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The Influence of Water Content and Water Activity on the Sugar–Amino Browning Reaction in Model Systems Under Various Conditions

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Nonenzymatic browning due to the Maillard reaction between reducing sugars and glycine was studied in systems containing varying amounts of water, glycerol, and hydrophilic polymers. We observed that the browning rate decreased with increasing water content, except in systems in which mobility of reactants became substantially impeded at low water contents. The effect of water was complex and depended on the presence of various water-binding agents among other factors. The inhibitory effect of high water contents could be due to the fact that water is a product of several condensation steps in the browning reaction.

Sugar–amino browning reactions in foods and model systems of low moisture content occur over a wide range of water activities (Karel, 1960; Heiss, 1968). A maximum browning reaction occurs in most foods between water activities (a_w) 0.3 and 0.7. The position of this maximum depends on the type of food. Therefore, water activity, though it reflects the effect of water being bound to specific polar groups in the food and other factors limiting the availability of water molecules for chemical reaction, cannot be used to predict optimum browning conditions. Furthermore, in some studies the browning rate increased with increasing water *without a maximum* (Jones, 1954, 1956), and in others increased with *decreasing* moisture content (Rosen *et al.*, 1953; Loncin *et al.*, 1965).

Water's influence on the rate of the sugar–amino browning reaction in a food system is unclear. At higher water activities the reaction rate decrease has generally been attributed to dilution of the reaction partners. The decreased reaction rate at low water activities when the amount of mobile water lowers has been ascribed to an *increasing diffusion resistance* which lowers the mobility of the reaction partners (Labuza *et al.*, 1970).

We performed this study to elucidate the influence of water content, water activity, dilution of the reactants, and of viscosity on the browning rate of reducing sugars and amino acids. By varying the glycerol content in a sugar–amino acid–glycerol–water system, we changed the water content of this system while maintaining a constant water activity and *vice versa*. In this way we investigated the action of water content and water activity on the browning rate separately. We demonstrated the influence of dilution of the reactants by adding increasing amounts of glycerol to the above mentioned system. In order to show the influence of the diffusion resistance on the browning rate, we increased the viscosity of the system by adding water-soluble polymers and lowering the water activity. The viscosity was decreased by the plasticizing effect of glycerol.

MODEL SYSTEMS

The model systems studied contained glucose (glucose and fructose) and glycine as the reactants, as well as additives allowing water activity and water content control.

Materials. Microcrystalline cellulose (Avicel-PH-105), FMC Corp. Methylcellulose (Methocel), visc. 4000 cps, Type MC, Dow Chemical Co. Gum arabic (Hallmark), Stein, Hall & Co., Inc. Polyvinyl alcohol (Elvanol), grade 50-42, and grade 51-05, E. I. du Pont de Nemours & Co., Inc.

Preparation of Sugar–Amino Acid Solutions. 1. 10 g (55.5 mmol) of glucose were dissolved in: a. 10 ml of water; b. 10 ml of a glycerol–water mixture containing 20% glycerol (w/w); c. 10 ml of a glycerol–water mixture containing 40% glycerol (w/w); d. 10 ml of a glycerol–water mixture containing 60% glycerol (w/w); and e. 10 ml of a glycerol–water mixture containing 80% glycerol (w/w). In each solution 2.085 g (27.8 mmol) of glycine were dissolved; in solution e, part of the glycine remained undissolved.

2. Same as solutions 1a–1e, only 1.043 g (13.9 mmol) of glycine were used.

3. Same as solutions 2a–2e, only 5 g of glucose plus 5 g of fructose were used instead of 10 g of glucose.

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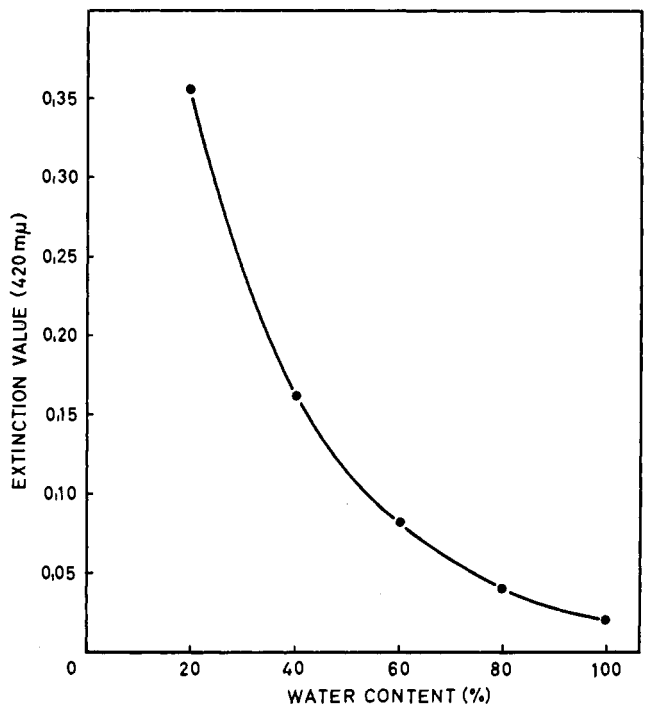

Figure 1. Browning rates of a glucose–glycine–glycerol–water system as influenced by the water content (w/w) of the solvent (Run 1)

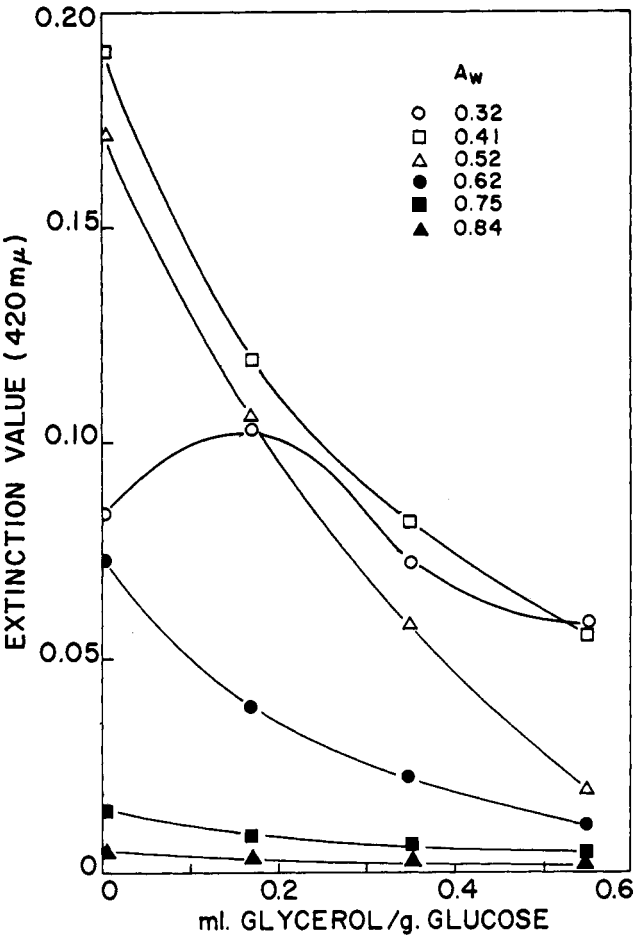

Figure 2. Influence of increasing amounts of glycerol in a glucose–glycine–glycerol–water system on the browning rate at constant water activities (Run 2)

EXPERIMENTAL

Run 1. Influence of water content on the browning rate in a glucose–glycine–glycerol–water system (total volume of the solvent being constant). 2.0–2.5 g of solutions 2a–2e (containing approximately 1 g of glucose) were weighed into serum-cap bottles on an analytical balance, closed heated to 50°C for 70 hr, and diluted with water in volumetric flasks.

The samples were diluted to obtain extinction values mainly between 0.1 and 1.0. We measured the extinction values at 420 mμ for calculation of the extent of browning, and related the extinction values to 1 g of glucose in a browned mixture diluted to 100 ml for data presentation (Figure 1). All following runs were calculated similarly.

We estimated water activities of solutions 2a–2e by storing them over different salt solutions at 37°C, which provided constant water activities (Table I). The water activities of the sugar–amino acid solutions were calculated by graphic interpolation of weight changes after exposure to suitable humidities (Landrock and Proctor, 1951). The resulting water activities are presented in Table II.

Run 2. Influence of the amount of glycerol and water in a glucose–glycine–glycerol–water system on the browning rate at constant water activities. 2.0–2.5 g of solutions 1a–1d were weighed in bottles and stored at 37°C for 70 hr over salt solutions providing relative humidities between 32 and 84% (Table I). We calculated the amount of water in the samples during the reaction by the changes of the sample weights after equilibration (Table III). (The equilibration time could be neglected compared to the reaction time.) The extinction data are presented in Figures 2 and 3.

Run 3. Dilution effect of glycerol in a glucose–glycine–glycerol–water system on the browning rate at constant water contents. Increasing amounts of glycerol were added to the solutions 2a–2e. The browning reaction was performed as described in Run 1. The results are presented in Figures 4 and 5.

Run 4. The influence of water-soluble and insoluble polymers in a glucose–glycine–water system on the browning rate

Table I. Constant Humidity Solutions

Saturated salt solutions	Water activity 37°C, a_w
CH_3COOK	0.23
$MgCl_2$	0.32
K_2CO_3	0.41
$Mg(NO_3)_2$	0.52
$NaNO_2$	0.62
NaCl	0.75
K_2CrO_4	0.84

Table II. Water Activities of Glucose–Glycine–Glycerol–Water Systems at 37°C

Solution	Water activity, a_w
2a	0.86
2b	0.81
2c	0.71
2d	0.58
2e	≅0.40

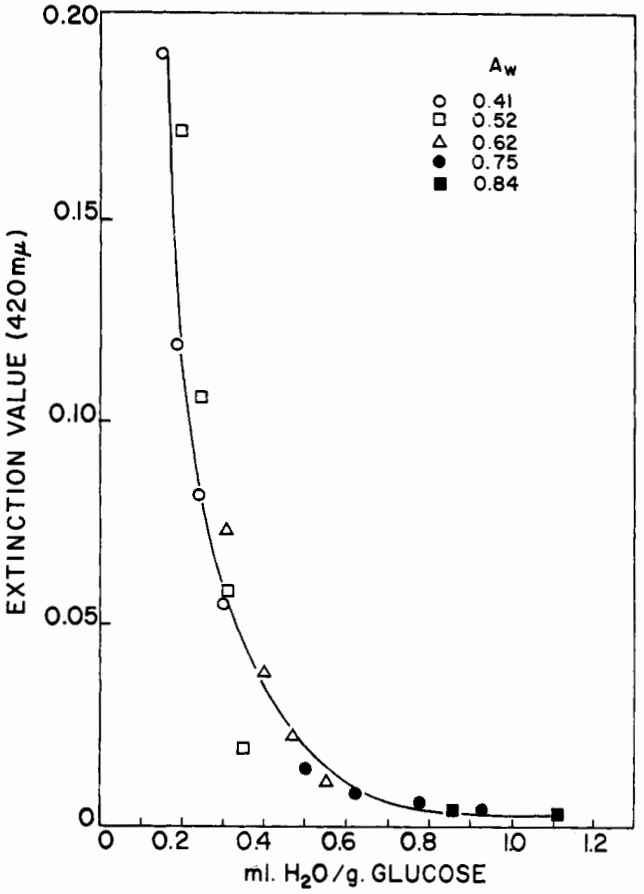

Figure 3. Influence of increasing amounts of water in a glucose–glycine–glycerol–water system on the browning rate at constant water activities (Run 2)

at different water activities. One-third (w/w) microcrystalline cellulose (Avicel), methylcellulose (Methocel), and gum arabic were added to aliquots of solution 2a and mixed thoroughly with a glass rod. The mixtures were stored at 37°C for 70 hr at relative humidities between 41 and 75%. The samples were diluted with water in volumetric flasks and centrifuged for determination of the extinction values. Nonheated reaction mixtures were used as blanks. The results are presented in Figure 6.

Run 5. Browning rates of a glucose–glycine–glycerol–water system as influenced by water-soluble and insoluble additives and by the water content of the solvent. Two-thirds (w/w) Avicel, Methocel, gum arabic, glycerol, and sorbitol were added to aliquots of solutions 2a, 2c, and 2e, and mixed thoroughly. The mixtures were stored in serum-cap bottles at 50°C for 70 hr. The samples were treated as in Run 4 for measurement of the extinction values. The results are presented in Figure 7.

Run 6. The influence of high viscosity and low viscosity polyvinyl alcohol (Elvanol) in a glucose–fructose–glycine–glycerol–water system on the browning rate at constant water activities with increasing amounts of glycerol. One-third (w/w) high viscosity Elvanol (grade 50-42) or low viscosity Elvanol (grade 51-05) dissolved in the fivefold (w/w) amount of water was added to proportions of solutions 3a–3e and mixed with a Sorvall omnimixer. Aliquots of the mixtures and the solutions without Elvanol were weighed in bottles and stored at 37°C for 142 hr at relative humidities between 23 and 62%. The results are presented in Figures 8, 9, and 10.

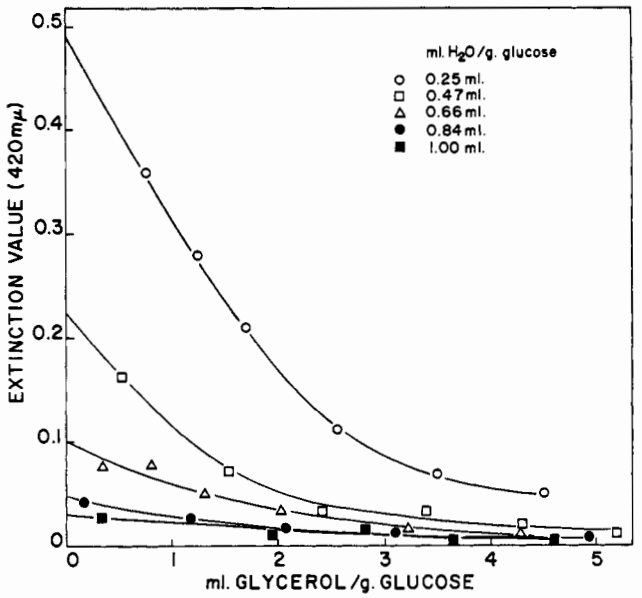

Figure 4. Dilution effect of glycerol in a glucose–glycine–glycerol–water system on the browning rate at constant water contents (Run 3)

Table III. Amounts of Water and Glycerol in Glucose–Glycine–Glycerol–Water Systems at Different Water Activities (Run 2)

Water activity	Solution	ml H_2O/g glucose	ml glycerol/g glucose
0.32	1a	0.09	0.00
	1b	0.13	0.17
	1c	0.17	0.35
	1d	0.20	0.55
	1e	0.22	0.77
0.41	1a	0.15	0.00
	1b	0.19	0.17
	1c	0.24	0.35
	1d	0.30	0.55
	1e	0.37	0.77
0.52	1a	0.20	0.00
	1b	0.25	0.17
	1c	0.31	0.35
	1d	0.35	0.55
	1e	0.41	0.77
0.62	1a	0.31	0.00
	1b	0.40	0.17
	1c	0.47	0.35
	1d	0.55	0.55
	1e	0.63	0.77
0.75	1a	0.50	0.00
	1b	0.62	0.17
	1c	0.78	0.35
	1d	0.93	0.55
	1e	1.10	0.77
0.84	1a	0.86	0.00
	1b	1.11	0.17
	1c	1.37	0.35
	1d	1.73	0.55
	1e	2.07	0.77

RESULTS AND DISCUSSION

The browning rate is increased by a decrease of water content in a glucose–glycine–glycerol–water system (Figure 1; the total volume of the solvent was constant). This finding implies that water is slowing the net reaction in some important steps of browning by laws of mass action (water being formed during the browning reaction). Similar effects

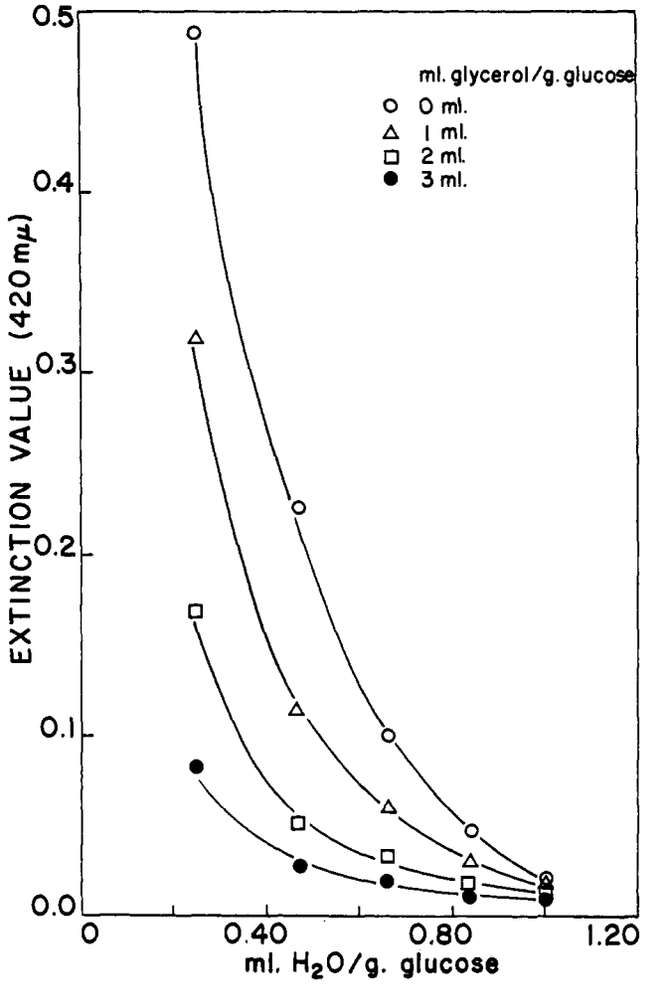

Figure 5. Influence of the amount of water in a glucose–glycine–glycerol–water system on the browning rate at constant glycerol contents (Run 3)

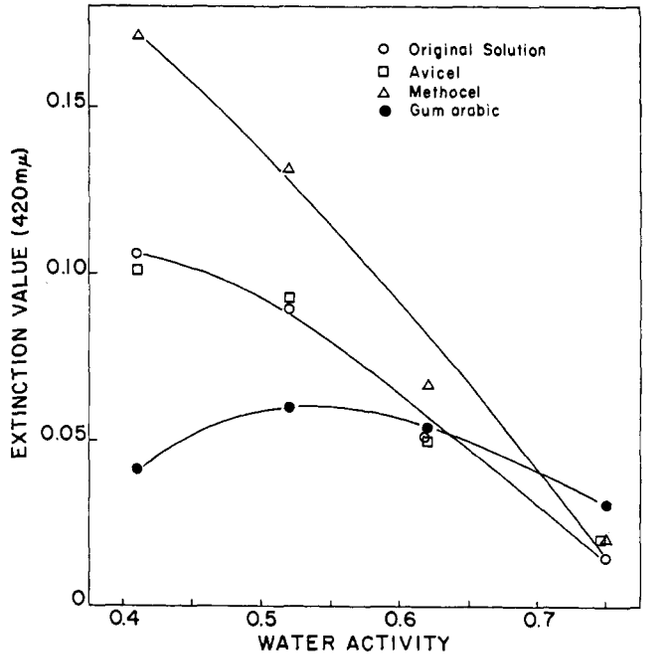

Figure 6. Influence of water-soluble and insoluble polymers in a glucose–glycine–water system on the browning rate at different water activities (Run 4)

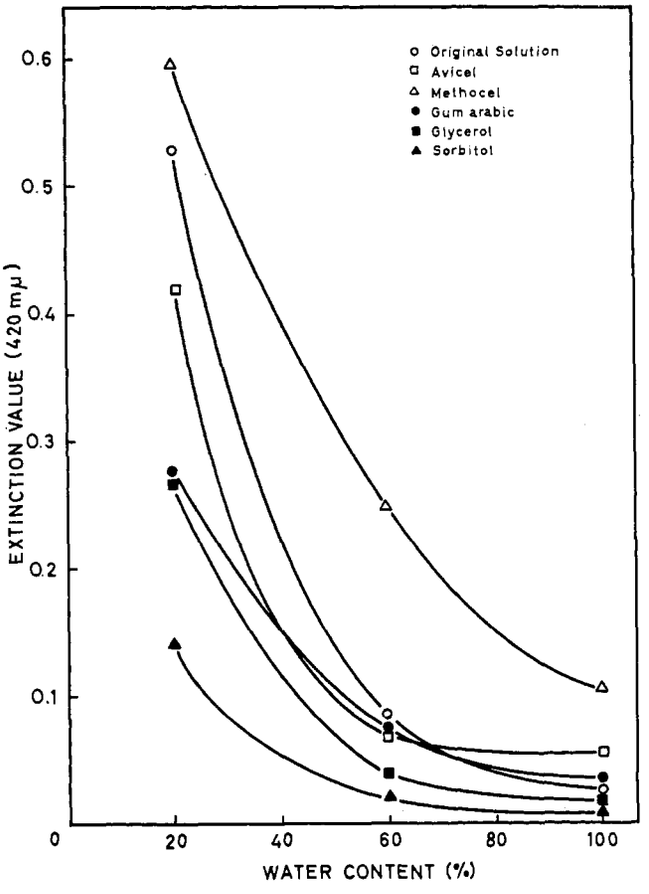

Figure 7. Browning rates of a glucose–glycine–glycerol–water system as influenced by water-soluble and insoluble additives and by the water content (w/w) of the solvent (Run 5)

in foods can be explained on the same basis as the above results obtained in a model system.

In Run 2 (Figures 2 and 3) systems containing different amounts of glycerol were stored at constant relative humidities. Increasing the amount of glycerol lowered the reaction rate more than simply diluting reactants in the solvent (Figure 4).

Increasing the amount of glycerol at a constant water activity increases the amount of water in the present system (Table III). Therefore the observed decrease in the browning rate is due both to the product-inhibition of the forward reaction by water and the diluting influence of water and glycerol, decreasing the concentration of the reactants. As we show in Figure 3, the reversing effect of water is much higher than the diluting effect, compared with Figure 4.

Decreasing water activity at constant glycerol contents *increases* the browning rate down to a_w 0.4. At a_w 0.32 the browning rate is *decreased*, especially at a glycerol concentration of zero due possibly to the decreased mobility of reactants and products at the very high viscosity of the sugar solution under these conditions (the sugar remained in a glassy state solution without crystallization). With increasing amounts of glycerol, two opposite effects are causing a maximum in the rate of browning. First, in the presence of relatively low water contents (Table III), the plasticizing effect of glycerol improves the mobility of the reactants, thus increasing the reaction rate. Second, the increasing amount of water and glycerol lowers the reaction rate due to factors described above. The low mobility at a_w 0.32 results in a reaction rate lower than at a_w 0.41 at the corresponding water contents.

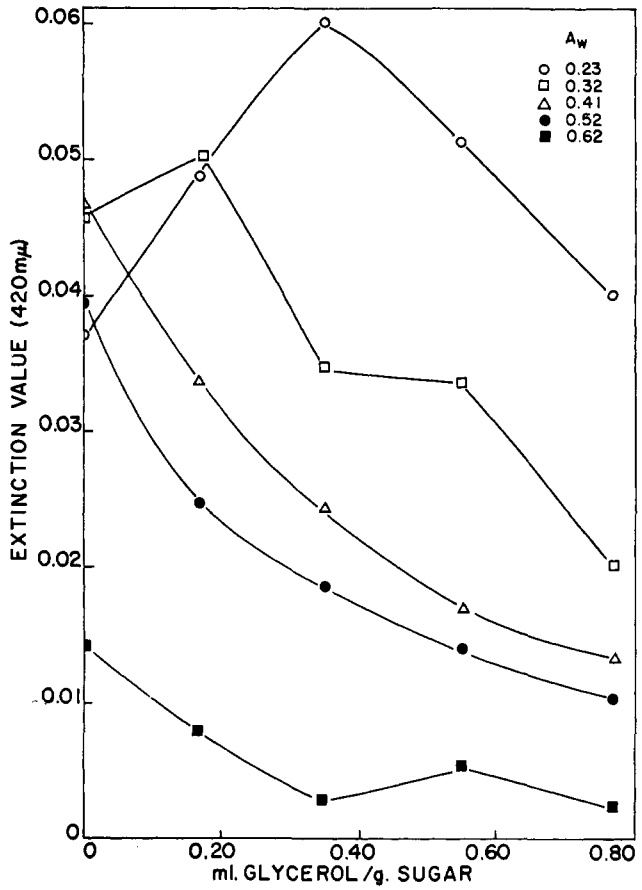

Figure 8. Browning rates of a glucose–fructose–glycine–glycerol–water system at constant water activities with increasing amounts of glycerol (Run 6)

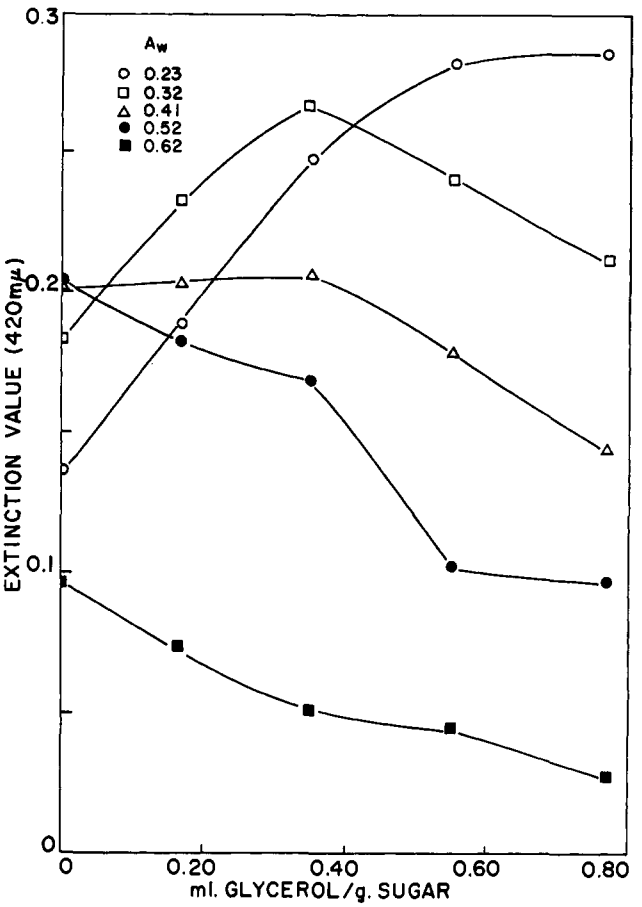

Figure 10. Influence of low-viscosity polyvinyl alcohol (Elvanol 51-05) in a glucose–fructose–glycine–glycerol–water system on the browning rate at constant water activities with increasing amounts of glycerol (Run 6)

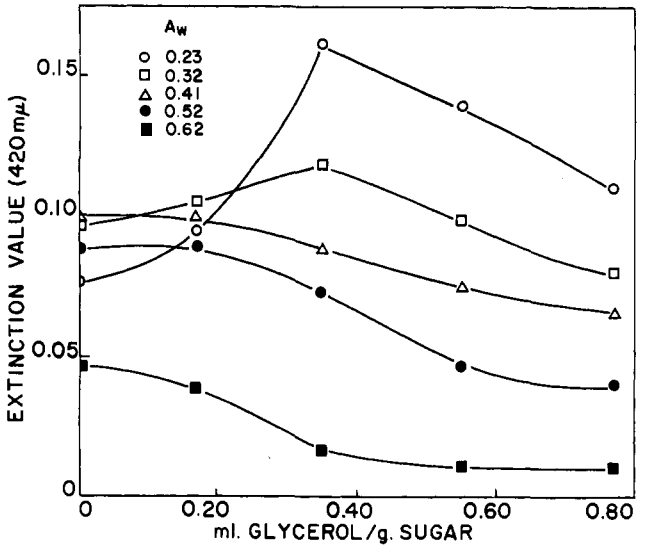

Figure 9. Influence of high-viscosity polyvinyl alcohol (Elvanol 50-42) in a glucose–fructose–glycine–glycerol–water system on the browning rate at constant water activities with increasing amounts of glycerol (Run 6)

In Run 3 (Figures 4 and 5) we investigated the diluting effect of glycerol at constant water contents. Figure 5 shows the effect of water at constant glycerol contents. At low water contents (high browning rates) the diluting effect of glycerol is very significant, whereas at higher water contents this effect becomes much smaller. This experiment shows again that inhibition of browning by water is greater than the effect of decreasing the concentration of the reactants.

Further studies concerned effects on the browning rate at low water activities of increased diffusion resistance due to addition of various polymers. We added Avicel, Methocel, and gum arabic to a solution of glucose and glycine in water and equilibrated to different relative humidities (Run 4, Figure 6). The browning rate was increased by Methocel, but decreased by gum arabic at the lower water activities. A higher viscosity provided by the gum arabic may cause this decrease, whereas Methocel seems to reaggregate to insoluble aggregates before higher viscosities are reached, and does not decrease the browning rate. On the contrary, the water-binding properties of Methocel may be responsible for the increase in browning rate compared with the original sugar–glycine solution without additives.

The addition of Avicel, which is insoluble in water, does not show any influence on the browning rate.

In Run 5 (Figure 7) the water activities were determined by the ratio of glycerol to water. At lower water activities, Methocel and gum arabic reacted as in Run 4; Avicel lowered the browning rate to a small degree. The action of sorbitol which lowers the browning rate more than glycerol is remarkable.

In Run 6 (Figures 8, 9, and 10) we studied the influence of viscosity on the browning rate, using high and low viscosity polyvinyl alcohol (Elvanol). Figure 6 shows the behavior of the solutions 3a–3e without Elvanol at different water activities. The browning rate of the samples without glycerol was in-

creased up to a_w 0.41 without further increase at a_w 0.32, but decreased at a_w 0.23. This action may again be due to the increasing viscosity of these solutions at low water activities.

Addition of glycerol at a_w 0.23 and 0.32 increased the browning rate first (plasticizing effect of glycerol) but decreased it again with higher proportions of glycerol (diluting effect of glycerol and water, inhibitory action of water by the laws of mass action; Figures 8, 9, 10).

Comparing the high and low viscosity Elvanol reaction mixtures (Figures 9 and 10) we see that the samples with the high viscosity Elvanol showed only about 50% of the browning of the low viscosity Elvanol samples. Furthermore, the browning rate in the Elvanol reaction mixtures was higher than in those without Elvanol, due perhaps to the water-binding properties of Elvanol overcoming even the influence of higher viscosities. The browning maximum in most of the Elvanol samples was shifted towards higher glycerol concentrations, indicating that more glycerol was necessary to attain the optimal plasticizing effect than in samples without Elvanol.

In the high viscosity Elvanol reaction mixture, (Figure 9) the differences of the extinction values at different water activities and different amounts of glycerol are smaller than in the samples without Elvanol (Figure 8) and with low viscosity Elvanol (Figure 10). This smaller difference indicates that in high viscosity systems the influence of viscosity is predominant over a wider range of water activities, thus diminishing the influence of water activity and water content.

CONCLUSIONS

Lowering the water content of a sugar–amino acid system increased the browning rate, except in systems in which reactant mobility became limited in high viscosity solutions of low water activities. Partial restoration of the mobility through the plasticizing effect of glycerol increases the browning rate at low moisture contents.

Browning rate in a sugar–amino system is not simply related to water activity. Optimum browning conditions are determined by the amount of water and state of water binding in a distinct system, and by the mobility of reactants in the system. The maximum browning depends on the extent to which these conflicting influences affect the reaction.

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Viscosimetric Studies of Alkaline Degradation of Ovomucin

John W. Donovan,* John Gorton Davis, and Mary B. Wiele

Thinning or decrease in viscosity of the gel-like thick portion of the white of chicken eggs is produced by exposure of the high molecular weight glycoprotein ovomucin to alkaline pH. Studies of the rate of change of viscosity of solutions of egg white and of ovomucin as a function of pH, temperature, concentration of hydroxide ion, and concentration of ovomucin indicate that the reaction producing thinning is first-order in hydroxide ion activity and first-order in ovomucin concentration. The activation energy is approximately 7 kcal/mol. The rate of thinning appears to have no relation to the lysozyme concentration.

The thinning of the white of chicken eggs held in storage is a well-known phenomenon (for a review, see Romanoff and Romanoff, 1949). Thinning is a decrease in the viscosity of the thick, gel-like portion of the white of the egg which contains the high molecular weight glycoprotein ovomucin (Almquist and Lorenz, 1932; McNally, 1933; Almquist *et al.*, 1934). Ovomucin, a virus-hemagglutination inhibitor (Lanni and Beard, 1948) of high intrinsic viscosity, has been characterized by Lanni *et al.* (1949), Sharp *et al.* (1950, 1951), and by Donovan *et al.* (1970). A review of the earlier work on isolation and characterization has been given by Warner (1954).

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Balls and Hoover (1940) showed that the amount of ovomucin precipitated from fresh eggs was the same as that precipitated from naturally-thinned eggs; that is, that thinning did not change the amount of ovomucin present. They precipitated ovomucin from fresh eggs and allowed it to be digested by trypsin. The precipitability properties of the proteolytically digested ovomucin were markedly different from those of ovomucin either from fresh eggs or from eggs which had thinned naturally. Thus, although Neumann and Sela (1960) and Hasegawa (1961) have reported proteolytic activity in egg white, thinning does not appear to be produced by proteolytic enzymes.

Hoover (1940) suggested that a reducing agent naturally present in egg white caused thinning, and *in vitro* showed a large decrease in viscosity when reducing agents were added to solutions of ovomucin. These experiments were confirmed