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THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF ALDEHYDES ENCOUNTERED IN FOODS AS DIMETHONES, OCTAHYDROXANTHENES AND BARBITURATES

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SUMMARY

Aldehydes in foods can conveniently be characterized and determined by thin-layer chromatography (TLC) after converting them into their dimethones. For further confirmation the dimethones can be cyclized on the TLC plates or in solution to give octahydroxanthenes. Aldehydes also can be determined as their barbiturates. The aldehydes could be determined as dimethones and octahydroxanthenes in amounts up to 5 μg and as barbiturates in amounts up to 7.5 μg at their UV maxima.

INTRODUCTION

Carbonyls influence significantly the aroma and flavour of foodstuffs and, in order to gain an insight into the freshness of foods and the changes undergone during processing and storage, the carbonyls have to be isolated and characterized. The micro amounts present have been determined by gas chromatography-mass spectrometry (GC-MS) after converting them into phenylhydrazones¹, 2,4-dinitrophenylhydrazones^{2,3} and oximes⁴. Using thin-layer chromatography (TLC) and paper chromatography (PC) they have been determined as 2,4-dinitrophenylhydrazones⁵⁻⁷, oximes⁸, semicarbazones⁹, azines¹⁰ and nitroguanylhydrazones¹¹.

This paper describes equally effective procedures that are specific for aldehydes. After reaction with dimedone (I), the aldehydes present in different food materials can be quantitatively converted into dimethones (II). The latter are easily cyclized to their corresponding octahydroxanthenes (III) by treatment with hydrochloric acid in solution or on TLC plates. The aldehydes also can be converted into barbiturates (IV). Dimethones, octahydroxanthenes and barbiturates have sharp melting points and characteristic UV maxima.

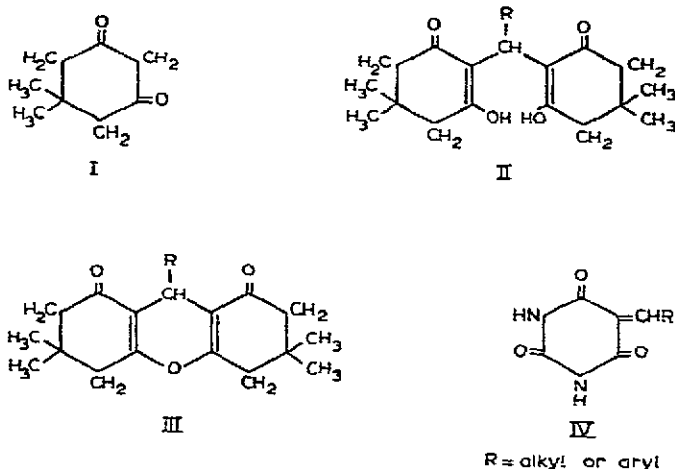
The procedure has been applied to the micro-determination of aldehydes produced during caramelization of glucose and in canned fish, canned meat, cucumber, rancid milk and ice cream. The aldehydes can be determined as II and

TABLE I

R_F VALUES OF VARIOUS DERIVATIVES OF ALDEHYDES WITH DIFFERENT ADSORBENTS AND SOLVENT SYSTEMS AND THEIR λ_{max} VALUES IN 80% ETHANOL

Aldehyde	R_F values					λ_{max} values (nm)						
	I*	II*	III*	I*	II*	III*	IV*	V*	Dimethones	Octahydroxanthones	Barbiturates on silica gel	Barbiturates
Acetaldehyde	0.74	0.87	0.70	0.91	0.87	0.56	—	—	260	290, 300	—	—
Acrylaldehyde	0.68	0.80	0.68	0.68	0.66	0.42	0.74	0.62	262	228, 298	260	260
Propionaldehyde	0.72	0.76	0.61	0.47	0.61	0.40	—	—	260	232, 300	—	—
<i>n</i> -Butyraldehyde	0.64	0.73	0.53	0.38	0.51	0.32	0.70	0.58	258	233, 290	256	256
Isobutyraldehyde	0.61	0.70	0.50	0.44	0.54	0.35	0.68	0.55	260	230, 282	258	258
<i>n</i> -Valeraldehyde	0.57	0.66	0.48	0.64	0.60	0.38	0.65	0.52	265	233, 285	258	258
Isovaleraldehyde	0.52	0.63	0.45	0.60	0.56	0.34	0.61	0.50	264	232, 292	256	256
<i>n</i> -Hexaldehyde	0.50	0.58	0.41	0.41	0.51	0.30	0.56	0.44	257	231, 295	258	258
<i>n</i> -Heptaldehyde	0.48	0.55	0.36	0.30	0.43	0.25	0.51	0.40	260	232, 294	258	258
Furfuraldehyde	0.69	0.67	0.54	0.75	0.64	0.39	0.38	0.29	268	225, 284	260, 370	260, 370
Glyoxal	0.63	0.53	0.48	0.61	0.51	0.29	0.43	0.31	269	260, 310	275	275
DL-Glycerinaldehyde	0.59	0.61	0.39	0.64	0.63	0.41	0.48	0.34	282	230, 285	259	259
Pyruvaldehyde	0.64	0.65	0.50	0.59	0.58	0.36	0.40	0.32	265	290, 230	255	255
Glutaraldehyde	0.82	0.75	0.58	0.64	0.58	0.43	0.70	0.58	265	232, 295	258	258
Benzaldehyde	0.91	0.83	0.66	0.80	0.78	0.53	0.57	0.41	271	232, 295	256	256
<i>o</i> -Hydroxybenzaldehyde	0.82	0.76	0.61	0.88	0.71	0.49	0.56	0.43	275	224, 274	230, 255	230, 255
<i>p</i> -Hydroxybenzaldehyde	0.76	0.78	0.57	0.71	0.62	0.43	0.47	0.35	275	229, 295	260, 460	260, 460
<i>o</i> -Methylbenzaldehyde	0.86	0.85	0.65	0.73	0.65	0.47	0.41	0.30	278	226, 280	256	256
Anisaldehyde	0.94	0.87	0.68	0.76	0.71	0.48	0.88	0.80	270	227, 290	281	281
3,4-Dimethoxybenzaldehyde	0.46	0.53	0.37	0.88	0.42	0.26	0.54	0.41	289	230, 297	260	260
<i>p</i> -Dimethylaminobenzaldehyde	0.88	0.85	0.69	0.74	0.61	0.39	0.48	0.35	280, 450	232, 294	250	250
Cinnamaldehyde	0.35	0.34	0.29	0.65	0.35	0.21	0.30	0.27	269, 380	231, 271	270, 370	270, 370
Vanillin	0.76	0.40	0.46	0.53	0.51	0.29	0.40	0.31	281	230, 290	259, 480	259, 480
Piperonal	0.76	0.59	0.61	0.59	0.50	0.27	0.69	0.52	280	232, 295	259, 315	259, 315
Phenylacetaldehyde	0.49	0.78	0.56	0.38	0.41	0.26	0.80	0.68	264	232, 298	258	258
<i>n</i> -Nonaldehyde	0.42	0.46	0.29	0.26	0.37	0.20	0.43	0.26	262	233, 295	259	259

* Solvents: I = 80% ethanol; II = acetone-ethanol-water (30:30:20); III = ethyl acetate-acetone-water (40:20:30); IV = isopropanol-chloroform-ammonia solution (35%) (40:10:20); V = *n*-butanol-ethylene dichloride-ammonia solution (35%) (30:40:15).



III in amounts up to 5 μg and as IV in amounts up to 7.5 μg by employing TLC and UV spectroscopy.

EXPERIMENTAL

Preparation of dimethones from known aldehydes

The dimethones were essentially prepared according to the procedure of Horning and Horning¹². The pure aldehyde (1 mole) and dimedone (2 mole) were dissolved in 80% ethanol. The solution was warmed at 50° for 1 h and water was added drop by drop until it became cloudy. On cooling, the dimethones were obtained as a crystalline mass, which was recrystallized from aqueous methanol. They had sharp melting points which corresponded with those reported in the literature. Their λ_{max} values in absolute ethanol are given in Table I.

Cyclization of dimethones to octahydroxanthenes

The dimethones were cyclized according to the method of Horning and Horning¹². The crystalline dimethone was dissolved in 50% ethanol, a few drops of concentrated hydrochloric acid were added and the solution was kept for 20 min at 70°. On cooling the octahydroxanthene derivative precipitated. The precipitate was filtered and recrystallized from aqueous methanol. The compounds had distinct melting points. Their λ_{max} values in absolute ethanol are given in Table I.

Liberation of aldehydes during caramelization of glucose and preparation of dimethones

Dry glucose (100 g) was heated in a 250-ml flask at 150° for 2.5 h on an oil-bath and nitrogen was gently passed through to sweep out the vapour. The volatiles were trapped in a 1-l round-bottomed flask containing 0.1% dimedone in ethanol (300 ml) kept in a freezing mixture. After sweeping nitrogen through the flask for a further 0.5 h, the contents were left at room temperature for 1 h. The solution was then warmed at 50° for 0.5 h. The solvent was removed under vacuum, the residue dissolved in 80% ethanol and the volume made up to 5 ml with 80% ethanol to give a solution of the dimethones.

Extraction of aldehydes from canned fish, canned meat, cucumber and preparation of dimethones

Canned fish, canned meat and cucumber were separately reduced to fine pastes in a waring blender kept at 2–5°. The material (500 g) was extracted in the cold with ethyl acetate (3 × 200 ml). To the combined extracts a solution of dimedone (0.6 g) in ethanol (100 ml) was added. The solution was left at room temperature for 1 h, then warmed at 50° for 0.5 h and the solvent removed under reduced pressure. The residue was taken up in ethanol to give a volume of 5 ml.

Extraction of aldehydes from rancid milk and ice cream and preparation of dimethones

Rancid milk (500 ml) or ice cream (250 g) was extracted with ethyl acetate (3 × 200 ml). The extracts were combined and a solution of dimedone (0.6 g) in ethanol (50 ml) was added. The solution was left at room temperature for 1 h and then warmed at 50° for 0.5 h. After removal of the solvents under vacuum, the residue was dissolved in 80% ethanol and the volume made to 5 ml with 80% ethanol.

Cyclization of dimethones obtained from the aldehydes of food materials into octahydroxanthenes

To a portion of an ethanol solution containing a dimethone mixture obtained from the aldehydes of food materials, a few drops of concentrated hydrochloric acid were added. The solution was kept at 70° for 20 min and then cooled. The resulting solution, which contained a mixture of octahydroxanthenes, was made-up to a known volume with 80% ethanol and spotted on the TLC plates.

Conversion of dimethones into octadihydroxanthenes on TLC plates

Each dimethone spot on the TLC plate was treated with one drop of concentrated hydrochloric acid. The plate was left at room temperature for 2 h and then warmed at 70° for 15 min; this resulted in their complete cyclization.

Preparation of barbiturates from the aldehydes

The procedure was essentially that of Weinschenk¹³ and Conrad and Reinbach¹⁴.

A known aldehyde (1 mole) and barbituric acid (1 mole) were separately dissolved in 80% ethanol and their solutions mixed. With aliphatic aldehydes the mixture was left at room temperature overnight and then warmed at 70–80° for 6–7 h. On the other hand, aromatic aldehydes soon gave the crystalline barbiturates on mixing their solutions with that of barbituric acid. The crystals were filtered, washed with water and recrystallized from 80% ethanol to give shining coloured compounds. The compounds had sharp melting points. Their maxima in both the UV and visible spectral regions are given in Table I.

Preparation of barbiturates of aldehydes obtained from foods

The aldehydes produced during the caramelization of glucose were trapped in a 0.1% solution of barbituric acid in 80% ethanol (200 ml) kept in a freezing mixture (as with dimedone derivatives). The solution was kept at room temperature overnight and then warmed at 70–75° for 7 h. After removal of the solvents under vacuum, the solution was made up to 5 ml with 80% ethanol.

The aldehydes from canned fish, canned meat and cucumber were extracted in the cold with ethyl acetate (3×200 ml) as above and to the extracts barbituric acid (0.7 g) dissolved in 80% ethanol (100 ml) was added. The solution was left at room temperature overnight and then warmed at 70–75° for 7 h. After removal of the solvents the residue was dissolved in 80% ethanol and the solution made to 5 ml with 80% ethanol.

Rancid milk or ice cream was extracted with ethyl acetate (as with dimedone derivatives). To the combined extracts, barbituric acid (0.7 g) in 80% ethanol (100 ml) was added. The solution was left overnight, worked up as above and 5 ml of the solution was prepared in 80% ethanol.

Preparation of barbiturates on TLC plates

The authentic aldehydes together with the aldehyde mixture from the foods were spotted on silica gel G plates, followed by a known volume of barbituric acid solution (0.1% in 80% ethanol). The plates were left at room temperature overnight, then warmed at 70° for 7 h when the barbiturates appeared as deep coloured spots.

Preparation of the plates, spotting of the compounds, development and determination

Photographic glass plates (40 × 20 cm) were used. A homogeneous slurry of the adsorbent (50 g) in water (100 ml) was poured on the glass plates and uniformly spread by tilting them from side to side. The plates were left overnight at room temperature for drying and activated at 110° for 1 h. The average coating of the adsorbent was recorded by weighing the plates. The coatings were silica gel G (Merck, Darmstadt, G.F.R.) (5.81 mg/cm²) and basic alumina (Merck) containing 10% of calcium sulphate (7.0 mg/cm²). A known volume of the solution containing dimethones/octahydroxanthenes/barbiturates were spotted together with the standards. Development was carried out by ascending technique at room temperature. After development and drying of the plates at room temperature the compounds were located under UV light (260 nm) and marked. The R_F values obtained using different solvent systems are given in Table I.

The compounds were scraped off the plates with a micro-spatula onto a sintered-glass funnel and extracted with 80% ethanol. The volume was made to 5 ml with 80% ethanol and the percentage transmission was recorded with a Perkin-Elmer Model 124 UV spectrophotometer at the absorption maxima of the compounds.

RESULTS AND DISCUSSION

The aldehydes associated with foods can be converted into dimethones, octahydroxanthenes and barbiturates, which are crystalline compounds with specific UV spectra. The aldehyde mixture, after extraction from the food material, can be quantitatively converted into dimethones and barbiturates. These compounds can also be prepared directly on TLC plates by spotting the two reactants, thus making the identification of aldehydes easier. The dimethones could easily be cyclized on TLC plates as well as in solution to octahydroxanthenes for further characterization of aldehydes.

Of the various adsorbents tried, basic alumina containing 10% of calcium sulphate was found to be the best for the separation of both dimethones and octahydroxanthenes, whereas for alkylidene/arylidene barbiturates silica gel G gave better resolutions. In the developing solvent mixtures for the first two series of derivatives water was important and for barbiturates ammonia solution was important as one of the components.

The procedure is useful for directly converting the micro-amounts of aldehydes associated with foods into high-molecular-weight derivatives for their spectroscopic microdetermination after resolution on TLC plates. The first two series of products can conveniently be determined down to a lower limit of 5 μg and those of the third series down to 7.5 μg .

All three series of compounds have characteristic absorption maxima in UV light; barbiturates also absorb in visible light. Unknown amounts of the compounds could be determined from the percentage transmissions of known derivatives at specific wavelengths. Their resolutions are shown in Figs. 1-3.

It was observed that the conversion of aldehydes into dimethones gave the highest percentage recovery, followed by octahydroxanthenes and then barbiturates. Hence the best procedure for the determination of aldehydes is via their conversion into dimethones.

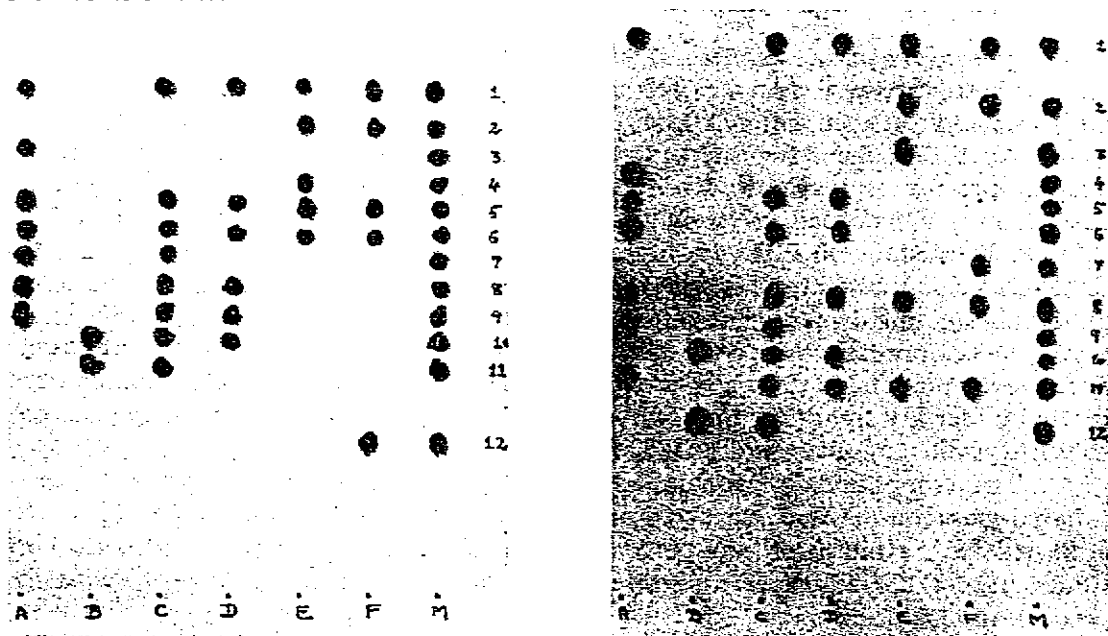


Fig. 1. TLC separation of dimethones of aldehydes from different food materials. A, Glucose caramel; B, cucumber; C, canned fish; D, canned meat; E, rancid milk; F, ice cream. M, mixture of standard dimethones of: 1, acetaldehyde; 2, benzaldehyde; 3, acrylaldehyde; 4, *p*-hydroxybenzaldehyde; 5, propionaldehyde; 6, *n*-butyraldehyde; 7, isobutyraldehyde; 8, *n*-valeraldehyde; 9, isovaleraldehyde; 10, *n*-hexaldehyde; 11, *n*-heptaldehyde; 12, vanillin. Adsorbent: basic alumina with 10% CaSO_4 . Solvent system: acetone-ethanol-water (30:30:20). Development: ascending technique.

Fig. 2. TLC separation of octahydroxanthenes of aldehydes from food materials obtained after cyclization of their dimethones. A, Glucose caramel; B, cucumber; C, canned fish; D, canned meat; E, rancid milk; F, ice cream. M, mixture of the standard derivatives of: 1, acetaldehyde; 2, benzaldehyde; 3, *p*-hydroxybenzaldehyde; 4, acrylaldehyde; 5, valeraldehyde; 6, isovaleraldehyde; 7, vanillin; 8, propionaldehyde; 9, isobutyraldehyde; 10, *n*-hexaldehyde; 11, *n*-butyraldehyde; 12, *n*-heptaldehyde. Adsorbent: basic alumina with 10% CaSO_4 . Solvent system: 80% ethanol. Development: ascending technique.

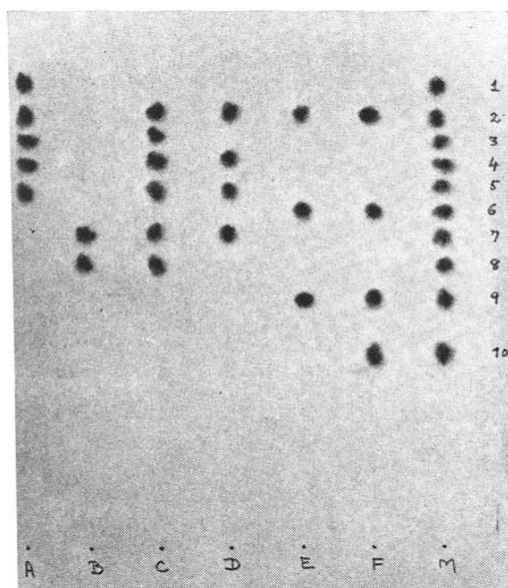


Fig. 3. TLC separation of alkylidene/arylidene barbiturates of aldehydes from various foods. A, Glucose caramel; B, cucumber; C, canned fish; D, canned meat; E, rancid milk; F, ice cream. M, mixture of standard barbiturates of: 1, acrylaldehyde; 2, *n*-butyraldehyde; 3, isobutyraldehyde; 4, valeraldehyde; 5, isovaleraldehyde; 6, benzaldehyde; 7, *n*-hexaldehyde; 8, *n*-heptaldehyde; 9, *p*-hydroxybenzaldehyde; 10, vanillin. Adsorbent: silica gel G. Solvent system: isopropanol-chloroform-ammonia solution (35%) (40:10:20). Development: ascending technique.

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