free S(IV). The solutions were then saturated with sodium hydrogen carbonate to release the reversibly bound S(IV) and the mixtures again titrated with iodine solution (5 mmol l^{-1}).

3. Results and discussion

3.1. Preliminary studies

The effect of adding ga at 10 mmol l^{-1} to a glucose + glycine browning reaction is illustrated in Fig. 3. Whereas the browning of glucose + glycine shows the expected induction phase (the absorbance varies with t^3), the behaviour of the glucose + glycine + ga system is characteristically different from that of glucose + glycine and ga + glycine reactions. There is no longer an induction phase and the behaviour of the mixed system is strongly synergistic, i.e. the extent of browning in the 3-component system is greater than the sum of browning in the individual reactions. It is striking that the absorbance of the glucose + glycine + ga reaction increases well beyond the maximum absorbance of the ga + glycine reaction. We believe this to be the first time that these kinetics have been reported.

3.2. The glyceraldehyde + glycine reaction

The starting point for a systematic investigation of the kinetics of the glucose + glycine + ga reaction is to identify the kinetics of the individual browning reactions. The kinetics of the glucose + glycine reaction are now well established (Davies et al., 1997), but those of the ga + glycine reaction have not been investigated in detail. The effect of [ga] on A_{470} is illustrated in Fig. 4, where the lines drawn through the experimental points represent a fit of the data to the integrated first order equation. Whereas individual runs conform well to first order kinetics over a major part of the reaction, the value of the first order rate constant is 0.0747, 0.0831, 0.0849 and 0.106 h^{-1} for [ga] = 4, 6, 8 and 10 mmol l^{-1}, respectively. Since [gly] = 0.5 mol l^{-1}, its concentration can be considered to be constant throughout each kinetic run, and the overall rate of browning can be said to be approximately of first order with respect to the minor reactant, ga. The small but significant dependence of the first order rate constant on concentration suggests that the actual order is greater than 1, and that the fitting of the integrated rate equation to the experimental data was not sufficiently discriminating to show systematic deviations between the data and the fits. When the data are re-plotted as second order plots ($1/A_{470}$ vs t) the graphs are non-linear and the true order of reaction is closer to 1 than 2. On the other hand, the initial rate of reaction varies accurately with $[ga]^2$ over a

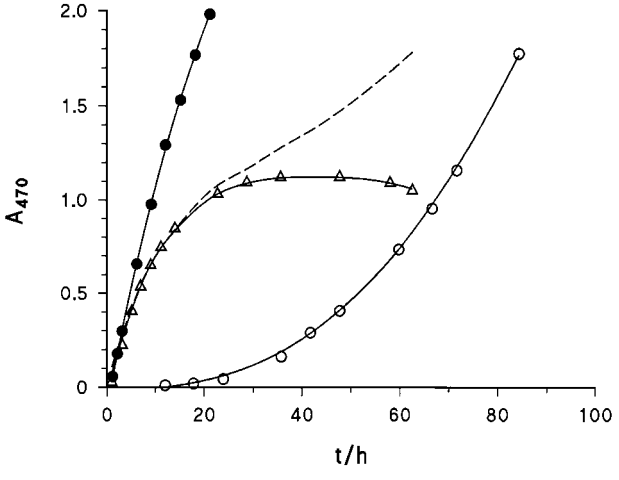


Fig. 3. Effect of glyceraldehyde (ga) on the browning of glucose + glycine mixtures. Reaction conditions: [glucose] = 0 or 1 mol l^{-1}; [gly] = 0.5 mol l^{-1}; [ga] = 0 or 10 mmol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C. Key to symbols: ○, glucose + glycine; △, ga + glycine; ●, glucose + glycine + ga. The broken line represents the sum of absorbances from the glucose + glycine and the ga + glycine reactions.

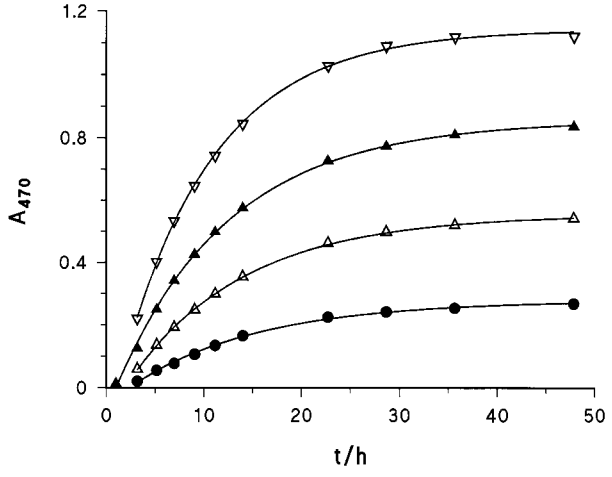


Fig. 4. Effect of glyceraldehyde (ga) concentration on the reaction of ga, with glycine. Reaction conditions: [gly] = 0.5 mol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C. Key to symbols: [ga]/mmol l^{-1} = 4 ●; 6 △; 8 ▲; 10 Δ. The lines which are drawn through the experimental points are calculated from the integrated first order rate equation.

5-fold concentration range as illustrated in Fig. 5, indicating that the very early reaction is bimolecular with respect to ga. Neither the initial rate nor the overall reaction depend on [gly] in the range 0.2–1 mol l^{-1}. Thus, the mechanism of the ga + glycine reaction involves a spontaneous conversion of ga to reactive intermediates without the assistance of glycine. The amino acid is required for the subsequent formation of colour since the browning of ga in the absence of glycine is negligible on the timescale of these experiments.

In attempting to reconcile these data with a chemical mechanism, we note with interest the work of Cämmerer, Wedzicha, and Kroh (in press) who describe the formation of hydroxymethylfurfural, HMF, from ga under caramelisation conditions or in its reaction with glycine (pH 4-5, reflux temperature). The yield of HMF, whilst small (typically mmol per mol of carbonyl reactant), is nevertheless an order of magnitude greater when from ga than from glucose under caramelisation conditions, and the same order of magnitude at short reaction times in the presence of glycine. In order to form HMF, it is necessary for a 6-carbon species to be constructed, presumably by the combination of two molecules of ga. Cämmerer et al. propose that the mechanism could proceed by way of the conversion of ga to 3DH and subsequently DDH. It is suggested that this reaction involves the dehydration, by β-elimination, of ga to pa, which then reacts, by aldol condensation, with a second molecule of ga to form 3DH as the precursor of HMF. Such a reaction could be of first or second order with respect to ga depending on whether the single rate limiting step is the conversion of ga to pa, or the reaction of ga with pa, respectively.

The kinetics of colour formation in the DH + glycine reaction are overall of first order with respect to DH (Davies et al., 1997). Under the same conditions as used in the present investigation, Khandelwal and Wedzicha (1997) give the value of the first order rate constant as 0.00752 h^{-1}, i.e. the reaction is slower than the overall ga + glycine reaction by an order of magnitude. It is unlikely, therefore, that 3DH is an intermediate in the browning of ga + glycine in the present system. The main reaction appears to be a unimolecular conversion of ga to the activated complex in the reaction.

3.3. The glucose + glycine + glyceraldehyde reaction

The effects of varying the concentrations of reactants in the glucose + glycine + ga reaction are illustrated in Figs. 6–8. Detailed kinetic analysis of this complex system is not possible at this stage, but it is important to see the progressive effects of the reactants as their concentrations are increased. Thus, the initial rate of the 3-component reaction increases linearly with [ga] and Fig. 9 shows this behaviour to be accurately of first order with respect to that reactant. Similarly, we see that both glycine (Fig. 7) and glucose (Fig. 8) exert a kinetic effect on the new reaction. In the case of glycine, the initial rate increases linearly with concentration. Glucose has no effect on the rate strictly at $t = 0$ but, shortly after the start of the reaction (e.g. 5 h) a clear kinetic dependence emerges. It is inferred that the effective reactant is some (possibly early) intermediate formed by the conversion of glucose. The major effect of glucose is on the rate of the reaction once it is well under way. A noteworthy feature of this reaction is that glucose appears to exert a considerable effect on rate after reaction times at which the glucose + glycine reaction (in the absence of ga) is still in its induction phase. It is seen, therefore, that the kinetics of the glucose + glycine + ga

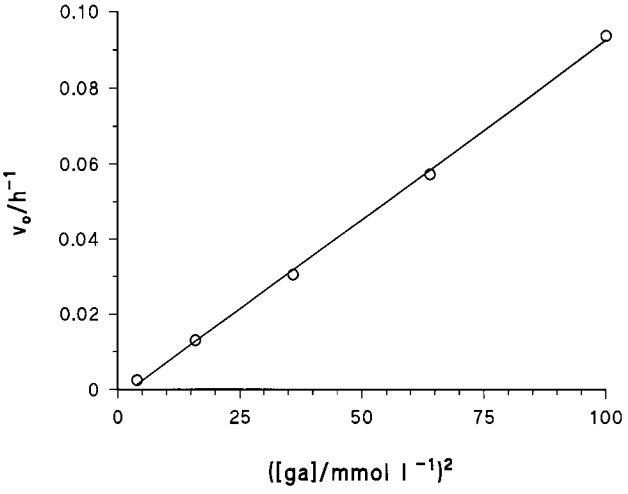


Fig. 5. The effect of glyceraldehyde (ga) concentration on the initial rate, v_o, of the ga + glycine reaction, to demonstrate second order behaviour. Reaction conditions: [gly] = 0.5 mol l^{-1}; [ga] = 2–10 mmol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C.

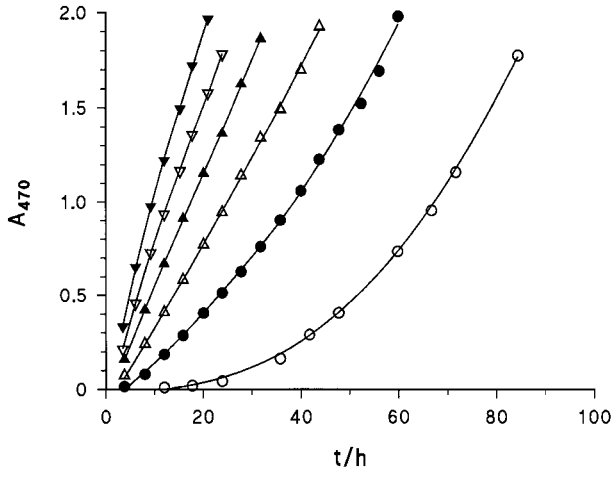


Fig. 6. Effect of glyceraldehyde (ga) concentration on the kinetics of colour formation in the glucose + glycine + ga reaction. Reaction conditions: [glucose] = 1 mol l^{-1}; [gly] = 0.5 mol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C. Key to symbols: [ga]/mmol l^{-1} = none ○; 2 ●; 4 △; 6 ▲; 8 Δ; 10 ▼.

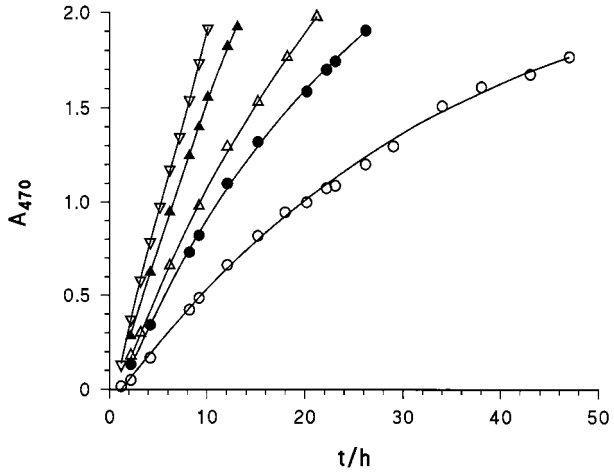


Fig. 7. Effect of glycine concentration on the kinetics of colour formation in the glucose + glycine + glyceraldehyde reaction. Reaction conditions: [glucose] = 1 mol l^{-1}; [glyceraldehyde] = 10 mmol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C. Key to symbols: [gly]/mol l^{-1} = 0.2 ○; 0.4 ●; 0.6 △; 0.8 ▲; 1 Δ.

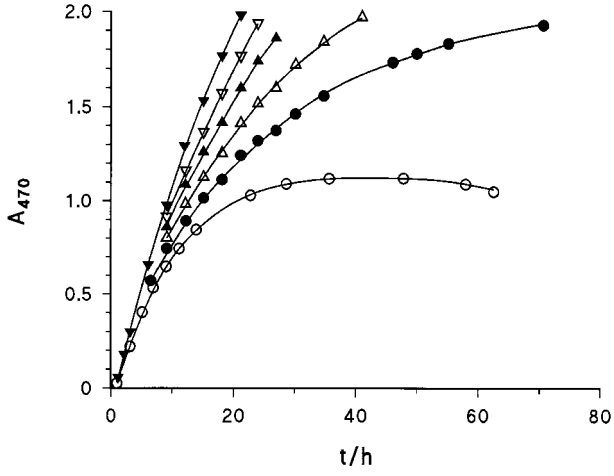


Fig. 8. Effect of glucose concentration on the kinetics of colour formation in the glucose + glycine + glyceraldehyde reaction. Reaction conditions: [gly] = 0.5 mol l^{-1}; [glyceraldehyde] = 10 mmol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C. Key to symbols: [glucose]/mol l^{-1} = none ○; 0.2 ●; 0.4 △; 0.6 ▲; 0.8 Δ; 1 ▼.

reaction are quite different from the kinetics of the individual reactions and the mechanism is likely to involve all 3 reactants in the rate-limiting step. Regardless of this detail, the data clearly demonstrate the importance of low concentrations of glyceraldehyde on the progress of browning.

3.4. The glyceraldehyde-S(IV) reaction

Reaction mixtures were found to have reached equilibrium (constant ratio of [bound S(IV)] to [free S(IV)]) after heating for 24 h. The reaction between a carbonyl compound and S(IV) is given by the following stoichiometric equation,

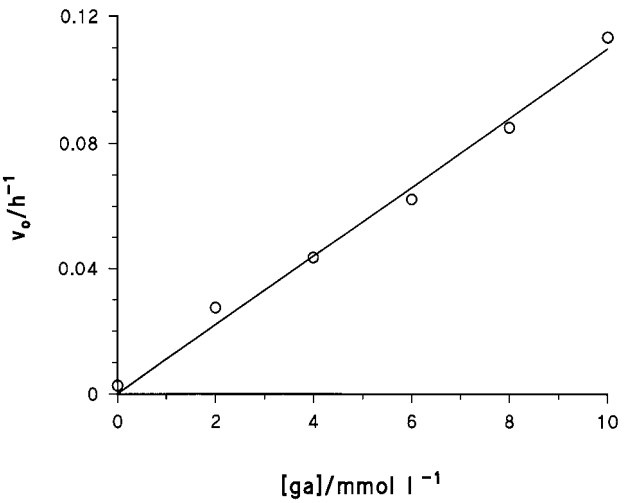
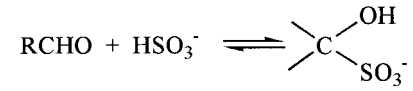


Fig. 9. Effect of glyceraldehyde (ga) concentration on the initial rate of colour formation in the glucose + glycine + ga reaction. Reaction conditions: [glucose] = 1 mol l^{-1}; [gly] = 0.5 mol l^{-1}; [ga] = 0-10 mmol l^{-1}; pH 5.5; [buffer] = 0.2 mol l^{-1} with respect to acetate ion; 55°C.



The dissociation constant for the hydroxysulphonate adduct is given by the law of mass action as follows,

$$K = \frac{[\mathrm{RCHO}][\mathrm{HSO_3^-}]}{[\text{hydroxysulphonate}]}$$

Since the concentration of carbonyl compounds at equilibrium can be expressed in terms of the initial carbonyl concentration and the amount which has been converted to the hydroxysulphonate [the bound S(IV)], this equation is equivalent to

$$K = \frac{([\mathrm{ga}]_0 - [\text{boundS(IV)}])[\text{freeS(IV)}]}{[\text{boundS(IV)}]}$$

and may be rearranged to give

$$[\text{boundS(IV)}] = [\mathrm{ga}]_0 - K\frac{[\text{boundS(IV)}]}{[\text{freeS(IV)}]}$$

Thus, a graph of the concentration of bound S(IV) vs the ratio of bound S(IV) to free S(IV) concentration allows the initial concentration of ga and the equilibrium constant to be obtained. The approach has been used successfully in the past (Burroughs & Sparks, 1973) and has a particular advantage in that it is not neccessary to know the actual concentration of the carbonyl compound provided it is maintained constant throughout the series of experiments. The corresponding graph is shown as Fig. 10, and is found to be linear over a 20-fold range of [bound S(IV)]/[free S(IV)] ratio. Thus, we calculate the dissociation constant of glyceraldehyde

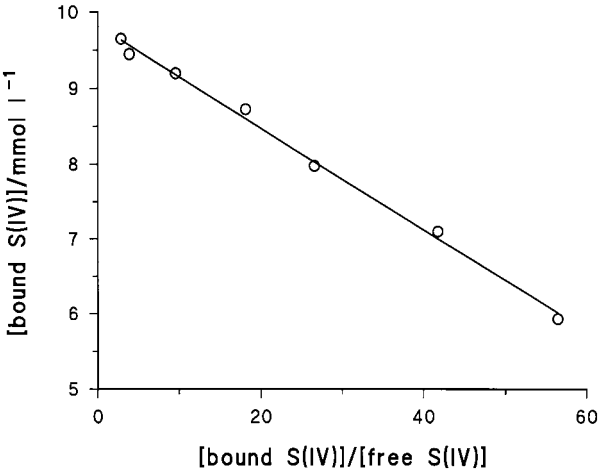


Fig. 10. Graph of bound S(IV) concentration vs the ratio of bound S(IV) to free S(IV) concentration, to determine the dissociation constant of glyceraldehyde hydroxysulphonate. Reaction conditions: pH 5.5, [buffer] = 0.2 mol l^{-1} with respect to sodium acetate, 55°C.

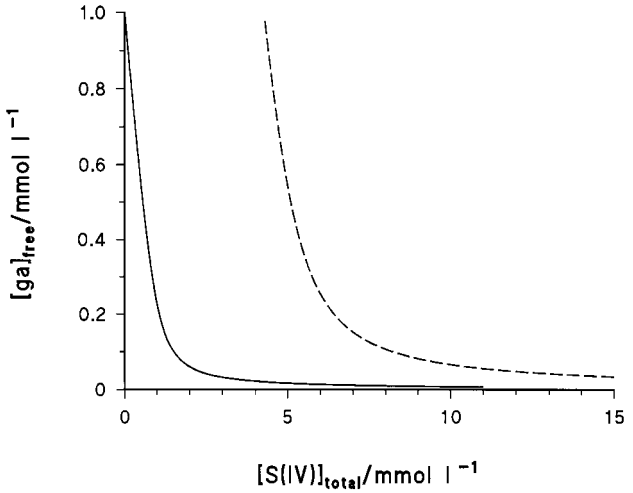


Fig. 11. Calculated equilibrium concentrations of free glyceraldehyde (ga) as a function of the total (free + bound) S(IV) concentration given that the dissociation constant of glyceraldehyde hydroxysulphonate is 6.76×10^{-5} mol l^{-1}. The total concentration of glyceraldehyde is 1 mmol l^{-1} (solid line) and 5 mmol l^{-1} (broken line).

hydroxysulphonate to be 6.76×10^{-5} mol l^{-1}. Since the S(IV) species are measured collectively, this value is the *apparent* dissociation constant at pH 5.5, 55°C and an ionic strength of approximately 0.2 mol l^{-1}, provided largely by the acetate buffer. The intercept on the *y*-axis is 9.83 mmol l^{-1}. The solutions of ga were prepared to be 10 mmol l^{-1} by weight and this result indicates that the purity of the ga sample is very acceptable.

In a typical model system to investigate the inhibition of browning by S(IV), the concentration of inhibitor is in the range 20–100 mmol l^{-1} with [reducing sugar] = 1 mol l^{-1} and [amino acid] = 0.5 mol l^{-1}. We are not aware of any reported measurement of the concentration of ga in such Maillard systems. We find, above, that there is a noticeable effect of ga on the rate of browning at 1 mmol l^{-1}. The maximum rate of browning measured in glucose + glycine reactions without additional ga is reported as 0.05 absorbance units h^{-1}. If this browning were caused entirely by the formation of ga in situ, then the concentration of ga required to cause browning at this rate is 4.6 mmol l^{-1} together with glucose (1 mol l^{-1}) + glycine (0.5 mol l^{-1}). Thus, we suggest that the absolute maximum concentration of ga formed in situ during the observation period (80 h) is 4.6 mmol l^{-1}, but is likely to be only a fraction of this concentration, because other aldehydes will no doubt also participate in promoting the browning reaction. Fig. 11 shows the calculated equilibrium concentrations of free ga when the total concentration of ga is 1 and 5 mmol l^{-1} with S(IV) in the range 0–15 mmol l^{-1}, and the results are plotted for $[ga]_{free} < 1$ mmol l^{-1}. Thus, for a total S(IV) concentration as low as 10 mmol l^{-1}, the residual concentration of free ga is very small (< 0.1 mmol l^{-1}), and below the level at which it exerts a significant effect on the rate of browning. The binding of ga to S(IV) is even greater at higher S(IV) concentration.

4. Conclusion

Glyceraldehyde has a marked effect on the kinetics of browning in the glucose + glycine reaction. At short reaction times, when the glucose + glycine reaction is still in its induction phase, low concentrations of ga serve to promote a browning reaction which has a kinetic dependence on glucose, glycine and ga. Glyceraldehyde forms a very stable hydroxysulphonate and this aldehyde would be rendered unreactive in sulphited foods.

Acknowledgements

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necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

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