

Reactivity of Sorbic Acid and Glycerol in Non-Enzymatic Browning in Liquid Intermediate Moisture Model Systems

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ABSTRACT

The kinetics of brown pigment formation in a water-glycerol-sorbate-glycine (WGSG) model system at pH 4 were studied as a function of water activity (0.55-0.90) and temperature (40-60°C). The rate of browning was found to follow zero-order kinetics and to increase with decreasing a_w . There was no evidence of any browning rate maximum over the a_w range studied. Activation energy values ranged from 15.5 to 22.2 kcal/mol, being higher at the lower a_w levels. Glycerol was found to actively participate in the formation of brown pigments by reaction with sorbate or glycine, the rates also following zero-order kinetics and obeying the Arrhenius equation. Comparison was made of the rates of browning of WGSG, water-glycerol-sorbate (WGS), water-glycerol-glycine (WGG), and water-glycerol (WG) systems at the same a_w level of 0.80 under steady state conditions of 40, 50 and 60°C. The rate of browning was found to increase in the order $WGG < WGS < WGSG$. The WG system did not exhibit any browning over the time span of the experiment even at 60°C.

INTRODUCTION

Sorbic acid (*trans, trans*-2,4-hexadienoic acid) and its potassium salt are effective antimicrobial agents used to enhance the stability of a wide variety of food products, including soft-moist or intermediate moisture foods (IMF). Although sorbic acid is generally known to undergo

relatively rapid degradation in aqueous solutions (Arya, 1980; Saxby *et al.*, 1982; Seow & Cheah, 1985), comparatively little is known about its stability and reactivity in actual food systems, especially over the intermediate water activity (a_w) range between 0.60 and 0.90. Instability of sorbic acid may adversely affect the shelf-life of a product to which it has been added as a preservative. Bolin *et al.* (1980) have shown that sorbic acid in high moisture prunes is relatively unstable, the rate of loss increasing with an increase in moisture content. A higher level of the preservative would thus have to be applied to higher moisture prunes to achieve the same or desired degree of protection against microbial proliferation.

In studies of the stability of sorbic acid in aqueous glycerol solutions at intermediate a_w levels, Seow & Cheah (1985) have shown that the presence of glycerol not only accelerated the rate of degradation of sorbic acid but also promoted brown pigment formation during storage. Arya (1980) had earlier observed that the rate of browning in aqueous solutions of sorbic acid was accelerated by certain amino acids. In a more recent study, Vidyasagar & Arya (1984) have found that addition of sorbic acid at the 0.2% level tended to increase the rate of browning in stored mango squash. However, the precise mechanism of browning reactions involving sorbic acid awaits elucidation. The kinetics and significance of sorbate-induced browning in intermediate moisture systems have also yet to be studied. It is a well-known fact that non-enzymatic browning in most foods and model systems generally proceeds at faster rates over the intermediate a_w range (Loncin *et al.*, 1968; Eichner & Karel, 1972; Eichner, 1975; Warmbier *et al.*, 1976). Sorbate-induced browning would probably behave similarly and, if significant at the concentrations of sorbic acid normally used in foods, could lead to undesirable changes in quality, acceptability and stability of certain IMF containing sorbate as an antimycotic agent.

This paper presents the findings of a study into the kinetics of sorbate-induced browning in model aqueous solutions over the a_w range normally associated with IMF. Glycerol, a humectant commonly used in the development of IMF, was employed to adjust a_w .

MATERIALS AND METHODS

Water-glycerol (WG), water-glycerol-glycine (WGG), water-glycerol-sorbate (WGS), and water-glycerol-sorbate-glycine (WGSG) systems

at different a_w levels were prepared. The concentrations of potassium sorbate (ICN Pharmaceuticals) and glycine (BDH Analar) used were 0.02M and 0.05M, respectively. Adjustment of a_w was achieved by varying the concentration of glycerol (Ajax Univar) used in the systems. The actual a_w of each system was determined at 40°C using a NOVASINA equi-Hygroscope comprising a DAL-20 Digital Humidity Indicator and an enZFBA-4 Sensor. All solutions were adjusted to pH 4.0 using HCl.

Each solution was then apportioned (*ca.* 10 ml aliquots) into air-tight, screw-capped Universal glass bottles and stored in the dark at 40, 50 and 60°C. Duplicate bottles of each solution were withdrawn periodically and the intensity of browning determined by measuring the absorbance at 420 nm.

RESULTS AND DISCUSSION

Browning in the WSGG system

Figure 1 shows the changes in intensity of browning of WSGG solutions at different a_w levels during storage at 60°C. The browning reaction in such a system (which for convenience is termed sorbate-induced browning) appeared to exhibit zero-order kinetics with browning intensity increasing linearly with time, as is true also for many other non-enzymatic browning systems (Mizrahi *et al.*, 1970; Warmbler *et al.*, 1976; Labuza & Saltmarch, 1982; Stamp & Labuza, 1983). This was also found to be the case at storage temperatures of 40 and 50°C. There was no evidence of any induction period in all cases.

Rate constants for the browning reaction in WSGG systems were calculated from the slopes of the plots using linear regression analysis and are given in Table 1. It is apparent that the rate of brown pigment formation decreased with an increase in a_w from 0.55 to 0.90 at any particular storage temperature. Thus sorbate-induced browning appears to behave similarly to reducing sugar-amino acid reactions in glycerol-water mixtures as observed by Eichner & Karel (1972) and Eichner (1975). The same explanation as suggested by those authors could possibly be applied here to account for the effect of a_w , i.e. water may cause a slowing of condensation stages of the browning reaction as a result of end-product inhibition by the law of mass action. However, no maximum in rate of browning was observed over the range of a_w studied in

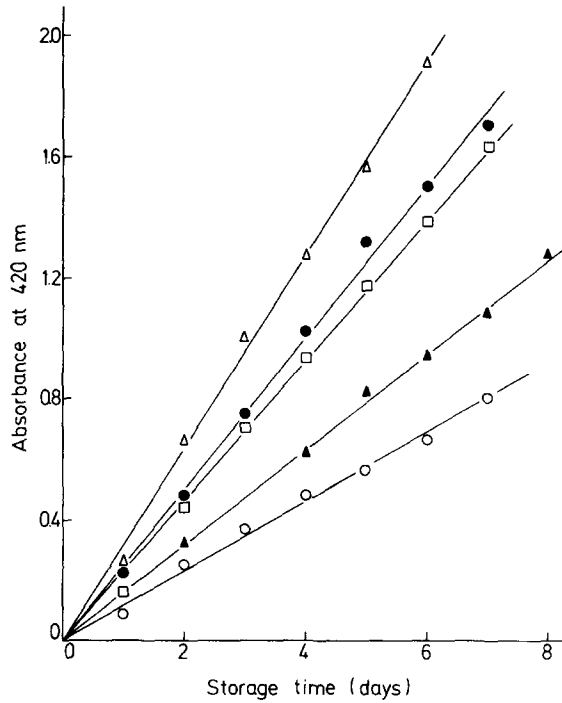


Fig. 1. Browning in the WGS system during storage at 60°C as a function of a_w .
 Δ , 0.55; \bullet , 0.65; \square , 0.71; \blacktriangle , 0.80; \circ , 0.90.

TABLE 1
 Rate Constants (k) and Activation Energy (E_a) Values of Browning in
 Water-Glycerol-Sorbate-Glycine (WGS) Systems as a Function of
 Water Activity

a_w (at 40°C)	$k \times 10^2 (\Delta \text{ Abs./day})$			E_a (kcal/mol)
	40°C	50°C	60°C	
0.55	3.76	13.14	31.9	22.2
0.65	3.23	8.30	25.1	21.2
0.71	2.81	6.75	23.7	22.0
0.80	2.57	6.64	15.1	18.3
0.90	2.54	5.81	11.4	15.5

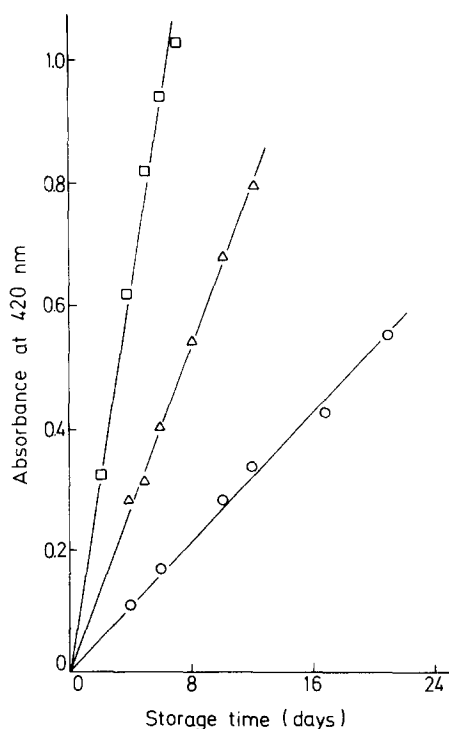


Fig. 2. Effect of temperature on rate of browning in the WGSG system at $0.80 a_w$. ○, 40°C ; △, 50°C ; □, 60°C .

the present investigation. It is possible that such a maximum could occur at an a_w lower than 0.55 . Eichner & Karel (1972), Eichner (1975) and Warmbier *et al.* (1976) have shown that the presence of glycerol could shift the maximum in rate of non-enzymatic browning to a lower a_w in both liquid and solid model systems.

The temperature dependency of the browning reaction in the WGSG system was studied over the temperature range 40 – 60°C . The results obtained at $0.80 a_w$ are shown in Fig. 2 as a typical example. As expected, the rate of browning at any particular a_w was observed to increase with an increase in storage temperature and conformed to the Arrhenius equation as shown in Fig. 3. Activation energy (E_a) values, calculated from the slopes of the Arrhenius plots, ranged from 15.5 to 22.2 kcal/mol (Table 1). The E_a values of sorbate-induced browning are thus of the same order of magnitude as those previously reported for

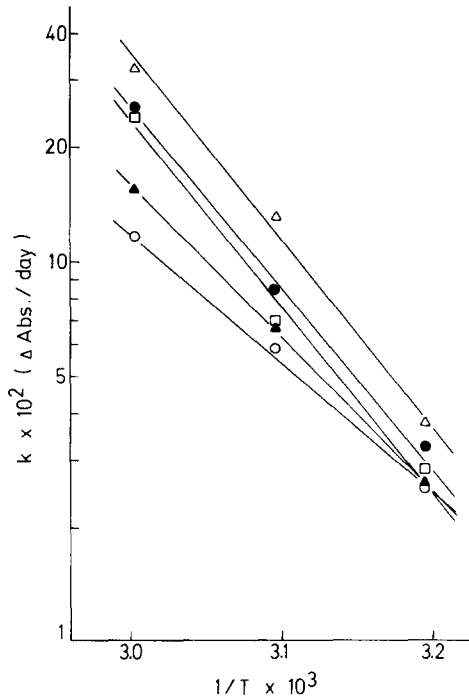


Fig. 3. Arrhenius rate plots of browning in the WGS system at different a_w levels. Δ , 0.55; \bullet , 0.65; \square , 0.71; \blacktriangle , 0.80; \circ , 0.90.

certain other non-enzymatic browning model systems (Song *et al.*, 1966; Stamp & Labuza, 1983).

There appeared to be a general increase in E_a of sorbate-induced browning with decreasing a_w , a trend which was also observed in the non-enzymatic browning of potatoes (Hendel *et al.*, 1955) and freeze-dried cabbage (Mizrahi *et al.*, 1970). The temperature sensitivity of the browning reaction involving sorbate thus appears to increase as a_w is decreased.

Reactivity of glycerol

In studies of non-enzymatic browning reactions in model systems containing glycerol as the humectant or a_w -adjusting agent, it is generally tacitly assumed that glycerol itself does not actively participate in reactions leading to brown pigment formation or that such reactions, if any, are insignificant compared with the Maillard-type reactions between

reducing sugars and amino acids. Obanu *et al.* (1977) have, however, shown that non-enzymatic browning reactions could occur between glycerol and amino acids or proteins, and that other polyhydric alcohols appear to behave in the same way as glycerol. Prolonged exposure to air can also result in the oxidation and browning of glycerol itself. In the light of these findings, experiments were designed and carried out to determine the relative browning potential of the interactions between glycerol and glycine (WGG system) and between glycerol and sorbate (WGS system) in aqueous solutions. The browning potential of aqueous glycerol solutions (WG system) in the absence of glycine and sorbate under the same storage conditions was also investigated.

Figure 4 shows the changes in intensity of browning of WGS, WGS and WGG systems at the same a_w level of 0.80 during storage at 40, 50 and 60°C. Induction periods were observed in WGS and WGG systems at 40°C, but these appeared to diminish with an increase in temperature. The browning reactions in the three systems appeared to proceed via zero-order kinetics after the induction period (if any). The rates of browning were again calculated from the slopes of the plots. It is apparent from the Arrhenius rate plots (Fig. 5) that the reactions leading to brown pigment formation in all three systems conform to the Arrhenius equations. E_a values were calculated from the slopes of the linear plots. Table 2

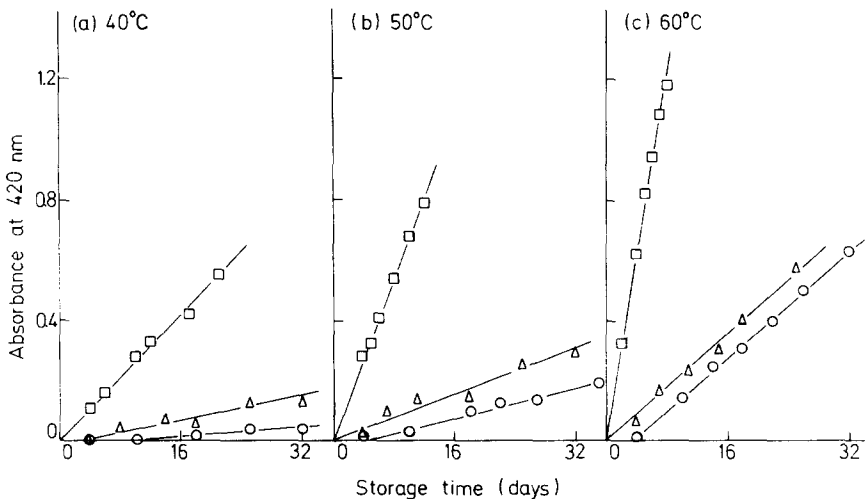


Fig. 4. Browning in WGS (□), WGS (△) and WGG (○) systems at 0.80 a_w during storage at 40, 50 and 60°C.

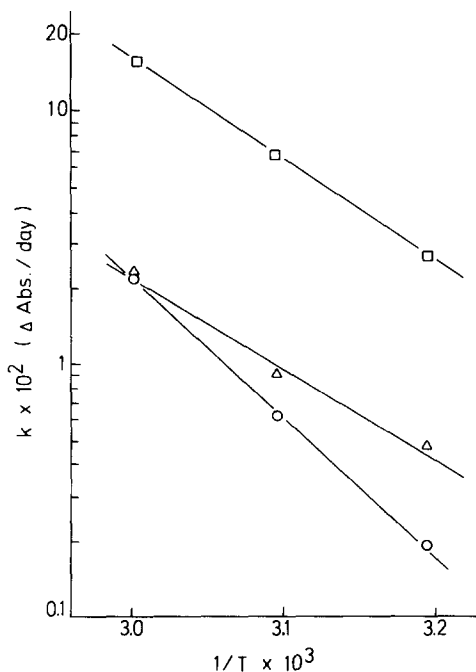


Fig. 5. Arrhenius rate plots of browning in WGS (□), WGS (△) and WGG (○) systems at $0.80 a_w$.

compares the rate constants and the E_a values of the browning reactions in the three systems. The rate of browning was found to be in the order $WGG < WGS < WGS$ at any particular storage temperature. The E_a of browning in the WGG system appeared to be the highest of the systems tested. It should be noted that the WG system at $0.80 a_w$ did not exhibit any browning over the time span of the experiment, even at 60°C . The results

TABLE 2
Rate Constants (k) and Activation Energy (E_a) Values of Browning in WGS, WGS and WGG Systems at $0.80 a_w$

System	$k \times 10^2 (\Delta \text{Abs./day})$			E_a (kcal/mol)
	40°C	50°C	60°C	
WGS	2.57	6.64	15.1	18.3
WGS	0.47	0.91	2.26	16.2
WGG	0.19	0.61	2.17	25.2

of the present study corroborate the findings of Obanu *et al.* (1977) concerning the ability of glycerol to react directly with amino acids (in this case glycine) to produce brown pigments. The sum of the individual contributions of the glycerol-sorbate and the glycerol-glycine browning interactions at any one time cannot, however, account for the very much enhanced browning potential of the WSG system at any particular temperature. It can thus be inferred that the reaction between glycine and sorbic acid (or its oxidation products) possesses a very much greater browning potential than the glycerol-sorbate or the glycerol-glycine interactions. Nevertheless, the browning potentials of the last two reactions are by no means insignificant, especially at high temperatures.

Meaningful mechanistic interpretations of these various reactions cannot be given at this stage. Under the conditions of the present study, oxidation of sorbic acid will very likely take place (Seow & Cheah, 1985). It is highly probable that the carbonyl compounds formed on oxidation of sorbic acid would actively participate in the formation of brown pigments in the WSG system by reaction with glycine. The present results indicate that such reactions can probably lead to significant browning during storage of food systems at intermediate a_w levels. The role of glycerol as a reactant (and not merely as a humectant or solvent) in reactions of significance to IMF bears further investigation.

REFERENCES

- Arya, S. S. (1980). Stability of sorbic acid in aqueous solutions. *J. Agric. Food Chem.*, **28**, 1246-9.
- Bolin, H. R., King, A. D. & Stafford, A. E. (1980). Sorbic acid loss from high moisture prunes. *J. Food Sci.*, **45**, 1434-5.
- Eichner, K. (1975). The influence of water content on non-enzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In: *Water relations of foods* (Duckworth, R. B. (Ed.)). Academic Press, London, pp. 417-34.
- Eichner, K. & Karel, M. (1972). The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *J. Agric. Food Chem.*, **20**, 218-23.
- Hendel, C. E., Silveira, V. G. & Harrington, W. O. (1955). Rates of nonenzymatic browning of white potato during dehydration. *Food Technol.*, **9**, 433-8.
- Labuza, T. P. & Saltmarch, M. (1982). Kinetics of browning and protein quality loss in whey powders during steady state and non-steady state storage conditions. *J. Food Sci.*, **47**, 92-6, 113.

- Loncin, M., Bimbenet, J. J. & Lenges, J. (1968). Influence of the activity of water on the spoilage of foodstuffs. *J. Food Technol.*, **3**, 131–42.
- Mizrahi, S., Labuza, T. P. & Karel, M. (1970). Computer-aided predictions of extent of browning in dehydrated cabbage. *J. Food Sci.*, **35**, 799–803.
- Obanu, Z. A., Ledward, D. A. & Lawrie, R. A. (1977). Reactivity of glycerol in intermediate moisture meats. *Meat Sci.*, **1**, 177–83.
- Saxby, M. J., Stephens, M. A. & Reid, R. G. (1982). Degradation of sorbic acid in model food systems. *Food Chem.*, **9**, 283–7.
- Seow, C. C. & Cheah, P. B. (1985). Kinetics of degradation of sorbic acid in aqueous glycerol solutions. *Food Chem.*, **17**, 95–103.
- Song, P-S., Chichester, C. O. & Stadtman, F. H. (1966). Kinetic behaviour and mechanism of inhibition in the Maillard reaction. I. Kinetic behaviour of the reaction between D-glucose and glycine. *J. Food Sci.*, **31**, 906–13.
- Stamp, J. A. & Labuza, T. P. (1983). Kinetics of the Maillard reaction between aspartame and glucose in solution at high temperatures. *J. Food Sci.*, **48**, 543–3, 547.
- Vidyasagar, K. & Arya, S. S. (1984). Degradation of sorbic acid in fruit squashes and fish paste. *J. Food Technol.*, **19**, 447–54.
- Warmbier, H. C., Schnickels, R. A. & Labuza, T. P. (1976). Effect of glycerol and nonenzymatic browning in a solid intermediate moisture model food system. *J. Food Sci.*, **41**, 528–31.