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Problems in Investigation of the Aroma of Foodstuffs and the Production of Imitations

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General principles of the investigation of volatile components of foodstuffs are discussed, as well as the state of the problem of flavoured food additives. Aspects of the conferment of taste and odour on new types of food are considered. A list of 82 references is included.

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I. INTRODUCTION

In the majority of developed countries industrial methods for the production of food products are continually being intensified. The consequent acceleration of technical processes for the manufacture of various types of nutritional products frequently results in loss of flavour. The insufficiency of full-value protein in feeding the population (the annual world protein deficit exceeds three million tonnes[1]) has made it necessary to obtain by industrial methods new types of food based on protein concentrates, and both taste and odour must be entirely created for these products.

II. DEVELOPMENT OF THE PRODUCTION OF FOOD ADDITIVES AND FLAVOURINGS

Table 1 demonstrates the consumption of protein concentrates in the manufacture of various food products in the USA based on statistics for 1972.[2] During 1969 protein concentrates were added in almost all categories of products, especially in the manufacture of conserved meats and intermediate products. It is predicted that by 1980 a new artificial product—a milk substitute—will have appeared, and it is planned to consume another 188 million lb of protein concentrates for this purpose. A total of about a million tons of protein additives will be used in the USA foodstuffs industry in 1980. According to forecasts made in 1971 by a long-term planning service, the 1985 production of meat and poultry substitutes and of milk products in Western Europe will be respectively 2.8 and ~3.5 million tonnes[3].

Loss of flavour in food manufacture and the necessity of obtaining products from protein concentrates are the chief reasons for the sharp increase in the demand for flavourings and flavour and odour intensifiers. Demand doubled during the decade from 1955 to 1966, and continues to grow. Even the term "flavour renaissance" has now appeared (the complex of tactile, gustatory, and olfactory sensations caused by foods is denoted by the word *flavour*).

Aromatising and flavouring additives constitute 15–20% of the total cost of a foodstuff from a protein concentrate[2,4]. A vivid example of the economic advantage of the development of scientific and applied research, as well as the production of flavourings and taste and odour intensifiers, is provided by returns (Table 2) for 1971 and the forecasts for 1980 made by the American firm Frost and Sullivan Inc.[4]

Table 1. Growth of consumption of protein concentrates in the manufacture of various foodstuffs in the USA.

Product category	Planned annual increase, %	Protein additions, 10^6 lb	
		1969	1980
Baby foods	1.0	3.5	3.9
Bread and bakery products	7.5	101.0	126.1
Convenience meals	9.7	17.9	36.0
Confectionery	3.0	16.6	23.0
Cooked meats and semimanufactures	19.3	92.2	642.4
Coffee whiteners	6.0	12.0	22.8
Milk substitutes	—	—	188.2
Synthetic ice cream	5.0	3.8	6.5
Other types of milk products	1.0	98.1	109.1
Desserts	6.0	31.7	60.0
Dietetic products	2.0	8.7	105.0
Frozen foods	3.6	3.8	5.6
Macaroni and pasta products	3.0	1.5	2.1
Animal fodder	5.4	229.3	426.0
Soups	0.0	1.5	1.5
Other uses	9.3	207.3	555.0
Total quantity		828.6	2218.5≃10^6 tons

Table 2. Income from use of food additives (millions of dollars).

Year	Food additives	
	total	flavourings and taste intensifiers
1971	635.97	254.00
1975	804.19	326.90
1980	1108.81	476.40

Since 1958 the Flavoring Extract Manufacturers Association (FEMA) has conducted a programme aimed at establishing a statute on flavourings. All known natural and synthetic compounds employed in the food industry up

to 1958 have been tested for toxicity, pharmacological effect, and metabolism in the organism both of the substance itself and of its derivatives, in six academic institutions independent of industry. A list of GRAS substances ("generally recognised as safe") has been published systematically; it includes 1121 natural and synthetic compounds, with the permissible dosage for each category of foodstuffs (drinks, ice cream, confectionery, bread and bakery products, jellies, puddings, meat, sauces, soups, milk products, pickles, seasonings, etc.)[5,6]. The number of compounds used in the food industry before 1958 that failed the test was 267, including some natural substances[7]. Of the 1121 GRAS substances 859 are used in the food industry (at a maximum dosage of 10 ppm—by volume or by weight) to flavour the foodstuff. The list of GRAS substances is extended systematically and published annually.

The modern flavourings industry is based on substances detected in the odour of natural foodstuffs, in contrast to the earlier period, when restrictions were imposed by similarity of odour and inferred toxicity. The new approach has led in several cases to qualitatively new types of imitations, indistinguishable from the natural products, since they include the key substances responsible for the odour of the latter. For example, ethyl $\alpha\beta$-epoxy-α-methyl-β-phenylpropionate has the odour of strawberries[8], 4-*p*-hydroxyphenylbutan-2-one that of raspberries[9,10], allyl phenoxyacetate pineapple[11], 2-isobutyl-3-methoxypyrazine pepper[12-14], and diallyl trisulphide garlic[15]; this last compound also possesses bactericidal properties.

III. ISOLATION AND ANALYSIS OF ODOUR COMPONENTS

Substantial difficulties arise in the investigation of the odour of natural foodstuffs. Man is able to distinguish up to 17 000 odours[16]. Since a theory of olfaction is lacking, aroma cannot be predicted from the physical and chemical properties of the molecule. The minuteness of the content of odour condensate in a specimen is responsible for the difficulties in investigating flavour. But according to Weurman the amounts of odour condensate associated with 1 kg of meat, strawberries, and bread are respectively 35, 2–8, and 8–10 mg.[17,18] The number of different substances in an odour condensate is immense—several hundreds of components belonging to various classes of organic compounds—which in practice always include acids, alcohols, esters, sulphur-containing compounds, amines, ketones, aldehydes, lactones, phenols, hydrocarbons, etc. with a wide range of boiling points, from gases to compounds boiling at 300°C.

The substances determining the character of the odour often have low threshold concentrations—of the order of 10^{-12}–10^{-14} part per unit volume or weight—undetectable by present-day analytical apparatus. The odour threshold for 2-isobutyl-3-methoxypyrazine is 10^{-12}.[14] Clearly, therefore, it is necessary to concentrate the odour in order to study it[19-21].

It must be emphasised also that analytical instruments are like a biological detector—man's olfactory organ—in being unable to estimate the quantity of odour in a complex. Moreover, there is no correlation between the sensory sensitivity of man to individual components and the sensitivity of an analytical instrument. For example, the gas chromatograph is more sensitive to acetone, whereas to vanillin the nose is more sensitive than this instrument by several powers of ten[22] (Table 3).

Table 3. Comparison between the sensitivity of the human nose and that of a chromatograph with a flame-ionisation detector[22].

Compound (in aq. soln.)*	B.p., [°C]	Limit of detection, ppm	
		chromatograph	nose
Propanal	61	0.0025	0.17
Butanal	75.7	0.12	0.07
Hexanal	131	0.3	0.03
Acetone	56	0.3	500
Methanethiol	7.6	0.013	0.002
Methyl salicylate	222	**	$0.1 \cdot 10$
Vanillin	285	**	$0.1 \cdot 10^{-9}$

* Analysis made on 10 ml of saturated vapour above the solution.
** Undetectable.

The investigation of odour involves mostly study of the composition of volatile components in the vapours above a foodstuff, actually in condensates of these vapours. In the scientific literature the special term "head-space analysis" has become attached to such an approach[23]. The quantity of a substance in the vapour above a food is not equivalent to its actual content in the latter. The vapour pressure P_{ia} of a substance i in the gas phase over a foodstuff is given by the formula[23]

$$P_{ia} = \gamma_i X_i P_i, \qquad (1)$$

where P_i is the saturated vapour pressure of pure i at the same temperature, X_i the mole fraction of i in the foodstuff, and γ_i its activity coefficient at this concentration.

At a constant mole fraction X_i the value of γ_i may vary by several powers of ten depending on the nature of the substance and of the medium in which it is situated[24]. In the aqueous phase of a foodstuff, for example, the activity coefficient may depend on the concentration of metal salts, their capacity for complex formation with the test substance, and also the pH. The proportions of components in the vapour over a product varies with the content of water, lipids, proteins, and other ingredients of the sample. Furthermore, several substances are present in a foodstuff in a combined state, as "precursors" from which they pass into the gas phase as a result of enzymatic or chemical reactions. Therefore samples of the same foodstuff prepared at different times from different batches of raw material may exhibit both qualitative and quantitative differences in the volatile components of the odour concentrate.

Equation (1) is valid under equilibrium conditions. On prolonged collection of volatile components the rate of diffusion from the specimen to the gas phase changes, which may distort the proportions of volatiles in the condensate in comparison with the gas phase above the specimen. For this reason volatiles are collected above the specimen only as long as the latter retains its full flavour, and the condensate has the same odour.

The influence of the above factors on the process of concentrating the aromatic components makes it almost impossible to obtain quantitative data on the total composition of odour components in a test product. However, analysis of the odour condensate enables the substances involved in forming the flavour to be studied, and their proportions in the composition to be determined. Gas-chromatographic and organoleptic investigations enable "key" components of the odour to be detected and identified. More than 2500 compounds have now been identified in the odour of various foodstuffs[25], the number increasing every year.

Separation and *identification* are necessary stages in the investigation of an odour concentrate. Gas–liquid chromatography is the only method suitable for the separation of multicomponent mixtures of volatiles of unknown composition. The detection of individual compounds and their identification can be accomplished gas-chromatographically, mass-spectrometrically, and by infrared and nuclear magnetic resonance spectroscopy. The last two methods are generally employed when components can be isolated and accumulated by preparative chromatography. Difficulties are aggravated by the fact that the majority of flavour-forming substances studied have odour thresholds in the range 10–100 pg.[23] Hence there is hardly a single present-day analytical method that can ensure reliable determination of all the organic compounds in the volatile components of natural mixtures. The absence of direct methods of analysis of the required sensitivity makes the stages of extraction and concentration of the odour components especially important. A critical approach is required to the choice of method for these stages, for treating the odour concentrate right until the specimen to be analysed is introduced into the chromatograph. Every stage requires maximum protection of the mixture from contamination and possible loss of components, with especially careful purification of solvents. Sources of error in obtaining a sample for analysis have been discussed[26]. It must also be borne in mind that if the whole of the odour concentrate undergoes gas-chromatographic analysis, it is difficult to exclude artefacts and the formation of new substances by the interaction of reactive compounds directly in the gas-chromatographic column, or even in the vaporiser under the influence of the temperature, the material of the column, and its adsorbent.

Inspection of published information has shown that special gas-chromatographic methods of identification are required for the examination of complicated natural flavouring mixtures. It is impossible to prepare a gas-chromatographic column capable of separating simultaneously diverse classes of organic compounds. Furthermore, identification by means of standard substances, in the study of mixtures containing up to 400 or more components, presents considerable difficulties, even with the use of mass-spectrometric detection.

An odour concentrate can be investigated in two ways: it can be either chromatographed as a whole or first divided into different classes of organic compounds, which are chromatographed separately. In order to diminish the artefacts and to utilise most fully the specific donor–acceptor interactions between the test substance and the adsorbent in the gas-chromatographic column it is more advisable to separate complicated odour compositions into individual classes of organic compounds having the same type of functional groups. Methods of gas-chromatrographic identification using a set of columns of differing polarity are found for each class of organic compounds[27–43].

In the separation and analysis of multicomponent mixtures the reproducibility of the gas-chromatographic results over a long period of time, which is necessary in the study of different specimens of a test material, becomes especially important. Experience of analysing the odour of natural foodstuffs has permitted comparison of the reproducibility of different gas-chromatographic characteristics[27]. The most convenient and reproducible gas-chromatographic parameter—the Kovats index—has been selected and recommended, and a theoretical justification provided for the use of an ideal series of n-alkanes in its calculation[27]. In the analysis of multicomponent mixtures it is almost impossible to place every test substance between two n-alkanes, as the Kovats formula requires[44]. I therefore propose to calculate the indices on the basis of an "ideal" series of n-alkanes constructed from three or four experimental values of the logarithms of corrected retention volumes of n-alkanes containing $n \geqslant 6$ carbon atoms, i.e. to find the coefficients in the linear equation

$$\lg V_n' = a + bn. \qquad (2)$$

The legitimacy of employing an "ideal" series of straight-chain hydrocarbons for the calculation of Kovats indices was examined separately on the basis of experimental data in McReynolds' tables[45] on the retention of n-alkanes by 13 stationary phases. The accuracy of Eqn. (2) and of known non-linear equations[46,47] describing the gas-chromatographic behaviour of n-alkanes was estimated statistically on an electronic computer. It was shown that the linear approximation (2) provided quite satisfactory accuracy (0.8%) abd could be used in gas-liquid chromatography for identification purposes and for the calculation of thermodynamic functions from retention indices. Use of an "ideal" series of n-alkanes simplifies the calculation and makes the system of retention indices extremely convenient for the study of natural mixtures. It was shown also that the time required for analysis could be shortened by the use of an arithmetic retention index, which was converted by means of the "ideal" series of n-alkanes into the Kovats index[48] in order to obtain more information on the structure of the compound.

Natural mixtures can be investigated by gas chromatography either under isothermal conditions or with temperature programming. The most accurate gas-chromatographic parameters required for identification can be obtained by isothermal analysis. With linear temperature programming the only reliable retention parameter is the index I_{lin} calculated by means of the formula in Ref. 49. In order to obtain information on the structure of a compound, the dependence of this index on the experimental conditions was examined, and a correlation was found with the isometric index. A formula was deduced theoretically for the equivalent isothermal index $I_{T_0/\beta}$, so that it became possible to identify the components of an odour from an analysis with a programmed column temperature[36]. The formula enables $I_{T_0/\beta}$ to be calculated in terms of parameters characterising the experimental conditions:

$$I_{T_0/\beta} = I_{T_0} + \frac{\beta \tau_x}{2} \cdot \frac{\partial I}{\partial T},$$

where I_{T_0} is the isothermal index with T_0 the initial temperature of the analysis, β (deg min^{-1}) the rate of the

programmed variation of temperature, τ_X (min) the retention time of the substance in the column, and $\partial I/\partial T$ the temperature gradient of the index.

Retention indices are convenient in being directly related to the free energy of sorption of the test substance during chromatography. Equations have been found which enable experimental indices to be used for a quantitative estimate of the gas-chromatographic sorption process based on thermodynamic functions—partial molar enthalpies, entropies, and free energies of dissolution, as well as the differences in the corresponding excess thermodynamic functions of mixing ΔH^E, ΔS^E, and ΔG^E.[50-54] The proposed set of equations, relating the indices with the thermodynamic functions of sorption, enabled optimum conditions to be selected for gas-chromatographic separation and methods of identification to be developed without the use of standard substances[27].

IV. METHODS OF IDENTIFICATION WITHOUT THE USE OF STANDARD SUBSTANCES

The name "identification without standards" is applied to a method involving the special selection, for a mixture of substances having the same type of functional groups, of a set of columns of ensure optimum conditions of separation and to permit mathematical expression of the dependence of the gas-chromatographic behaviour of a substance on its physicochemical properties. The chosen system of columns enables relative retention parameters to be used for identification, without the help of standard substances. Scheme 1 lists the set of columns recommended for the identification of various classes of organic compounds. Each system is selected to diminish the probability of errors due to the overlapping of chromatographic zones in the column.

Scheme 1

Amines

$T = 100°C$

10% tristearin
5% mineral oil + 2% KOH
10% Tween-80
10% PEG-1000
on Novator detergent at pH 11.5

$T = 100°C$

4% Apiezon-L + 1% Na_3PO_4
5% Triton X-305 + 0.5% Na_3PO_4
5% PEG-1000 + 0.5% Na_3PO_4
3% tetrakishydroxyethylaziridine + 2% PEG-1000 + 0.5% Na_3PO_4
on Chromosorb G

Monocarbonyl organic compounds

$T = 50, 125°C$

10% Apiezon-M
10% PEGA
10% oxy-$\beta\beta'$-dipropionitrile

Programmed temp.

10% Apiezon-M
10% PEGA

Sulphur-containing compounds

$T = 60, 130°C$

10% Apiezon-M
10% Triton X-305
10% silicone OV-17
10% PEG-1000
on Chromosorb W

The set of four columns of differing polarity listed on the left of Scheme 1 was originally proposed for analysing a mixture of aliphatic amines and heterocyclic bases. The support used was Novator detergent. The donor-acceptor interaction observed between amines and phosphates[55,56] permitted the development of a practically new method for the analysis of amines[34]. The alkali was replaced by trisodium phosphate, and the detergent by Chromosorb G. The set of four columns listed on the right of Scheme 1 was proposed for the identification of amines among the components of an odour. Organic monocarbonyl compounds among the volatile components of natural materials were to be identified by the set of columns on the left (also the list on the right for analysis under isometric conditions) with temperature progamming[35-38]. A system of four stationary phases on Chromosorb W (see Scheme 1) has also been selected for the analysis of complicated mixtures containing thiols, sulphides, disulphides, and sulphur heterocycles together; this method enables substances having boiling points up to 280°C to be identified[41-43].

Table 4. Equations correlating retention indices I^* with the number of carbon atoms n in a straight-chain aliphatic amine for analysis at 100°C (Na_3PO_4)**, where m the ordinal number of the homologue.

Primary amines		Secondary amines		Tertiary amines
$I^{Ap}=100n+234+45/n$	$m\geqslant1$	$I^{Ap}=100n+134+104/n$	$m\geqslant2$	$I^{Ap}=85n+149$; $m\geqslant3$
$I^{Ap}=100n+241$	$m\geqslant3$	$I^{Ap}=100n+146$	$m\geqslant3$	
$I^{Tr}=100n+460-28/n$	$m\geqslant2$	$I^{Tr}=111n+84+744/n$	$m\geqslant2$	$I^{Tr}=86n+177$; $m\geqslant3$
$I^{Tr}=100n+455$	$m\geqslant3$	$I^{Tr}=100n+270$	$m\geqslant3$	
$I^{PEG}=100n+566-36/n$	$m\geqslant2$	$I^{PEG}=100n+319+90/n$	$m\geqslant2$	$I^{PEG}=85n+188$; $m\geqslant3$
$I^{PEG}=102n+547$		$I^{PEG}=100n+330$	$m\geqslant3$	
$I^{TH+PEG}=96n+686+6/n$	$m\geqslant2$	$I^{TH+PEG}=100n+329+46/n$	$m\geqslant2$	$I^{TH+PEG}=86n+200$; $m\geqslant3$
$I^{TH+PEG}=96n+686$		$I^{TH+PEG}=94n+435$	$m\geqslant3$	

* Stationary phases: Ap = Apiezon-L; Tr = Triton X-305; PEG = polyethylene glycol 1000; TH + PEG = tetrakishydroxyethylaziridine with polyethylene glycol 1000.
** Columns treated with trisodium phosphate as in Ref.34.

Table 5. Equations correlating retention indices I with number of carbon atoms n in carbonyl compounds*.

Temperature of analysis 50°C		
Alkanals	**Alkan-2-ones**	**Alk-2-enals**
$I^{Ap\text{-}M}=100n+170$	$I^{Ap\text{-}M}=100n+153$	$I^{Ap\text{-}M}=100n+228$
$I^{DNP}=100n+285$	$I^{DNP}=100n+278$	$I^{DNP}=100n+369$
$I^{PEGA}=100n+497$	$I^{PEGA}=97n+524$	$I^{PEGA}=90n+690$
$I^{TTP}=100n+397$	$I^{TTP}=100n+399$	$I^{TTP}=100n+513$
$I^{\beta\beta'}=87n+806$	$I^{\beta\beta'}=80n+876$	
$I^{\beta\beta'}=815+86,6n-13/n$ $n\geqslant2$	$I^{\beta\beta'}=677+99,3n+499/n$ $n\geqslant3$	

Temperature of analysis 125°C		
Alkanals	**Alkan-2-ones**	**Alk-2-enals**
$I^{Ap\text{-}M}=100n+177$	$I^{Ap\text{-}M}=100n+157$	$I^{Ap\text{-}M}=100n+235$
$I^{DNP**}=100n+295$	$I^{DNP**}=100n+283$	$I^{DNP**}=100n+378$
$I^{PEGA}=100n+555$	$I^{PEGA}=100n+558$	$I^{PEGA}=97n+734$
	$I^{Ap\text{-}M}=148+100n+64/n$; $n\geqslant3$	
	$I^{PEGA}=520+101n+225/n$; $n\geqslant3$	

Symmetrical ketones	Alka-2,4-dienals
$I^{Ap\text{-}M}_{125}=100n+140$	$I^{Ap\text{-}M}_{125}=100n+297$
$I^{DNP}_{125}=100n+251$	
$I^{PEGA}_{125}=100n+492$	$I^{PEGA}_{125}=100n+874$

* IAp-M, IDNP, IPEGA, ITTP, and $I\beta\beta'$ are the retention indices on columns of Apiezon-M, dinonyl phthalate, polyethylene glycol adipate, tritolyl phosphate, and oxy-$\beta\beta'$-dipropionitrile respectively.
**Temperature of analysis 100°C.

A study of 50 amines, 52 monocarbonyl compounds, and 42 sulphur-containing organic compounds has shown that the gas-chromatographic behaviour of homologues is described by a set of equations relating retention indices with numbers of carbon atoms. As an example, the equations for aliphatic amines show (Table 4) that dependence of the index I on the number of carbon atoms n in a compound is described by a set of linear and partial linear equations for each homologous series. Equations of the latter type are convenient for purposes of identification by means of an electronic computer, since they permit the coverage of a further 200 scale units of the indices, whereas linear equations are more convenient for visual interpretation of the chromatograms. Analogous equations for determining numbers of carbon atoms from gas-chromatographic data have been obtained for monocarbonyl compounds (saturated and α-unsaturated aldehydes, methyl ketones, alkadienals, and symmetrical ketones—Table 5), and also for sulphur-containing compounds (thiols, sulphides, and disulphides—Table 6).

Table 6. Equations correlating retention indices I with number of carbon atoms n in sulphur-containing compounds* for analysis at 130°C.

n-Alkanethiols	n-Alkyl sulphides	n-Alkyl disulphides
$I^{Ap\text{-}M}=100,3n+336-50/n$; $n\geqslant 2$	$I^{Ap\text{-}M}=98,9n+280+90/n$; $n\geqslant 2$	$I^{Ap\text{-}M}=93,6n+539+74/n$; $n\geqslant 2$
$I^{OV}=100,9n+411-30/n$; $n\geqslant 2$	$I^{OV}=99,4n+361+116/n$; $n\geqslant 2$	$I^{OV}=96,0n+639+120/n$; $n\geqslant 2$
$I^{Tr}=100,0n+526-54/n$; $n\geqslant 3$	$I^{Tr}=98,5n+434+188/n$; $n\geqslant 2$	$I^{Tr}=88,5n+793+136/n$; $n\geqslant 2$
$I^{PEG}=99,8n+590-45/n$; $n\geqslant 3$	$I^{PEG}=98,7n+481+230/n$; $n\geqslant 2$	$I^{PEG}=87,3n+873+155/n$; $n\geqslant 2$
	Isoalkanethiols	
	$I^{Ap}_{130}=103,3n+256$; $n\geqslant 3$	$I^{OV}_{130}=105,7n+316$; $n\geqslant 3$

* I^{Ap}, I^{OV}, I^{Tr}, and I^{PEG} are the retention indices on columns of Apiezon-M silicone OV-17, Triton X-305, and polyethylene glycol 1000.

Relations have also been found for all the series by means of which boiling points of homologues can be calculated from experimental values of the indices. The identity of boiling points determined from data on all the columns is a criterion of the correctness of an identification. Table 7 gives as example equations [for amines] together with boiling points derived from the indices, and shows good agreement with published boiling points. Similar sets of equations have been obtained for carbonyl compounds (Table 8). The equations set out in Scheme 2 were found (temperature of analysis 130°C) for determining the boiling points of sulphur-containing compounds from their indices on Apiezon M and OV-17 silicone columns. Table 9 lists boiling points for several sulphur-containing compounds calculated from experimental indices by means of the equations for Apiezon M.

Table 7. Equations correlating retention indices I* of n-alkylamines with their boiling points T_b (Na_3PO_4)**.

Equation	Alkylamine	Boiling point claculated from I^{Ap}	I^{Tr}	I^{PEG}	I^{TH+PEG}	Published b.p.
$T_b=\frac{I^{Ap}}{4.0}-81$	propyl	54.5	57.1	54.6	52.5	49—50
$T_b=\frac{I^{Tr}}{4.1}-127$	butyl	79.8	81.5	79.9	78.6	78—79
	pentyl	104.8	105.9	105.3	104.6	104
$T_b=\frac{I^{PEG}}{4.1}-153$	hexyl	128.8	130.3	129.9	129.9	129—130
	heptyl	154.5	154.7	154.6	154.6	155
$T_b=\frac{I^{TH+PEG}}{3,8}-203$	octyl	179.1	179.1	179.2	179.4	179.6
$T_b=666-\frac{1.1084\cdot10^6}{I^{Ap}+1249}$	dimethyl	7.4	6.7	6.5	7.4	7.0
$T_b=500-\frac{4.968\cdot10^5}{I^{Tr}+400}$	diethyl	53.3	53.6	51.9	52.8	55.5
	dipropyl	111.0	110.1	109.7	107.7	110.5
$T_b=437-\frac{3.293\cdot10^5}{I^{PEG}+73}$	dibutyl	161.3	161.4	162.8	163.7	159.0
	dipentyl	203.0	202.0	202.0	199.7	202.5
Tertiary amines :						
$T_b=363-\frac{1.7\cdot10^5}{I^{TH+PEG}-334}$		$T_b=876-\frac{2.0183\cdot10^6}{I^{Ap}+1890}$				

* Indices determined at 100°C.
** See footnote to Table 4.

Table 8. Equations correlating retention indices I* of monocarbonyl compounds with their boiling points T_b.

Equation	Compound	Boiling points calculated from I^{Ap-M}	I^{DNP}	I^{PEGA}	$I^{\beta\beta'}$	Published b.p.
1	2	3	4	5	6	7
$T_b=539-\frac{7.2\cdot10^5}{982+I^{Ap-M}}$	propanal	44	45	45	50	49.5
	butanal	75	75	73	75	75.7
$T_b=563-\frac{8.216\cdot10^5}{1002+I^{DNP}}$	pentanal	105	104	104	104	103.4
	hexanal	129	129	129	130	129
$T_b=531-\frac{6.836\cdot10^5}{546+I^{PEGA}}$	heptanal	152	152	152	155	155
	octanal	171	171	172	—	168.5
$T_b=2883-\frac{2.546\cdot10^7}{7884+I^{\beta\beta'}}$	nonanal	189	189	190	—	185
	decanal	206	205	206	—	208.5
$T_b=1098-\frac{3.587\cdot10^6}{2553+I^{Ap-M}}$	propenal	53	53	53	—	52.5
	but-2-enal	98	101	104	—	102.2
$T_b=6380-\frac{1.7144\cdot10^8}{27230-I^{DNP}}$	pent-2-enal	126	126	125	—	125
	hex-2-enal	151	—	149	—	150
$T_b=-730+\frac{3.019\cdot10^6}{4746-I^{PEGA}}$	hept-2-enal	176	176	175	—	175
	propanone	56	57	56	56	56
$T_b=674-\frac{1.317\cdot10^6}{1661+I^{Ap-M}}$	butan-2-one	83	85	81	80	79.6
	pentan-2-one	105	106	103	101	101
$T_b=721-\frac{1.583\cdot10^6}{1800+I^{DNP}}$	hexan-2-one	129	131	129	127	127.5
	heptan-2-one	151	152	151	151	151.5
$T_b=582-\frac{8.56\cdot10^5}{729+I^{PEGA}}$	octan-2-one	171	172	172	—	173
$T_b=771-\frac{1.403\cdot10^6}{823+I^{\beta\beta'}}$	undecan-2-one	223	224	223	—	223
	pentan-3-one	103	105	104	—	101.7
$T_b=2097-\frac{1.691\cdot10^7}{7814+I^{Ap-M}}$	heptan-4-one	144	143	143	—	144.2
$T_b=2316-\frac{2.161\cdot10^7}{8994+I^{DNP}}$	nonan-5-one	188	186	189	—	187.7
$T_b=707-\frac{1.253\cdot10^6}{1022+I^{PEGA}}$	undecan-6-one	227	227	226	—	228

* Indices determined at 125°C, except in the case of oxy-$\beta\beta'$-oxydipropionitrile (50°C).

Table 9. Comparison of boiling points of sulphur-containing compounds calculated from the retention indices $I^{Ap\text{-}M}$ with published values*.

Compound	Boiling point, °C		Compound	Boiling point, °C	
	calculated	published		calculated	published
Thiols			Butyl ethyl	144.4	144.2
Ethane-	35.0	35.0	Di-isopropyl	116.8	120.2
Propane-	68.8	67.6	Di-isobutyl	168.4	170.1
Butane-	99.5	98.5	Di-t-butyl	150.1	146.0
Hexane-	153.3	151.5	Di-isopentyl	213.4	215.2
Heptane-	177.5	176.2	Diallyl	134.5	138.0
Nonane-	220.9	220.2	Ethyl vinyl	91.5	91.5
Dodecane-	277.0	277.3			
Propane-2-	52.4	52.6	**Disulphides**		
Isopentane-	113.0	118.0	Dimethyl	110.0	109.7
Isobutane-2-	63.9	64.2	Diethyl	154.0	154.0
Neopentane-	101.2	99.0	Dipropyl	195.3	195.8
Prop-2-ene	63.6	67.5	Dibutyl	233.8	235.0
Benzene-	169.6	168.7	Di-isopropyl	175.4	174.0
			Di-t-butyl	197.5	200.5
Sulphides			**Cyclic**		
Dimethyl	32.8	37.3	Thiophen	84.0	84.2
Diethyl	92.4	92.1	Thiophan	121.1	121.1
Dipropyl	143.1	142.8	2-Methylthiophen	112.6	112.6
Dibutyl	189.0	188.9	2,5-Dimethylthiophen	136.7	136.7
Dipentyl	229.7	229.0			
Dihexyl	266.2	260.0			
Ethyl methyl	67.8	66.7			
Methyl propyl	96.7	95.5			

* Indices determined at 130°C.

Scheme 2

Straight-chain and isomeric thiols, sulphides, and disulphides	$T_b = 896.3 - \frac{2.255 \cdot 10^6}{I_{130}^{Ap\text{-}M} + 2106}$
Cyclic compounds	$T_b = 899.1 - \frac{2.255 \cdot 10^6}{I_{130}^{Ap\text{-}M} + 2106}$
Isoalkanethiols and isoalkyl disulphides	$T_b = 872.6 - \frac{2.153 \cdot 10^6}{I_{130}^{OV} + 1998}$
Symmetrical and unsymmetrical straight-chain sulphides	$T_b = 726.2 - \frac{1.359 \cdot 10^6}{I_{130}^{OV} + 1356}$
n-Alkyl and isoalkyl disulphides	$T_b = 847.3 - \frac{1.889 \cdot 10^6}{I_{130}^{OV} + 1670}$

The identification of a multicomponent mixture from chromatograms on three or four columns is very tiresome and time-consuming. The accumulated experimental data have been used to set up a computer programme. Fig. 1 shows the logical sequence of operations involved in deciphering an analysis. The programme was set up in computer codes in collaboration with Grigor'eva and Zhuravleva[27]. Experimental values of the indices I from all the chromatograms were fed into the computer, and for each group of organic compounds the coefficients in the equations by means of which the numbers of carbon atoms n and the boiling points were determined. The data fed into the computer and their notation are given for the analysis of amines as example.

1. Series of compounds having functional groups of the same type q, where q_1 represents primary amines, q_2 secondary amines, q_3 tertiary amines, q_4 pyridine derivatives, and q_5 other amines.

2. The sequence of processing data from the columns, with i the number of the column and j the number of the peak on the chromatogram; $i = 1$–4 represent respectively tristearin, mineral oil, Tween-80, and polyethylene glycol 1000.

3. The coefficients α, σ, B, and L for the calculation of n and T_b by means of the equations

$$n = \frac{I_{ji} - B_{q_i}}{L_{q_i}}; \quad T_b = \frac{I_{ji}}{\alpha_{q_i}} - \sigma_{q_i}.$$

4. Retention indices and their differences $I_{j,i}$ and ΔI. The permissible errors in the comparison of I, ΔI, and T_b are

$$\theta_I = \pm 5\text{deg}; \quad \theta_T = \pm 5\text{deg}; \quad \theta_{\Delta I} = \pm 20\,\text{deg}.$$

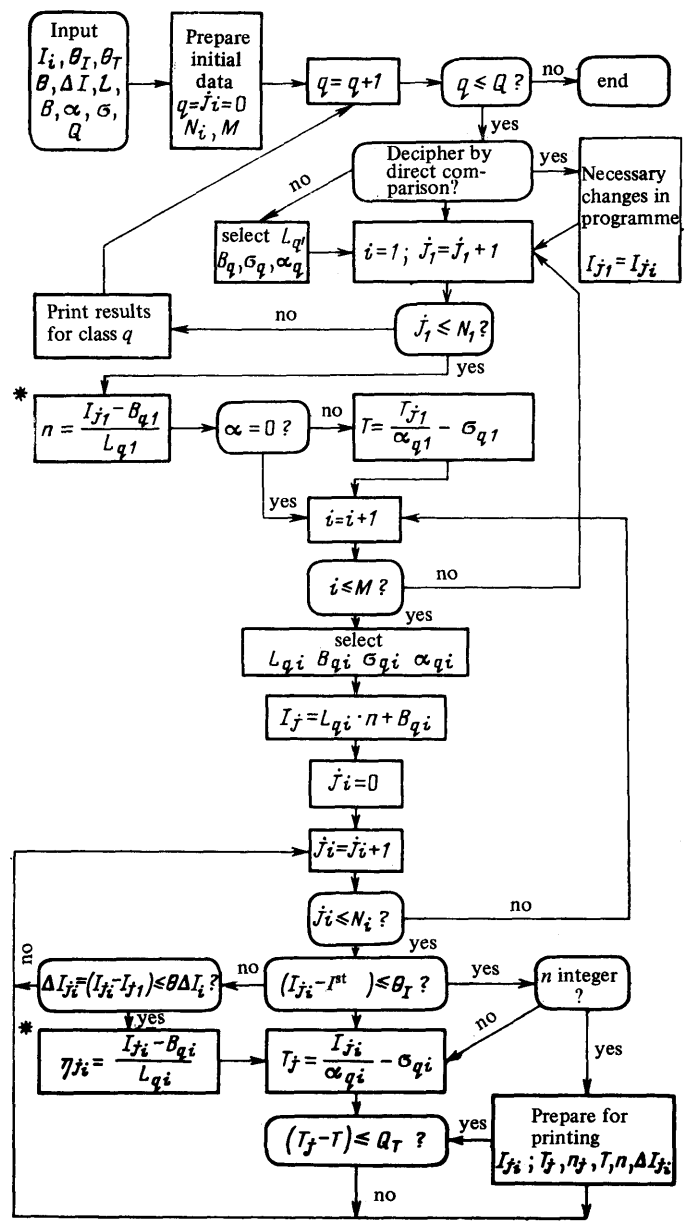


Figure 1. Block diagram of computer programme for identifying complicated mixtures from gas-chromatographic analysis without use of standard substances. For notation see text.

5. Several other parameters are also used by the computer: Q is the maximum number of series of compounds, M the maximum number of columns, and N_i the maximum number of peaks on each chromatogram. The programme permits the simultaneous processing of analytical data for a mixture of 12 types of compounds on 8 columns giving chromatograms each of which may contain up to 200 peaks[27].

The methods developed make possible the identification not only of members of the homologous series studied. In the case of unsymmetrical aliphatic and heterocyclic compounds the character of the functional group can be determined accurately, the number of carbon atoms tentatively predicted, and the boiling point of the compound estimated. The computer prints the results as a list of provisionally recognised compounds. The block scheme in Fig. 1 is of fundamental importance. It can be used to elucidate the components not only of an odour but also of other natural mixtures, e.g. hydrocarbon fractions of petroleum, essential oils, etc. The logical sequence of operations in the block diagram remains unchanged even if the gas-chromatographic behaviour of a whole series of substances on a system of columns differing in polarity is expressed in other parameters than the indices. With the accumulation of experimental information on the structural contributions to the indices the programme can be extended by calculating the indices of structures of provisionally recognised compounds. This will guide the choice of standard substance to confirm the structure of such compounds, which will facilitate identification. If the preliminary operations of calculating the indices from retention times according to previously published programmes[57] are introduced into the block diagram in Fig. 1, the task of the experimentalist is reduced to obtaining chromatograms, feeding retention times of peaks directly from the integrator into the computer, and identifying merely the peaks provisionally recognised by the computer.

V. ODOUR COMPONENTS OF CERTAIN FOODSTUFFS

Methods of identification without use of standards have been used to study the odours of several foodstuffs containing protein of animal origin—soft roe from salmon and sturgeon, salmon fillets, Russian and Dutch cheeses, volatile components of the Maillard reaction, simulating the odour of cooked meat.

The sequence of operations adopted in investigating volatile components was to obtain an odour condensate, to determine the classes of organic compounds of significance for reproducing the flavour of the product under investigation, to concentrate these classes of compounds as derivatives, to regenerate them, and then to undertake identification by gas-chromatographic analysis without the use of standard substances.

It was found that amines and monocarbonyl compounds were the most significant for the odours of sturgeon and salmon roe and of salmon fillet. The odour of cheeses is determined primarily by organic carbonyl compounds, acids, and to some extent organic bases. Sulphur-containing compounds are important in the odour of cooked meat. The results of the identification are set out in Tables 10–12.

There are several important differences in composition between the amines of sturgeon and salmon roes, and these affect their organoleptic properties. Thus the former contain more heterocyclic compounds, in particular pyridine, and the latter trimethylamine, which is characteristic of a fishy odour. It must be stressed that it has not hitherto proved possible to detect such a large number of volatile organic bases in any foodstuff. The results indicate that differences in odour between fish and fish products are probably due mainly to different quantitative relations among nitrogen bases.

Table 10. Proportions of amines in volatile components of foodstuffs expressed as relative percentages*.

Amine	Sturgeon roe	Salmon roe	Dutch cheese (4-month)	Russian cheese (4-month)	Russian cheese (10-month)
Methylamine	2 2	3.2	7.0	7.4	0.2
Dimethylamine	2.8	1.0	2.9	2.5	2.5
Trimethylamine	3.4	87.5	13.5	14.3	3.0
Ethylamine	2.5	—	—	—	0.3
Diethylamine	2.8	0.4	1.0	2.5	1.1
Triethylamine	9.0	0.3	4.6	4.8	2.8
Propylamine	12.0	—	—	0.6	—
Isopropylamine	2.0	0.1	0.5	0.2	0.1
Dipropylamine	2.6	0.3	5.5	2.9	0.5
Di-isopropylamine	8.6	2.3	8.9	9.0	8.8
Tripropylamine	—	0.1	1.5	1.2	0.1
Butylamine	3.1	0.3	1.4	—	—
Isobutylamine	—	1.8	11.8	12.8	0.5
Dibutylamine	2.4	0.3	1.9	1.2	0.4
Di-isobutylamine	1.8	0.1	1.1	0.3	0.1
Pentylamine	2.8	0.1	—	—	—
Isopentylamine	—	0.2	—	0.6	0.1
Pyridine	40.4	0.3	29.2	29.6	4.9
2-Methylpyridine	1.4	0.6	6.0	7.5	52.4
Piperidine	—	0.5	1.2	0.4	15.4
Pyrrolidine	—	0.1	0.2	—	0.2
3-Methylpyridine	—	—	—	—	0.3
Perhydroazepine	—	—	—	—	0.3
Unidentified amines	0.4	0.5	2.0	2.3	6.4

* Sum of areas of peaks of all amines, including those that remain unidentified, taken as 100%.

Lactic acid bacteria are widely used in the manufacture of various foodstuffs, and several of them are found in the alimentary canal. Investigation of the volatile components of the metabolism of such bacteria is therefore of especial interest. Analysis of the organic bases in volatile products of *Streptococcus lactis* shows that amines, including secondary and tertiary, accumulate during the logarithmic growth phase[58,59].

Comparison of the qualitative composition of the amines identified in various foodstuffs shows in all cases the presence of aliphatic amines, including secondary and tertiary, as well as heterocyclic compounds. Straight-chain amines are accompanied by many compounds containing isoalkyl groups. These results are of interest not only in the investigation of odour but also in the study of the carcinogenic nitrosamines contained in convenience meals or formed in the organism from precursors—amines and sodium nitrite.

Organic monocarbonyl compounds as well as amines have been studied in the odour condensates from the foodstuffs investigated. Table 11 lists the compounds found and their relative contents in the odour. With programming of the column temperature identification by means of my proposed equivalent isothermal index $I_{T_0/\beta}$ has revealed 4–9 additional compounds (indicated by an asterisk). Thus the formula for this index can be successfully applied to the analysis of a natural mixture of unknown composition with linear programming of the column temperature.

Table 11. Proportions (in relative percentages) of monocarbonyl compounds identified among volatile components of foodstuffs.

Compound	Sturgeon roe	Salmon roe	Salmon fillet	Russian cheese
Alkanals				
Ethanal	4.5	2.36	3.2	5.4
Propanal	3.5	3.75	8.29	0.15
Butanal	2.8	1.65	0.44	2.0
Pentanal	38.3	0.28	5.82	0.7
Hexanal	2.4	0.82	2.14	0.95
Heptanal	0.5	1.76	0.29	0.2
Octanal	0.8	0.67	12.4	0.9
Nonanal	1.5	1.21	5.1	0.6
Decanal	1.7	0.46	1.21	0.7
Undecanal	0.06	1.21	4.52	0.12
Dodecanal	—	3.24	4.78*	—
Isobutanal	4.3	3.74	1.89	0.16
Isopentanal	6.2	33.4	0.9	1.9
Alk-2-enals				
Propenal	4.9	1.1	0.72	3.0
Butenal	0.2	0.04	0.05	0.17
Pentenal	0.5*	0.09*	0.05*	—
Hexenal	0.07*	0.17*	0.68*	—
Heptenal	—	0.59	1.21	0.1
Octenal	1.8	0.5	0.63	0.01
Nonenal	0.1	0.13	3.96	0.13
Decenal	0.06	0.81	1.84	0.05
Undecenal	—	1.6	0.45*	—
Alka-2,4-dienals				
Hexadienal	0.7	—	0.19	0.3
Heptadienal	0.06	0.09	0.29	0.01
Octadienal	0.07	0.07	1.03	0.04
Nonadienal	0.07	0.44	0.46	0.04
Decadienal	—	0.89	1.05*	—
Alkan-2-ones				
Propanone	4.8	8.43	5.08	42.0
Butanone	0.7	0.44	0.26	13.7
Pentanone	12.8	1.11	3.47	5.6
Hexanone	0.4	0.16	0.08	0.3
Heptanone	0.06*	—	0.19	4.6
Octanone	0.04*	0.14	8.7	0.03
Nonanone	0.7	0.72	0.81	1.6
Decanone	0.4	0.05	5.23	0.06
Undecanone	0.1	10.73	2.82	0.5
Dodecanone	—	2.54	2.44*	—
Isopentanone	0.48	0.16	0.09	0.02
Totals in relative %				
Alkanals	67.6	54.3	51.0	13.8
Alk-2-enals	8.3	5.1	9.6	3.5
Alk-2,4-dienals	0.9	1.5	3.0	0.4
Alkan-2-ones	20.9	24.6	29.1	68.4
Unidentified	3.2	14.6	7.3	13.8

* Further identified by means of the isothermal index obtained in gas chromatography with linear programming of the column temperature.

Comparison of the quantitative proportions of various monocarbonyl compounds in the odours showed the predominance (50–70%) of alkanals in fish products, and a relatively high content of unsaturated aldehydes in salmon fillets, whereas in the odour of cheese only 13.8% of alkanals was detected, but the quantity of alkan-2-ones reached almost 70%. This represents the first observation of so many monocarbonyl compounds in the odour of such foodstuffs.

Organic sulphur compounds have been studied in the volatile components of the Maillard reaction with the odour of cooked meat[42]. Table 12 lists 25 sulphur-containing compounds identified in the odour condensate. This is probably the first case in which a large number of organosulphur compounds have been obtained from a single amino-acid (cysteine) as source of sulphur[42]. Study of the condensate of the vapour phase from the Maillard reaction is promising from the present point of view, since it permits the examination of a smaller number of components than with odour condensates from the natural product, which contains far more compounds not involved in the formation of flavour.

Table 12. Sulphur-containing compounds identified in condensate of volatile components of the Maillard reaction.

Hydrogen sulphide	Dimethyl disulphide
Methanethiol	Diethyl disulphide
Ethanethiol	Dipropyl disulphide
Propane-1-thiol	Ethyl propyl disulphide*
Propane-2-thiol	Di-t-butyl disulphide
Butanethiol	Di-isobutyl disulphide
Neopentanethiol	Butyl propyl disulphide*
Hexanethiol	Thiophen
Dipropyl sulphide	2-Methylthiophen
Di-isopropyl sulphide	2,5-Dimethylthiophen
Butyl propyl sulphide	Benzenethiol
Dibutyl sulphide	2,5-Dimethyltrithiolan
Butyl pentyl sulphide*	

* Provisionally identified.

According to the literature the odour of cooked natural meat is determined by carbonyl- and sulphur-containing organic compounds, the most significant role being assigned to thiocarbonyl derivatives[60–62]. The Reviewer's investigations have shown unambiguously that both carbonyl and thiocarbonyl compounds have only a subordinate role[42]. The presence of acids and amines is also unimportant. The odour is determined by sulphur-containing compounds.

The gas-chromatographic method of identification can be used to investigate the aroma of bread[63, 64].

VI. APPLICATION OF ODOUR ANALYSIS TO THE SOLUTION OF PRACTICAL PROBLEMS

The approach to the investigation of odour proposed and developed by the Reviewer and its application to the analysis of volatile components have enabled a simulator to be made for artificial-protein soft caviar produced in the Institute of Organoelementary Compounds of the USSR Academy of Sciences. The odour simulator was included in the technical regulations for manufacture of the caviar.

The extremely laborious investigations on the composition of the odour of natural foodstuffs can be used in solving several problems, e.g. to confer a lost flavour on products obtained by industrial methods. Thus it has been found indirectly that ethyl $\alpha\beta$-epoxy-α-methyl-β-phenylpropionate can be used in the preparation of foodstuffs smelling of strawberries. According to 1970 statistics sales in the USA were such that, if the flavouring had been natural, the size of the strawberry crop would have enabled only the demand in the single state of New Jersey to be met, although the addition of the above ester to intensify the odour was not reported to consumers[65].

Data on the composition of natural odours are used also in eliminating undesirable aftertastes with the aim of recovering valuable products for the nutrition of the population. Thus the work of Patterson in the USA revealed in 1968 the reason for the intense "smell of cats" developed in meat when stored for a long time. It was found that 0.4% of mesityl oxide contaminating the solvents

for the paints previously applied to the meat-store will react with hydrogen sulphide evolved from the meat to form 4-mercapto-4-methylpentan-2-one, which has an intense "catlike" odour and a low sensitivity threshold[66,67].

Knowledge of the composition of the odour components of natural products is used in the fight against their adulteration, as well as in the manufacture of products for medical nutrition. Dietary problems in obesity and in the post-operative period can be solved by the use of flavourings and taste and odour intensifiers.

During recent years an intensive search has been made for correlations which will enable the expensive sensory assessment of the quality of foodstuffs to be replaced by objective physicochemical measurements. Such work is necessary for the establishment of objective criteria for assessing the quality of foodstuffs and for their standardisation. An example of the successful solution of this problem is provided by correlations, obtained in the Scandinavian Centre for Odour Research, for assessing the quality of canned meat from gas-chromatographic data, which required the elaboration of a verbal description of quality. Mathematical statistics was then applied to the organoleptic data, and an electronic computer used to find from these and gas-chromatographic data equations evaluating canned meat of good quality and with various defects. In this way it was shown that an objective assessment of the quality of a product can be obtained from gas-chromatographic results without the help of tasters[68-70].

Finally, data on the composition of components of the odour of natural products are required for solving the problem of flavouring new forms of food. The types of foodstuffs that supply the organism with proteins, carbohydrates, fats, vitamins, and mineral salts vary considerably and depend on the life-style, national traditions, and cultural peculiarities of each country. A unified set of foodstuffs, acceptable to all nations and peoples, cannot be envisaged in the foreseeable future. For this reason the food industry of each country is increasing its range and giving preference to forms customary in the national cuisine. The preparation of specific products of tasteless powdered protein concentrates requires both the imitation of a complex of structural and physical characteristics and a method of introducing flavourings that ensures their uniform distribution and prolonged fixation of the odour in the product.

The production of new types of food requires not only nutritional value but also a harmonious combination of texture, taste, and smell. Sour, salt, bitter, and sweet tastes can be conferred on a textured product by a combination of chemicals, e.g. common salt, citric acid, sugar, and piperine (the last isolated from pepper).

Intensifiers—natural substances minute additions of which reinforce particular taste sensations and produce a pleasant aftereffect—are used to improve taste properties. The formulae of some of them are given in Fig. 2. Since 1962 such compounds as sodium L-glutamate[71] and disodium 5′-inosinate and 5′-guanylate[72-74] have been widely used to improve the taste properties of meat and fish products, broths, vegetable courses, tomato juice, and sauces. They are able to suppress undesirable taints in the odour of foodstuffs, such as "sulphide", "greasy", "grassy", "chemical", etc. The most astonishing effect is the ability of nucleotides to create the illusion of substantiality: such sensations arise e.g. on the addition of 50–200 ppm to a broth.

Nutritionists have pointed out the unfavourable consequences of excess of carbohydrates, in particular sugar. Addition of 15 ppm of a sweet taste intensifier—Maltol (3-hydroxy-2-methyl-4-pyrone)—enables the consumption of sugar in fruit juices and drinks to be reduced to 15%. The methyl ester of the dipeptide L-aspartyl-L-phenylalanine, which is 200 times as sweet as sugar, has also been recommended as a substitute for the latter[75].

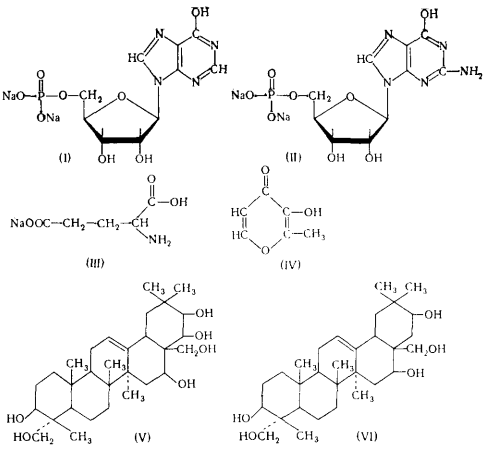


Figure 2. Some taste and odour intensifiers: (I) sodium 5′-inosinate; (II) sodium 5′-guanylate; (III) sodium glutamate; (IV) Maltol; and inhibitors: (V) gymnemagenin; (VI) gymnoestrogenin[76].

Not only taste intensifiers but also taste inhibitors have been found. For example, gymnoestrogenin[76], isolated from an African plant, suppresses a sweet taste and to some extent a bitter taste for several hours: sugar seems as tasteless as sand. The berries of the Nigerian plant *Synsepalum dilcificum* suppress a sour taste and strengthen a sweet taste (lemons taste like oranges)[77]. A thorough search is being undertaken for new inhibitors and intensifiers of taste and smell[77,78].

It is a far more complicated task to imitate the flavour of food. The creation of flavouring compositions involves deciphering the odour of natural foodstuffs. Among the several hundreds of odour components it is necessary to determine the specific, key compounds responsible for the characteristic flavour. An odour simulator for a foodstuff should be harmless and contain substances present in the flavouring composition of the natural product or obtained by reactions simulating processes occurring in the natural foodstuff (e.g. by the Maillard reaction).

The most successful examples of the conferment of odour on artificial products are provided by drinks and wines[65,79]. The reason is that flavouring compositions based on study of the odour of natural juices and wines do not change their properties on addition to aqueous and aqueous–alcoholic solutions. In this case the "texture" of the product does not appreciably affect the balance in the initial composition. Numerous attempts to impart a flavour to structured products by means of artificial compositions having a flavour closely similar to that of the natural product have given a positive result only when 95–50% of the natural product was added as foundation to the artificial product. Protein soft caviar is probably the first artificial product flavoured with an imitator

without addition of the natural roe. By itself this imitation does not possess the odour of sturgeon roe, the flavour being formed in the product after introduction of the odour imitation in 2–3 h after the establishment of adsorption–desorption equilibrium[27].

The flavouring of a structurised protein usually involves a change in odour owing to disturbance of the quantitative relations among the components. Selective adsorption of the components of a flavouring mixture disturbs the quantitative proportions of the ingredients with loss of the specific odour. The selection of imitations for new forms of food may be considerably complicated by cases of synergism—the suppression or strengthening of the odour of a substance in the presence of one or more other substances—when the concentrations of the compounds may be less than the threshold values, so that each of them separately could not be perceived by man.

For two substances at concentrations x and y synergism can be expressed both as $x + y$ and as xy, and even x^y.[80] This is why the selection of flabourings cannot yet be based solely on instrumental data, but depends on the intuition and experimental skill of the chemist. Thus cases of synergism have made it extraordinarily difficult to produce a flavouring mixture for protein soft caviar, by increasing the number of variations to 200. The imitation itself does not possess the odour of roe, which appears in the culinary processing of the product and, in the opinion of tasters, imparts to protein caviar "an aromatic and gustatory character closely resembling the natural roe of fishes of sturgeon species".

A chemical imitation of an odour usually comprises 10–20 reactive compounds, which change readily not only on storage in air but also by interaction among the components. It is therefore necessary to preserve such mixtures by encapsulation or by "sealing" them into edible polymers—e.g. alginates, dextrans, polyvinyl alcohol, dextrins, etc.[81,82]—from which the components are easily displaced when required by the action of water. These powders are convenient as ingredients of products for prolonged storage. They are used also in the flavouring of fodder for cattle. In Japan, for example, special flavouring mixtures in powder form are added to improve the appetite, which promotes better anabolism of the fodder.

We may note in conclusion that the development of investigations on the odour of foodstuffs and the production of imitators are economically advantageous, despite the expensive equipment and highly qualified experts required for research on this problem. The combination of a gas-chromatographic method of separation, developed for each class of organic compounds, with mass-spectrometric detection constitutes the optimum procedure enabling maximum information to be obtained on the composition of an odour.

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