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Reactivity of Sorbate and Glycerol in Intermediate Moisture Meat Products

C. E. M. Webster, S. E. Allison, I. O. Adelakun, Z. A. Obanu & D. A. Ledward

Department of Applied Biochemistry and Food Science, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, Great Britain

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ABSTRACT

Intermediate moisture meats were prepared by cook–soak equilibration with glycerol/salt, sucrose/salt or salt only solutions to an a_w of 0·85. Sorbate or propionate was added to some products and all were stored at 38°C in the presence of air. It was found that sorbate, but not propionate, caused increased rates of protein aggregation and accelerated loss of haemoprotein character. Glycerol may also take part, albeit more slowly, in these deteriorative reactions. However, sorbate did not appear to lead to any marked increase in the rate or extent of the reactions leading to solubilisation of the collagen present in these samples; glycerol, however, significantly increased the rate and extent of such reactions. The results are discussed in the light of the results obtained with the model systems described in the previous paper.

INTRODUCTION

During aerobic storage at 38°C glycerol/salt desorbed intermediate moisture (im) meats undergo several types of deteriorative reaction, leading to loss of eating quality (Obanu *et al.*, 1975*a*,*b*; Ledward, 1981, 1985). Such changes are manifested as a progressive decrease in solubility in sodium dodecyl sulphate (SDS) and β-mercaptoethanol, suggesting that stable, non-disulphide covalent linkages are formed and that these

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increase solubility of the collagenous material present. Marked decreases in pH and loss of haemoprotein character in the samples also take place. We have shown that at least some of these changes may be related to the reaction of sorbate, used as an antimycotic, or glycerol oxidation products, with the proteins (Obanu *et al.*, 1977; Webster *et al.*, 1982; Ledward, 1985). Model systems, based on single amino acids or proteins, have shown that both sorbate and glycerol, at the levels present in im foods, can take part in non-enzymic browning (NEB) reactions (Seow & Cheah, 1985*a*,*b*; Obanu & Ledward, 1986). The present work was undertaken to elucidate the relative rôle of these chemicals in the reactions undergone in im meats and to determine whether or not the results obtained on the model systems described earlier (Obanu & Ledward, 1986) can be related directly to a more complex food system.

MATERIALS AND METHODS

For each experiment a post-rigor bovine *longissimus dorsi* muscle, trimmed of all visible fat and connective tissue, was cut into pieces ($\sim 1\ cm^3$) and the portions desorbed in 1·5 times their weight of infusing solution, at 77 °C, as described previously (Obanu *et al.*, 1975*a*). All meats were desorbed to an a_w of $0{\cdot}85 \pm 0{\cdot}01$, as measured by an equisinahygroscope, by use of the infusing solutions shown in Table 1.

After processing, all samples were sealed in the presence of air, in Cryovac PVDC bags (W. R. Grace Ltd., London, Great Britain) and stored at 38 ± 1 °C. Samples were removed at regular intervals and

TABLE 1
Composition of the Infusing Solutions (%wt)

Water	*Glycerol*	*Salt*	*Sucrose*	*K-Sorbate*	*Ca-Propionate*
51·0	39·0	9·5	—	0·5	—
51·0	39·0	10·0	—	—	—
67·0	—	33·0	—	—	—
66·5	—	33·0	—	0·5	—
66·8	—	33·0	—	—	0·2
10·0	—	10·0	80·0	—	—
10·0	—	9·5	80·0	0·5	—
10·0	—	9·8	80·0	—	0·2

analysed for moisture content, solubility in 3% SDS plus 1% β-mercaptoethanol and amount of soluble hydroxyproline present. All such analyses have been described previously (Obanu *et al.*, 1975*a*,*b*). The pH and reflectance spectra of all samples were also determined (Obanu *et al.*, 1975*a*; Obanu & Ledward, 1975). Sorbate was separated from the meats by steam distillation and, following purification by anion exchange high pressure liquid chromatography (Bennett & Petrus, 1977), its concentration was determined from the absorption of the solution at 256 nm (Melnick & Luckmann, 1954).

RESULTS

Although storage was in Cryovac bags, some loss of moisture occurred in all three experiments, due to the finite permeability of this plastic at 38 °C. This decrease was from about 49% to 41% in the first experiment, i.e. the glycerol-based system, from about 56% to 44% in the second experiment, i.e. the salt-based system, and from about 30% to 15% in the third experiment, i.e. the sugar/salt-based system. Such changes lead to some decrease in the measured a_w ($\leq 0{\cdot}05$). Such decreases have little or no effect on the rates of haemoprotein and collagen degradation but may lead to slight decreases in the rate of protein crosslinking (Webster, 1980; Ledward, 1985). However, within each experiment, all samples exhibited very similar decreases in a_w.

Effect of omission of sorbate

In the samples desorbed to 0·85 in glycerol/salt solution not containing sorbate and stored at 38 °C, surface mould growth became evident after 2–4 weeks. Thus, the experiment was terminated after 7 weeks. The presence of sorbate led to the differences shown in Table 2. It is seen that marked decreases in pH were observed in both samples but that the decrease was immediately apparent in those samples containing no sorbate whereas those containing sorbate showed little change over the first 2 weeks. Nitrogen solubility in SDS/β-mercaptoethanol decreased very rapidly in the presence of sorbate although little change was apparent in the sorbate-free systems. There was a marked increase in soluble hydroxyproline in both samples although the changes appeared to be inhibited in the presence of sorbate.

TABLE 2

Changes in pH, Per Cent Nitrogen Soluble in SDS/β-Mercaptoethanol (ME) and Per Cent Soluble Hydroxyproline (OHP) During Aerobic Storage at 38°C of Glycerol Salt-Desorbed Intermediate Moisture Meats Processed to an a_w of 0·85 in the Presence and Absence of Potassium Sorbate. The Initial Sorbate Concentration was 0·18% (w/w)

Storage time (weeks)	*pH*	*With sorbate*		*pH*	*Without sorbate*	
		Solubility in SDS/ME	*Sol. OHP*		*Solubility in SDS/ME*	*Sol. OHP*
0	5·80	79	7	5·70	79	9
2	5·70	66	6	5·42	83	29
4	5·33	60	18	5·09	81	30
7	5·21	57	27	4·98	59	32

All values are the means of duplicate determinations that differed by less than 0·05 (pH) or 2% (solubility).

These results suggest that sorbate actively participates in the protein aggregation (crosslinking) reactions (increasing rates of insolubilisation in SDS/β-mercaptoethanol) but does not actively catalyse the degradation of collagen. These results are as expected since, under these conditions in model systems, sorbate is known to participate in NEB reactions (Seow & Cheah, 1985*a*; Obanu & Ledward, 1986), but not to any marked extent in collagen degradation reactions (Obanu & Ledward, 1986). Increased crosslinking, with no concomitant increase in the rate of degradation, would account for the decreased release of soluble hydroxyproline in the sorbate-containing system. It is of interest that the increased crosslinking found in the presence of sorbate does not lead to accelerated rates of pH fall (due to loss of amino groups); however, degradation of sorbate is known to lead to significant increases in pH in model systems (Obanu & Ledward, 1986) and this probably explains the observed differences. It is also apparent that, even in the absence of sorbate, some crosslinking reactions and very marked decreases in pH still take place, possibly due to glycerol oxidation products (Obanu *et al.*, 1977; Webster *et al.*, 1982; Ledward, 1985; Obanu & Ledward, 1986).

Reflectance spectra of the interior of the meats were recorded and, as previously found, the typical cooked meat spectrum was lost on storage (Obanu & Ledward, 1975). The rate of loss, however, was faster in the

sorbate-containing samples as the characteristic peaks at 540 and 640 nm were lost in less than 4 weeks whereas they were still apparent in the sorbate-free samples after 7 weeks. Thus, as anticipated, sorbate would appear to accelerate the destruction of the haemoproteins in the meats (Obanu & Ledward, 1986).

Although mould growth was apparent in the sorbate-free systems, those containing sorbate were stable and thus these samples were kept for 17 weeks and the sorbate concentration monitored (Fig. 1). It is seen that the sorbate concentration halves over this time period, as would be expected if it participated in the above reactions.

Although these results strongly suggest that sorbate is a major reactant in some of the deteriorative reactions taking place in im meats, the proliferation of mould on the sorbate-free samples complicated the issue.

Thus, experiments were set up using an alternative antimycotic and, as glycerol may also take part in browning and affect the reactivity of sorbate, humectants other than glycerol were used.

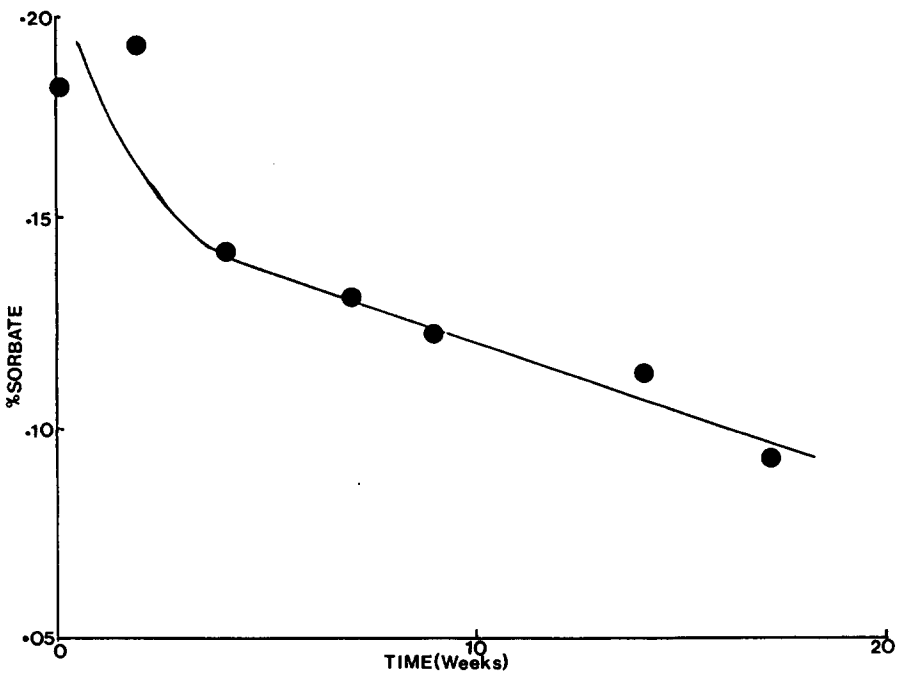

Fig. 1. Concentration of sorbate (as a percentage of the total weight) during storage at 38 °C of glycerol/salt desorbed intermediate moisture meats of initial a_w 0·85.

Effect of different humectants and antimycotic

In the first experiment meat was desorbed in salt alone, salt plus sorbate and salt plus propionate to an a_w of 0·85 and their properties followed during aerobic storage at 38 °C for 12 weeks. In all these meats, no significant change in pH occurred during storage. The mean values (± standard errors) were 5·23 ± 0·04, 5·30 ± 0·04 and 5·27 ± 0·6 for the salt alone, salt plus sorbate and salt plus propionate desorbed meats, respectively.

The solubility of the nitrogen in SDS/β-mercaptoethanol, however, differed quite markedly between the products, those containing sorbate having much lower solubilities at all storage times, although initial solubilities were all similar (Fig. 2). Table 3 shows that all samples exhibited increases in soluble hydroxyproline during storage but the

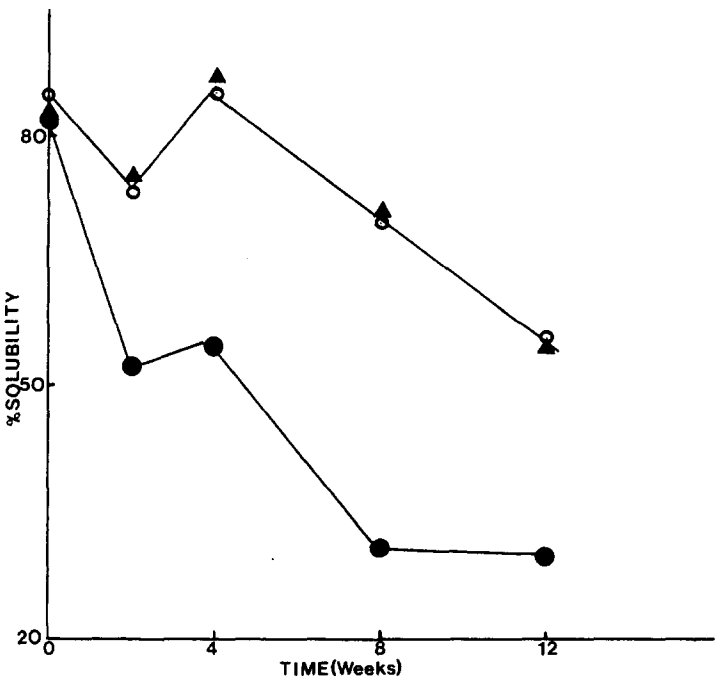

Fig. 2. Nitrogen solubility (as a percentage of total nitrogen) in 3% SDS + 1% β-mercaptoethanol of salt desorbed intermediate moisture meats of a_w 0·85 (○) and similar products containing potassium sorbate (●) or calcium propionate (▲) during storage at 38 °C.

TABLE 3

Changes in Soluble Hydroxyproline (Per Cent of Total) During Aerobic Storage at 38 °C of Salt Desorbed Intermediate Moisture Meats Processed to an a_w of 0·85 in the Presence of no Antimycotic, Propionate or Sorbate

Storage time (weeks)	*No antimycotic*	*+ Sorbate*	*+ Propionate*
0	2·4	2·3	3·4
2	4·1	3·3	3·9
4	11·5	7·5	7·4
8	8·5	7·8	7·3
12	8·5	6·2	9·2

Each value is the mean of duplicate determinations which differed by 1 % or less.

changes were much less than those usually observed in glycerol-based systems (Ledward, 1981).

Reflectance measurements made on the meats indicated that, after 2 weeks' storage, the characteristic cooked meat spectra with reflectance minima (absorption peaks) at about 540 and 640 nm had disappeared in the sorbate-containing samples. In the sorbate-free meats there was still some evidence for these reflectance minima after this time. After 4–8 weeks' storage, these reflectance minima had been lost from all samples.

In the second experiment, meat was desorbed to an a_w of 0·85 in sucrose/salt solutions with and without sorbate and/or propionate and their properties followed during storage at 38 °C. Again, there was little consistent change in pH in any of the meats; mean values ($\pm$ standard errors) were 5·49 $\pm$ 0·08, 5·56 $\pm$ 0·07 and 5·51 $\pm$ 0·08 for the meats with no antimycotic, with sorbate and with propionate, respectively. The per cent nitrogen soluble in SDS/β-mercaptoethanol decreased in all samples during storage and although, after 2 weeks, the sorbate samples had far lower solubilities, over longer periods differences were minimal (Fig. 3). The amount of soluble hydroxyproline in all samples increased slightly during storage (Table 4) and, although there was some evidence that those containing sorbate generated less soluble hydroxyproline, the differences were slight.

Reflectance spectra of all samples indicated complete loss of cooked

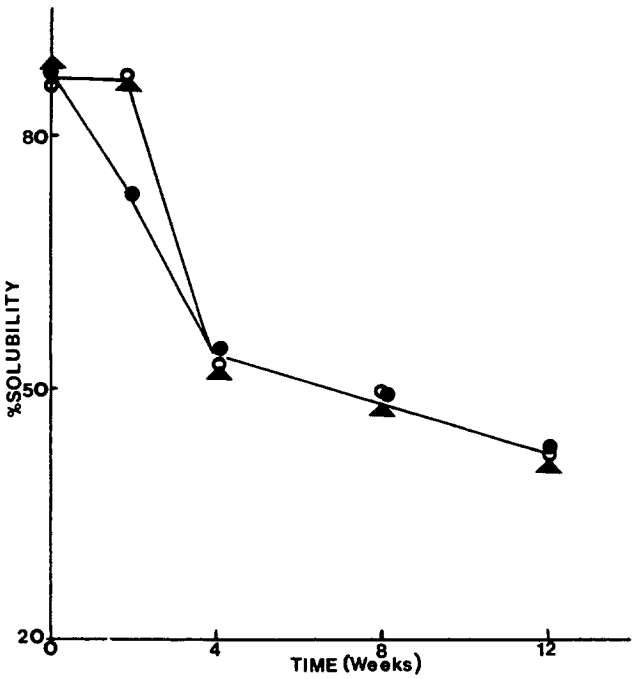

Fig. 3. Nitrogen solubility (as a percentage of total nitrogen) in 3% SDS + 1% β-mercaptoethanol of sugar/salt desorbed intermediate moisture meats of a_w 0·85 (○) and similar products containing potassium sorbate (●) or calcium propionate (▲) during storage at 38 °C.

TABLE 4

Changes in Soluble Hydroxyproline (Per Cent of Total) During Aerobic Storage at 38 °C of Meat Samples Desorbed in Sucrose/Salt Solutions to an a_w of 0·85 in the Presence of No Antimycotic, or Sorbate or Propionate

Storage time (weeks)	*No antimycotic*	*+ Sorbate*	*+ Propionate*
0	5·2	6·5	4·3
2	7·7	7·2	10·4
4	16·0	10·2	11·2
8	11·2	6·9	10·1
12	11·7	9·6	9·1

All values are the mean of two determinations that differed by 1% or less.

meat character during 2 to 4 weeks' storage; there were no measurable differences between the meats.

In these latter two experiments no mould growth was evident even on the samples containing no antimycotic.

DISCUSSION

The present work demonstrates that, even at levels of 0·2% or less, sorbate makes a significant contribution to NEB type reactions in both salt and glycerol/salt based meats. It also governs, to some extent, the loss of haemoprotein character. Propionate, however, appears to be inactive in both crosslinking and haemoprotein breakdown reactions. These results are as expected from earlier model studies (Obanu & Ledward, 1986).

In the model system studies it was found that, at a_w 0·85, sorbate/lysine solutions browned more readily in a system based solely on salt than in one containing salt, 30% glycerol and sorbate (Obanu & Ledward, 1986). Although different meats were used in this work, the salt/sorbate desorbed meats appear to become insoluble in SDS/β-mercaptoethanol far more rapidly than in glycerol-based products (*cf.* Fig. 2 and Table 2). In fact, the rate and extent of the loss in solubility shown in Fig. 2 is greater than that observed in numerous previous studies (Obanu *et al.*, 1976; Ledward, 1982; Webster, 1980). These results therefore suggest that 'NEB reactions' at a_w 0·85 are slower in a glycerol-based system than in one based on salt, in much the same way as happens in casein–glucose (Labuza, 1975) or amino acids–sorbate (Ledward & Obanu, 1986) model systems.

Ledward & Madden (1981) have previously shown that the release of soluble hydroxyproline in im stored meats is significantly less in a salt/sorbate system than in a salt/glycerol/sorbate system. The present results tend to confirm this as, in glycerol/salt desorbed meats stored at 38 °C, soluble hydroxyproline increases to about 30% in 7 weeks (Table 2) whereas, in the absence of glycerol, the concentration reached is only 10% or less (Tables 3 and 4). In typical products containing glycerol and stored at 38 °C, values of from 28% to 60% soluble hydroxyproline have been reported after 12 weeks (Obanu *et al.*, 1976; Ledward, 1982). Thus, glycerol would appear in some way to accelerate these breakdown reactions. What the mechanism is is not clear as these reactions also take

place even at very low oxygen tensions (Ledward, 1985) although model system studies suggest glycerol oxidation products may be of importance (Obanu & Ledward, 1986). That the rôle of glycerol is not passive is also indicated from the pH changes. Invariably, glycerol/salt-based im meats exhibit a decrease in pH of between 0·4 and 0·9 of a unit during 12 weeks' aerobic storage at 38 °C (Webster, 1980, Table 2). However, in both glycerol-free systems studied, no such change was observed. As discussed previously, browning reactions involving glycerol lead to marked decreases in pH (Obanu & Ledward, 1986) and these may well contribute to the observed changes.

Although sorbate and glycerol may well be the major contributors to the changes taking place on storage, it should be noted that, even in their absence, crosslinking, collagen degradation and haemoprotein breakdown occur, albeit more slowly (Figs 2 and 3 and Tables 3 and 4), indicating that other components, such as lipid oxidation products, are also involved.

When sucrose was used as one of the humectants it appeared to nullify, at least to some extent, the sorbate effect on nitrogen solubility in SDS/β-mercaptoethanol and on haemoprotein breakdown. This may be because, on storage, some inversion of the sucrose occurs, yielding potential browning reactants.

In conclusion, it is seen that:

(i) Sorbate, or, rather, its degradation products, can lead to accelerated loss of quality in im meats due to its participation in NEB reactions and haemoprotein breakdown.
(ii) Glycerol may also play an active rôle in some of these reactions and is of major importance in relation to those involving collagen degradation.
(iii) To some extent, results from model systems can be extended to more complex, real foods.
(iv) In intermediate moisture foods it is misleading to talk about the a_w dependence of chemical reactions since their rate, at a given a_w, is dependent to a very large extent on the nature of the humectants.

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