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Reactivity of Sorbate and Glycerol in Some Model Intermediate Moisture Systems

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ABSTRACT

Model systems were developed to study the rôle of selected humectants and antimycotics in non-enzymic browning, haemoprotein breakdown and collagen degradation reactions at a_w 0·85 and initial pH 5·5. a_w adjustment was made using sodium chloride and the solutions contained 0·5% glucose, 0·5% sorbate, 0·5% propionate, 30% glycerol or 30% glycerol plus 0·5% sorbate. During aerobic storage at 38°C or 65°C, 10% lysine or glutamate solutions all exhibited increased browning and 0·01% or 0·02% haemoglobin solutions increased loss of haemoprotein from solution in the presence of glycerol and/or sorbate. During anaerobic storage only the glucose-containing solutions exhibited any reactivity.

The pH and concentration of reactive carbonyls in the systems were also monitored and the rôle of sorbate and glycerol oxidation products in non-enzymic browning is discussed in the light of these results.

Breakdown of the collagen in heat-treated tendon during storage at 38°C under similar conditions was also studied. Although the results were not unequivocal, it was apparent that, at least initially, storage in 30% glycerol caused increased degradation.

INTRODUCTION

In the preparation of novel intermediate meat products it is usual to use, as the humectants, salt (to the level of normal seasoning) and a polyol

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such as glycerol (Ledward, 1981) to lower the water activity, a_w, to the required level, usually 0·85. In addition, sorbate is usually added to prevent mould growth. It has been shown that, in model systems (Obanu *et al.*, 1977; Seow & Cheah, 1985*a*,*b*) both glycerol and sorbate can, following oxidation, take part in non-enzymic browning (NEB) by reacting with the amino groups of amino acids or proteins. These types of reaction may well be responsible, at least in part, for the covalent linkages established between the proteins of intermediate moisture meats during storage at 38 °C (Ledward, 1981; 1985). Although these types of reactions do take place in intermediate moisture meats during storage at elevated temperatures, it is well established that degradation of the haemoproteins and extensive proteolysis of the collagen of the connective tissue also occurs (Ledward, 1981). The rôle, or not, of the humectants and antimycotic in these last two reactions is not known. The present study was designed to elucidate the rôle of selected humectants and antimycotics in the rate of these browning and degradative reactions in some model intermediate moisture systems.

MATERIALS AND METHODS

Three types of model system, designed to follow non-enzymic browning (I), reaction with haemoproteins (II) and collagen degradation (III) at a_w 0·85 and pH 5·5 (*cf.* intermediate meat products) were designed. In all cases adjustment to pH 5·5 was by addition of 1 M HCl or 1 M NaOH and sodium chloride was used to adjust the a_w to $0{\cdot}85 \pm 0{\cdot}02$. Initial adjustment to a_w 0·85, whilst keeping the concentration of the reactants at the desired level, was achieved by trial and error using an equisina-hydroscope to monitor the a_w.

Non-enzymic browning reactions

The systems consisted of 10 % lysine or 10 % glutamic acid (50 g/500 ml) adjusted to a_w $0{\cdot}85 \pm 0{\cdot}02$ and pH 5·5 in the solutions shown in Table 1. After taking initial readings, the solutions were divided into four equal portions and placed in 250 ml conical flasks. One aliquot was placed at 65 ± 1 °C and one at $38° \pm 1$ °C, the aerobic samples. The remaining aliquots were flushed with oxygen-free nitrogen for 5 min and then evacuated for about 15 min. These latter samples were assumed to be

anaerobic and were also stored at 65 ± 1 or 38 ± 1 °C. Aliquots (~ 10 ml) were analysed at regular intervals. After each sampling the anaerobic samples were reflushed with N_2 and re-evacuated. To examine the concentration-dependence with sorbate, 10% lysine solutions were also stored aerobically at 65 °C and pH 5·5, in 40% glycerol, 9·5% salt and 0% to 0·5% sorbate.

TABLE 1
Composition of the Solutions used to Follow the Rôle of Selected Reactants in Non-enzymic Browning (NEB) (g/500 ml)

Model system	*Antimycotic/ Humectant*	*Salt*	a_w
10% lysine (control)	0	109	0·84
10% lysine + 0·5% K sorbate	2·5	100	0·84
+ 0·5% Ca propionate	2·5	100	0·85
+ 0·5% Glycerol	2·5	100	0·84
+ 0·5% Glucose	2·5	100	0·85
+ 30% Glycerol	150	42	0·84
+ 30% Glucose	150	66	0·84
+ 30% Glycerol + 0·5% Sorbate	150	35	0·84
10% Glutamate (control)	—	107	0·84
+ 0·5% K sorbate	2·5	100	0·85
+ 0·5% Ca propionate	2·5	100	0·85
+ 0·5% Glycerol	2·5	100	0·86
+ 0·5% Glucose	2·5	100	0·85
+ 30% Glycerol	150	42	0·84
+ 30% Glucose	150	68	0·85
+ 30% Glycerol + 0·5% Sorbate	150	35	0·84

At each sampling time the absorbance at 420 nm was determined (against distilled water) using a Perkin–Elmer Model 124 double beam spectrophotometer (to monitor browning) and the pH was determined. To determine the presence of carbonyl oxidation products, especially malonaldehyde, 5·0 ml of the solution was mixed with 5·0 ml of 0·2M thiobarbituric acid (TBA) in 90% glacial acetic acid. The mixture was heated in a boiling water bath for 35 min, cooled in tap water for 10 min and the absorbance at 538 nm determined (Tarladgis *et al.*, 1960).

All storage trials were performed in duplicate.

Haemoprotein reactivity

Initial experiments indicated that storage at 65 °C led to very rapid precipitation of the protein (haemoglobin) and that flushing with N_2 led to frothing and surface denaturation of the protein. Thus, in these trials, storage was only at 38 °C and selected samples were made 'anaerobic' by evacuation for 15–30 min. Bovine haemoglobin (No. H3760, Sigma Chemical Co., Poole, Dorset, Great Britain) was chosen as the test protein and all solutions were made 0·01 % or 0·02 % (w/v) with respect to it. The composition of the solutions is shown in Table 2.

At intervals during storage, aliquots (~ 10 ml) were taken for analysis. Analysis consisted of recording the spectra of the solutions (from 550 nm to 360 nm) with a Perkin–Elmer double beam Model 124 spectrophotometer and measuring the pH.

Collagen degradation

Achilles tendon from heifer (first experiment) or steer (second experiment), 9–12 months old, was freeze-dried and 1·0 g suspended in 200 ml solutions of composition identical to that shown in Table 1 (excluding the amino acid), i.e. at 0·5 % concentration. The suspensions were heated in a boiling water bath for 90 min to ensure the collagen was in the denatured form. In both experiments, two such systems, in each solution, were made up and stored, aerobically and anaerobically at 38 °C.

At intervals during storage, aliquots of the solution were removed with

TABLE 2

Composition of the Solutions Containing 0·01 % or 0·02 % Haemoglobin (Hb) (w/v) used to Monitor the Rôle of Selected Reactants with this Protein (g/500 ml)

Model system	*Antimycotic/ Humectant*	*Salt*	a_w
0·01 or 0·02 % Hb	0	110	0·86
+ 0·5 % K sorbate	2·5	105	0·86
+ 0·5 % Ca propionate	2·5	105	0·86
+ 0·5 % Glucose	2·5	105	0·85
+ 30 % Glycerol	150	65	0·81

a fine bore Pasteur pipette (~ 10 ml) and the concentration of soluble hydroxyproline determined by the method of Woessner (1961). The pH of the solution was also determined.

RESULTS

Non-enzymic browning of amino acids

At an a_w of 0·85 and initial pH of 5·5 both 30 % glycerol and 0·5 % sorbate led to increased rates of browning in 10 % lysine and 10 % glutamate solutions held at 65 °C (Figs 1 and 2), sorbate being the most reactive. On prolonged storage at this temperature (> 30 days), all the solutions ultimately browned but the rates of those containing 0·5 % propionate, 0·5 % glycerol and lysine or glutamate alone were very slow (Figs 1 and 2).

All the browning curves exhibited a lag phase followed by a rapid, approximately linear increase in absorbance with time ('zero-order' reaction). Seow & Cheah (1985*b*) also found that, at pH 4·0, sorbate and

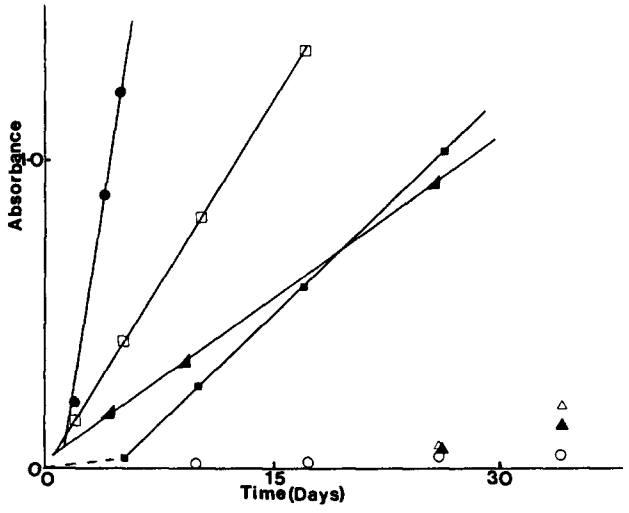

Fig. 1. Absorbance at 420 nm of solutions of 10 % lysine at a_w 0·85 and initial pH 5·5 (○) and similar solutions containing 0·5 % glucose (●), 0·5 % potassium sorbate (□), 30 % glycerol (■), 0·5 % calcium propionate (△), 0·5 % glycerol (▲) and 0·5 % sorbate plus 30 % glycerol (◢) during storage at 65 °C. The solutions were adjusted to a_w 0·85 by the addition of sodium chloride as shown in Table 1. A second experiment yielded similar, but not identical, results.

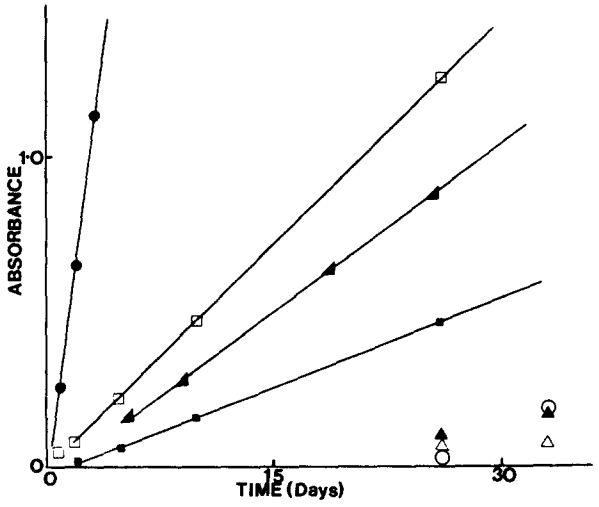

Fig. 2. Absorbance at 420 nm of solutions of 10 % glutamate at a_w 0·85 and initial pH 5·5 (○) and similar solutions containing 0·5 % glucose (●), 0·5 % potassium sorbate (□), 30 % glycerol (■), 0·5 % calcium propionate (△), 0·5 % glycerol (▲) and 0·5 % sorbate plus 30 % glycerol (◢) during storage at 65 °C. The solutions were adjusted to a_w 0·85 by the addition of sodium chloride as shown in Table 1. A second experiment yielded similar, but not identical, results.

glycerol model systems browned with zero order kinetics. Attempts to rationalize the reaction profiles in terms of the length of the lag phase, rate of increase in absorbance of the linear phase and the time to reach an absorbance of 1·0 are shown in Table 3. It is apparent that, no matter what criteria are chosen, the glutamate reacts far more slowly than the lysine and the sorbate system is far more liable to browning than one containing 30 % glycerol. Interestingly, a system containing 30 % glycerol plus 0·5 % sorbate appears to brown less rapidly than one containing only 0·5 % sorbate (Table 3). However, a_w adjustment in the solutions was carried out by the addition of sodium chloride and thus those containing 30 % glycerol contained far less of this humectant than those not containing glycerol (Table 1). Sodium chloride is believed to catalyse NEB reactions involving polyols (Obanu *et al.*, 1977) and this may account for this apparent anomaly, i.e. salt, *in the presence of a potential browning carbonyl*, is a more effective catalyst than glycerol at an a_w of 0·85. Warmbier *et al.* (1976) found that, in model amino acids–reducing sugars systems, the a_w of maximal browning was at 0·48 in a glycerol-based system but at 0·83 when a solid humectant was used, i.e. at a_w

TABLE 3
Kinetics of NEB at 65 °C

System	*Lag phase (days)*	*Rate (units/day)*	*Time to reach an absorbance of 1·0 (days)*
10 % lysine + 0·5 % glucose	< 1	0·35	3
	< 1	0·30	4
10 % glutamate + 0·5 % glucose	< 1	0·35	3
	< 1	0·40	3
10 % lysine + 0·5 % sorbate	< 1	0·084	12
	< 1	0·085	9
10 % glutamate + 0·5 % sorbate	10	0·055	25
	4	0·050	21
10 % lysine + 30 % glycerol	9	0·060	23
	5	0·050	26
10 % glutamate + 30 % glycerol	> 10	0·010	> 60
	16	0·019	60
10 % lysine + sorbate + glycerol	< 1	0·035	28
10 % glutamate + sorbate + glycerol	4	0·036	29

0·83, the rate of browning decreased by a factor of 4 or 5 in a glycerol-based, compared with a salt-based, system. This supports the above contention. In Table 3 the results obtained for 0·5 % glucose solutions are shown and it is seen that, in this case, there is little difference between the reactivity of the glutamate and lysine.

At 38 °C the rates of browning are very much slower and there is little apparent difference between the lysine and glutamate systems. Typical results are shown in Fig. 3. There was no evidence for any browning in those solutions containing no sorbate, with the exception of those containing 30 % glycerol or 0·5 % glucose which browned slowly and only achieved absorbances of about 0·1 after 60 days' storage (Fig. 3).

In the solutions from which it was attempted to exclude air, browning was inhibited in the sorbate and glycerol systems. However, glucose systems browned at approximately the same rates as in air. Typical results for samples stored at 65 °C are shown in Table 4.

The pH of all solutions was monitored as a function of time and, as expected, in the lysine solutions browning in those containing glucose led to a marked decrease in pH as the amino groups became unavailable due to their active participation in the browning reactions. The lysine–glycerol systems exhibited similar, if less marked, behaviour at 65 °C but,

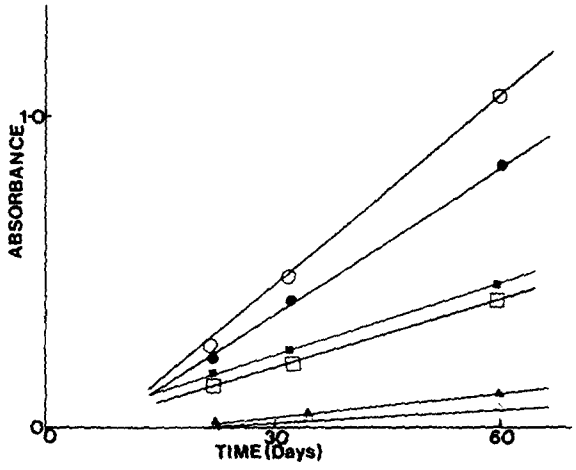

Fig. 3. Absorbance at 420 nm of 10% solutions of lysine (○, ■, △) and glutamate (●, □, ▲) in the presence of 0·5% sorbate (●, ○), 30% glycerol (△, ▲) and 0·5% sorbate plus 30% glycerol (■, □) during aerobic storage at 38 °C, a_w 0·85 and initial pH 5·5. All other solutions shown in Table 1, except 30% glucose which had an absorbance of greater than 2 after 20 days, exhibited little browning. After 60 days the absorbances of both control solutions was less than 0·01, those containing calcium propionate less than 0·04 in glutamate and less than 0·01 in lysine, those containing 0·5% glucose less than 0·05 in lysine and about 0·12 in glutamate.

at 38 °C, the decrease in 30% glycerol was more marked than in the 0·5% glucose systems. In the sorbate solutions there was a marked *increase* in pH, due presumably to oxidation of the sorbate which, under these circumstances, must override the loss of amino groups in the browning reactions; this was observed at both 38 °C and 65 °C but the change was far slower at the lower temperature and, after about 40 days at 38 °C, a

TABLE 4

Absorbance at 420 nm After 3 or 59 Days' Storage at 65 °C of 10% Lysine Solutions of a_w 0·85 when Stored in the Presence and Absence of Air

System	*Aerobic*	*Anaerobic*
0·5% sorbate	>6·0[a]	0·152[a]
0·5% glucose	1·0[b]	1·25[b]
30% glycerol	2·1[a]	0·209[a]

[a] After 59 days.
[b] After 3 days.

decrease was noted in the sorbate systems, indicating that the loss of amino groups in browning may become the dominant reaction. The results are summarised in Figs 4 and 5. In the anaerobic systems, apart from the glucose systems, which showed similar decreases to those observed in air, only the sorbate and glycerol systems exhibited small, but measurable, changes in pH. Thus, the solutions containing 0·5 % sorbate increased by about 0·8 of a pH unit after 59 days at 65 °C and by about 0·5 of a unit after this time at 38 °C. The solutions containing 30 % glycerol decreased by about 0·1 and 0·2 of a unit after 59 days at 38 °C and 65 °C, respectively.

In the glutamate systems the changes in pH on storage were far less than in the lysine systems. Even in the glucose systems at 65 °C, which brown as readily as the lysine systems, the decrease over 35 days was less than 0·4 of a pH unit compared with about 1·8 units in the lysine solutions (Fig. 4). The 0·5 % sorbate–glutamate solutions increased in pH by about 0·6 of a unit over 35 days (*cf.* 1·5 units in the lysine solutions) at 65 °C but no measurable differences were observed in any of the other glutamate-containing systems at 65 °C. At 38 °C all the changes were less than 0·1 of a

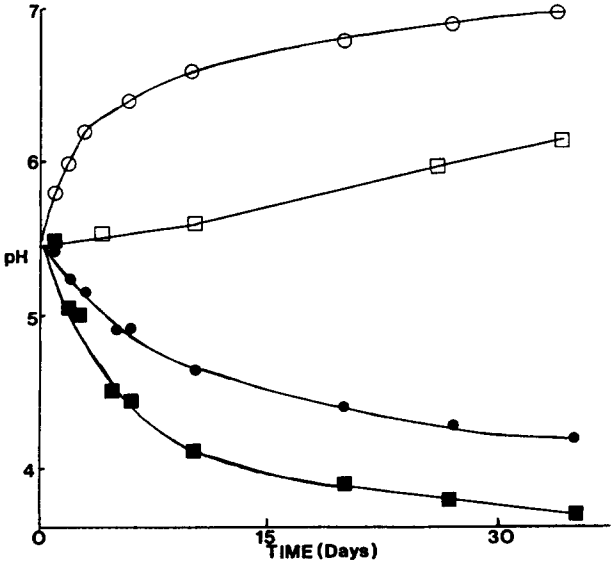

Fig. 4. pH of 10 % solution of lysine at a_w 0·85 during aerobic storage at 65 °C in the presence of 0·5 % sorbate (○), 30 % glycerol (●), 30 % glycerol + 0·5 % sorbate (□) and 0·5 % glucose (■). Combined results from two different experiments analysed at different times.

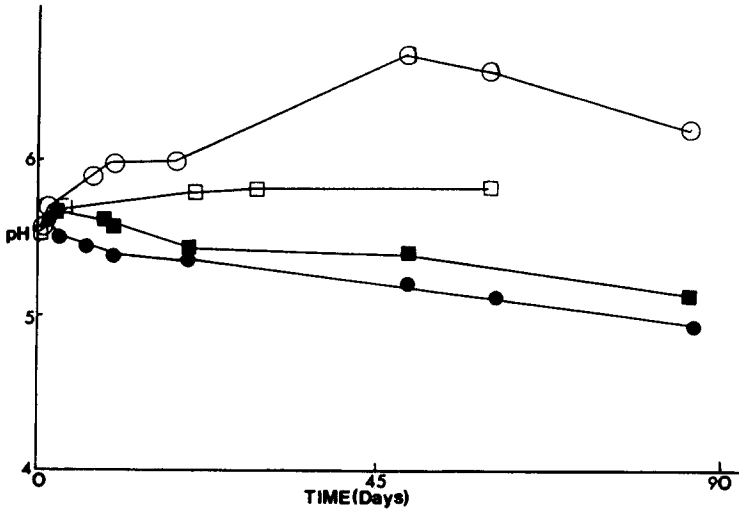

Fig. 5. pH of 10% solution of lysine at a_w 0·85 during aerobic storage at 38 °C in the presence of 0·5% sorbate (○), 30% glycerol (●), 30% glycerol + 0·5% sorbate (□) and 0·5% glucose (■). A second experiment gave similar, but not identical, results.

unit except for the sorbate solutions which increased by about 0·2 of a unit over 40 days. These results are undoubtedly due to the buffering capacity of the glutamate in this pH range.

It has been claimed that malonaldehyde and other such reactive carbonyls derived from the oxidising sorbate and polyols are the major reactants in the browning reactions (Ledward, 1985). These compounds react with TBA reagent to yield a pink complex with maximal absorbance at 538 nm. Analysis of the solutions during storage indicated that these types of compounds were present at far higher levels in the sorbate systems than the others (Table 5). However, the results did not appear to vary in any consistent manner with the time of storage, in air. Sodium glutamate solutions in the presence of salt all gave relatively high TBA reactivities (absorbances in the range 0·2 to 1·2 at 65 °C) and although those containing sorbate always yielded higher values (absorbances in the range 0·4 to 3·0), the results were difficult to quantify. Lysine alone gave no such TBA reactivity (absorbance less than 0·1) and in both experiments during storage at 65 °C the concentration of TBA-reactive species in the lysine/sorbate solutions decreased during 1 day of storage, the concentration remaining relatively constant during further storage. No such changes were apparent in the other solutions. At 38 °C there was

TABLE 5
Absorbance at 538 nm of TBA Complex in 10% Lysine Solutions Stored at 65 °C and 38 °C and Adjusted to an a_w of 0·85 ± 0·01 With Sodium Chloride

Solution	*Time (days)*	*Temperature (°C)*	*Absorbance*
Control	0–60	65	0·03 ± 0·01[b]
Control	0–34	65	0·01 ± 0·01
Control	0–60	38	0·02 ± 0·01
Control	0–34	38	0·00 ± 0·00
+ 0·5% sorbate	0[a]	—	2·30
+ 0·5% sorbate	0[a]	—	2·76
+ 0·5% sorbate	0[a]	—	2·80
+ 0·5% sorbate	1–34	65	1·70 ± 0·09
+ 0·5% sorbate	1–23	65	1·34 ± 0·015
+ 0·5% sorbate	3–20	38	4·70 ± 0·35
+ 0·5% sorbate	23–34	38	4·95 ± 0·20
+ 30% glycerol	0–52	65	0·27 ± 0·02
+ 30% glycerol	0–34	65	0·28 ± 0·06
+ 30% glycerol	0–60	38	0·20 ± 0·02
+ 30% glycerol	0–34	38	0·07 ± 0·03
+ 0·5% propionate	0–52	65	0·07 ± 0·02
+ 0·5% propionate	0–34	65	0·04 ± 0·01
+ 0·5% propionate	0–60	38	0·03 ± 0·01
+ 0·5% propionate	0–34	38	0·01 ± 0·01
30% glycerol	0–25	65	0·52 ± 0·50[b]
+ 0·5% sorbate	0–25	38	0·82 ± 0·60[b]

[a] Within 4 h of preparation at room temperature (~ 20 °C). All values are means ± standard errors from 2 to 12 determination taken over the time interval indicated.
[b] Some evidence for slight increase during storage, but effect small and difficult to quantify on the number of samples studied.

an apparent increase in the concentration of such compounds during storage of the sorbate solutions but no apparent change in the other solutions. These results are summarised in Table 5.

The relatively low concentration found in the sorbate/glycerol system indicates that the nature of the humectant (salt or glycerol) markedly affects their rate of formation in much the same way as the browning reactions themselves are humectant-dependent (Figs 1 and 2).

During anaerobic storage the sorbate solutions initially contained high

concentrations of TBA-reactive compounds (absorbance at 538 nm > 2) and no decrease in concentration was apparent during 30 days' storage at either 38 °C or 65 °C. In the 30 % glycerol systems, the concentration varied little with time at 38 °C although a slight decrease was apparent at 65 °C (from 0·17 to 0·04).

To gain further information regarding the sorbate reactivity, the browning of a typical humectant mix containing lysine was followed at 65 °C at different sorbate concentrations. It was found that the time of the lag phase and the subsequent rates were directly proportional to the initial sorbate concentrations (Fig. 6). In these experiments the a_w was 0·69, i.e. 10 % lysine, 40 % glycerol and 10 % salt. It is seen that the rate of browning of these solutions is much faster than in 10 % lysine solutions containing 0·5 % sorbate and 30 % glycerol (a_w 0·85, Fig. 1). This supports the Warmbier *et al.* (1976) finding that, in a glycerol-based system, the a_w of maximum browning is shifted to lower a_w values. They found that,

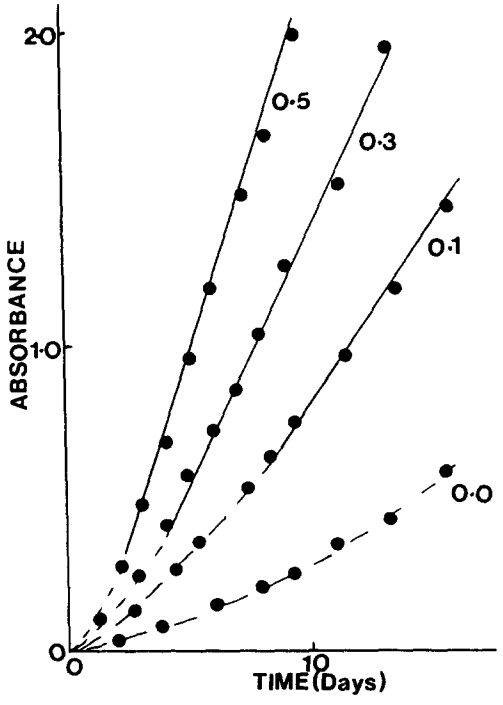

Fig. 6. Absorbance at 420 nm of solutions containing (w/w) 10 % lysine, 40 % glycerol, 9·5 % salt (a_w = 0·69) and potassium sorbate at the levels indicated during aerobic storage at 65 °C.

for an amino acid–reducing sugars system the a_w of maximal browning was at 0·48 in a glycerol system and 0·83 in one based on sucrose. Webster (1980) found that, in 10% lysine, 10% salt and 0·5% sorbate and varying glycerol concentrations, the a_w of maximal browning was at 0·69, i.e. the conditions used in the experiment reported in Fig. 6.

Reactions with haemoglobin

At both 0·01% and 0·02% haemoglobin loss of the characteristic spectrum, indicative of the presence of haematin in solution, as judged by the loss of absorption at 410 nm, was observed in all systems during storage at 38 °C. However, the losses were far more rapid in the 0·5% sorbate and 30% glycerol solutions stored in air (Figs 7 and 8). On prolonged storage marked precipitation was apparent in these solutions, due presumably to NEB, and was undoubtedly the major cause of the apparent loss of haematin character in solution. However, in some of the

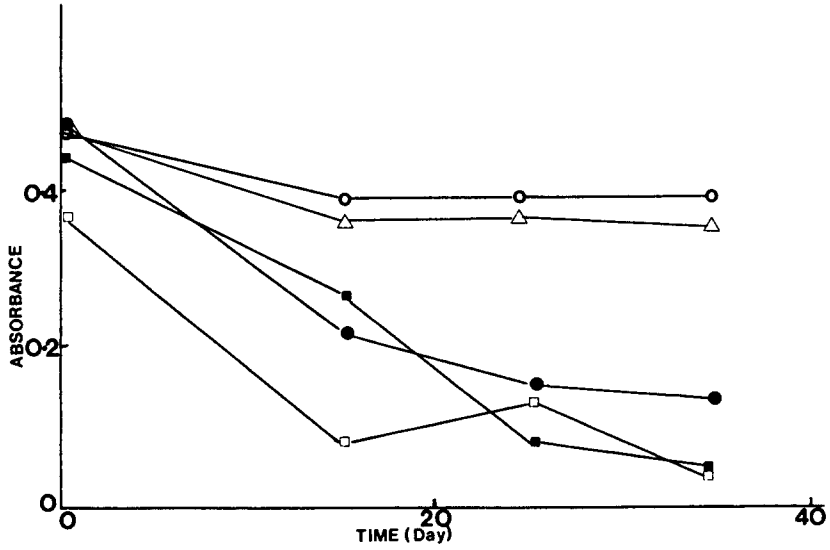

Fig. 7. Absorbance at 410 nm of approximately 0·01% haemoglobin at a_w 0·85 during aerobic storage at 38 °C (○) and in the presence of 0·5% sorbate (●), 30% glycerol (□), 0·5% glucose (■) and 0·5% propionate (△). Most solutions stored anaerobically yielded similar profiles to those given by the haemoglobin solutions stored aerobically, i.e. little change was observed. However, sorbate- or glycerol-containing solutions occasionally exhibited slightly increased loss of absorbance, although the inconsistency of the decrease suggested it was due to failure to maintain anaerobic conditions.

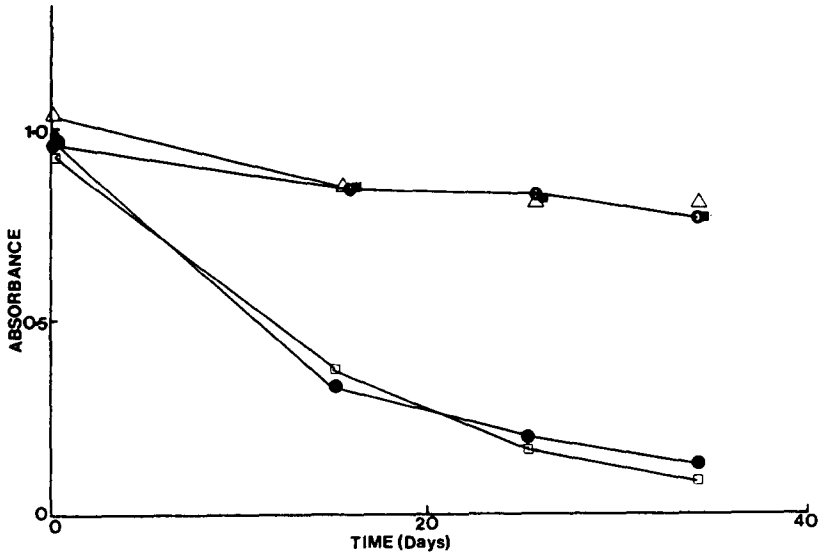

Fig. 8. Absorbance at 410 nm of approximately 0·02 % haemoglobin at a_w 0·85 during aerobic storage at 38 °C (○), and in the presence of 0·5 % sorbate (●), 30 % glycerol (□), 0·5 % glucose (■) and 0·5 % propionate (△). Most solutions stored anaerobically yielded similar profiles to those given by the haemoglobin solutions stored aerobically, i.e. little change was observed; however, sorbate- and glycerol-containing systems occasionally exhibited slightly increased loss of absorbance; the inconsistent nature of the decrease suggested it was due to failure to maintain 'anaerobic' conditions.

glycerol-containing systems the spectra of the haemoprotein remaining in solution also appeared to change, with the Soret peak at 410 nm shifting to lower wavelengths (400–406 nm). Bello & Bello (1976) found that the oxidation products present in glycerol could lead to these types of spectral change in myoglobin and this loss of haematin character has also been observed in glycerol/sorbate-desorbed intermediate moisture meats (Obanu & Ledward, 1975). Whatever the cause, it is interesting to note that the rate of loss of 'haematin' from solution is similar for 0·5 % sorbate and 30 % glycerol whereas the rates of browning with both lysine and glutamate, are very different for these two systems (Figs 1 and 2). It is also noteworthy that the reactions with glucose are apparently very dependent on slight differences in experimental conditions. The extremes of behaviour are shown in Fig. 7 (large decrease in absorbance at 410 nm) and Fig. 8 (little apparent difference compared with haemoglobin alone). These differences were not dependent on the presence of oxygen. In six experiments (three at 0·01 % haemoglobin and three at 0·02 %

haemoglobin) the decrease in absorbance varied from 5 % to 84 % over 30 days at 38 °C.

The pH changes observed in these systems were similar to those observed in the amino acids solutions with the sorbate-containing solutions exhibiting increases in pH (from 5·5 to 5·8 in the 0·02 % solution and to 5·9 in the 0·01 % solution over 30 days at 38 °C) and the solutions containing 30 % glycerol exhibiting decreases in pH (from 5·5 to 4·6 in the 0·2 % solution and from 5·5 to 4·2 in the 0·01 % solutions over the same time).

During anaerobic storage it is seen that, to a large extent, the reactions involving sorbate and glycerol were inhibited (Figs 7 and 8).

Degradation of collagen

The per cent of collagen solubilised from the freeze-dried tendons at 38 °C is shown in Fig. 9 and Table 6. It is seen that degradation does occur and, at least initially, appears to be more rapid in the systems containing glycerol. Samples were stored both anaerobically and aerobically and, at least initially, the anaerobic samples displayed lower levels of soluble

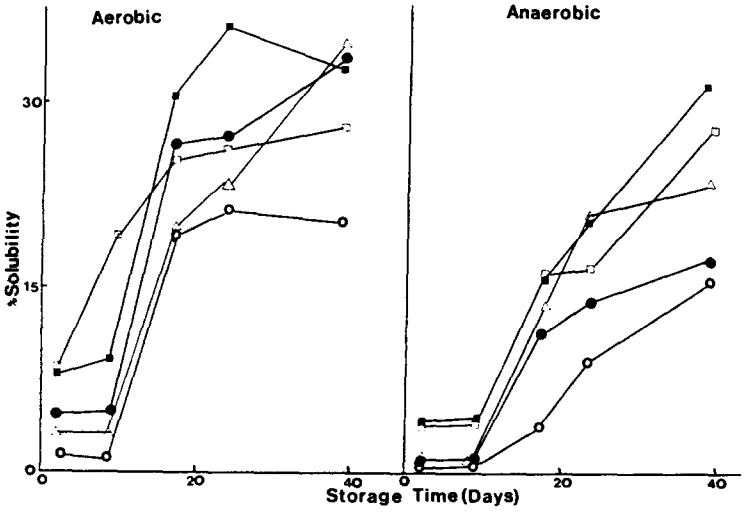

Fig. 9. Soluble hydroxyproline as a percentage of the total in heat-denatured tendon stored aerobically and anaerobically at 38 °C and a_w 0·85 (○) and in the presence of 0·5 % sorbate (●), 0·5 % propionate (△). 30 % glycerol (□) and 30 % glycerol plus 0·5 % sorbate (■).

TABLE 6

The Per Cent Soluble Hydroxyproline Derived by Storing 1·0 g of Freeze-Dried Tendon, at a_w 0·85 and Initial pH 5·5, in Salt Solutions (200 ml) Containing Different Potential Reactants at 38 °C in the Presence and Absence of Air

Time (days)	*None*		*Reactant*							
			0·5% Sorbate		*0·5% Propionate*		*30% Glycerol*		*30% Glycerol + 0·5% sorbate*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
0	3·5	—	—	—	3·6	—	9·6	—	15·0	—
11	1·0	—	16·0	—	6·7	—	—	—	21·5	—
20	8·8	29·1	20·4	17·0	28·0	23·8	18·6	20·0	21·5	31·5
32	8·9	31·0	20·5	18·8	26·4	25·0	20·8	33·3	23·4	20·0
43	14·6	39·0	28·0	28·4	32·5	30·5	26·0	38·0	25·2	26·2
50	16·7	41·0	28·5	31·9	—	33·6	27·8	49·5	28·5	28·8

A, Aerobic.
B, Anaerobic.
—, Not determined.

hydroxyproline in one experiment (Fig. 9) but not in the other (Table 6). Although all the samples were taken from one tendon (in each experiment), it is readily apparent that there is some inherent difference between samples and this must complicate any interpretation.

DISCUSSION

The present work confirms that, at 65 °C, pH 5·5 and a_w 0·85, lysine solutions brown far more rapidly in the presence of glycerol than do those containing glutamate (Obanu *et al.*, 1977) and that sorbate (0·5%) behaves similarly. However, at 38 °C, or in solutions containing both sorbate and glycerol at 38 °C and 65 °C and in 0·5% glucose solutions, both amino acids react at similar rates. The reasons for these differences are not obvious.

Of more interest to the practical situation is the observation that sorbate, at 0·5%, is far more reactive than glycerol at 30% although oxygen (air) is apparently essential if either is to take part in browning reactions. Although some slight browning did take place in the sorbate

solutions during supposed 'anaerobic' storage, the results showed that, at least initially, these systems contained TBA-reactive carbonyls and thus the potential for browning was presumably present.

In intermediate moisture meats the pH normally drops during aerobic storage (Obanu *et al.*, 1976) and this type of behaviour was observed when glucose or 'oxidised' glycerol was used to supply the reactive carbonyl groups. However, oxidation of the unsaturated sorbic acid to malonaldehyde and other such compounds led to an increase in pH. This is not unexpected as not every degraded sorbic acid molecule is likely to react with a free amino group. However, in a complex system such as an intermediate moisture meat product it would be difficult to predict the rate, or even the direction, of pH change likely to occur during storage. This will account for the great variation found in the literature (Obanu *et al.*, 1976; Webster, 1980). The rates of the various reactions may, of course, themselves vary with pH. The problems of extrapolating model results to more complex systems is highlighted by the haemoglobin data as it is seen that the 0·5 % sorbate and 30 % glycerol react at similar rates whilst, with the single amino acids (lysine and glutamic acid), sorbate reacts far more rapidly.

Temperature is obviously a key determinant of the changes undergone in these systems and in 0·05M glycine and 0·2M sorbate systems at pH 4·0 the activation energy of the browning reaction is relatively high, so that, at 65 °C, the rate is about 30 times faster than at 38 °C (Seow & Cheah, 1985*b*). Sorbic acid degradation, at pH 4·0 and a_w 0·80 has an activation energy of 7·8 kcal mole^{-1} (Seow & Cheah, 1985*a*), i.e. the rate is about three times faster at 65 °C than at 38 °C. In the present work the relative rates of sorbate-induced browning at 38 °C and 65 °C differed by a factor of about 2 to 3 (Figs 1 to 3). This suggests that sorbate degradation may be the rate-limiting step when the amino groups are in large excess (10–20-fold). It should be noted that, at 38 °C, there is an apparent accumulation of TBA reactive species during storage, suggesting that subsequent reactions may dictate the overall rate (Table 5) whilst, at 65 °C, the concentration declines, indicating that the formation of these compounds may be rate limiting.

The suggestion that the rate of sorbate degradation is not, at these concentrations, the rate-limiting step at 38 °C, may explain the observations that in sorbate/glycerol solutions, the formation of TBA reactive compounds (Ledward, 1985) and loss of sorbate (Seow & Cheah, 1985*b*) increase with decreasing a_w whilst the reactions leading to

insolubilization in SDS/β-mercaptoethanol of the proteins in intermediate moisture meats occur less rapidly at lower a_ws (Ledward, 1985). However, this interpretation must be viewed with some reserve since, in the meats, the content of TBA reactive species decreases on storage at 38 °C (Ledward, 1985), suggesting that other reactions are also taking place, leading to their destruction, and the pH usually declines, showing that sorbate is not the only browning agent present. Also, even if sorbate degradation is rate-limiting at 65 °C, the kinetics are still, apparently, 'zero' order (Figs 1 and 2) although sorbate degradation is, at pH 4·0, first order (Seow & Cheah, 1985*a*).

Table 5 also indicates that 30% glycerol solutions generate low concentrations of TBA-reactive intermediates (in agreement with previous studies; Ledward, 1985) and these may well be responsible for the slow rates of browning observed with amino acids stored in these solutions.

The results obtained with the collagen degradation within the infused tendons are not clear cut. There is evidence that, at least initially, glycerol accelerates the rate of degradation (Table 6, Fig. 9). Degradation still appears to take place, at least to some extent, in the 'anaerobic' samples but the differences between the two experiments may merely reflect differences in the oxygen levels. Certainly, the reaction would appear to be less sensitive to the presence of limited oxygen than the NEB reactions. More work is necessary on this type of system to clarify the situation.

Although the model studies highlight some of the potential reactants in intermediate moisture foods, it is desirable to evaluate the reactants in such systems before their practical significance can be established.

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