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Degradation Products of Sorbic Acid in Aqueous Solutions

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ABSTRACT

Carbonyl compounds from autoxidising sorbic acid solutions were isolated as 2,4-dinitrophenyl hydrazones (DNPHs) and separated by TLC on silica gel G. Acetaldehyde and β-carboxylacrolein were found to be the major degradation products. Crotonaldehyde and acetone were also detected but in very small proportions. In the presence of amino acids, β-carboxylacrolein polymerises rapidly to brown pigments and its proportion decreases in the reaction mixture.

INTRODUCTION

Sorbic acid is widely used in cheese, bakery and processed fruits and vegetables to prevent microbial spoilage (Luck, 1976). Literature on safety of sorbic acid is well documented but information on its stability and the role of degradation products of sorbic acid on the sensory properties of preserved foods is rather scanty. In previous publications from this laboratory the role of various additives and packaging materials in the degradation of sorbic acid in aqueous solutions and fruit squashes and fish paste was reported (Arya, 1980; Vidyasagar & Arya, 1984). It was observed that degradation of sorbic acid in aqueous systems was accompanied by a concomitant increase in the concentration of carbonyls. Hildegard & Sabalitschka (1965) have also reported the formation of crotonaldehyde and malonaldehyde in stored solutions of sorbic acid. Saxby *et al.* (1982) reported the formation of α-angelicalactone and 2-methyl-5-acetylfuran in aqueous sorbic acid solution containing sulphur dioxide and sulphuric acid. In wines, geranium

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type off-flavours have been reported to be associated with the formation of 2-ethoxy-3,5-hexadiene (Crowell & Guymon, 1975). In the present study carbonyls from stored aqueous solutions of sorbic acid were isolated as DNPHs and characterised by TLC and spectrophotometric methods and the role of carbonyls in sorbic acid-mediated browning is discussed.

MATERIALS AND METHODS

Twenty millilitres of 2% ethanolic solution of sorbic acid were diluted to 200 ml with distilled water and stored at $37 \pm 1°C$ in a loosely stoppered flask both with and without added glycine (2 g) and copper sulphate (100 ppm). After 10 and 20 days of storage, 100 ml of the solutions were taken and treated with 100 ml 0·1% 2,4-dinitrophenylhydrazine solution in 1N HCl. The mixture was left for 2 h and the precipitated 2,4-dinitrophenylhydrazones were filtered and washed first with 0·1N HCl and then with distilled water to remove unreacted dinitrophenylhydrazine.

Separation of phenylhydrazones into neutral and acidic fractions

DNPHs (500 mg) were dissolved in 50 ml chloroform:ethylacetate (80:20) mixture and treated with 20 ml of chilled 1N sodium carbonate solution. The mixture was shaken vigorously for 2 min in a separating funnel and the two layers were allowed to separate. The lower chloroform layer containing the neutral fraction was separated, washed repeatedly with water and concentrated under reduced pressure. The upper sodium carbonate layer was treated with 6N HCl and the precipitated DNPHs of the acidic carbonyls were filtered, washed with water and dried under vacuum.

Thin-layer chromatographic separation of DNPHs

Total and neutral carbonyls were separated on silica gel G plates activated at 120°C for 2 h using carbon tetrachloride:hexane:ethylacetate (100:20:10) and hexane:diethylether (80:20) as developing solvents. Acidic carbonyls were separated by using chloroform:methanol:ethylacetate:ammonia (80:20:10:2). For quantitative estimation, bands were scraped off and the DNPHs were extracted with chloroform or chloroform–methanol mixture and filtered through a sintered funnel. The filtrate was made up to 10 or 25 ml and absorbance measured at 340 nm for determining concentration. Various DNPHs bands were characterised from their R_f values, co-chromatography with authentic compounds, absorbance maxima in chloroform and in alkaline medium, IR spectra and elemental analysis.

Total carbonyls, unreacted sorbic acid and browning in stored sorbic acid solution were measured as described by Arya (1980).

RESULTS AND DISCUSSION

In aqueous solutions, degradation of sorbic acid is accompanied by a simultaneous increase in the concentration of carbonyl compounds (Table 1). In the present study, carbonyls were isolated as DNPHs and separated on silica gel G plates into seven bands using carbon tetrachloride–hexane–ethylacetate as developing solvent. The R_f values, absorbance maxima in chloroform and alkaline medium, tentative identification and relative concentration of each band are given in Table 2. As is evident, bands Nos 1 and 5 having R_f values of 0·0 and 0·50, respectively, were present in largest concentrations and accounted for 75–80% of the total carbonyls formed in autoxidising sorbic acid solutions. The absorbance maxima at 365 and 430 nm of the band No. 5 in chloroform and methanolic potassium hydroxide, respectively, are characteristic of saturated aldehydes and its R_f value (0·50) was the same as that of the DNPH of pure acetaldehyde in the above solvent systems. This band was isolated by preparative TLC, crystallised in alcohol, dried and subjected to elemental analysis. The experimental and theoretical elemental analyses were C, 42·1 (42·8); H, 3·68 (3·57); N, 24·8 (25·0). The IR spectra of the isolated compound and that of acetaldehyde DNPH were superimposable and had bands at 3290 cm^{-1} characteristic of —NH— and at 1620 cm^{-1} characteristic of the —C=N— bond (Fig. 1).

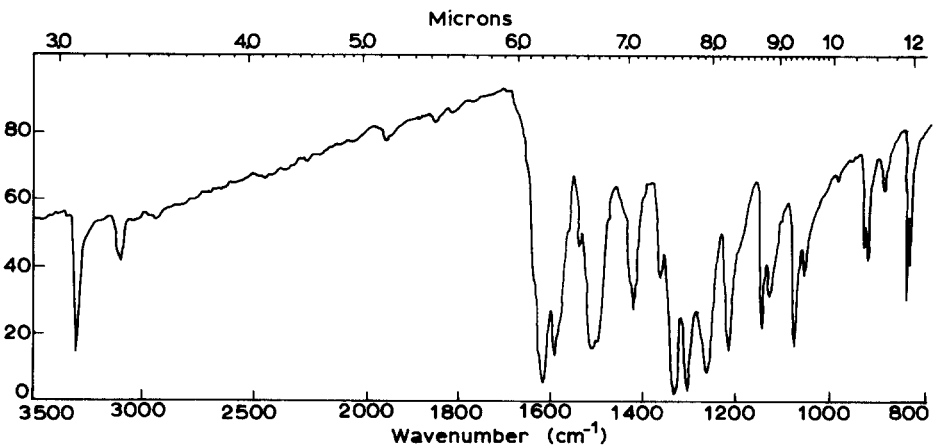


Fig. 1. IR spectra of DNPH of acetaldehyde isolated from autoxidising sorbic acid solution.

TABLE 1
Changes in Sorbic Acid Concentration, Browning and Carbonyls in Aqueous Sorbic Acid Solutions at 37 ± 1°C

	0	*10*	*16*	*23*	*0*	*10*	*16*	*23*	*0*	*10*	*16*	*23*
		(days)				*(days)*				*(days)*		
	Changes in SA %				*Browning intensity (absorbance at 400 nm)*				*Carbonyls (mg glyoxal per 100 ml solution)*			
SA	0·20	0·186	0·162	0·123	0·02	0·02	0·03	0·10	2·5	30·1	38·7	49·2
SA + Gly	0·20	0·166	0·125	0·106	0·02	0·40	0·75	1·06	2·5	21·5	28·3	39·5
SA + Gly + Cu^{2+}	0·20	0·200	0·200	0·200	0·03	0·02	0·02	0·03	2·6	2·6	2·8	3·0
SA + PG	0·20	0·200	0·200	0·192	0·02	0·02	0·02	0·03	2·5	2·8	3·0	3·1

SA, Sorbic acid; Gly, glycine; PG, propylgallate.

TABLE 2
R_f Values, Absorption Maxima, Tentative Identification and Relative Concentration of Carbonyls in Stored Sorbic Acid Solution

Band no.	R_f *value*	*Absorption maxima (nm) in:*		*Tentative identification*	*Percentage of total DNPHS*
		Chloroform	*Methanolic KOH*		
1	0·00	375	475	Acidic carbonyls	45·6
2	0·11	375	480	—	4·0
3	0·18	395	515	Dicarbonyl	1·4
4	0·24	365	375	—	1·7
5	0·50	355	430	Acetaldehyde	39·4
6	0·63	360	435	Acetone	6·3
7	0·78	365	465	Crotonaldehyde	1·9

The bands Nos 6 and 7 could not be isolated in sufficient quantities for elemental analysis and IR spectra. However, from their R_f values and absorbance maxima, these were tentatively characterised as acetone and crotonaldehyde, respectively.

The band No. 1, which did not move from the base line, constituted about 50% of total DNPHs. It was insoluble in pure chloroform but soluble in methanol and dilute sodium-carbonate solution, indicative of acidic carbonyls having a free carboxyl group. Accordingly, total DNPHs were further separated into acidic and non-acidic derivatives by dissolving them in chloroform and ethyl acetate mixture (80:20) and extracting acidic carbonyls with sodium carbonate solution (1N). The DNPHs were precipitated with dilute HCl, filtered and dried. The acidic DNPHs were further separated by TLC on silica gel G plates into four bands using chloroform–methanol–ethyl acetate–ammonia mixture as developing solvent. Their R_f values, absorbance maxima and tentative identification are given in Table 3. All the bands had absorbance maxima at 375 nm in chloroform–methanol and at 475 nm in alkaline methanol, a characteristic of the DNPHs of α,β-unsaturated aldehydes. Among these, band No. 1 (R_f value, 0·04) constituted more than 70% of the total acidic DNPHs and was further separated on preparative TLC crystallised in methanol. Its IR spectra (Fig. 2) (in KBr pellet) had absorbance maxima at 3290, 1700 and 1620 cm^{-1}, characteristics of —NH—, α,β-unsaturated —COOH and —C=N— groups, respectively. Elemental analysis of the isolated DNPH (C, 43·2; H, 2·98; N, 19·8) tallied with that of DNPHs of β-carboxylacrolein (C, 42·84; H, 2·85; N, 20·0). The DNPH of acrolein has absorbance maxima at 368 nm while in the case of β-carboxylacrolein this was found to be at 375 nm. This bathochromic shift is as anticipitated because an additional

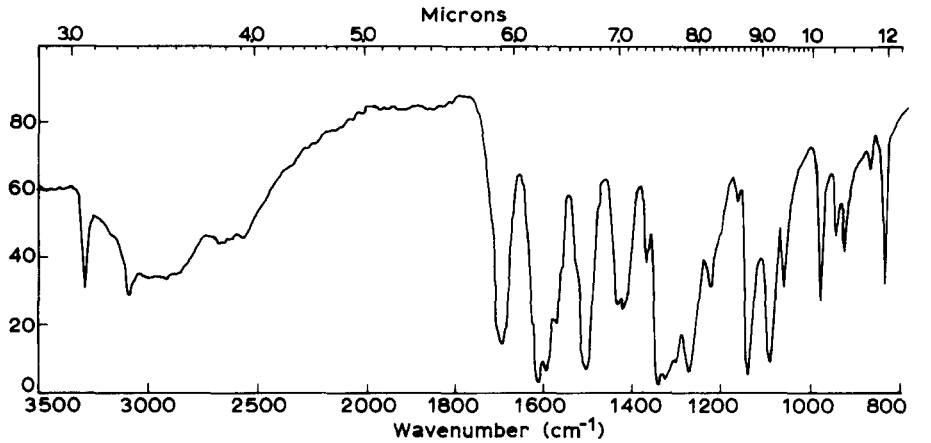


Fig. 2. IR spectra of DNPH of β-carboxyl acrolein isolated from autoxidising sorbic acid solution.

carboxyl group in carboxylacrolein is expected to shift the maxima to longer wavelengths.

Other bands could not be crystallised, but from their migration behaviour on TLC plates these seemed to be more hydrophobic in nature and may have been formed due to the polymerisation of β-carboxylacrolein and other aldehydes. Progressive increase in their proportion in solutions stored for longer periods would tend to support the above assumption.

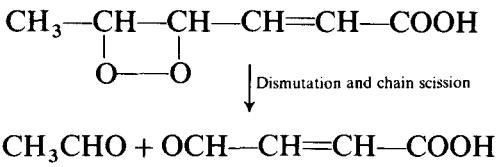
It is evident from the above discussion that acetaldehyde and β-carboxylacrolein constitute about 80% of the total carbonyls found in stored aqueous solutions of sorbic acid. Formation of these compounds would suggest that, in aqueous systems, the double bond in the 4,5-position of the sorbic acid molecule is prone to oxidative attack leading to the formation of a 4,5-cyclic peroxide. Degradation of the cyclic peroxide by

TABLE 3

R_f Value, Absorption Maxima, Tentative Identification and Relative Concentration of Acidic Carbonyls in Stored Aqueous Sorbic Acid Solution

Band No.	R_f *value*	*Absorption maxima (nm)*		*Tentative identification*	*% of total acidic DNPHS*
		Methanol	*Methanolic KOH*		
1	0·09	375	475	β-carboxyl acrolein	74·5
2	0·21	375	475	—	10·2
3	0·44	375	475	—	6·1
4	0·93	375	480	—	6·0

dismutation and chain scission would form acetaldehyde and β-carboxylacrolein as indicated below:



Absence of measurable level of hydroperoxides in autoxidising sorbic acid solution also supports the above mechanism.

Effect of storage and amino acids on carbonyl profile

The carbonyl profile of autoxidising sorbic acid solution is likely to undergo changes because of susceptibility to further oxidation and condensation reactions leading to browning. Carbonyl profiles of autoxidising sorbic acid solutions, after 10 and 20 days storage both with and without glycine, are given in Table 4. Interestingly, there were only slight differences in carbonyl profile of aqueous sorbic acid solution after 10 and 20 days of storage. After 20 days storage, proportions of β-carboxylacrolein and other minor acidic carbonyls slightly increased. On the other hand, there were striking changes in the carbonyl profile of sorbic acid solution in the presence of glycine. Proportions of β-carboxylacrolein and other minor acidic carbonyls and crotononaldehyde were considerably less while that of acetaldehyde was significantly higher. Since β-carboxylacrolein forms the major component of acidic carbonyls, the changes in carbonyl profile resulting from incorporation of amino acids may be interpreted by the differences in the susceptibilities of unsaturated and saturated aldehydes to

TABLE 4
Changes in the Concentration of Carbonyls in Autoxidising Sorbic Acid with and without added Glycine

	Sorbic acid % of total carbonyls after		*Sorbic acid + glycine % of total DNPHs after*	
	10 days	*20 days*	*10 days*	*20 days*
β-Carboxyl-acrolein + minor acidic carbonyls	45·6	51·6	23·7	16·6
Acetaldehyde	39·4	38·7	56·9	75·0
Acetone	6·3	7·3	5·8	8·3
Crotonaldehyde	1·90	1·2	1·1	0·4

condensation and polymerisation reactions. Burton *et al.* (1963) have reported that unsaturated aldehydes, especially α,β-unsaturated aldehydes in the presence of amino acids, polymerise at very rapid rates, forming brown pigments, while saturated aldehydes polymerise at relatively insignificant rates. Apparently, in the presence of glycine, β-carboxylacrolein polymerises very rapidly, forming brown pigments while acetaldehyde, another major degradation product of autoxidising sorbic acid, polymerises relatively very slowly. The proportion of acetaldehyde would therefore be considerably higher than that of β-carboxylacrolein in autoxidising sorbic acid solution in the presence of amino acids. Also, because of rapid depletion of unsaturated aldehydes in polymeric reactions, the concentration of total carbonyls in autoxidising sorbic acid solutions is expected to be significantly less in the presence of amino acids which has been found to be the case (Arya, 1980). The concentration of brown pigments found in autoxidising sorbic acid was considerably higher in the presence of glycine, thus supporting the involvement of unsaturated carbonyls and amino acids in the formation of brown pigments. In order to confirm the involvement of autoxidation products of sorbic acid in the formation of brown pigments in the sorbic acid–glycine system, formation of brown pigments was followed both with and without Cu^{2+} (100 ppm) which has been shown to exhibit a strong inhibitory effect in sorbic acid oxidation (Arya, 1980). It is interesting to observe that in the presence of Cu^{2+} there was no increase in browning intensity up to 23 days while in the control system without Cu^{2+}, browning intensity increased to 1·06. In the presence of propylgallate, too, formation of brown pigments was negligible, confirming the role of β-carboxylacrolein in the sorbic acid-mediated browning.

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