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A Kinetic Model for the Glucose–Fructose–Glycine Browning Reaction

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The browning of glucose−fructose−glycine mixtures involves parallel glucose−glycine and fructose−glycine reactions, which share a common intermediate, the immediate precursor of melanoidins in the kinetic model. At pH 5.5, 55 °C glucose is converted into this intermediate in a two step process where $k_1 = (7.8 \pm 1.1) \times 10^{-4}$ mol L^{-1} h^{-1} and $k_2 = (1.84 \pm 0.31) \times 10^{-3}$ h^{-1} according to established kinetics, whereas fructose is converted into this intermediate in a single step where $k_4 = 5.32 \times 10^{-5}$ mol L^{-1} h^{-1}. The intermediate is converted to melanoidins in a single rate limiting process where $k_{mix} = 0.0177$ h^{-1} and the molar extinction coefficient (based on the concentration of sugar converted) of the melanoidins so formed is 1073 ± 4 mol^{-1} L cm^{-1}. Whereas the value of k_{mix} is the same when the individual sugars undergo browning, the value of the molar extinction coefficient is similar to that for melanoidins from the glucose−glycine reaction (955 ± 45 mol^{-1} L cm^{-1}) but it is approximately double the value for melanoidins from the fructose−glycine reaction (478 ± 18 mol^{-1} L cm^{-1}). This is the reason that the effects of glucose and fructose on the rate of browning are synergistic.

KEYWORDS: Nonenzymatic browning; Maillard reaction; glucose; fructose; invert sugar; kinetics; melanoidins; reaction rate

INTRODUCTION

Model systems for the study of Maillard browning have usually involved only one reducing sugar component. Browning in foods is unlikely to involve a single carbohydrate reactant; foods contain mixtures of reducing sugars, and it is interesting to speculate as to whether the browning of these sugars occurs independently or whether there is some (and as yet unexpected) interaction between the reaction pathways. The most common mixture of simple reducing sugars found in foods must surely be that of glucose + fructose; both are found widely in plant foods.

There is some uncertainty regarding claims as to the ease with which fructose browns in foods. This appears to be related to whether the systems are buffered (*1*); unbuffered fructose−amino acid mixtures allegedly brown faster than glucose−amino acid mixtures while buffered solutions brown at comparable rates. On the other hand, Kato et al. (*2*) showed that at low concentrations of glycine, fructose browned more rapidly than glucose, but the situation was reversed at high amino acid concentration. Thus, in a glucose−fructose−amino acid system, fructose and glucose are likely to contribute to the overall development of color, but the relative importance of the two reactions depends on the composition of the reaction medium. Also evident from the results was an almost linear increase of the optical density with time for the fructose−glycine reaction, whereas a pronounced induction period was observed for the glucose−glycine reaction. Similar findings were reported by Buera et al. (*3*), who studied the relative browning rates of fructose and glucose in high water activity systems.

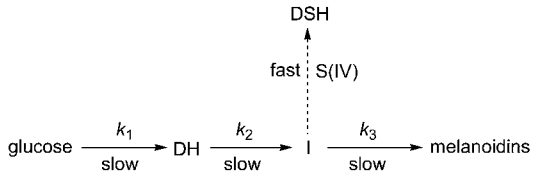

Figure 1. Kinetic model of the glucose–glycine reaction and its inhibition by S(IV). k_1, k_2, and k_3 are rate constants, 3-DH is 3-deoxyhexosone, DSH is 3,4-dideoxy-4-sulfohexosone, and I is an unspecified intermediate.

The kinetics of the glucose−glycine reaction (*4*, *5*) can be described successfully in three rate determining steps as shown in **Figure 1**. Studies of the kinetics of sugar−amino acid−sulfite systems can lead to a successful approach to the modeling of the browning reaction (*6*). This is due to the fact that sulfur(IV) oxospecies (S(IV)) react irreversibly with an early intermediate, which is a precursor of melanoidins. Thus, in the glucose−glycine−S(IV) reaction, the reaction of intermediate I is diverted toward the relatively unreative 3,4-dideoxy-4-sulfohexosone (DSH) (*7*). The kinetics of the glucose−glycine−S(IV) reaction reveal two rate determining steps (*4*), and the kinetic parameters k_1 and k_2 can be extracted by fitting the integrated rate equation to the experimental data (*5*) using multiple nonlinear regression.

Wedzicha and Swales (*8*) report the kinetics of the fructose−glycine−S(IV) reaction to be much simpler than those of the glucose−glycine−S(IV) reaction. Fructose appears to be con-

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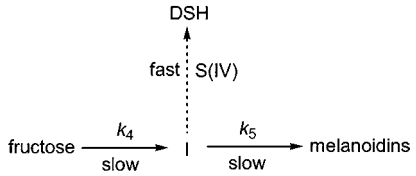

Figure 2. Proposed kinetic model of the fructose–glycine reaction and its inhibition by S(IV). k_4 and k_5 are rate constants while DSH and I have the same meaning as in **Figure 1.** It is proposed here that the intermediate I is converted to melanoidins in a process with a single rate determining step.

verted into the intermediate, which reacts with S(IV) in a process that involves only one rate determining step, but the major final reaction product is the same as in the glucose−glycine−S(IV) reaction, i.e., DSH. Thus, it was speculated that the kinetics of the fructose−glycine−S(IV) reaction is explained by the conversion of fructose into intermediate I, which is the same as, or closely related to, the corresponding intermediate in the glucose−glycine−S(IV) reaction. This suggestion is consistent with the observations made by Kato et al. (*2*) who found that at pH 5.5, the main pathway for the degradation of fructose involves 1,2-enolization, without the participation of amino acid. If the browning of fructose was to proceed in the same way as the browning of glucose beyond intermediate I, the kinetics of the fructose−glycine−S(IV) and fructose−glycine reactions would fit the kinetic scheme given in **Figure 2**.

Here, we describe an investigation into the kinetics of the formation of color in a Maillard system containing glucose + fructose + glycine by studying the kinetics of the individual reactions of glucose and fructose with glycine and the reaction of a mixture of both sugars with glycine, in the presence and absence of S(IV) for the following reasons.

There is a need to extend simple model Maillard systems to those that resemble better the situation that exists in foods, where there is usually a mixture of reactants capable of browning. The fructose + glucose system is of particular importance to a wide range of fruit and vegetable products and to foods containing added sucrose where inversion occurs.

The kinetics of the fructose−glycine reaction are expected to be different from those of the glucose−glycine reaction; yet, the two reactions are postulated to share a common intermediate. Thus, the modeling of the mixed system presents an important intellectual challenge and a critical test of our understanding of the mechanisms of the individual browning reactions.

MATERIALS AND METHODS

All chemicals were of AnalaR grade and were obtained from Sigma Company Ltd. or Aldrich Company Ltd. The reaction mixtures were prepared by dissolving solid glucose, fructose, and glycine in water to give a final concentration of 0.5 M glycine and 1.0 M sugar. Before the mixture was made up to the final volume with water, an aliquot (10% of the final volume) of a 2.0 M sodium acetate/acetic acid buffer (pH 5.5) stock solution was added and the pH of the mixture was adjusted to 5.5 using acetic acid/sodium hydroxide. For the reactions involving S(IV), the correct mass of solid sodium metabisulfite was dissolved in the reaction mixtures to give a final concentration of S(IV) in the range of 0.02−0.1 M before it was made up to the final volume.

All reaction mixtures were placed in a water bath and heated at 55 ± 0.1 °C. To determine the extent of browning, aliquots of the reaction mixtures were withdrawn at timed intervals and their absorbance was measured at 470 nm against a blank containing water. The S(IV) concentration was determined spectrophotometrically at 412 nm as described by Humphrey et al. (*9*), using 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent).

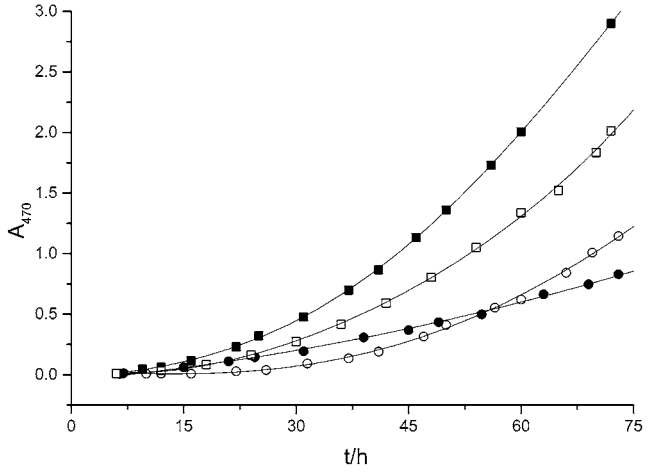

Figure 3. Absorbance–time data for the glucose–glycine (○), fructose–glycine (●), and glucose–fructose–glycine (■) browning reactions together with the calculated sum (□) of absorbances in the glucose–glycine and fructose–glycine reactions. Reaction conditions: [glucose] = [fructose] = 1.0 M, [glycine] = 0.5 M, pH 5.5, 0.2 M acetate buffer, T = 55 °C.

RESULTS AND DISCUSSION

Formation of Color. Absorbance−time data for the glucose−glycine, the fructose−glycine, and the glucose−fructose−glycine reaction together with the calculated sum of absorbances of the individual browning reactions of glucose and fructose are shown in **Figure 3**.

As expected (*2*−*4*), the glucose−glycine reaction showed a characteristic "induction" phase, when little browning occurred followed by a rapid increase in absorbance. The absorbance increased approximately in proportion to the cube of reaction time.

The optical density of the fructose−glycine system increased without a significant induction period, and it was found that the rate of increase was approximately proportional to the square of reaction time. A comparison of the sum of absorbances due to the reactions of the individual sugars with those obtained experimentally for the glucose−fructose−glycine reaction shows that the mixture of sugars browns more rapidly than expected from additive behavior of the components, indicating a significant synergistic effect of the two sugars in the mixed system.

Determination of the Rate Constants in the Individual Reactions. *Glucose−Glycine Reaction.* It was found that for the glucose−glycine reaction, the reported three step model can be applied to the experimental data obtained here and the rate constants $k_1 = (7.8 \pm 1.1) \times 10^{-4}$ mol L^{-1} h^{-1}, $k_2 = (1.84 \pm 0.31) \times 10^{-3}$ h^{-1}, and $k_3 = (1.71 \pm 0.29) \times 10^{-2}$ h^{-1} together with the apparent extinction coefficient $E = 955 \pm 45$ mol^{-1} L cm^{-1} were obtained by multiple nonlinear regression of the integrated rate equations to the experimental data as described by Wedzicha and Leong (*5*). The basis on which the extinction coefficient is given was critically discussed by these authors. The integrated rate equation for the loss of S(IV) in the glucose−glycine reaction is given by

$$[\mathrm{S(IV)}]_t = [\mathrm{S(IV)}]_0 - k_1 t + \frac{k_1}{k_2}(1 - e^{-k_2 t}) \qquad (1)$$

[S(IV)]−time data for such reaction mixtures are shown in **Figure 4**, which demonstrates the quality of the fit of eq 1 to the data. Whereas the values of k_2 and E are in excellent agreement with those reported by Wedzicha and Leong (*5*) ($k_2 = (1.92 \pm 0.22) \times 10^{-3}$ h^{-1}, $E = 972 \pm 29$ mol^{-1} L cm^{-1}),

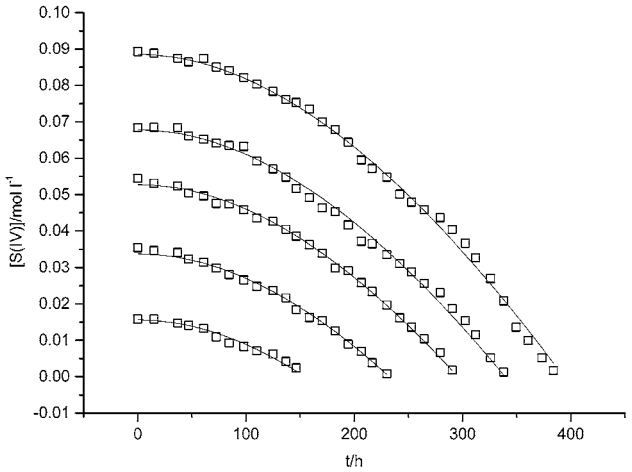

Figure 4. [S(IV)]–time data for the glucose–glycine–S(IV) reaction at different initial concentrations of S(IV). Reaction conditions: [glucose] = 1.0 M, [glycine] = 0.5 M, T = 55 °C, pH 5.5. The lines drawn through the data points are calculated after multiple nonlinear regression to the integrated rate equations.

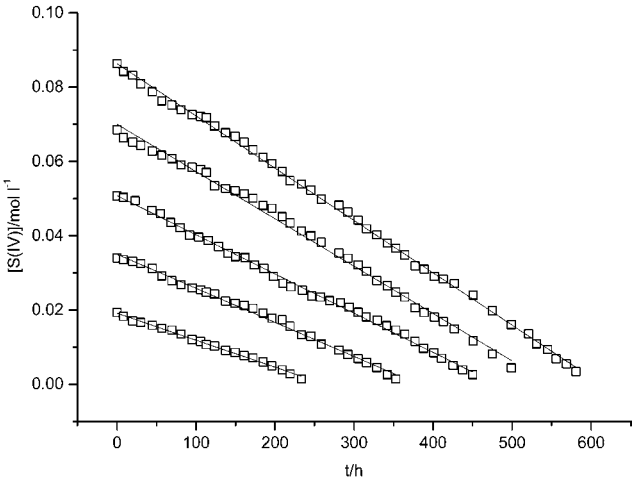

Figure 5. [S(IV)]–time data for the fructose–glycine–S(IV) reaction at different initial concentrations of S(IV). Reaction conditions: [fructose] = 1.0 M, [glycine] = 0.5 M, T = 55 °C, pH 5.5. The lines drawn through the data points are calculated after linear regression to the integrated rate equation.

the values of k_1 and k_3 were found to be within 3−4 standard deviations of the previously published values (k_1 = (5.34 ± 0.48) × 10^{-4} mol L^{-1} h^{-1}, k_3 = (2.59 ± 0.15) × 10^{-2} h^{-1}). Our experience indicates that values of these rate constants are very sensitive to the way that reaction mixtures are prepared. While they are highly reproducible and self-consistent within a given investigation, their values tend to differ slightly from study to study.

Fructose−Glycine Reaction. As found by Wedzicha and Swales (*8*), [S(IV)]−time plots for the fructose−glycine−S(IV) reaction are essentially linear as shown in **Figure 5**. This suggests that the reaction of fructose + glycine is of zero kinetic order. Because [fructose] ≫ [S(IV)], this behavior was interpreted in terms of a single rate limiting step for the conversion of fructose to the intermediate, which reacts with S(IV). The concentration of S(IV) varies with time as follows:

$$[S(IV)]_t = [S(IV)]_0 - k_4 t \quad (2)$$

The graph also reveals that the rate of loss of S(IV) was found

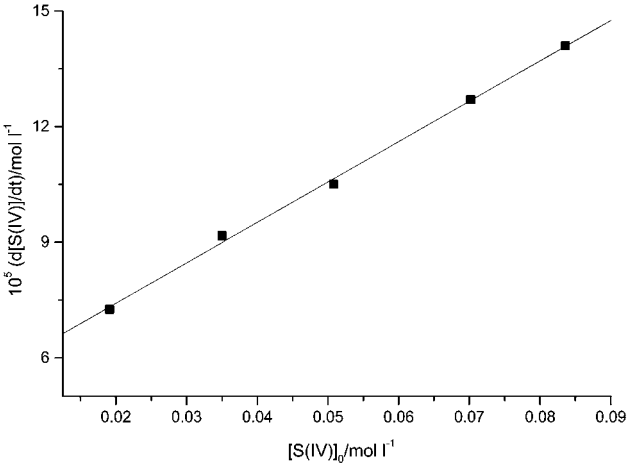

Figure 6. Plot of the rate of loss of S(IV) vs $[S(IV)]_0$ for the fructose–glycine–S(IV) reaction. Concentrations and reaction conditions are as for **Figure 5**.

to be dependent on the initial [S(IV)], and current thinking suggests that in the fructose−glycine−S(IV) reaction, S(IV) may in some way catalyze the reaction step involving k_4. This effect is clearly illustrated in **Figure 6**.

The apparent value of k_4 (5.32 × 10^{-5} mol L^{-1} h^{-1}) for a system without S(IV) can be obtained from the intercept on the y-axis of the graph of k_4 vs $[S(IV)]_0$. This is in excellent agreement with the earlier reported value of 5.34 × 10^{-5} mol L^{-1} h^{-1} of Swales (*10*).

The minimum requirement for a kinetic model to describe absorbance−time data for the fructose−glycine browning reaction is one additional rate limiting step. By hypothesis, we will assume a two step model for the conversion of fructose to melanoidins in the presence of glycine. The rate constant for the final step will be k_5, and the browning of fructose F is therefore suggested to be described by the following rate equations:

$$-\frac{d[F]}{dt} = k_4 \quad (3)$$

$$\frac{d[I]}{dt} = k_4 - k_5[I] \quad (4)$$

$$\frac{d[M]}{dt} = k_5[I] \quad (5)$$

Integration, in sequence, gives the absorbance as a function of time as

$$(A_{470})_t = E\left\{k_4 t - \frac{k_4}{k_5}(1 - e^{-k_5 t})\right\} \quad (6)$$

where I and the rate constants k_4 and k_5 are defined in **Figure 2**. [M] is the concentration of melanoidins, and E is the corresponding molar extinction coefficient.

Nonlinear regression of eq 6 to the experimental absorbance−time data (shown in **Figure 7**) of the fructose−glycine reaction after setting k_4 = 5.32 × 10^{-5} mol L^{-1} h^{-1} gave values of k_5 = (1.83 ± 0.12) × 10^{-2} h^{-1} and E = 478 ± 18 mol^{-1} L cm^{-1}. The fitted line is in remarkable agreement with the experimental data demonstrating the accuracy of the fit of eq 6 to the data and removing the need for a more complicated model.

It is interesting to note that the extinction coefficient of the melanoidins from the fructose−glycine reaction obtained in this

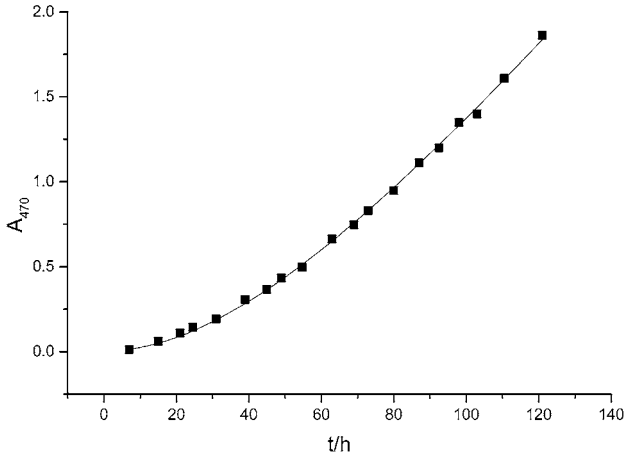

Figure 7. Absorbance–time data for the fructose–glycine reaction. Reaction conditions: [fructose] = 1.0 M, [glycine] = 0.5 M, T = 55 °C, pH 5.5. The line drawn through the data points is calculated after regression to eq 6.

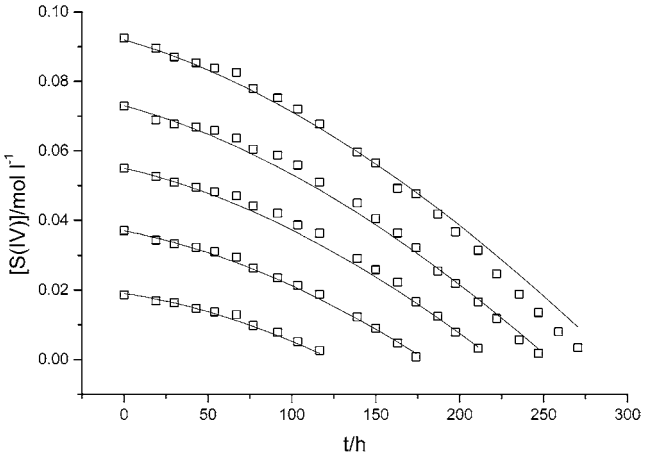

Figure 8. [S(IV)]–time data for the glucose–fructose–glycine-S(IV) reaction at different initial concentrations of S(IV). Reaction conditions: [glucose] = [fructose] = 1.0 M, [glycine] = 0.5 M, T = 55 °C, pH 5.5. The lines drawn through the data points are calculated by inserting the rate constants obtained for the individual reaction into eq 7.

way is approximately half the value of that for glucose−glycine melanoidins despite the value of k_5 being indistinguishable from that of k_3 within the experimental error. This suggests that the conversion of the "final" intermediate to melanoidins occurs at the same rate in both reactions, i.e., the intermediate could have similar structures, but some feature of the melanoidins causes them to have different absorbances. One possibility is that the melanoidins could contain different proportions of "unreacted" sugars. Wedzicha and Leong (*5*) confirmed the theoretically calculated value of E for the glucose−glycine melanoidins by studying the incorporation of ^{14}C-labeled glucose into the glucose−glycine melanoidins, but no such published data are available for fructose−glycine melanoidins. Preliminary studies (*11*) of the absorbance characteristics of melanoidins prepared at 75 °C, but otherwise under the same conditions as used in the kinetic studies reported here, confirm the value of the extinction coefficient for glucose−glycine melanoidins, but two measurements on fructose−glycine melanoidins gave E = 574 and 497 mol^{-1} L cm^{-1}. This suggests that the value calculated using the kinetic model is reliable. These additional measurements were carried out according to the method published by Leong and Wedzicha (*5*). The latter result could be accounted for by the incorporation of "unreacted" fructose to a greater extent than unreacted glucose into the melanoidins.

Kinetics of the Glucose−Fructose−Glycine Reaction. If the overall loss of S(IV) in the glucose−fructose−glycine−S(IV) reaction (i.e., containing all four components) is simply the sum of the [S(IV)]−time behavior in the individual glucose−glycine−S(IV) and fructose−glycine−S(IV) reactions, the overall concentration of S(IV) with time is given by

$$[\mathrm{S(IV)}]_t = [\mathrm{S(IV)}]_0 - k_4 t - k_1 t + \frac{k_1}{k_2}(1 - e^{-k_2 t}) \qquad (7)$$

It is found that when the appropriate rate constants for the individual reactions are inserted into eq 7, the calculated lines fit the experimental data very well (**Figure 8**) confirming that the behavior of glucose and fructose in the mixed system, up to the point at which S(IV) reacts irreversibly, is indeed additive. The synergy observed in the mixed sugar system must, therefore, originate from the step that converts the final intermediate to melanoidins. An assumption in the model for the mixed sugar reaction is that this intermediate is common to both sugars. A possible reaction scheme is given in **Figure 9**.

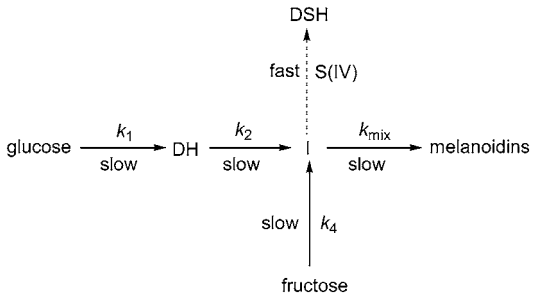

Figure 9. Kinetic model for the glucose–fructose–glycine reaction and its inhibition by S(IV). The rate constants have the same meaning as in **Figures 1** and **2**, while k_{mix} is numerically equal to k_3 and k_5.

If the kinetics of the conversion of intermediate I to melanoidins is the same as that proposed for the conversion of the final intermediate in the individual reactions, i.e.,

$$\frac{\mathrm{d[M]}}{\mathrm{d}t} = k_{\mathrm{mix}}[\mathrm{I}] \qquad (8)$$

the absorbance due to melanoidins in the glucose−fructose−glycine reaction is given by

$$(A_{470})_t = E\left\{(k_1 + k_4)t - \frac{k_1}{k_2} - \frac{k_1 + k_4}{k_{\mathrm{mix}}} + \frac{k_1 k_{\mathrm{mix}}}{k_2(k_{\mathrm{mix}} - k_2)} e^{-k_2 t} - \frac{k_1 k_2 + k_2 k_4 - k_{\mathrm{mix}} k_4}{k_{\mathrm{mix}}(k_{\mathrm{mix}} - k_2)} e^{-k_{\mathrm{mix}} t}\right\} \qquad (9)$$

The values of k_1, k_2, and k_4 are known from the experiments on the individual sugar−glycine−S(IV) reactions. Because the final intermediate is proposed to be common to the reactions of both sugars because of the similarity in values of k_3 and k_5, it is assumed here that this intermediate is the same in the mixed sugar system. The best available value of k_{mix} is the average of k_3 and k_5, i.e., k_{mix} = 0.0177 h^{-1}. Absorbance−time data were fitted to eq 9 with all rate constants set to the appropriate values, with only parameter E allowed to vary. Nonlinear regression gave E = 1073 ± 4 mol^{-1} L cm^{-1}, which is similar to the value obtained for the glucose−glycine browning reaction. The high quality of the fit of experimental data to eq 9 with these parameters is illustrated in **Figure 10**.

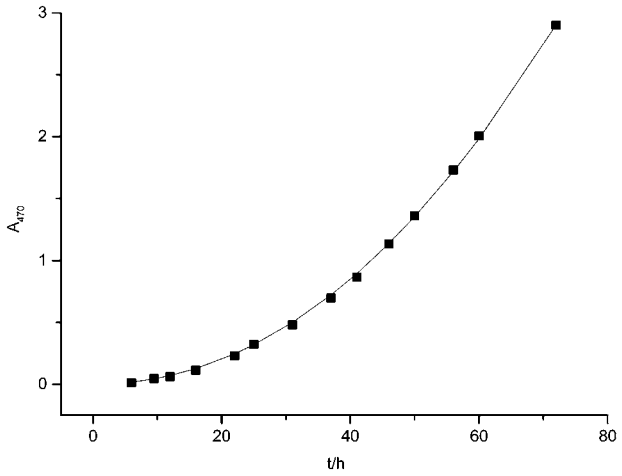

Figure 10. Regression of absorbance–time data to the integrated rate equation for the browning of glucose + fructose + glycine according to the model shown in **Figure 9**. Reaction conditions: [glucose] = [fructose] = 1.0 M, [glycine] = 0.5 M, T = 55 °C, pH 5.5. The line drawn through the data points is calculated after regression of absorbance–time data to eq 9 with E = 1073 mol^{-1} L cm^{-1}.

Figure 3 indicates that during the observation period of the mixed sugar−glycine reaction, there is a similar contribution to the overall color from both glucose and fructose. Thus, the two processes that make up the mixed sugar reaction have an impact on the concentration of the final intermediate, and the overall acceptability of the model is sensitive to the parameters used for both contributing reactions. Despite the extinction coefficient of melanoidins formed in the fructose−glycine reaction being half that in the glucose−glycine reaction, in the mixed sugar system, the value appears to be dominated by that due to glucose. This is the main reason for the synergistic behavior.

CONCLUSIONS

Despite the different kinetic behavior of the Maillard reaction of either fructose or glucose with glycine, the kinetics of the glucose−fructose−glycine reaction can be described as a combination of the two parallel reactions of glucose + glycine and fructose + glycine, which share a common intermediate.The reaction of the mixed sugar system is given by the following rate equations:

$$-\frac{d[G]}{dt} = k_1 \quad (10)$$

$$-\frac{d[F]}{dt} = k_4 \quad (11)$$

$$\frac{d[DH]}{dt} = k_1 - k_2[DH] \quad (12)$$

$$\frac{d[I]}{dt} = k_4 + k_2[DH] - k_{mix}[I] \quad (13)$$

$$\frac{d[M]}{dt} = k_{mix}[I] \quad (14)$$

The synergistic behavior of the two sugars stems from the fact that the extinction coefficient of melanoidins in the mixed sugar system is equal to that of melanoidins from the glucose−glycine reaction, whereas the extinction coefficient of melanoidins from the fructose−glycine reaction alone is half that value.

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