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A METHOD FOR ANALYZING VOLATILE ALKANALS FROM AUTOXIDIZING FATS

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SUMMARY

A procedure was developed for the determination of the composition of important "off-flavour alkanals" formed in the autoxidation of fats.

The volatile carbonyls were isolated from the fat (5–10 g) by steam distillation by using an inert stripping gas and then trapped as their 2,4-dinitrophenylhydrazones on a solid support.

Qualitative and quantitative evaluations of the alkanals were carried out by gas chromatography via the adsorbed 2,4-dinitrophenylhydrazones.

The proposed procedure was tested on chicken fat autoxidized to various extents. In this study, a close correlation was obtained between the peroxide value (16–118) and the *n*-hexanal content (0.05–1.9 μg per gram of fat).

INTRODUCTION

Substances of different types are formed as secondary degradation products in the autoxidation of fats. Of these substances the volatile aldehydes have attracted special attention owing to their significant contribution to the off-flavour known as fat or rancid.

GADDIS *et al.*[1] and other workers have shown that the aldehydes formed in this process are those which can be predicted from the cleavage of the intermediate hydroperoxides derived from unsaturated fatty acids, *e.g.*, oleic acid, linoleic acid and linolenic acid, present in fats as triglycerides. The spontaneously formed aldehydes in this process are mainly *n*-alkanals[1–3].

The extent of autoxidation of fats is commonly expressed as the peroxide value or TBA value*. Although these values are of practical use and often are descriptive enough, they do not necessarily give a true picture of the off-flavour quality. In special investigations it is therefore more appropriate to use the concentrations of one or more of the "off-flavour alkanals" to characterize the rancidity of fats. However, this statement is relevant only if these substances can be qualitatively and quantitatively analyzed in head-space concentrations, where the rancid flavour is relatively low.

This paper describes a method for characterizing the extent of autoxidation of a fat by determining the content of some volatile alkanals.

* TBA = thiobarbituric acid.

EXPERIMENTAL

Principles

Our aim was to develop a relatively simple and rapid method to determine some important off-flavour alkanals qualitatively and quantitatively from 5–10 g of fat with peroxide values of 0–100. This procedure consists of an isolation and an analysis step. In designing these steps, the following aspects were considered to be of importance. The isolation of volatile aldehydes from fat alters more or less the actual composition. However, it is reasonable to assume that the mechanism of the break-down of hydroperoxides under standard conditions is approximately the same for a given fat at the beginning of the autoxidation. Aldehydes of this type are often trapped as their 2,4-dinitrophenylhydrazones so as to increase the selectivity and to reduce their volatility, and are further analyzed as these derivatives.

The use of large amounts of organic solvents in the working-up of mixtures of 2,4-dinitrophenylhydrazones necessitates the use of highly pure solvents. Purification methods described in the literature[4,5] are not always sufficient.

GADDIS AND ELLIS[6] and SCHWARTZ *et al.*[7,8], amongst others, have developed liquid–solid and liquid–liquid chromatographic systems for the resolution of complex mixtures of 2,4-dinitrophenylhydrazones. These methods are hardly applicable in the present work owing to the very low aldehyde concentrations present and the time-consuming multi-separation steps involved.

Procedures

The isolation of the volatile carbonyls from fat is illustrated in Fig. 1. Ultra-pure nitrogen, used as an inert stripping gas, is swept at a flow-rate of 25 ml/min through a sintered-glass disc into carbonyl-free and oxygen-free water (B). The water-saturated nitrogen is led into the fat sample (C) through a polyethylene tube and a fine glass capillary that ends just above the surface of the fat. A magnetic stirrer (D) spreads the fat into a thin film on the walls of the erlenmeyer flask during distillation, which is carried out at 100° for 3 h. Vapour from C passes through a reaction column (E).

The presence of water in the stripping gas makes it possible to carry out steam distillation of the volatile compounds in the fat, and is also necessary for the step in which the 2,4-dinitrophenylhydrazones are formed.

The reaction column is as described by SCHWARTZ AND PARKS[4], but modified in the following way: Celite (Johns-Manville) was conditioned overnight at 275°

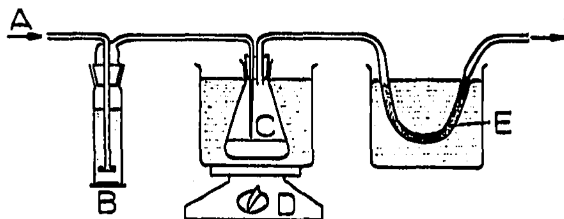


Fig. 1. Schematic diagram of the system used for trapping volatile carbonyls from fat. A = Nitrogen source; B = wash bottle containing carbonyl-free water; C = erlenmeyer flask containing fat sample immersed in a thermostated water-bath; D = magnetic stirrer; E = reaction column, containing Celite impregnated with 2,4-dinitrophenylhydrazine and anhydrous phosphoric acid, immersed in ice-water.

with a flow of nitrogen. The Celite was then impregnated with a solution containing 0.5 g of 2,4-dinitrophenylhydrazine dissolved in a mixture of 6 ml of 85 % phosphoric acid and 4 ml of carbonyl-free water by immersing the powder in the solution. After filtration, the impregnated Celite was dried under vacuum over phosphorus pentoxide and kept there until required.

When used as a reaction column, the bright yellow Celite powder was packed in a PTFE tube (3 mm I.D.) to give a column about 5 cm in length. After use, the reaction column was worked up as follows. After keeping the column in darkness at room temperature overnight, the reddened reaction zone was scraped out into a small beaker. Then 2.0 ml of carbonyl-free water was added, together with a spatula-tip of activated charcoal (BDH, activated at 275° with a flow of nitrogen). After about 15 min, the suspension was filtered through a "glass-nail" (about 0.5 cm^2 filter area), whereupon the solid material was dried over phosphorus pentoxide under vacuum. The dried Celite–charcoal mixture containing the 2,4-dinitrophenylhydrazones was then ready for gas chromatographic (GC) analysis. The weight of the dried powder was usually about 70 mg.

The GC analysis of the adsorbed 2,4-dinitrophenylhydrazones is based on a regeneration technique described by HALVARSON[9,10], modified as follows. An aliquot of the worked-up sample containing the hydrazones was mixed with α-ketoglutaric acid. This mixture was placed in the PTFE tube attached to the reactor described earlier[9] (Fig. 2) and the carrier gas was admitted. When a stable baseline was obtained, the carbonyls were regenerated and swept into the GC system by heating the PTFE tube. The reproducibility of this regeneration method is about the same as that reported[9] for pure 2,4-dinitrophenylhydrazone mixtures. No significant GC peaks were registered when conditioned Celite or activated charcoal was heated in the reactor.

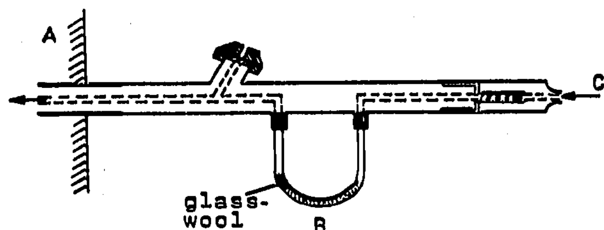


Fig. 2. Reactor used for the regeneration of volatile carbonyls. A = Gas chromatograph; B = PTFE tube containing 2,4-dinitrophenylhydrazones trapped on a Celite–charcoal mixture and α-ketoglutaric acid; C = carrier gas flow.

Alkanals of interest were quantitatively evaluated with the use of the 2,4-dinitrophenylhydrazone of *n*-pentanal as the internal standard. When the internal standard was not used, the worked-up samples were mixed with α-ketoglutaric acid in the ratio 5:1, and 10.0 mg of the mixture was heated in the PTFE tube attached to the reactor (Fig. 2) at 250° for 15 sec. When the internal standard was used, an aliquot of the sample was mixed with a mixture of α-ketoglutaric acid and the 2,4-dinitrophenylhydrazone of *n*-pentanal (100:1, w/w) in the ratio 5:1, after which 10.0 mg was regenerated as above. The carbonyls were subjected to GC under the following conditions.

A Varian 1400 gas chromatograph equipped with a flame ionization detector (FID) was used, together with a Varian Aerograph, Model 20, recorder. The detector responses were calculated with a Varian 475 electronic integrator connected to a printer or evaluated by area determinations. The following two columns were used.

(1) A Porasil C column, 1.00 m × 2 mm I.D., coated with 9% Carbowax 20M, packed in a PTFE tube, pre-conditioned overnight at 20° above the highest working temperature, with a nitrogen flow-rate of 10 ml/min. The column temperature was programmed from 75° to 175° at 6°/min, or maintained isothermally at 120°. The injector temperature was 210°, the detector temperature 210° and the nitrogen flow-rate 25 ml/min.

(2) A Porapak S column, 0.50 m × 1.35 mm I.D., packed in a PTFE tube, pre-conditioned at 220° for at least 24 h with a nitrogen flow-rate of 25 ml/min. The column temperature was 150° and the other conditions were the same as above. The GC system was calibrated as follows.

The 2,4-dinitrophenylhydrazones of the C_3, C_5, C_6, C_7, C_8 and C_9 *n*-alkanals were mixed in weighed proportions. This standard was mixed with α-ketoglutaric acid in the ratio 1:3 and diluted with conditioned Celite in the ratio 1:30, and 10 mg of this diluted standard was regenerated in each calibration as described above.

The resolution of some *n*-alkanals on the Porasil column is shown in Fig. 3. This column separates all *n*-alkanal homologues and the straight-chain 2-alkenals from the alkanals up to C_9. The resolution of important carbonyls on the Porapak column has been demonstrated earlier[9,10]. The Porasil column was used for quantitative analysis, the Porapak column for supplementary identification purposes.

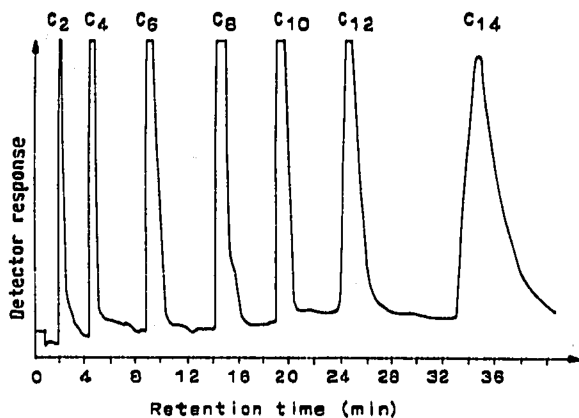


Fig. 3. Gas chromatogram showing the resolution of some *n*-alkanals on the Porasil C column. Column temperature programmed from 75° to 175° at 6°/min; range 10^{-11}; attenuation 16. For other GC parameters, see text. In this analysis, the regenerated 2,4-dinitrophenylhydrazone mixture contained about 0.1 mg of each component.

The following procedure was used in order to establish the formation of 2,4-dinitrophenylhydrazones in the reaction column used. The worked-up reaction zone was eluted with carbonyl-free benzene[6] until the eluate was colourless. After evaporation of the solvent until the solution became saturated, the solution was spotted on to Whatman No. 1 filter-paper impregnated with dimethylformamide together

with solutions of model substances. The paper was impregnated by descending development with a solution of 30% dimethylformamide in diethyl ether for at least 6 h. After equilibration overnight, the chromatogram was developed by the descending technique with hexane saturated with dimethylformamide as the mobile phase. After drying, the chromatogram was sprayed with a 4% solution of potassium hydroxide in ethanol.

Experiments with chicken fat

The method was tested on chicken fat. The visible fat from ten chickens was trimmed off, pooled, melted at 80° and filtered. The fat was separated from water by centrifugation at 50° at 5000 r.p.m. for 20 min. The clear, bright yellow fat was transferred by pipette in 20-ml portions into glass pots. After bubbling nitrogen through for 2 min, the pots were sealed with tight plastic caps and stored at −25° until required.

These fat samples were autoxidized to different extents by bubbling air through them at 100°. The air was washed with sodium dichromate and carbonyl-free water and dried before use. The extent of autoxidation was followed by determination of peroxide value. A second peroxide value was determined after the trapping of the volatile carbonyls. The mean value of these determinations was used as a measure of the extent of autoxidation.

The volatile alkanal content of the autoxidized chicken fat was determined by subjecting 10-ml samples (about 8.5 g) to the above isolation and evaluation procedures.

RESULTS AND DISCUSSION

Some of the *n*-alkanals to be expected in the autoxidation of the chicken fat studied are given in Table I.

TABLE I

EXPECTED *n*-ALKANALS FROM AN AUTOXIDIZED CHICKEN FAT

Fatty acid present	*Fatty acid composition* (%)	*n-Alkanals expected*
$C_{16} = 1$ (palmitoleic)	7.2	C_7
$C_{18} = 1$ (oleic)	42.3	C_9
$C_{18} = 2$ (linoleic)	14.3	C_6
$C_{18} = 3$ (linolenic)	1.2	C_3
$C_{20} = 4$ (arachidonic)	0.1	C_6

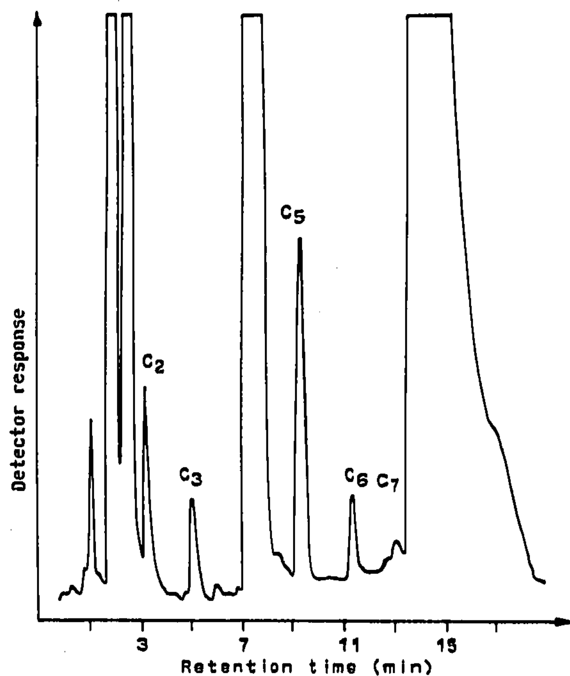
It has been shown that *n*-hexanal is one of the major constituents of the *n*-alkanal fraction in autoxidized animal fat. We therefore determined the content of this substance in the chicken fat at various autoxidation levels according to the methods described. The results are given in Table II.

There is evidently a close correlation between the extent of autoxidation and the *n*-hexanal concentration, indicating that the methods used are adequate for the

TABLE II

n-HEXANAL CONCENTRATIONS IN CHICKEN FAT OF VARIOUS AUTOXIDATION LEVELS IN RELATION TO PEROXIDE VALUES

Peroxide value	*n-Hexanal concentration in fat* ($\mu g/g$)
16	0.05
39	0.3
51	0.5
82	0.8
118	1.9



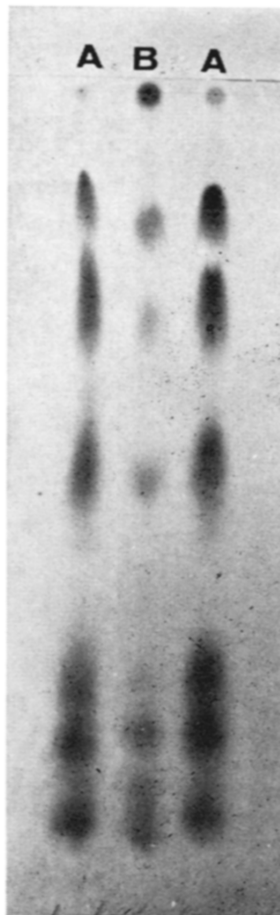


Fig. 4. Gas chromatogram showing n-alkanals isolated from chicken fat (peroxide value 51) after the addition of 19 μg of the 2,4-dinitrophenylhydrazone of C_8 as internal standard. Column, Porasil C coated with 9% Carbowax 20M; column temperature programmed from 75° to 175° at 6°/min; range 10^{-11}; attenuation 16. For other details, see text.

Fig. 5. Paper chromatogram verifying the formation of 2,4-dinitrophenylhydrazones on the reaction column used. A denotes a mixture of the 2,4-dinitrophenylhydrazones of the following n-alkanals (from top to bottom): C_1, C_2, C_3, C_5, C_6, C_9. A faint spot is also visible on the starting line. B denotes the composition of substances eluted from a worked-up reaction zone after trapping volatile carbonyls from the chicken fat. The peroxide value of the distilled fat was 450. A 50-μl volume of a saturated solution of B was spotted on to the paper.

intended purpose. The peroxide value of the "unoxidized" chicken fat was 16, which explains why we were not able to study any fat samples with peroxide values in the range 1–10.

A gas chromatogram showing some of the *n*-alkanals isolated from the fat samples is given in Fig. 4. It can be seen that some other peaks besides the alkanal peaks are registered in this type of chromatography. These peaks do not originate from the Celite and charcoal material, but are derived either from non-carbonylic fat volatiles or from α-ketoglutaric acid and/or 2,4-dinitrophenylhydrazine degradation products. Depending on the column used, these peaks may interfere with some alkanal peaks. This disadvantage can be overcome only by using a more suitable column.

The presence of 2,4-dinitrophenylhydrazones formed by trapping the volatile carbonyls was verified by paper chromatography as described earlier. By comparison with model substances (R_F values and colours of the alkali forms), the presence of C_1, C_2, C_3, C_5 (very faint), C_6, C_7 (faint) and C_9 *n*-alkanals could be established (Fig. 5).

The results obtained seem to indicate that the technique described for the determination of some important off-flavour alkanals is fairly simple, rapid and reproducible.

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