CHREV. 159

SOME ANALYTICAL PROBLEMS IN FLAVOUR RESEARCH*

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The study of the volatile components of biological specimens involves the more general problem of analysing trace amounts of organic compounds in complex mixtures of unknown composition which occur in the vapour phase over the specimen. Biogenic volatiles play an important role in the plant and animal kingdoms, particularly as a means of communication, *viz.*, pheromones[1,2]. During man's evolution the original chemical language was replaced by speech; however, the senses of smell and taste are still important in choosing the foods most suitable for the organism. According to Amoore *et al.*[3], trimethylamine and isovaleraldehyde are man's pheromones that communicate the quality of food.

The composition and content of biological volatiles are usually determined by analysis of the vapour phase, or its condensate, over the specimen. This method has been termed headspace analysis[4].

The versions of headspace analysis** developed to date[5] can be divided into two major groups: those dealing with vapour in a state of thermodynamic equilibrium with the specimen and those in which equilibrium is not reached and the specimen belongs to so-called open systems. The analysis of biological volatiles, including food odours, falls into the latter group.

The major problems encountered in studying odours may be summarized as follows.

(1) The aroma-carrying material consists of substances belonging to different classes of compounds. Many of them result from natural biological processes or arise during subsequent treatment of the product. Organic acids, alcohols, esters, amines, aldehydes, ketones, lactones, phenols, sulphur compounds, hydrocarbons and various others are almost always present. The smell of boiled meat, for example, consists of over 500 components[6].

(2) Within each class there is also a considerable concentration range, the difference between the content of individual compounds sometimes being several orders of magnitude, from 10^{-3} to 10^{-12}. The latter circumstance decreases the sensitivity of mass spectrometry (MS) owing to the "memory" of substances present at high concentrations.

(3) Another difficulty is a wide boiling range of aroma-carrying substances, which include gases and compounds boiling at 300°C.

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** Headspace analysis is often identified with analysis of the equilibrium vapour phase (the EVP method), which is incorrect as headspace analysis also involves the study of non-equilibrium systems.

TABLE I

RECOVERY OF ISOPROPYL MERCAPTAN FROM HEAVY METAL MERCAPTIDES

Precipitating agent	Retention time (min)	Products recovered (%)	
		Isopropyl mercaptan	Diisopropyl disulphide
AgNO$_3$	1	40	43
AgC$_2$H$_3$O$_2$	2	36	4
Pb(C$_2$H$_3$O$_2$)$_2$	180	44	2
Hg(CN)$_2$	180	36	9

(4) A final problem is the very low odour threshold of some principal components. According to Dravnieks and O'Donnel[4], the thresholds of some compounds are of the order of 10^{-10}–10^{-11} g/ml, and for mercaptans and sulphides even lower, and they depend on the medium.

Hence the identification of the components of odours, whose concentration in food may be as low as 0.001 %, cannot be accomplished directly even with the use of the most sophisticated techniques of analysis. Table I shows that the threshold values are so low that they cannot be measured by gas chromatography (GC) with a flame-ionization detector (FID) or by gas chromatography–mass spectrometry (GC–MS). The practically achievable sensitivity of MS analysis is three orders of magnitude poorer with natural flavour specimens than with the individual pure substances. Investigation of odour-related substances, therefore, necessitates the development of methods for (i) extraction and concentration, (ii) essentially complete gas chromatographic separation of the mixture and (iii) identification of the individual components.

Before considering in brief each of these, it should be noted that the determination of flavour substances in food as such is impossible, as these substances cannot be completely extracted without destroying the quality of odour. What can actually be determined is the qualitative composition and the relative amounts of the components in the vapour phase or its condensate. This limitation is due to a number of factors. Some substances enter the air as a result of enzymatic or chemical reactions. The odour of onions or garlic, for example, appears only after cutting these vegetables, as alliin itself is odourless but produces disulphides under the action of alliinase. On the other hand, some substances are localized in the texture of specimen. An aqueous extract of cheese, for example, has an acidic pH. We have shown, however, that volatile amines from the vapour phase can easily be concentrated, and 40–60 mg of amines can be obtained as salts from 1 kg of cheese[7].

It is worth noting that the aroma-carrying vapour phase is not at equilibrium in the thermodynamic sense, and only a certain quantitative composition of volatiles, the proportions of which may change, may produce the sensation of a full-value odour. Concentration of caviar odour under vacuum, for instance, may be carried out for 6 h; after 10 h some defects may be noted, but if one adds water and leaves the mixture closed for several hours the odour will be restored completely[8]. This operation can be repeated several times with the same specimen. Hence we are dealing here with an open system where the required proportions of volatiles are maintained only for a certain period of time.

Exact values of absolute concentrations of volatiles in food cannot be calculated from vapour phase composition data[8]. The vapour pressure of a compound i in the gaseous phase over a biological specimen, including food, is described by the following equation[4,9]:

$$P_{i\alpha} = P_i X_i \gamma_i$$

where $P_{i\alpha}$ is the vapour pressure of the compound over the specimen, P_i is the saturated vapour pressure of the compound at a given temperature, X_i is a molar fraction of the compound in the specimen and γ_i is activity coefficient of the compound in the specimen. The activity coefficient varies greatly with the nature and environment of the compound and therefore assumes different values in different micro-areas of the specimen:

$$X_i \gamma_i = X_f \gamma_f + X_m \gamma_m + X_c \gamma_c + X_{lp} \gamma_{lp} + X_{sw} \gamma_{sw} + \ldots$$

where the subscripts denote the micro-areas: fatty (f), muscle (m), connective (c) tissues, lipoprotein (lp), structured water (sw) layers, etc.

The gaseous phase over the specimen is continuously supplied with water, which evaporates together with the volatiles. As water in the specimen is usually in the structured state, the rate of evaporation is largely a function of texture of the micro-area, and the X and γ values in different micro-areas change very irregular. For this reason the content of a compound in the specimen does not correspond to and cannot be inferred from that in the vapour phase.

In our opinion, the development of a theory for the study of saturated vapour phases over complex, non-equilibrium and non-stationary open systems is one of the major objectives in the analytical chemistry of biological specimens. Such a theory should provide information about the initial state of the system and predict its changes within reasonable time intervals in the future.

An unavoidable stage in flavour investigations is the concentration of aroma-carrying components. This is usually carried out by the following methods:

(1) distillation, including sublimation under vacuum or with sample water vapour;

(2) extraction with low-boiling solvents and carbon dioxide;

(3) bubbling through with inert gases;

(4) adsorption on carbon, silica gel or polymeric material with a low water vapour absorption such as Tenax, Amberlites, Norit and Porapak R and Q. Desorption is carried out with a stream of inert gas at 200°C and above or with a solvent, followed by distillation. The use of Polysorbimide-I and Carbochrom-K5 sorbents for the analysis or organic compounds in Leningrad air has also been reported[10]. Literature data on the adsorption capacities of sorbents and the decomposition and transformation of organic compounds during desorption at high temperatures are scarce and limited mainly to model systems composed of simple monofunctional compounds, usually unable to react with one another[5,11]. For this reason, the method of extraction from polymeric sorbents with a solvent seems to have considerable advantages[11].

Every method of concentrating and preparing a sample for analysis suffers from the risk of introducing impurities or losing some components. Taking into

account that sometimes substances present at the 10^{-10}–10^{-12} g level are essential components, exceptionally high demands are placed on the solvent purity. Thermal treatment and contact with oxygen from the air should be kept to a minimum. The solvent is eliminated in the cold or at room temperature with the use of a rotory evaporator or, with isopentane, by vacuum freezing from $-80°C$ into traps at $-196°C$.

The sample activity should be biologically controlled at each stage. The methods chosen for extraction and concentration of food odours should be controlled by the olfactory test.

After making certain that the concentration method has been chosen correctly, i.e., the concentrate possesses a full-value aroma, one may proceed to analyse it. There are several commonly used methods for the analysis of concentrates: either it is applied to a capillary column with temperature programming and the components are identified by MS, or it is divided into fractions using preparative GC, each fraction subsequently being rechromatographed on capillary or packed columns coupled to a mass spectrometer. This would appear to be a most convenient procedure. However, as practice has shown, it has produced only modest results during the last 25 years. In our opinion, a number of important considerations must be taken into account:

(1) First, the odour concentrate represents a mixture of very active substances that can react with one another even in the cold, and particularly in the chromatograph evaporator or in a column at high temperature, giving rise to secondary products. Aldehydes plus mercaptans or aldehydes plus amines may produce cyclic and sulphur- and nitrogen-containing compounds[12,13]. Catalytic transformations and irreversible adsorption to metal parts of the equipment are also possible.

(2) A second consideration is the absence of chromatographic columns, packed or capillary, that can ensure good separation of the several hundreds of organic compounds belonging to different classes that are usually present in an odour concentrate.

In the 1960s our experience showed that peak overlapping cannot be completely excluded even for compounds with a similar functional group when one column is used, and three or four columns with different polarity characteristics should be employed[7,14]. This point of view was supported in 1979 when, based on a mathematical assessment of various experimental settings in gas–liquid chromatography[15], it was shown that a system of four columns with different polarities is the best.

The complex character of natural aroma-carrying compositions necessitated the development of a new approach to the identification of their component parts[16–19].

Our first step in analysing an odour is a qualitative estimation, using chemical reactions, of classes of organic compounds that are essential to the odour. This procedure is controlled organoleptically.

The next step is the trapping of these classes from the headspace. Trapping of selected classes of compounds using stable derivatives has several advantages: it increases the relative concentration of each component and decreases the probability of peak overlap, as each sample contains compounds with similar functional groups. Interaction with other reactive organic substances is essentially excluded. Our experience has shown, however, that the known reactions developed for milligram

amounts should not automatically be applied to microgram and nanogram amounts without special testing. A common technique in studying sulphur-containing compounds, for example, is trapping of hydrogen sulphide, mercaptans, sulphides and disulphides with solutions of lead(II) acetate, mercury(II) cyanide and sometimes with silver salts and mercury(II) chloride.

Our results on testing the applicability of these reagents for concentrating microgram and nanogram amounts (Table 1) indicate that the recovery of mercaptans from mercury and silver mercaptides is not quantitative. Lead(II) acetate absorbs not only hydrogen sulphide but also mercaptans but recovers only 44% of them. The method described for the regeneration of mercaptans from mercury mercaptide[20] yields only 38% of n-butylmercaptan. As is evident from Table 1, none of the salts listed, can ensure quantitative and selective trapping of mercaptans.

Our further experiments have shown that the recommended method for the regeneration of mercaptans, sulphides and disulphides from the mercury(II) chloride precipitate (upper line in Table 2) does not yield a quantitative recovery[21]. However, when the temperature was increased and the reaction time decreased, especially after addition of a reducing agent, we obtained almost complete recoveries of mercaptans and sulphides.

These modification were successfully applied to the analysis of sulphur-containing compounds in the flavour of boiled meat, to the products of the Maillard reaction and other samples[12,22].

We now consider another example stressing the necessity to apply test reactions recommended for compound trapping. Table 3 lists several substances that are generally used for the recovery of carbonyl compounds precipitated as 2,4-dinitrophenylhydrazones. It can be seen that the most promising reagent is n-dimethylaminobenzaldehyde, and that the recovery decreases with increasing number of carbon atoms in the carbonyl molecule[18]. Recently we have found that 2,4-dinitrophenylhyd-

TABLE 2

REGENERATION OF n-BUTYL MERCAPTAN, DI-n-PROPYL SULPHIDE AND DIETHYL DI-SULPHIDE FROM MERCURY(II) CHLORIDE PRECIPITATE

Regeneration conditions	Recovery (%)			
	n-Butyl mercaptan	Di-n-propyl sulphide	Diethyl disulphide*	Di-n-butyl disulphide**
HCl, 20°C, 40 min	38	92	28	13
HCl, 50°C, 10 min	49	91	28	2
HCl, 60°C, 8 min	81	92	17	2
HCl, 70°C, 4 min	97	95	23	3
HCl, 70°C, 4 min, $K_2C_2O_4$***	98	97	51	3
C_6H_5SH, 20°C, 20 min	52	67	11	8
C_6H_5SH, 100°C, 3 min	63	99	11	27

* Ethyl mercaptan was determined chromatographically as a product of diethyl sulphide decomposition, but not quantitatively at the analysis temperature.

** Di-n-butyl sulphide is the product of n-butyl mercaptan oxidation.

*** An aqueous solution of potassium oxalate (100 μl in this experiment) was added before the addition of concentrated hydrochloric acid.

TABLE 3

RECOVERY OF ALDEHYDES AND KETONES FROM 2,4-DNPH MONOCARBONYL COMPOUNDS

Reagent	No. of carbon atoms in the carbonyl compound	Recovery (%)	Formation of byproducts
Levulinic acid	C_1–C_7	20–80	+
Levulinic acid + H_2SO_4	C_1–C_7	80–95	+
α-Ketoglutaric acid	C_2–C_6	50–60	+
α-Ketoglutaric acid + 2,4-DNPH of formaldehyde	C_2–C_6	60–100	+
N,N-Dimethylamino-benzaldehyde +	C_2–C_6	100	–
oxalic acid	C_7–C_{12}	50–80	–

razones may produce both *syn*- and *anti*-isomers when analysed directly on capillary columns and thus give two peaks instead of one. The proportion of *syn*-isomers depends markedly on solvent and temperature[23].

The above examples demonstrate that GC and MS analysis of microgram and smaller amounts of compounds require the testing of existing procedures and the development of special techniques and approaches. This is, in fact, an important area of organic analytical chemistry, not only with respect to flavour chemistry but particularly with regard to monitoring environmental pollution.

Extraction of specific compound classes from the volatiles of a specimen is necessarily followed by the separation of these classes into individual compounds and their identification. We have developed standardless methods for the GC identification of compounds within several classes. The method is based on the maximum utilization of intermolecular interaction energy.

An advantage of GC for studying intermolecular interactions is that retention characteristics are affected by many features of these interactions. As retention characteristics are closely related to thermodynamic parameters of sorbent–compound interactions, they offer some information about the molecular structure of sample compounds. The character of the interaction in this instance should be chosen so as to reveal essentially the structural peculiarities of the compound. The establishment of molecular structure by means of GC was given the name "chromatoscopy" by Kiselev[24].

A series of studies on the calculation of thermodynamic parameters from Kováts retention indices performed in our laboratory has demonstrated that intermolecular interactions during GC do provide certain information on compound structure[25,26]. The conditions for the identification of the components of a mixture of unknown composition are as follows:

(i) choice of the most selective and effective columns;
(ii) good reproducibility of retention characteristics;
(iii) sensitivity of the retention parameters to structural changes and the avail-

TABLE 4

COMPARISON-OF KOVÁTS RETENTION INDICES OF SULPHUR COMPOUNDS FROM THREE BOILED MEAT SAMPLES

Samples: A. Longissimus dorsi, 2-year-old cow, Moscow, 1973; B. Longissimus dorsi, 1-year-old bull, Potsdam, G.D.R., 1974; C, shoulder-blade, 3-year-old cow, Potsdam, G.D.R., 1975. Analytical conditions: 10 % Apiezon M on Chromosorb W, glass column (210 × 0.4 cm I.D.), temperature, 130°C, FID.

No.	A (1973)	B (1974)	C (1975)	No.	A (1973)	B (1974)	C (1975)
1	541	546	554	20	—	1018	—
2	600	603	600	21	1026	1030	1025
3	—	648	650	22	1040	1037	1036
4	676	—	—	23	—	1044	—
5	701	690	687	24	1051	1054	1053
6	727	720	736	25	1071	1072	1070
7	770	762	768	26	1083	1080	1088
8	796	799	802	27	1103	1102	1105
9	839	841	839	28	1115	1113	1120
10	853	—	853	29	1127	1127	1129
11	872	875	873	30	1145	1153	1147
12	889	889	—	31	1162	—	1156
13	905	907	902	32	1171	1173	1168
14	919	—	922	33	1179	1187	1182
15	933	—	936	34	1201	1201	1199
16	946	—	—	35	—	1218	1213
17	971	972	976	36	1227	1235	1230
18	—	982	—	37	1246	1251	1248
19	1002	1002	999				

ability of a simple mathematical relationship between retention and molecular structure.

Specialists concerned with the investigation of volatiles know that it is virtually impossible to compare the data in the literature on the separation of odour components and their identification, as the GC parameters in most instances are not reported.

After extensive testing, we recommended the Kováts retention index system for identification purposes[7,14]. These indices are directly proportional to the free energy of dissolution (or adsorption, in gas-adsorption chromatography), and therefore represent an additive function of the number of functional groups and structural fragments of a molecule. For this reason, the Kováts retention indices seem to be the most convenient identification parameter.

A comparison of the GC separation of sulphur-containing substances from three samples of boiled meat differing in the time of sampling (1973–76) and prepared in two laboratories[12] demonstrate that the retention indices provide individual characteristics of a biological specimen even without identification of all the components (Table 4). The use of Kováts retention indices allows one to trace the fate of each peak and its interpretation and to compare the results with literature data.

The next important stage is the choice of the most selective columns for separating a mixture of compounds with a similar functional group. In doing this we aim to exploit to the maximum extent the differences in intermolecular interactions be-

TABLE 5

COLUMN SYSTEMS USED FOR ANALYSIS OF SOME CLASSES OF ORGANIC COMPOUNDS

Compounds	Conditions	Columns
Amines	100, 150°C; packed columns	4% Apiezon L + 1% KOH 5% Triton X-305 + 0.5% Na$_3$PO$_4$ 5% PEG-1000 + 0.5% Na$_3$PO$_4$ 3% Tetraoxyethyleneamine + 0.5% Na$_3$PO$_4$ + 2% PEG-1000 on Chromosorb G
Monocarbonyl compounds	120°C; packed columns	5% Apiezon M 5% Triton X-305 5% PEG-1000 10% TCEP
	temperature programming (3–5°C/min)	10% Apiezon M 10% PEG-1000
Monocarbonyl compounds, in the form of 2,4-DNPH	225°C; capillary column	SE-30
Sulphur compounds	60, 130°C; packed columns	10% Apiezon M 10% Triton X-305 10% Silicone OV-17 10% PEG-1000 in Chromosorb W
Methyl esters of fatty acids	200°C; packed and capillary columns	3% SE-30 5% OV-225 3% Silar 5 CP 10% Silar 10C

tween the compounds studied and the selective stationary phase, including the donor--acceptor interactions. For the determination of column selectivity we proposed a simple method based on calculating the difference in the excess free energies of mixing, $\delta(\Delta G^E)$, between compounds with similar boiling points and belonging to different homologous series. The calculation is based on Kováts retention indices and has been described in detail for the selection of columns for sulphur-containing compounds and monocarbonyls[27–29].

It should be noted that choosing columns according to differences in excess free energies of mixing allows one to select not only the most selective stationary phase but also the optimal temperature for analysis.

Based on $\delta(\Delta G^E)$ values for the classes of organic compounds of interest, we have selected optimum conditions for analysis on three or four columns which reduce to a minimum the number of possible mistakes due to peak overlapping. Table 5 lists column systems proposed for the analysis of amines, carbonyl- and sulphur-containing compounds and methyl esters of fatty acids.

Over 250 equations of the general form shown below, describing GC behaviour

for each series as a function of number of carbon atoms and boiling temperature, have been proposed for standardless identification:

$$I = A + Bn \qquad\qquad I = A + Bn + C/n$$
$$T_{boil.} = a + bI \qquad\qquad T_{boil.} = \gamma + \beta/(I + \gamma)$$

where I = Kováts retention index, n = number of carbon atoms in the radical R and $A, B, C, a, b, \gamma, \beta$ = coefficients. The equations were constructed on the basis of GC data for 800 compounds in 60 homologous series:

Amines: n-R-NH$_2$; iso-R-NH$_2$; $(n$-R)$_2$NH; CH$_3$NH-n-R; C$_2$H$_5$NH-n-R; C$_3$H$_7$NH-n-R; $(n$-R)$_3$N; (CH$_3$)$_2$N-n-R; iso-C$_3$H$_7$NH-n-R; $tert.$-C$_4$H$_9$NH-n-R; $sec.$-C$_4$H$_9$NH-n-R; iso-C$_4$H$_9$NH-n-R; iso-C$_5$H$_{11}$-NH-n-R; C$_6$H$_{11}$NH-n-R; (C$_2$H$_5$)$_2$N-n-R; (C$_3$H$_7$)$_2$N-n-R;
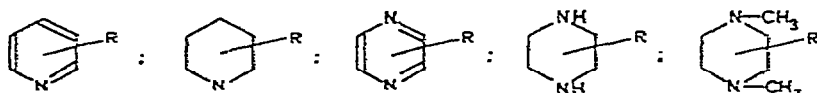
Sulphur compounds: n-R-SH; iso-R-SH; HS-(CH$_2$)$_n$-SH; $(n$-R)$_2$S; n-R-S-iso-R; CH=CH-S-n-R; CH$_2$=CH-CH$_2$-S-n-R; CH$\equiv$CH-CH$_2$-S-R; HS-(CH$_2$)$_n$-S-n-R; $(n$-R)$_2$S$_2$; n-R-S-CH$_2$-S-n-R; n-R-S-n-R'; iso-C$_3$H$_7$-S$_2$-n-R; iso-C$_4$H$_9$-S$_2$-n-R; $sec.$-C$_4$H$_9$-S$_2$-n-R; C$_4$H$_9$-S$_2$-n-R; iso-C$_5$H$_{11}$-S$_2$-n-R; $tert.$-C$_5$H$_{11}$-S$_2$-n-R; CH$_2$=CH-CH$_2$-S$_2$-n-R; CH$_3$-CO-S-n-R; C$_2$H$_5$-CO-S-n-R; C$_3$H$_7$-CO-S-n-R; CH$_3$-S-CH(n-R)-S-CH$_3$; CH$_3$-S-CH(n-R)-S-C$_2$H$_5$; C$_2$H$_5$-S-CH(n-R)-S-C$_2$H$_5$;
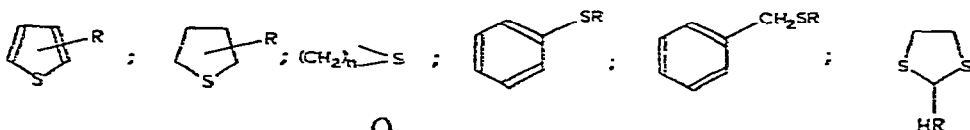
Carbonyl compounds: n-R-C$\overset{O}{\underset{H}{\diagup}}$; R-CH=CH-COH; n-R-CH=CH-CH$_2$CH =CH-COH; CH$_3$-CO-n-R; $(n$-R)$_2$CO; $(iso$-R)$_2$CO.

By the term "standardless identification" we mean a method that consists of a column system specially designed for a mixture of compounds with a similar functional group, selected to ensure optimum separation, and allowing a mathematical relationship to be established between the GC behaviour of a compound and its physico-chemical properties. The identification may be performed on a computer by a special program developed on the basis of chromatographic data[8,16].

The relationship between retention index and number of carbon atoms may be expressed in the form of linear or fractional-linear equations. The linear form is used for identification by an operator whereas the fractional-linear form is more convenient for computer analysis. Table 6 shows similar equations for sulphur-containing compounds.

An additional test of correct identification in all instances may be coincidence of boiling points calculated from experimental retention indices obtained on all columns.

Table 7 presents equations for calculating boiling points on columns of Apiezon L, Triton X-305 and PEG for primary, secondary and tertiary amines. There s good correspondence between the calculated and experimental values. In some in-

TABLE 6

COEFFICIENTS OF THE EQUATION $I = A + Bn + C/n$ AND STANDARD DEVIATION (σ) OF I_{exp} FROM $I_{calc.}$

Stationary phase	Coefficient	Homologous series					
		n-Mercap-tan	α,ω-Dimer-captans	Methyl-n-alkyl-sulphides	Hexyl-n-alkyl-sulphides	Isopropyl-n-alkyl-sulphides	tert.-Butyl-n-alkyl-sulphides
Apiezon M	A	331.1	678.0	407.5	865.6	515.1	562.3
	B	100.6	100.8	101.0	99.9	101.4	99.6
	C	−30	−66	14	51	58	62
	σ	0.2	0.4	0.4	0.6	0.8	0.3
OV-17	A	409.4	881.0	498.9	952.7	604.0	638.1
	B	100.7	96.8	100.8	99.6	99.9	98.9
	C	−24	−137	11	54	58	66
	σ	0.3	0.4	1.3	0.6	0.1	0.2
Triton X-305	A	500.9	1121.1	589.5	1012.8	657.5	699.6
	B	101.8	93.1	100.7	100.6	101.4	97.5
	C	−17	−139	20	84	88	83
	σ	0.4	0.5	0.8	1.0	0.4	0.6
PEG-1000	A	564.3	1256.9	633.8	1065.4	717.8	737.2
	B	100.9	90.5	101.8	99.8	99.1	98.8
	C	−27	−180	39	86	92	102
	σ	0.6	0.6	0.8	1.4	1.5	0.5

stances these equations helped us to reveal some mistakes in the reported boiling points of amines and carbonyl- and sulphur-containing compounds.

The observed regularities form the basis for constructing a computer program aimed at the identification of volatiles in complex mixtures. Table 8 presents, as an example, data introduced into a computer when analysing an amine mixture[8,16]. The output from the computer consists of a list of identified substances, tentatively identified substances noting the nature of the functional group and boiling point, and a separate list of Kováts retention indices for unidentified compounds. Table 9 summarizes the results of computer-aided identification of amines in six foodstuffs, that we studied during the last 3 years. Results for previous years have been reviewed earlier[2].

An interesting problem was encountered in studying the unpleasant odour that arises during the storage of casein and coprecipitate (milk protein precipitated by $CaCl_2$). We have found that amines, but not carbonyl compounds as mentioned in the literature, are responsible for this odour. A correlation has been established between the accumulation of amines in casein and coprecipitate odour and the deterioration of their nutritional value: 84% and 57% for casein and coprecipitate, respectively, after storage for 1 year (ref. 30 and Table 10).

Table 11 presents our results on the identification of sulphur-containing compounds in volatile components of boiled beef and in Maillard reaction products[12]. The analytical scheme in both instances was identical (Fig. 1).

The techniques of standardless GC identification of odour components de-

TABLE 7

EQUATIONS RELATING KOVÁTS RETENTION INDICES OF ALIPHATIC n-AMINES WITH THEIR BOILING POINTS, T_b (Na$_3$PO$_4$)

Equation	Amine	T_b calculated from				T_b from literature
		I_{100}^{Ap}	I_{100}^{Ta}	I_{100}^{PEG}	I_{100}^{TN+PEG}	
$T_b = \dfrac{I_{100}^{Ap}}{4.0} - 81$	Primary Propyl	54.5	57.1	54.6	52.5	49–50
$T_b = \dfrac{I_{100}^{Ta}}{4.1} - 127$	Butyl	79.8	81.5	79.9	78.6	78–79
	Amyl	104.8	105.9	105.3	104.6	104
$T_b = \dfrac{I_{100}^{PEG}}{4.1} - 153$	Hexyl	128.8	130.3	129.9	129.9	129–130
	Heptyl	154.5	154.7	154.6	154.6	155
$T_b = \dfrac{I_{100}^{TN+PEG}}{3.8} - 203$	Octyl	179.1	179.1	179.2	179.4	179.6
$T_b = 666 - \dfrac{1.1084 \cdot 10^6}{I_{100}^{Ap} + 1249}$	Secondary Dimethyl	7.4	6.7	6.5	7.4	7.0
	Diethyl	53.3	53.6	51.9	52.8	55.5
$T_b = 500 - \dfrac{4.968 \cdot 10^5}{I_{100}^{Ta} + 400}$	Dipropyl	111.0	110.1	109.7	107.7	110.5
	Dibutyl	161.3	161.4	·162.8	163.7	159.0
$T_b = 437 - \dfrac{3.293 \cdot 10^5}{I_{100}^{PEG} + 73}$	Diamyl	203.0	202.0	202.0	199.7	202.5
$T_b = 363 - \dfrac{1.7 \cdot 10^5}{I_{100}^{TN+PEG} - 334}$						
$T_b = 876 - \dfrac{2.0183 \cdot 10^6}{I_{100}^{Ap} + 1890}$	Tertiary					

TABLE 8

DATA INTRODUCED INTO COMPUTER

Amine groups (q)		Columns (i)	Equation coefficients	Tolerable error in comparisons
Primary	Tertiary	$i = 1$,	$I_i = a_{qi} + b_{qi}n + \dfrac{C_{qi}}{n}$	$\theta_{qi}^{\Delta I} = \pm 5$ units
$q_1 = $ alkyl	$q_6 = $ symm. trialkyl	Apiezon L		
Secondary				
$q_2 = $ symm. dialkyl	$q_7 = $ dimethylalkyl	$i = 2$, Triton X-305		$\theta_{qi}^{T_b} = \pm 5^\circ C$
$q_3 = $ methylalkyl	$q_8 = $ diethylalkyl	$i = 3$,	$T_b = A_{qi} + B_{qi}I + \dfrac{C_{qi}}{I + D_{qi}}$	$\theta_q^{\Delta I} = \pm 20$ units
$q_4 = $ ethylalkyl	$q_9 = $ dipropylalkyl	PEG-1000		
$q_5 = $ propylalkyl	$q_{10} = $ others and heterocyclic compounds			

Values: $\Delta I = I_{q_1} - I_{q_i}$; $P = $ "max." number of classes; $M = $ "max." number of columns; $N_i = $ "max." number of peak on each column

TABLE 9

VOLATILE AMINES IN THE ODOUR OF FOOD PRODUCTS

Type	Amine	Boiled beef	Salmon	Bread Crust	Bread Crumb	Chanakh cheese	Casein	Coprecipitate
Primary	Methyl	+	÷	+	÷	+*	÷	+
	Ethyl	+*	—	+	+	+*	+	÷
	Isopropyl	÷	—	+	+	+	—	—
	Propyl	+	÷	+	÷	÷	—	—
	Isobutyl	+	÷*	+	+	+	+	+
	Butyl	÷*	+	—	—	÷	—	—
	Isoamyl	+	—	+	+	÷	÷	+
	Amyl	—	—	+	+	+*	—	—
	Hexyl	—	—	÷	+	—	—	—
	Heptyl	—	—	÷	÷	—	+*	÷
	Octyl	+*	—	—	—	—	+	—
Second-ary	Dimethyl	+	÷*	÷	+	÷	+	÷
	Diethyl	+	+	+	+	+	÷	+
	Di-n-propyl	+	+	÷	+	+	÷	—
	Diisopropyl	÷	÷	÷	+	+*	—	—
	Methyl-n-propyl	—	—	—	—	—	+	—
	Di-n-butyl	—	+	+	+	÷	—	—
	Diisobutyl	—	—	+	+	+	—	+
	Methyl-n-butyl	—	—	—	—	—	—	÷
	Diisoamyl	÷	—	—	—	+	—	+
	Methyl-n-butyl	—	—	—	—	—	—	÷
	N-Propyl-n-butyl	—	—	—	—	—	—	+
	Isopropyl-n-butyl	+	—	—	—	—	—	+
	Ethyl-n-hexyl	—	—	—	—	—	—	÷
	Ethyl-n-heptyl	+*	—	—	—	—	÷	+
	Methyl-n-octyl	—	—	—	—	—	÷	+
Tertiary	Trimethyl	+	+	+	+	+	+	÷
	Triethyl	÷	÷	÷	+	÷	+	+
	Tripropyl	—	—	+	÷	+	—	+
	Methyldipropyl	—	—	—	—	—	+	—
	Triisobutyl	—	—	÷	÷	—	—	—
	Diethyl-sec.-butyl	—	—	÷	—	—	—	—
	Dimethylformamide	—	—	—	—	—	—	—
	Dimethylamide	—	—	—	—	—	—	—
	Diethylbutyl	—	—	÷	÷	—	+	—
	Dipropylisopropyl	—	—	—	—	—	+	—
	Dipropylisobutyl	—	—	—	—	—	+	—
	Dimethylhexyl	—	—	—	—	—	+	÷
	Dipropyl-tert.-butyl	—	—	—	—	—	+	÷
	Dipropyl-sec.-butyl	—	—	÷	—	÷	—	—
	Dimethylheptyl	+	—	—	—	—	+	÷
	Dipropylamine	+	—	—	—	—	+	—
	Dimethylcyclohexyl	—	—	—	—	—	+	+
	Dipropylpropargyl	—	—	—	—	—	+	—
	Dimethyloctyl	—	—	÷	÷	—	+*	—
Hetero-cyclic	Piperidine	—	+	÷	+	÷	+	+
	Pyridine	÷	+	+	+	÷	+	+

TABLE 9 *(continued)*

Type	Amine	Boiled beef	Salmon	Bread Crust	Bread Crumb	Chanakh cheese	Casein	Copreci- pitate
	N-Methylpiperedine	+*	—	—	—	÷	+*	+
	N-Ethylpiperedine	+	÷	—	—.	—	+	÷
	N-*n*-Propylpiperedine	—	—	—	—	—	÷	÷
	Pyrrolidine	—	÷	+	÷	÷	—	—
	α-Picoline	+	÷	+	÷	÷	÷	÷
	β-Picoline	÷	+*	+	÷	÷	+	÷
	γ-Picoline	+	+*	+	+	+	÷	÷
	2,4-Dimethylpyridine	—	—	—	—	÷	—	÷
	2,5-Dimethylpyridine	+	—	÷	—	—	—	—
	2,6-Dimethypyridine	÷	—	—	—	÷	—	—
	4-Ethylpyridine	+	—	+	÷	+	÷	÷
	2,6-Lutidine	—	—	+	÷	—	—	—
	2,4-Lutidine	—	—	+	+	—	—	—
	2,3-Lutidine	—	—	+	÷	—	—	—
Pyra-zines	2,5-Dimethylpyrazine	+*	—	—	—	—	—	—
	2,6-Dimethylpyrazine	÷*	—	—	—	—	—	—
	2-Methoxypyrazine	÷*	—	—	—	—	—	—
	2-Methoxy-3-methyl-pyrazine	—	—	—	—	—	÷	÷
	2,3,5-Trimethyl-pyrazine	—	—	—	—	—	÷	÷
	2,3,5,6-Tetramethyl-pyrazine	—	—	—	—	—	÷	÷
	2-Methylpiperazine	—	—	—	—	—	÷	÷
	2,3-Dimethylpyrazine	—	—	—	—	—	—	÷

* Identified tentatively.

TABLE 10

CHANGES IN THE RELATIVE NUTRITIONAL VALUE (TEST ORGANISM *TETRAHYMENA PYRIFORMIS*) AND THE CONTENT OF SOME VOLATILES IN MILK-PROTEIN CONCEN-TRATES DURING STORAGE[30]

Product	Property	Storage time (months) 0	6	12
Casein	Nutritional value (%)	100	86	84
Coprecipitate		103	76	57
Casein				
Pyridine	Content of volatile	3.6	5.5	9.9
2,3,5-Trimethylpyrazine	amines (rel.%)	4.4	12.4	14.2
2,3,5,6-Tetramethylpyrazine		8.9	18.4	48.3
Coprecipitate				
Pyridine		7.1	15.6	17.2
Compounds of the pyridine series		41.8	60.2	60.2

TABLE 11

SULPHUR COMPOUNDS IDENTIFIED IN A BOILED MEAT ODOUR CONCENTRATE AND IN MAILLARD REACTION PRODUCTS

+++, ++ and + corresponds to a large, middle and small size peak; (+) indicates probable compound is present and has a small size of peak.

Type	Natural boiled meat		Maillard reaction	
	Compound	Peak size*	Compound	Peak size*
Mercaptans	Hydrogen sulphide	++, VCF	Hydrogen sulphide	+++
	Methyl mercaptan	++, VCF	Methyl mercaptan	+++
	Ethyl mercaptan	++, VCF	Ethyl mercaptan	++
	tert.-Butyl mercaptan	+		
	n-Octyl mercaptan	+		
	n-Nonyl mercaptan	+		
Polysulphides	Dimethyl disulphide	+++, MS, VCF	Dimethyl disulphide	++, MS
	Methyl ethyl disulphide	++, MS, VCF	Methyl propyl disulphide	+
	Methyl propyl disulphide	+	Methyl heptyl disulphide	(+)
	Diethyl disulphide	+, MS, VCF	Bis(methylthio)methane	(+)
	Methyl isopropyl disulphide	+	1,2-Bis(ethylthio)ethane	++
	Di-tert.-butyl disulphide	+	1,2-Bis(isopropylthio)ethane	+
	Dimethyl trisulphide	+++, MS, VCF	1,2-Bis(propylthio)ethane	++
	Methyl ethyl trisulphide	++	1-Methylthio-3-isopropylthiopropane	+
	Diethyl trisulphide	+	1,5-Bis(methylthio)pentane	(+)
	Dimethyl tetrasulphide	+, MS		
	Bis(methylthio)methane	++, VCF		
Sulphides	Methyl n-octyl sulphide	++	Dimethyl sulphide	++
	Methyl n-nonyl sulphide	+	Isopropyl propargyl sulphide	(+)
	Diisopropyl sulphide	+		
	Methyl benzyl sulphide	+		
Heterocyclic compounds	Ethylene sulphide	+, VCF	Ethylene sulphide	++
	Thiophene	+++, MS, VCF	Thiophene	+++, MS
	2-Methylthiophene	++, MS, VCF	2-Methylthiophene	++, MS
	2-Formylthiophene	+, MS, VCF	2-Ethyl-1,3-dithiolane	(+)
	2-Acetylthiophene	++, MS, VCF	2,2-Diethyl-1,3-dithiolane	(+)
	3,5-Dimethyl-1,2,4-trithiolane	+, VCF		
	1,4-Dithiocyclohexadiene	+		
	2,2,4-Trimethyl-1,3-dithian	+		
Miscellaneous	Ethyl thioacetate	+, VCF	Butyl thioacetate	+
	Carbon disulphide	+, VCF	Carbon disulphide	++
	1-Methylthioethanethiol	+, VCF		

* MS = identification confirmed by mass spectrometry; VCF = the compound is mentioned in ref. 6.
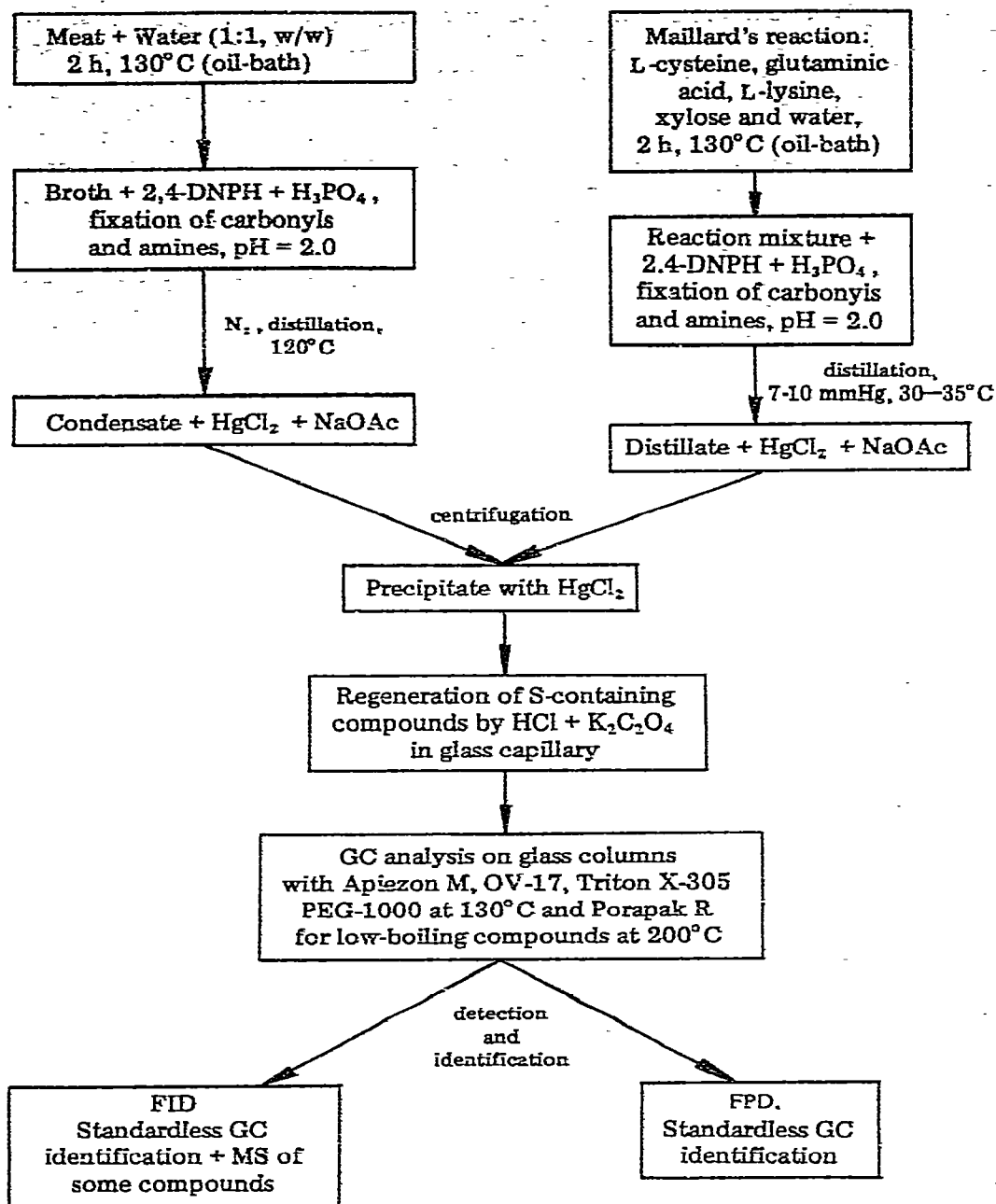
Fig. 1. Scheme of analysis of S-containing compounds in volatile compounds of boiled meat and Maillard's reaction.

veloped on the basis of Kováts retention indices were applied to a number of scientific problems. Over 70 organic bases including secondary aliphatic amines and heterocyclic compounds were discovered in various food products[8,31-35]. These studies have also revealed the presence in food products of secondary and tertiary amines which are possible precursors of carcinogenic N-nitrosamines[31,32].

The accumulation of organic bases, including heterocyclic bases, in volatile components of milk protein during storage has been shown to result in an unpleasant odour and to indicate a decrease in the nutritional value of the product.

A comparison of volatile compounds from boiled meat and Maillard reaction products has shown that the processes that occur during the Maillard reaction cannot be regarded as identical with those accompanying frying or boiling of food products[36]. An important conclusion that follows from the latter finding is that the commercial flavourings synthesized on the basis of the Maillard reaction cannot be used in the food industry without testing for toxicity.

In conclusion, this analytical approach to flavour research has general significance and may be recommended for the analysis of complex mixtures of biological volatile organic compounds and environmental contaminants.

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