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THE QUALITATIVE AND QUANTITATIVE EVALUATION OF THE LOW-MOLECULAR-WEIGHT MONOCARBONYLS IN MEAT PRODUCTS

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SUMMARY

A gas chromatographic analysis technique based on the regeneration of trapped 2,4-dinitrophenylhydrazones has been used to develop a method of evaluating the low-molecular-weight monocarbonyl composition in meat products.

Qualitative analysis was accomplished by combined regeneration–gas chromatography–mass spectrometry of dinitrophenylhydrazone mixtures and quantitative determination was carried out with the use of a dinitrophenylhydrazone as internal standard.

Experimental results are presented that show the precision of the regeneration method compared with that of the conventional injection method and the reproducibility that can be obtained by the use of a dinitrophenylhydrazone as internal standard.

Also demonstrated is the way in which the regeneration method can be applied to determine the efficiency of a 2,4-dinitrophenylhydrazine trap used for carbonyls isolated from aqueous systems.

Finally, one application is given to illustrate the use of the evaluation system with meat products.

INTRODUCTION

To design a procedure for the analysis of volatile monocarbonyl compounds in foods and beverages, the consecutive steps involved have to be treated separately. Some of the factors that have to be considered are as follows.

(1) The isolation step. Different techniques can be applied, depending on the type of system studied. Examples of such systems are aqueous solutions, fat solutions, fat–water emulsions and water–fat–protein systems.

If a non-quantitative isolation method such as "direct vapour analysis" is used, the distribution of the carbonyls studied between the actual phases must be known for adequate quantitative analysis.

(2) The concentration step. The method chosen, *e.g.*, freeze concentration, adsorption or precipitation, will depend on the techniques applied in the isolation and the succeeding analysis of the monocarbonyls. Sometimes isolation and concentration can be accomplished in one step. For quantitative analysis of the monocarbonyls, the losses during the concentration process chosen must be controlled.

(3) Since the volatile carbonyls often occur at p.p.m. or sub-p.p.m. levels, the risk of external contamination must be considered. At these low levels, even deionized water can contain appreciable amounts of contaminants.

The prerequisites of isolation and concentration techniques in food aroma research have been discussed by WEURMAN[1].

An effective and highly selective way to isolate and concentrate monocarbonyls is to trap them as their 2,4-dinitrophenylhydrazones (DNPHs). The quantitative aspects of this conversion have been described by SCHWARTZ *et al.*[2] for monocarbonyls isolated from fat systems.

The separation of volatile monocarbonyls trapped as DNPHs has usually been accomplished by adsorption and/or partition chromatography on columns or on thin layers. Thus, BADINGS AND WASSINK[3], SCHWARTZ *et al.*[4] and CRASKE AND EDWARDS[5] have outlined techniques for the resolution of such DNPH mixtures based on the principle that the monocarbonyl classes (alkanals, methyl ketones, etc.) are separated by adsorption chromatography while the homologues within each class are separated by partition chromatography.

This technique can, however, hardly be applicable in the quantitative analysis of monocarbonyls owing to the inevitable losses during the separation steps. HALVARSON[6] has also found the reproducible resolution of DNPH mixtures by liquid chromatography to be difficult owing to solute–solute interactions. However, we needed an analysis technique to study the quantitative formation of volatile monocarbonyls in certain meat products and therefore developed a reactor[7] for the gas chromatographic (GC) analysis of monocarbonyls trapped as DNPHs.

In this paper the application of the above GC technique is described for qualitative and quantitative analysis of volatile monocarbonyls up to C_8 from meat products, which, referring to the discussion above, can be classified as water–fat–protein systems.

EXPERIMENTAL

The experiments described were carried out on a type of fermented sausage.

Isolation and trapping of the volatile carbonyls

The volatile carbonyl fraction was isolated from the sausage by open-system steam distillation in a nitrogen atmosphere.

Before the steam distillation was started, 500 ml of carbonyl-free 2 *M* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine and 500 ml of carbonyl-free benzene were added to the receiver trap. During the distillation, the condensate was led into this two-phase system. Finely divided sausage (1000 g), 150 g of magnesium sulphate and 2000 ml of carbonyl-free water were mixed in the distillation flask and distilled as indicated above with continuous agitation until 2500 ml of condensate were collected in the receiver trap.

The formation of the DNPH derivatives was completed by shaking the receiver flask overnight at 37°. After separation of the two phases, the benzene layer was evaporated to dryness under reduced pressure. The solid residue was then dried over phosphorus pentoxide under vacuum to constant weight.

GC conditions

After regeneration of the trapped DNPHs in a reactor as described earlier[7], the liberated monocarbonyls were subjected to GC under the following conditions. A Varian gas chromatograph 1400, 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 A Porapak S column, 1.20 m × 1 mm, preconditioned at 220° for at least 24h was used. The column material was packed in a Teflon tube. The column temperature was programmed over the range 100–180° at 10°/min. The injector temperature was 210°, the detector temperature was 210°, and the nitrogen carrier gas flow-rate was 25 ml/min.

Identification of the DNPH components

The trapped monocarbonyls were identified by retention data and a combined regeneration – gas chromatography – mass spectrometry (MS) procedure in which the reactor was connected to an LKB 9000 instrument for combined GC–MS analysis. The temperature of the ion source was 290° and the separated substances were ionized at 70 eV.

Quantitative analysis of the DNPH components

The calibration of the GC system was carried out by regeneration of a standard DNPH mixture containing the following parent compounds: CH_3CHO, $(CH_3)_2CO$, C_2H_5CHO, n-C_3H_7CHO, i-C_3H_7CHO, n-$C_5H_{11}CHO$, *trans*-$CH_3(CH_2)_2CH{=}CHCHO$ and n-$C_7H_{15}CHO$.

The DNPH derivatives of these compounds were synthesized from commercial products of the highest purity. About 2.5 mg of this homogeneous mixture was used in each calibration. The resolution of this standard on the column used is demonstrated in Fig. 1.

The quantitative determination of the sample compositions was accomplished by using the DNPH of i-C_3H_7CHO as the internal standard. After a first run with

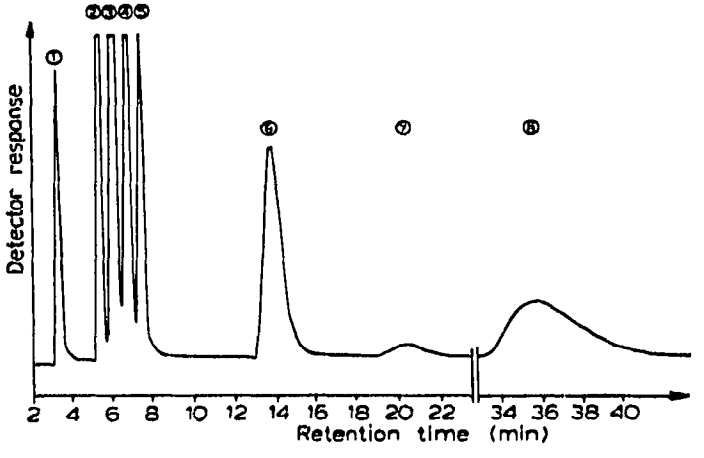

Fig. 1. Gas chromatogram showing the resolution of the calibration standard used. 1 = CH_3CHO; 2 = C_2H_5CHO; 3 = $(CH_3)_2CO$; 4 = i-C_3H_7CHO; 5 = n-C_3H_7CHO; 6 = n-$C_5H_{11}CHO$; 7 = *trans*-$CH_3(CH_2)_2CH{=}CHCHO$; 8 = n-$C_7H_{15}CHO$. For conditions, see text. Range, 10^{-11}; attenuation, 128.

a sample aliquot, a weighed amount of the internal standard was homogenized into another, after which this mixture was regenerated and subjected to GC in the usual way. The carbonyl composition was then evaluated relative to the amount of i-C_3H_7CHO added. The final calculations were carried out with an Olivetti Programma 101 electronic desk-top computer fed with detector response data. All the components in the samples were not included in the DNPH standard mixture, but were considered to give the same relative detector responses as related compounds. Thus, the methyl ketones were calculated as acetone, 2-enals as 2-hexenal and alkanals not included in the standard as the nearest lower homologue.

HCHO cannot be detected by the FID and was therefore determined in the following way. An aliquot of the sample was regenerated in a reactor similar to the one used for the GC analysis. During this time and for another 15 sec, the liberated carbonyls were led into 1.0 ml of carbonyl-free water. After the addition of a chromatropic acid reagent (0.6 g of $C_{10}H_6O_8S_2Na_2$ dissolved in 20 ml of water and diluted to 200 ml with concentrated sulphuric acid) and heating at 100° for 30 min, the HCHO content was spectrophotometrically evaluated at 570 nm.

Purification of the solvents

Doubly distilled water was used throughout these experiments, and thiophene-free benzene was rendered carbonyl-free according to the method of PARSONS[8].

RESULTS AND DISCUSSION

The efficiency of the DNPH-forming step

To trap the volatile carbonyls, we used a two-phase system consisting of 2 *M* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine and benzene. By using this system and introducing the shaking step, both water-soluble ($\leqslant$ 4C) and water-insoluble ($>$ 4C) monocarbonyls are trapped. As soon as the DNPHs are formed, they dissolve in the organic phase.

To test the efficiency of the trapping step, we added a mixture of monocarbonyls to this system and applied the procedure described above. After GC analysis of the isolated DNPHs, the yields could be calculated, and are given in Table I.

The recoveries are high and fairly constant up to C_6, then decrease with increasing carbon number. Correction factors compensating for non-quantitative DNPH formation can be introduced based on the results in Table I.

TABLE I

RESULTS SHOWING THE EFFICIENCY OF THE DNPH-FORMING TRAP USED

Carbonyl mixture tested	*Amount added (mg)*	*Amount found (mg)*	*Yield (%)*
CH_3CHO	0.78	0.65	85
C_2H_5CHO	0.78	0.69	89
n-C_3H_7CHO	0.80	0.80	100
n-$C_5H_{11}CHO$	0.85	0.73	86
n-$C_7H_{15}CHO$	0.83	0.38	46
$(CH_3)_2CO$	0.79	0.76	96
$C_3H_7CH=CHCHO$ (*trans*)	0.83	0.86	103

Comments on the quantitative–qualitative evaluation system used

The precision of the regeneration procedure prior to the GC analysis has been statistically demonstrated earlier[7].

To compare the reproducibility that can be obtained by the regeneration technique in relation to the conventional injection technique, we analysed a standard DNPH mixture and a standard solution of free carbonyls repeatedly and calculated the variations of the detector responses recorded. The results are given in Table II. The conditions were kept as constant as possible.

TABLE II

DEMONSTRATION OF THE ANALYSIS PRECISION OBTAINABLE BY THE REGENERATION METHOD

The data below are based on eight regenerations and eight injections. The standard solution consisted of 1 % w/w of each carbonyl in methanol.

DNPH or carbonyl mixture tested	*Relative S.D. of detector response*	
	Regeneration technique	*Injection technique*
C_2H_5CHO	5.9	3.0
n-C_3H_7CHO	3.6	2.9
i-C_3H_7CHO	3.1	3.1
n-$C_5H_{11}CHO$	3.2	3.6
n-$C_7H_{15}CHO$	11.1	9.9
$(CH_3)_2CO$	4.6	3.6
$CH_3(CH_2)_2CH=CHCHO$ (*trans*)	5.1	5.8

It is evident from Table II that the reproducibilities of the two techniques are about the same under the conditions used. Table II also shows that the conditions used are not ideal for molecules of the size of n-octanal (mol. wt. = 128).

From the above tests, three important advantages of the regeneration method became very clear, as follows.

(1) The composition of the carbonyl mixture is easily kept constant when the carbonyls exist as DNPHs. However, lifting the stopper of the vessel in which they are contained once or twice is sufficient to change significantly the carbonyl composition of a standard solution containing the free compounds.

(2) There is no influence of any solvent when the regeneration method is used. This means that no carbonyl will be overlapped (as CH_3CHO should have been in the solvent used) by a solvent peak and that the substances to be separated are freed from solute–solvent interactions.

(3) The calibration of the GC system is carried out more easily by the regeneration method owing to the higher purity of the components. The synthesis of DNPH derivatives is a well known technique for the purification of carbonyls. When standards of complex composition have to be used, confusion in interpreting GC data can be avoided if the regeneration technique is applied.

To calibrate the GC system, we used a mixture of the DNPHs of five n-alkanals, one i-alkanal, one methyl ketone and one straight-chain 2-alkenal. A typical gas

chromatogram of the standard mixture is shown in Fig. 1. As has previously been pointed out[7], the α,β-unsaturated aldehyde gives a much poorer relative detector response compared with those of saturated carbonyls. The quantitative analysis of the components was carried out with the addition of the DNPH of *i*-butanal as the internal standard.

Provided that the internal standard is carefully homogenized into the sample mixture, good agreements between double determinations can be obtained. An example of this is demonstrated in Table III.

The identification of the components was carried out by using GC retention data and combined regeneration–GC–MS of the sample mixtures. Fig. 2 shows a gas

TABLE III

RESULTS SHOWING THE AGREEMENT OBTAINED BETWEEN DOUBLE DETERMINATIONS OF THE VOLATILE MONOCARBONYL COMPOSITION IN MEAT PRODUCTS

The actual analyses were carried out on a smoked fermented sausage.

Qualitative carbonyl composition found	*Quantitative carbonyl composition found (mg/kg sausage)*	
	Analysis No. 1	*Analysis No. 2*
CH_3CHO	2.6	2.7
C_2H_5CHO	1.8	2.0
$(CH_3)_2CHCHO$ (internal standard)	0.20	0.20
$(CH_3)_2CH\ CH_2CHO$	0.47	0.45
$C_2H_5CH\ (CH_3)CHO$	0.24	0.23
n-C_4H_9CHO	0.20	0.21
n-$C_5H_{11}CHO$	0.32	0.26
n-$C_6H_{13}CHO$	0.17	0.14
$(CH_3)_2CO$	1.4	1.6
$CH_3COC_2H_5$	0.21	0.16
$CH_3COC_3H_7$	0.04	0.04
$(C_2H_5)_2CO$	0.02	0.02
C_4H_7CHO (2-enal)	0.62	0.65

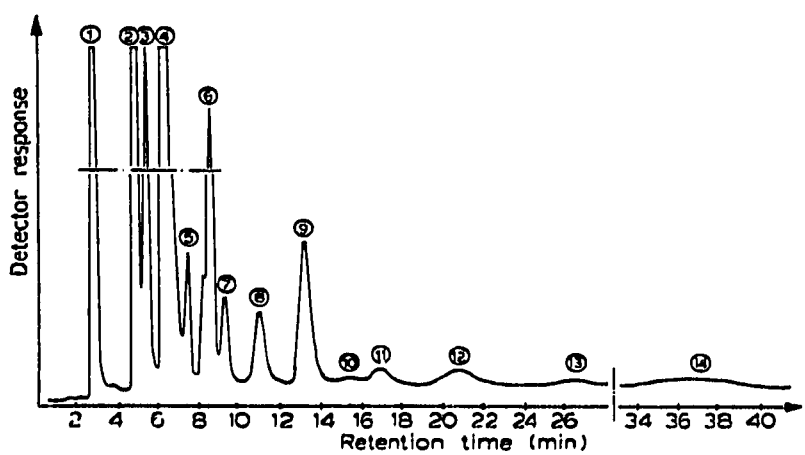

Fig. 2. Gas chromatogram showing the volatile monocarbonyl pattern in a fermented sausage after addition of the internal standard. 1 = CH_3CHO; 2 = C_2H_5CHO; 3 = $(CH_3)_2CO$; 4 = $(CH_3)_2CHCHO$; 5 = $CH_3COC_2H_5$; 6 = $(CH_3)_2CHCH_2CHO$ + $C_2H_5CH(CH_3)CHO$; 7 = n-C_4H_9CHO; 8 = C_4H_7CHO (2-enal, branched); 9 = n-$C_5H_{11}CHO$; 10 = C_5H_9CHO (2-enal, branched); 11 = n-C_5H_9CHO (2-enal); 12 = n-$C_6H_{13}CHO$; 13 = n-$C_6H_{11}CHO$ (2-enal); 14 = n-$C_7H_{15}CHO$. For conditions, see text. Range, 10^{-11}; attenuation, 128.

chromatogram of the volatile monocarbonyl content in a dried fermented sausage. For each of these peaks, a mass spectrum was recorded near the peak maximum. These spectra were compared with reference spectra given in the literature[9,10] or with our own reference spectra. We found that the Porapak S column used was very suitable for GC–MS analysis of regenerated carbonyls for the following reasons.

(1) Owing to the absence of a stationary phase, the column is suitable for programmed heating. Mass spectra taken on substances separated on a temperature-programmed column coated with a stationary phase are often difficult to interpret because of variations in the column bleeding. With the Porapak S column, very small changes in the MS background were recorded when it was programmed between 100° and 180° at 10°/min.

(2) It is easy to predict the carbonyl elution pattern by the use of this column. As it separates the substances according to molecular size, the carbonyls are separated according to chain length. In addition, they are eluted according to carbonyl class. For a fixed carbon chain-length, the monocarbonyls are eluted in the following sequence: branched alkanals, the *n*-alkanal, the methyl ketone, branched alkenals, *n*-alkenals, alkadienals.

All the monocarbonyls of these classes are not completely resolved on this column. With a mass spectrometer, however, the relative contribution of carbonyls of different classes in an unresolved peak can easily be calculated because of the widely different *m/e* fragmentation patterns characteristic of these classes. Better resolution of these monocarbonyls can most probably be obtained with the use of high efficiency capillary columns.

TABLE IV

THE FORMATION OF VOLATILE MONOCARBONYLS IN A DRIED SAUSAGE DURING STORAGE

Trace amounts ≃ 0.01 mg/kg. — = no indication of the substance; + = the substance was identified, but in an unknown amount.

Qualitative carbonyl composition	*Quantitative carbonyl composition (mg/kg sausage) at*	
	0 days	*21 days*
HCHO	Trace	Trace
CH_3CHO	3.6	2.3
C_2H_5CHO	1.8	2.4
$(CH_3)_2CHCHO$	0.1	0.4
n-C_4H_9CHO	0.2	0.3
$(CH_3)_2CHCH_2CHO$	1.1	1.0
$C_2H_5CH(CH_3)CHO$	0.6	0.5
n-$C_5H_{11}CHO$	0.2	0.5
n-$C_6H_{13}CHO$	0.1	0.1
n-$C_7H_{15}CHO$	—	0.1
$(CH_3)_2CO$	1.5	0.6
$CH_3COC_2H_5$	—	0.1
$CH_3COC_3H_7$	0.1	—
C_4H_7CHO (2-enal, branched)	0.8	1.9
n-C_5H_9CHO (2-enal)	—	0.6
C_5H_9CHO(2-enal, branched)	—	0.05
n-$C_6H_{11}CHO$ (2-enal)	Trace	0.3
C_5H_7CHO (2,4-dienal)	+	—
C_6H_9CHO (2,4-dienal)	+	—

(3) Carbon dioxide and water are evolved in the regeneration procedure. These compounds are not detected by the FID but are indicated on the mass spectrometer. On the Porapak S column, carbon dioxide and water only interfere with HCHO owing to the separation mechanism. These compounds may create separation problems when other types of columns are used.

Applications

The techniques described may have a wide application within organic chemical and biochemical research work. One example is the study of monocarbonyl formation in meat products during storage (Table IV).

Under the conditions used, the presence of about 10 p.p.b.* of each saturated carbonyl in the meat product could barely be detected. This limit can, if necessary, be lowered to 1 p.p.b. or perhaps even to 0.1 p.p.b. by increasing the load to be regenerated and the amplification of the detector signal.

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* Throughout this article the American (10^9) billion is meant.