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Instrumental analysis of volatile (flavour) compounds in milk and dairy products

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Summary — The present article reviews the most commonly used methods, techniques and equipments for instrumental analysis of volatile (flavour) compounds in milk and dairy products. After listing some previous important review articles, several methods commonly used for sample treatment are described, as well as the following techniques for extraction and concentration prior to gas chromatographic (GC) analysis: static and dynamic headspace, steam distillation, high-vacuum distillation, molecular distillation, direct extraction (liquid/liquid or liquid/solid), supercritical fluid extraction (SFE), simultaneous (steam) distillation extraction (SDE), dialysis, solid-phase extraction (SPE) and solid-phase microextraction (SPME). Two classical injection devices are also described: on-column injection and the so-called 'purge and trap' system. The main advantages and disadvantages of current commercially available types of fused silica capillary columns are briefly considered. The newly developed 'chiral' phases are also described. The article reviews some of the numerous detection systems used for qualitative and/or quantitative analyses such as FID or MS detection, FTIR detection, SCD, FPD and NPD detectors used for sulfur- and nitrogen-containing components, AED detection and the 'sniffing device'. Some useful library search systems such as PBM, INCOSTM and SISCOM (ie, MassLib[®]) are mentioned to complete the overview of this topic. Finally, this paper briefly points out some other methods (ie, photometric), capable of determining various specific chemical functions responsible for flavour (carbonyl compounds, etc), as well as promising techniques involving new electronic noses.

milk / dairy product / volatile compound / flavour / analytical method

Résumé — L'analyse instrumentale des composés volatils (de l'arôme) du lait et des produits laitiers. Le présent article passe en revue les méthodes, techniques et équipements les plus utilisés

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pour l'analyse instrumentale des composés volatils (parfois responsables de la flaveur du lait et des produits laitiers). Après avoir cité quelques articles de revue importants, certaines méthodes communément employées pour le traitement des échantillons sont décrites, de même que les techniques permettant l'extraction, la concentration et l'injection des composés de l'arôme en vue de leur chromatographie en phase gazeuse (CPG) : analyse statique et dynamique d'espace de tête ou d'effluves («headspace»), distillation à la vapeur, distillation sous vide poussé, distillation moléculaire, extraction directe liquide/liquide ou liquide/solide, extraction supercritique (SFE), extraction–distillation simultanée (SDE), extraction en phase solide (SPE) et microextraction en phase solide (SPME). Les deux techniques d'injection les plus classiques sont également présentées : l'injection sur colonne et le système appelé «purge and trap» (par entraînement et piègeage). Les principaux avantages et inconvénients des colonnes capillaires modernes en silice fondue sont brièvement cités. Les phases chirales récemment développées sont également décrites. Cet article cite quelques-uns des détecteurs utilisés pour l'analyse qualitative et/ou quantitative des composés de l'arôme : FID, MSD, FTIR, SCD, FPD et NPD (ces trois derniers détecteurs étant utilisés spécifiquement pour l'analyse des composés soufrés et azotés), AED ainsi que la détection par «sniffing». Les systèmes de recherche par bibliothèques de spectres tels que le PBM, INCOS™ et SISCOM (par exemple, Masslib®) sont mentionnés. Cet article fait un rappel de quelques autres méthodes (par exemple, photométriques) à même de déterminer des fonctions chimiques responsables de la flaveur (composés carbonylés, par exemple), ainsi que quelques techniques des plus prometteuses incluant les récents nez électroniques.

lait / produit laitier / composé volatil / arôme / méthode d'analyse

INTRODUCTION

Our civilisation is characterised by 'hedonism', which partially explains why flavours play an increasing role in human nutrition and why so much research has been carried out on this topic. Involved in the difference between gastronomy and nutrition, flavours have an impact on our senses of smell and taste, whereas food colours have an impact on our vision. Flavours are not really nutrients. They can stimulate our appetite or warn us of a lack of freshness, or of the presence of anti-nutrients, possibly preventing food poisoning.

There has been constant progress over the last 25 years in the development of analytical methods for detecting volatile, odiferous components such as flavours and perfumes. This research has been supported by the food industry on the one hand, and the perfume and cosmetics industry on the other. For the former, the

goal has often been to replace labile compounds or to concentrate interesting components, while for the latter, the aim has been to synthesise rare and expensive natural essences. Research on 'off-flavour compounds' in foods has covered all steps from raw materials to final products, including the manufacture, transformation, packaging, transportation and storage of foods.

The development of efficient analytical tools, such as gas chromatography (GC) and related techniques, has greatly contributed to progress in this area. Main accessories include 'on-column' or 'purge and trap' injectors, fused silica capillary columns with stabilised (crosslinked) stationary phases, highly efficient detectors, and fully automatic PC-analysers with software such as MassLib®, Systat®, Sysgraph®, etc for data collection and manipulation.

Some excellent review articles have been published on instrumental and analytical assessment of volatile (flavour) compounds (Day,

1967; Forss et al, 1967; Schwartz et al, 1968; Wong and Parks, 1968; Forss, 1969, 1971, 1972; Weurman, 1969; Paillard et al, 1970; Teranishi et al, 1971; Adda and Dumont, 1972; Dumont and Adda, 1972; Evans, 1972; Moinas et al, 1973; Ney, 1973; Lacrampe et al, 1975; Jennings and Filsoof, 1977; Bemelmans, 1979; Nursten, 1979; Benkler and Reineccius, 1980; Lamparsky and Klimes, 1981; Maarse and Belz, 1981; Cronin, 1982; Manning and Price, 1983; Jennings and Rapp, 1983; Leahy and Reineccius, 1984; Núñez and Bemelmans, 1984; Reineccius and Anandaraman, 1984; Sugisawa et al, 1984; Schreier and Idstein, 1985; Grosch, 1990; Klein et al, 1990; Vandeweghe and Reineccius, 1990; Careri et al, 1994; Xanthopoulos et al, 1994; Urbach, 1996) as well as a reference book (Marsili, 1996). The aim of this article is to complete and update existing literature on instrumental analysis of volatile (flavour) compounds from milk and dairy products.

PREPARATION AND TREATMENT OF THE SAMPLE, TECHNIQUES OF EXTRACTION, CONCENTRATION AND INJECTION OF THE VOLATILES

Sample preparation and treatment

General principles

Various procedures have been applied to the isolation of volatiles from complex matrices such as milk and dairy products. Since most volatiles are present in both the sample and vapour phase in small amounts or even traces (< 10 µg/kg), different steps for the extraction, concentration and injection of volatiles into the GC are required.

Due to the sensitivity of some compounds to heat and/or oxygen, precautions have to be taken during the preparation of the sample and the isolation of the volatiles to ensure that they remain unchanged and to minimise their losses. Moreover, the formation of new compounds (arte-

facts) should be prevented. Possible contamination from the atmosphere and laboratory personnel or their activities (eg, by smoking, cosmetics or solvent in the vicinity) must be avoided in trace analytical determinations by working under clean-room conditions.

Only materials and chemicals of high purity must be used. They should always be checked for contamination before use, and if necessary cleaned and purified. Distilled and boiled ultrapure water should be used, especially for 'purge and trap' techniques. Blank test values should be established on a regular basis to ensure the absence of contamination or, if not possible, to subtract the blank from the sample chromatogram.

Homogenisation of solid and semi-solid samples

Volatile (aroma) compounds in dairy products are generally distributed in a heterogeneous way. Trace analysis requires careful homogenisation of the sample prior to analysis.

A commonly used, convenient and mild technique for cheese is, for example, freezing followed by grating of the sample at low temperature. If necessary, the powder obtained can then be dispersed in water using a high speed homogeniser to obtain representative samples prior to taking an aliquot.

When using headspace techniques, the homogenate obtained will often be adjusted with alkali to pH 7.5 prior to headspace analysis to keep the high concentration of volatile acids dissolved in the aqueous solution as salts. The fraction to be analysed then mainly contains traces of the numerous neutral and alkaline components present (Imhof and Bosset, 1991). Making a homogenate alkaline can destroy some essential components, such as lactones (Urbach et al, 1972).

Separation into acidic, alkaline and neutral fractions

In general, flavour from dairy products is made up of a large number of volatile compounds

(Nursten, 1977; Maarse, 1983; Bosset et al, 1995; Molimard and Spinnler, 1996; Urbach, 1996). These may include significant amounts of free carboxylic acids, sulfur compounds, alkaline nitrogen-containing substances such as amines and substituted pyrazines (Nursten and Sheen, 1974) and pyridines and many neutral compounds such as carbonyl compounds (mostly methylketones and aldehydes), primary and secondary alcohols, esters, lactones, ethers, aliphatic and aromatic hydrocarbons (some of them polycyclic: Dafflon et al, 1995), as well as multifunctional components. In consequence, a preliminary separation into acidic, alkaline and neutral fractions can often help if it is included before their instrumental and sensory analysis (Cronin, 1982). Figure 1 highlights a commonly used scheme for such a separation.

Extraction and concentration techniques

In general, volatile (aroma) compounds are fairly lipophilic. They are consequently dissolved in the original fatty phase or bound to the proteins.

The first step in each procedure consists of their extraction from such a matrix and usually results in a dilute aqueous solution. Prior to GC-analysis, these volatiles must be separated from water and usually concentrated by using an organic solvent or solvent mixture. However, some modern techniques now allow capture of these compounds by direct adsorption onto a solid phase, so avoiding tedious and time-consuming steps.

Numerous techniques have been proposed for the extraction, concentration and injection of volatile food product components, especially of milk products. An update of the methods most frequently used may be summarised as follows:

Headspace techniques

Regarding headspace techniques, the sample to be analysed is a gas phase aliquot containing volatiles released from the condensed phase. In their classical static form (Görner et al, 1968; Hild, 1979; Kahlhofer, 1981; Price and Manning, 1983; Degorce-Dumas et al, 1986; McNeal and Hollifield, 1990; Ulberth, 1991; Gaafar, 1992; Linszen et al, 1995), these techniques are

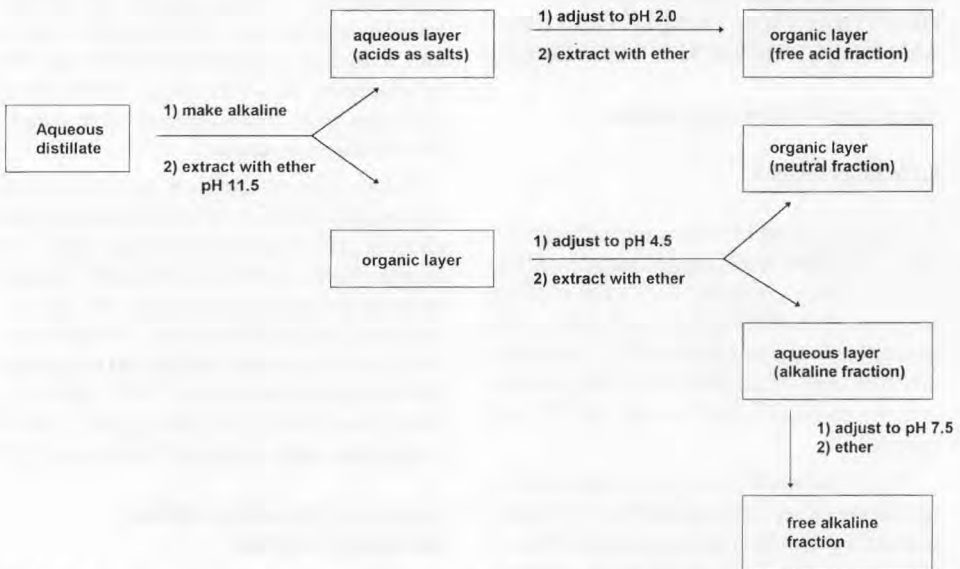


Fig 1. Technique used to isolate the acidic, neutral and alkaline volatile (flavour) compounds.
Technique utilisée pour séparer les composés volatils acides, neutres et alcalins (de l'arôme).

restricted to the most volatile components. The detection of flavour compounds greatly depends on their concentration and vapour pressure, as well as on the temperature and matrix of the food product. A very interesting application is the analysis of volatile acids of cheese, based on esterification in the gas phase prior to analysis (Badertscher et al, 1993). To obtain more concentrated extracts, dynamic headspace methods have been developed (Oria et al, 1987; Urbach, 1987; Horwood, 1989; Imhof and Bosset, 1991; Wijesundera and Urbach, 1993; Contarini and Leardi, 1994; O'Hare and Nursten, 1994; Wood et al, 1994; Yang and Min, 1994), in which the volatile components of the gas phase are continuously removed and concentrated in a cold trap or adsorbed onto an inert support, and finally recovered either by thermal desorption or by elution with a suitable solvent (Westendorf, 1985).

Steam distillation techniques

Regarding steam distillation techniques, the aqueous distillate is concentrated by liquid/liquid partitioning or by cryoconcentration. Although widely used, these very popular techniques present several problems. For example, highly volatile components may have low recoveries and/or may be masked by the chromatographic peak of the solvent. The solvent may contaminate the sample. In addition, unstable compounds may decompose thermally if the steam distillation and solvent extraction are not carried out under reduced pressure. This technique has been largely applied to cheese varieties such as Emmental (Vámos-Vigyázó and Kiss-Kutz, 1974), Limburger (Parliment et al, 1982), Fontina (Ney and Wirotama, 1978) and Cheddar (Vandeweghe and Reineccius, 1990), as well as to milk (Jeon et al, 1978; Alm, 1982) and other milk products such as butter (Stark et al, 1978), ghee (Jain and Singhal, 1969; Wadhwa et al, 1979), butter oil (Urbach et al, 1972) and milk powder (Ferretti and Flanagan, 1972). Steam distillation has been described recently (Xanthopoulos et al, 1994) as an advantageous method for isolating volatile substances like

acetaldehyde, ethanol, diacetyl and acetoin in dairy products.

High-vacuum distillation techniques

High-vacuum distillation techniques produce small volumes of concentrated aqueous extracts, which are in turn extracted with organic solvents such as methylene chloride and diethyl ether. These techniques prevent thermal degradation by working at ambient or even sub-ambient temperature, but usually require large sample amounts and the operations are very time-consuming. High-vacuum techniques have been applied to solid fat after ultracentrifugation at 25 000 g (McGugan et al, 1968; Manning, 1974) or liquid fat (Liebich et al, 1970) from a homogeneous suspension of fat in water (Bradley and Stine, 1968; Parliment et al, 1982), or even from fat/water/isopentane mixtures (Sloot and Harkes, 1975). Sour cream, as an aqueous suspension, can also be distilled under high vacuum (Mick et al, 1982). Moinas (1973) used a 'gas stripping' technique combined with high vacuum distillation by purging the volatiles with inert gas at low temperatures (Moinas et al, 1973; Groux and Moinas, 1974; Kowalewska et al, 1985). The alkaline volatile components of Swiss Gruyère cheese have been isolated by applying high vacuum gas stripping to grated cheese samples at room temperature, followed by ether extraction of the distillates after they were made alkaline (Liardon et al, 1982). The volatile odorous constituents of different French blue cheeses were isolated by high-vacuum distillation (Gallois and Langlois, 1990). Volatile compounds of different French Appellation d'Origine Contrôlée (AOC) cheeses were extracted by vacuum distillation (Guichard, 1994).

Molecular distillation techniques

Molecular distillation techniques are very similar to high-vacuum distillation and involve the direct transfer of volatile compounds from the matrix to a cold condenser. These techniques require very short distances between the food sample and condenser, as well as the use of high-vacuum pumping systems ($< 10^{-3}$ Pa). Such techniques have

been used to isolate volatiles from fats and oils, and necessitate water-free samples. Libbey et al (1963) called this 'cold-finger molecular distillation'. Day and Libbey (1964) used this technique to analyse cheese flavour (Cheddar). Stark et al (1976) for butter oil, and Urbach (1982) for milk fat. Dumont et al (1974) described this technique as 'distillation sous vide poussé' (translated as 'high-vacuum distillation'). Molecular distillation has also been carried out by Guichard et al (1987) for Comté cheese, and by Guichard (1995) for Parmigiano, Emmental, Comté and Beaufort cheeses. Volatile compounds from old Cheddar cheese were isolated by molecular distillation (Christensen and Reineccius, 1995).

Direct extraction techniques

Regarding direct extraction techniques, an extract is obtained by liquid/liquid or liquid/solid partitioning. While these techniques are generally rapid and efficient, they should only be applied to samples with a very low fat content, to keep simultaneous fat extraction low. Because of its low solvent power for triglycerides, acetonitrile can be used to extract cheese flavours. However, the relatively high boiling point of this solvent (82 °C) causes loss of volatile components during the concentration step. Several peaks in the chromatogram will be masked by this solvent (Wong and Parks, 1968; Lamparsky and Klimes, 1981). Preininger et al (1994) treated grated Swiss cheese directly with diethyl ether, filtered off the insoluble material and finally, under high vacuum, distilled the volatile compounds from the non-volatile material (ie, fat), which had dissolved in this solvent. In order to separate the neutral and alkaline fractions from the acidic fraction the distillate obtained, which smelled intensively of Emmentaler cheese, was treated successively with aqueous sodium bicarbonate and aqueous hydrochloric acid (fig 1).

Supercritical fluid extraction methods (SFE)

Supercritical fluid extraction methods (SFE) using carbon dioxide as solvent, avoid the problems of concentrating the extracts. Because of the simul-

taneous extraction of mono-, di- and triglycerides and the volatile compounds, the application of these techniques is limited to samples with a low fat content. Currently in the case of cheese samples, SFE-SFC (supercritical fluid chromatography) coupling (Gmür et al, 1987a,b,c) seems to be a possible way of making use of this promising technique; Gmür et al (1986) have already used this on a preparative scale. Commercially available SFE equipment (ie, Hewlett Packard type 7680A/T SFE module or Applied Separations type Spe-de SFE) now allows a release of pressure (after the restrictor) onto a cartridge containing a reverse phase material (similar to that of reverse-phase high performance liquid chromatography, RP-HPLC columns), which can be eluted stepwise by eluents of different polarities. This improvement in SFE systems now permits analysis of trace components in the presence of high amounts of glycerides without any interference or other difficulty.

Simultaneous (steam) distillation extraction methods (SDE)

Simultaneous (steam) distillation extraction methods (SDE), which were first developed by Nickerson and Likens (1966), use only very low-boiling solvents such as pentane for the concentration of the aroma volatiles. In a continuous process, the condensing water vapour is extracted by the condensing solvent vapour, yielding a high extraction rate. Both the water and the solvent are recirculated. A micro-scale modification, proposed by Godefroot et al (1981), and de Frutos et al (1988) has recently been applied to cheese volatiles. Maignial et al (1992) have proposed a system working under reduced pressure and at low temperature (20–40 °C) to prevent thermal generation of artifacts. Blanch et al (1993) have proposed a micro SDE apparatus working at normal pressure. Careri et al (1994) used this method for the extraction of volatiles in Parmesan cheese.

Dialysis techniques

Dialysis techniques, which separate volatiles according to their ability to diffuse through a

membrane, result in a concentration gradient. Although a high degree of concentration was obtained, the sample preparation and dialysis were very time-consuming. This technique was developed by Benkler and Reineccius (1980), modified by Chang and Reineccius (1985), and compared with other techniques by Vandeweghe and Reineccius (1990). A good review on the use of membranes in sample preparation is given by Majors (1995). Application of pervaporation (partial vaporisation through a non-porous permselective membrane) in food processing has been reported by Karlsson and Trägårdh (1996).

Solid-phase extraction methods (SPE)

Solid-phase extraction methods (SPE) have been used for the selective separation and concentration of analytes from liquid samples. Extraction of the analytes is based on the distribution of dissolved substances (ie, volatiles) between a solid-phase surface and the liquid sample. Separation may be a result of differences in polarity, molecular size, or even differences with respect to ion-exchange capacity. Solid-phase extraction or even micro-solid-phase extraction (SPME) techniques (Woolley and Mani, 1994) are relatively new methods for isolating volatiles. Takacs (1989) reported that flavour components in UHT processed milk could be extracted with C18 Sep-Pak materials and subsequently eluted with methylene chloride. A rapid and sensitive solid phase method was developed by Coulibaly and Jeon (1992) for the extraction of lactones at ppb levels. Analysis of volatiles in various wines using solid phase extraction is described by Sedláčková et al (1995). The use of an on-line SPE-GC-MSD instrument has been recently described by Brinkman and Vreuls (1995).

All aqueous extracts may be concentrated using the freeze-concentration technique, well described by Maarse and Belz (1981), and quoted from the literature by Urbach (1996). Commercial equipment had been proposed (eg, Virtis Co, model 3-100, ser 1049), but less expensive kitchen devices used for domestic ice cream preparation also seem to be convenient for such a purpose.

Injection techniques

Two types of injection are currently used: on-column injection and 'purge and trap' injection. One of the most popular and least expensive pieces of equipment used for many years for loading the volatile (flavour) components of foodstuffs onto a capillary column is the so-called 'cold' injection technique or on-column injection, where the liquid extract is injected directly onto the column. This prevents the loss of substances as well as the discrimination which occurs with the use of a split/splitless injector. Band broadening can be suppressed by stationary phase focussing via a retention gap consisting of a length of uncoated column material (Grob et al, 1985).

The other very common injection technique used is the so-called 'purge and trap' method already mentioned above (see 'dynamic headspace technique'), which is based on the adsorption of compounds onto a trap and/or condensation of the volatiles by low temperature ('cryofocussing') before injection (Imhof and Bosset, 1991).

CHROMATOGRAPHIC SEPARATION, DETECTION, IDENTIFICATION AND QUANTIFICATION

One-dimensional gas chromatography

The qualitative and quantitative analyses of complex mixtures of volatile (aroma) components require extremely efficient capillary columns (packed columns are no longer used). The GC separation should be performed exploiting both the volatility and the polarity of the analytes. It must be able to cover a wide spectrum of constituents from non-polar to very polar ones in line with the great variety of the chemical functions present in the mixture. Major progress in this area has been achieved by the introduction of capillary columns based on flexible fused silica tubing material, well known under the abbre-

viation FSOT (fused silica open tubular columns).

Most capillary columns used today contain a stationary-phase layer that has been cross-linked with the fused silica after coating. Today all traditional high molecular weight phases are available as 'bonded' or 'stabilised stationary phase' under various trade names. These modern cross-linked phases have polarities and separation performances at least comparable to or even better than those of the silicone gum or Carbowax-type phases they replaced. Capillary columns with internal diameters ranging from 0.10 mm (small bore) up to 0.53 mm (wide bore) are commonly used for the analysis of volatile (flavour) compounds.

Polyethyleneglycols (PEG, Carbowax) of various types have been the most popular highly polar phases and the best stationary phases for the separation of food flavour components for more than 25 years (Mick et al, 1982). However, the performance of polydimethylsiloxane as phases with a lower polarity came to be tested recently, especially in dynamic headspace analysis (ie, using 'purge and trap' equipment). These less polar phases prevent the haphazard shifts in retention time observed for some compounds (due to moisture or the high water content of aqueous samples) when PEG-coated capillary columns were used. Moreover, polydimethylsiloxane phases allow use of a much wider temperature range (from -30 up to 300 °C) than PEG phases (from +45 up to 250 °C) and are less sensitive to oxygen at high temperature (improved longevity).

The best performance for the GC analysis of samples of ripe Swiss Emmental cheese was obtained with a 30 m × 0.32 mm id capillary column coated with a 4 µm layer of 100% polydimethyl siloxane (SPB-1-SULFUR) (Imhof and Bosset, 1994a). The film thickness of 4 µm ensures better resolution and higher loading capacity of the column with samples containing many compounds in different concentrations (fig 2). This column made possible the identification of the highest number of compounds (ie, 58) in the cheese sample (table I). Furthermore,

highly volatile compounds such as ethanal could be easily detected. Sanz et al (1992) used a mixed-phase (43% FFAP: polar phase and 57% OV-1: non polar) and found it to be better for the analysis of volatile fractions from foods.

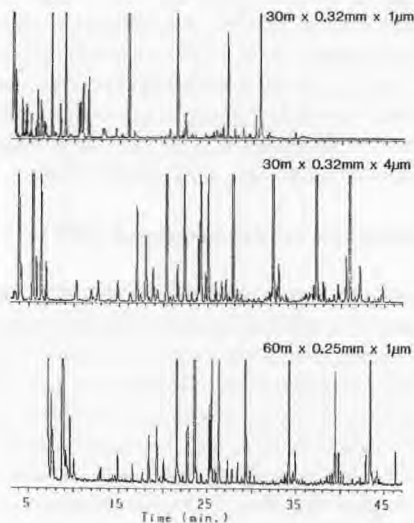


Fig 2. Influence of capillary column dimensions and layer thickness on the chromatograms obtained from a sample of ripe Swiss Emmental cheese using a polydimethylsiloxane stationary phase (SPB-1-SULFUR). The same sample was analysed with the same dynamic headspace method (purge and trap) using identical sample preparation but three different capillary columns. The temperature programme (45 °C for 13 min; 5 °C/min to 220 °C; 220 °C for 10 min) as well as the scale (abundance and retention time) used were identical for the three chromatograms. For further analytical details, see Imhof and Bosset (1994a).

Influence des dimensions de la colonne capillaire et de l'épaisseur du film sur les chromatogrammes d'un échantillon d'emmental suisse affiné, en utilisant une phase stationnaire en polydiméthylsiloxane (SPB-1-SULFUR). Le même échantillon a été analysé avec la même méthode (espace de tête dynamique par «purge and trap») en recourant à la même préparation de l'échantillon, mais à trois colonnes capillaires différentes. Le programme de température (13 min à 45 °C; 5 °C/min jusqu'à 220 °C; 10 min à 220 °C), de même que les échelles utilisées (abondance et temps de rétention), étaient identiques pour les trois chromatogrammes. Pour d'autres détails analytiques, voir Imhof et Bosset (1994a).

Table I. Influence of the characteristics of the separation column on the resolution (number of peaks separated*).
Influence des caractéristiques de la colonne de séparation sur la résolution (nombre de pics séparés).*

<i>No of compounds identified</i>	<i>Stationary phase</i>	<i>Dimensions (m × mm × μm)</i>	<i>Polarity</i>	<i>Composition</i>	<i>Supplier</i>
48	DB-wax	60 × 0.25 × 0.25	Polar	100% Polyethyleneglycol	J+W
33	Supelco wax	60 × 0.32 × 0.5	Polar	100% Polyethyleneglycol	Supelco
51	OV 1701	50 × 0.32 × 0.5	Intermediate	88% Methylsiloxane 7% Phenylsiloxane 5% Cyanopropylsiloxane	Macherey & Nagel
51	Ultra-2	50 × 0.32 × 0.5	Non polar	95% Dimethylpolysiloxane 5% Diphenylpolysiloxane	Hewlett-Packard
54	SE 54	50 × 0.32 × 0.5	Non polar	94% Methylsiloxane 5% Phenylsiloxane 1% Vinylsiloxane	Macherey & Nagel
58	SPB-1**	30 × 0.32 × 4.0	Non polar	100% Dimethylpolysiloxane	Supelco
47	SPB-1	30 × 0.32 × 1.0	Non polar	100% Dimethylpolysiloxane	Supelco
57	SPB-1	60 × 0.25 × 1.0	Non polar	100% Dimethylpolysiloxane	Supelco

* Swiss Emmental cheese sample (Imhof and Bosset, 1994a); ** SPB-1 SULFUR.

* *Échantillon d'emmental suisse (Imhof et Bosset, 1994a); ** SPB-1 SULFUR.*

Table II lists some chromatographic columns used for the analysis of volatile components in milk and dairy products.

Multidimensional gas chromatography (MDGC)

The recent development of chiral stationary phases such as cyclodextrins (Bicchi et al, 1992) brought about new highly efficient separation techniques, such as enantioselective multidimensional gas chromatography (MDGC). The use of chiral MDGC in the food industry has dramatically increased in the past few years. It has been applied to identify for example the adulteration of essential oils, fruit juices and many flavoured beverages (Woolley and Mani, 1994). An enantioselective fingerprint can be used to differentiate natural from synthetic raw materials and such a fingerprint is much more

difficult to imitate in an adulterated product (Pierce, 1995). The other method described recently by Casabianca et al (1995) deals with the combination of gas chromatography with isotope-ratio mass spectrometry (GC-IRMS). Significant differences were found between the ratio of stable isotopes (eg, $^{13}\text{C}/^{12}\text{C}$, $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$) of natural and synthetic flavours in food products. Scope and limitations of enantioselectivity and isotope discrimination are reported by Mosandl (1995).

Detection systems

Many detectors have already been proposed for the qualitative and quantitative analysis of volatile (flavour) constituents of foods. Although the following list is not exhaustive, it does indicate those that have been most frequently quoted in the literature:

Table II. Overview of some separation columns used for the analysis of volatile components of milk and dairy products.

Tableau synoptique de quelques colonnes de séparation utilisées pour l'analyse des composés volatils du lait et des produits laitiers.

<i>Stationary phase</i>	<i>Dimensions (m × mm × μm)</i>	<i>Polarity</i>	<i>Compounds/product investigated</i>	<i>Ref</i>
FFAP	60 × 0.3 × ?	Polar	Swiss Gruyère cheese	Liardon et al (1982)
DB-5	30 × 0.32 × 1.0	Non polar	Gruyère de Comté	Guichard et al (1987)
DB-1	30 × 0.53 × 1.5	Non polar	VOC in milk	McNeal and Hollifield (1990)
SE-30	22 × 0.3 × 0.1	Non polar	Artisanal cheese varieties	de Frutos et al (1991)
SP 1000	25 × 0.2 × 0.4	Polar		
DB-wax	60 × 0.25 × 0.25	Polar	Parmigiano-Reggiano Mahon, Comté, Beaufort, Appenzeller	Bosset and Gauch (1993)
SE-54	30 × 0.32 × 0.3	Non polar	Key odorants	Preininger et al (1994)
OV-1701	30 × 0.32 × 0.3	Intermediate	in Emmentaler cheese	
DB-FFAP	? ? ?	Polar		
DB 1701	60 × 0.32 × ?	Intermediate	AOC cheeses	Guichard (1994)
DB-5	30 × 0.25 × 1.0	Non polar	Cheddar cheese	Yang and Min (1994)
DB-wax	30 × 0.25 × 0.25	Polar	Parmesan cheese	Careri et al (1994)
SE-54	25 × 0.32 × 1.0	Non polar	Flavour defects in milk powder	Ulberth and Roubicek (1995)
DB-wax (1995)	30 × 0.25 × 0.25	Polar	Cheddar cheese	Christensen and Reineccius
CP Sil 5 CB	50 × 0.32 × 5.0	Non polar	Gorgonzola cheese	Contarini and Toppino (1995)

?: Not specified in the original publication. VOC: volatile organic compounds; AOC: appellation d'origine contrôlée.

? : Non spécifié dans la publication d'origine ; VOC : composés organiques volatils ; AOC : appellation d'origine contrôlée.

The flame ionisation detector (FID)

The flame ionisation detector (FID) is certainly the most popular detector used for GC analysis. Its main advantages are its low cost, universal applicability (except for carbon dioxide and formic acid), extremely wide linear range of concentration for quantitative determinations, and long-term stability (the response factors for analytes remain very constant over long periods). As a consequence of its universality, this type of detector is neither specific, nor selective. GC-FID systems have been widely applied to dairy products (Barbieri et al, 1994; Careri et al, 1994; Preininger et al, 1994). Grigoryeva et al (1994) used it for the analysis of Dutch cheese and Monnet et al (1994) to investigate volatile compounds in fermented milk by static

headspace techniques. Georgala et al (1995) also used this detector for the assay of flavour compounds in ewe's milk and yoghurt, Ulberth and Roubicek (1995) for detecting flavour defects caused by lipid oxidation, and Contarini and Toppino (1995) for volatile organic compounds (VOC) in Gorgonzola cheese during ripening.

The mass selective detector (MSD)

The mass selective detector (MSD) is much more expensive than the FID, but it allows both qualitative and quantitative determinations. Two main types are now commercially available: the quadrupole and the ion trap system. Both systems may be used in the full-scan mode (eg, total ion current, TIC) in order to identify compounds via their fragmentation pattern, and in

the selected ion storage (SIS) or selected ion monitoring (SIM) mode to quantify target compounds with high selectivity and sensitivity. In the scan mode, the identification is mostly performed by comparison with a library of reference spectra (eg, EPA/NIH library, Wiley library, TNO library). In the SIS or SIM mode, the voltages on the ion trap or quadrupole rods are adjusted stepwise to detect only a small number of selected ions (eg, molecular ion, heavy ions, etc) highly specific for the substances to be quantified. Applications of the MSD/SCAN mode to volatile compounds of Emmental cheese have recently been reported by Bosset et al (1995). The MSD/SIM mode was applied for the detection of sulfur-containing compounds in milk after various heat treatments (Bosset et al, 1994, 1996). The GC-MSD technique is the most popular technique used to identify volatile compounds in dairy products: neutral volatile compounds in fresh bovine, ovine, caprine and water buffalo raw milks (Moio et al, 1993a); off-flavour compounds in spray-dried skim milk powder (Shiratsuchi et al, 1994); the volatile components of Cheddar and Swiss cheese (Yang and Min, 1994); the volatile components of full-fat, reduced-fat and low-fat Cheddar cheese (Delahunty et al, 1994); the aroma fraction of Parmesan cheese (Barbieri et al, 1994), and volatile compounds in goat milk cheese (Vidal-Aragón et al, 1994). Dynamic headspace analysis with MS detection was also used by Laye et al (1995) to identify 33 volatile compounds from commercial whey protein concentrate (WPC).

Ion-trap instruments where ionisation and mass analysis both occur in the ion-trap produce non-standard mass spectra which cannot be compared with commercial data bases. In the latest ion-trap instruments, ionisation and mass analysis have been separated and it is now claimed that these instruments do produce standard spectra.

The Fourier transform infrared spectroscopy (FTIR) detector

The Fourier transform infrared spectroscopy detection (FTIR) is very expensive and less sen-

sitive than the MS detection due to its much higher dead volume and the secondary infrared emission from the heated cell (light-pipe). Similarly to MS, the recorded spectra can be compared with a library of FTIR reference spectra to aid in compound identification, but the commercially available databases are much smaller than those of mass spectra. The FTIR spectra are useful complements to mass spectra, especially for the differentiation of isomers for which MS is not usually helpful (Jackson et al, 1993). Terpenes are an example of substances that may have many isomers due to the position of double bonds. In GC/IR, however, all of these substances show specific absorption bands at different wave numbers (Kasalinsky and McDonald, 1983). The principal capability of the use of high-resolution gas chromatography (HRGC)-FTIR in the analysis of food flavours has been demonstrated by Schreier and Idstein (1985). Industrial applications of GC/FTIR have been described by Namba (1990) with some interesting examples relevant to foods and fragrances.

The flame photometric detector (FPD)

The flame photometric detector (FPD) is highly selective for sulphur-containing components when a suitable filter is used. Although reliable and sensitive, this detector also suffers from a number of major drawbacks, such as: the well known but unavoidable quenching effects (interferences, eg, from hydrocarbons and carbon monoxide); the non-linear (but sigmoidal) response of the output signal vs the concentration of sulphur species (Burdge and Farwell, 1994).

Prior to the introduction of the SCD (see the following detector), the dominant sulphur-selective detector used in GC was the FPD. This detector configured in parallel with an FID was used by Manning and Moore (1979) to determine hydrogen sulphide and methanethiol by headspace analysis of hard cheeses. Aston and Douglas (1981) described the detection of carbonyl sulphide in Cheddar cheese also using a headspace technique, whereas Parliment et al (1982) examined the VOC of Limburger cheese.

The sulphur chemiluminescence (SCD) detector

The sulphur chemiluminescence detector (SCD) is an universal sulphur detector. It has very high sensitivity and selectivity, a linear response to concentration of sulphur species, a large dynamic range, and is free from quenching effects over a wide range. A detailed description of the characteristics of this type of detector is given by Burdge et al (1994). Tuan et al (1995) recently published an interesting evaluation of the performances of FPD, MSD (working in SCAN and SIM mode) and SCD for the determination of sulphur in natural gas. They claimed that the SCD represented the best choice. The detection of sulphur flavour volatiles in cooked milk was reported by Steely (1994). Other applications using static headspace techniques are reported eg, by Nedjma and Maujean (1995) in the analysis of sulphur volatiles in water-alcohol solutions and brandies.

The atomic emission detector (AED)

The atomic emission detector (AED) is a new detector derived from the well-known atomic emission spectroscopy, used for the analysis of metals and metalloids. It can be tuned to detect any element in any compound that may be eluted by GC. The evaluation of the various detection systems for the determination of volatile sulphur compounds in foods was reviewed by Mistry et al (1994). The study of dynamic range, minimum detectable level and selectivity for a series of sulphur compounds showed that: the upper limit of the linear dynamic range for the AED is six to eight times greater than for the FPD or the SCD (ie, 1200–1550 ng of the compounds injected for the AED vs 200 ng for the FPD and SCD); the minimum detectable level of the sulphur compounds with the AED is as low as 1 pg (vs 200 pg with the SCD and FPD); the selectivity of the AED can be enhanced using the 'snapshot' option. A snapshot is a selected segment of the emission spectrum showing specific elemental emission wavelengths.

The nitrogen phosphorus detector (NPD)

The nitrogen phosphorus detector (NPD) is highly selective for nitrogen-containing compounds (amines, pyrazines, pyridines, etc). It has similar operating conditions to the FID detector, and is also of low cost. GC-NPD systems have been used in some investigations of flavour components. Nitrogen-containing volatiles of Swiss Gruyère cheese have been determined by Liardon et al (1982). Flavour defects in milk and dairy products due to pyrazines have been reported by Morgan (1976). Lund (1994) also used this detector for the determination of 2-methoxy-3-alkylpyrazines, eg, in carrots.

The 'sniffing device'

The 'sniffing device' uses the human nose as a detector. It plays a key role as a unique interface with sensory analyses due to its specificity for flavour and off-flavour compounds. Substances are sniffed individually as they are eluted from the GC column. Descriptors may be attributed to each retention time corresponding to an odour-active component (Sävenhed et al, 1985). Quantitative approaches were developed by Acree et al (1984) with the so-called CHARM (combined hedonic aroma response measurement). The analysis is based on sniffing of GC runs of serial dilutions of an odour essence, in order to determine the presence of odour-active zones. The sniffer points out the start and the end of each particular odour perception, and designates it with sensory descriptors. A CHARM value is calculated according to the formula $c = d^{n-1}$ where n represents the number of coincident responses and d the serial dilution factor. Ullrich and Grosch (1987) worked with a similar technique, aroma extract dilution analysis (AEDA), based on the dilution factor (DF), which represents the highest dilution where an odour-active component may still be detected. The resulting values are proportional to the odour number first defined by Rothe and Thomas (1963) as the 'aroma value' (ratio of the concentration of an odour-active compound in the matrix to the detection threshold of this com-

pound in the same matrix). Miranda-Lopez et al (1992) have developed a quantitative technique called gas chromatography-olfactometry (Osme or GC-O), based on the quantitative measurement of the perceived odour intensity of extracted components after separation on a GC column. Compared to the CHARM or AEDA tests (Etiévant et al, 1994), this method is not based on odour detection thresholds but on odour intensity. Guichard et al (1995) compared this latter technique with both former ones and found similar results. Moio et al (1994) used it for raw, pasteurised and UHT bovine milk. The powerful odorants in bovine, ovine, caprine and water buffalo milk had been previously determined by Moio et al (1993b) using GC-O. Guichard (1994) studied the key flavour compounds in Appellation d'Origine Contrôlée (AOC) cheese by a GC-sniffing technique.

Multiple detection of volatile (aroma) compounds

Multiple detection of volatile (aroma) compounds has also been proposed by some authors (table III). By using several detectors simultaneously, identification is made more reliable. Detectors may be configured in series or in parallel. Common destructive detectors such as FID, NPD, FPD and MSD must be mounted in parallel, or only as the final detector when configured in series. The FTIR detector, being non-destructive, is coupled in series with MSD in some commercial systems (GC-FTIR-MSD). The software then allows simultaneous recording of the infrared and mass spectra of the eluting compounds. The future in this respect definitely belongs to the spectroscopic detectors that allow selective recognition of the

Table III. Multiple detection of volatile compounds.
Détection multiple des composés volatils.

<i>Simultaneous detection</i>	<i>Investigated product</i>	<i>Ref</i>
FID/NPD	Nitrogen volatiles in Swiss Gruyère cheese	Liardon et al (1982)
Idem	Sulfur volatiles in Limburger cheese	Parliment et al (1982)
FID/MSD	VOC in office environment	Mogl et al (1995)
Idem	Fatty acid methyl esters	Traitler and Horman (1990)
FID/MSD FPD/MSD	Sulfur compounds in milk	Bosset et al (1996)
FTIR/MSD	Flavour compounds in wine	Buchbauer et al (1994)
FID/sniffing	Aroma of aged Cheddar cheese	Christensen and Reineccius (1995)
Idem	Sulfur volatiles in <i>Cucumis melo</i>	Wyllie et al (1994)
MSD/sniffing	Cheddar cheese	Arora et al (1995)
Idem	Blue crab meat volatiles	Chung and Cadwallader (1994)
FID/SCD/MSD	Flavour compounds in whiskey	MacNamara et al (1995)

FID: flame ionisation detector; NPD: nitrogen phosphorous detector; MSD: mass selective detector; FPD: flame photometric detector; FTIR: Fourier transform infrared spectroscopy detector; SCD: sulphur chemiluminescence detector; VOC: volatile organic compounds.

chromatographed compounds. Today, hyphenated techniques such as GC-MSD, GC-FTIR and GC-AED are the most powerful techniques available. Simultaneous detection allows the identification and quantification of almost all aroma compounds present in sufficient amounts after column effluent splitting.

Quantitative analysis

Two main routes have been described for the quantification of volatile compounds. The first technique is based on spiking the matrix with labelled compounds whose extraction rate and loss during sample treatment, response factor during detection, etc, are equal to those of the (unlabelled) substances to be quantified. Schieberle and Grosch (1987) described this method, called 'isotope dilution assay', for the analysis of aroma compounds. The quantification of a compound in the original matrix is based on the peak height-ratio (or area-ratio) of the unlabelled to the labelled compound (as an internal standard) using the MS detector in the SIM or SIS mode. Thirteen compounds were quantified by this method in Emmentaler cheese (Grosch et al, 1994; Preininger and Grosch, 1994; Preininger et al, 1996).

The second route uses a common standard addition method with increasing quantities of the constituents to be quantified (Imhof and Bosset, 1994b). Thirty-three VOC were quantitatively determined in this way in pasteurised milk and yoghurts in conjunction with dynamic headspace analysis and MS detection.

DATA COLLECTION AND PROCESSING, STATISTICAL ANALYSIS

Having acquired the mass spectra using electron-impact ionisation (EI), the interpretation of the data stored in the computer can begin. In the full scan mode, the mass spectra contain peak intensities on a mass to charge scale (m/z). Chromatograms from selected ion recordings (SIM or

SIS mode according to the type of equipment used) and data from multichannel analysers can also be handled. Various sophisticated software packages exist for the manipulation of data, display of spectra and chemical structure plots as well as for peak integration to obtain quantitative information. The main goal is the elucidation of the chemical structure of unknown compounds from their mass spectra. The fastest approach for extracting structural information from spectra is library search combined with the spectroscopist's expertise.

Dedicated software for the coding and searching of the relevant spectra exists in most modern GC-MS systems: a matching procedure compares unknown mass spectra and their intensities (m/z values) with those of the reference library. For all useful matches, some matching factors are calculated and a ranked list of the ' n ' best matches ('hit list') is presented, sometimes even as spectra together with their corresponding chemical structures (fig 3).

The following MS libraries are frequently used (nonexhaustive list): commercial libraries of electron-impact ionisation spectra like Wiley with over 275 000 spectra, National Institute of Standards and Technology (NIST) with \approx 63 000 spectra, the Central Institute for Nutrition and Food Research, TNO, Zeist (The Netherlands) library with \approx 1 600 spectra (especially from food volatiles), and the Chemical Concept (Germany) library with \approx 35 000 spectra; user libraries of certified spectra; user libraries of unknown or uncertified spectra.

The quality of some of the data contained in some databases is questionable: spectra are often strongly curtailed as to the number of peaks and hence are incomplete, and incorrect spectra taken from old collections may be reincluded in new compilations. Furthermore, threshold spectra frequently miss small but significant peaks.

Among the most powerful search algorithms currently in use, SISCO (Search for Identical and Similar COMpounds; Damen et al, 1978; Domokos et al, 1983), PBM (Probability Based Matching System; Pesyna et al, 1976; Atwater et al, 1985) and INCOSTM type searches could be

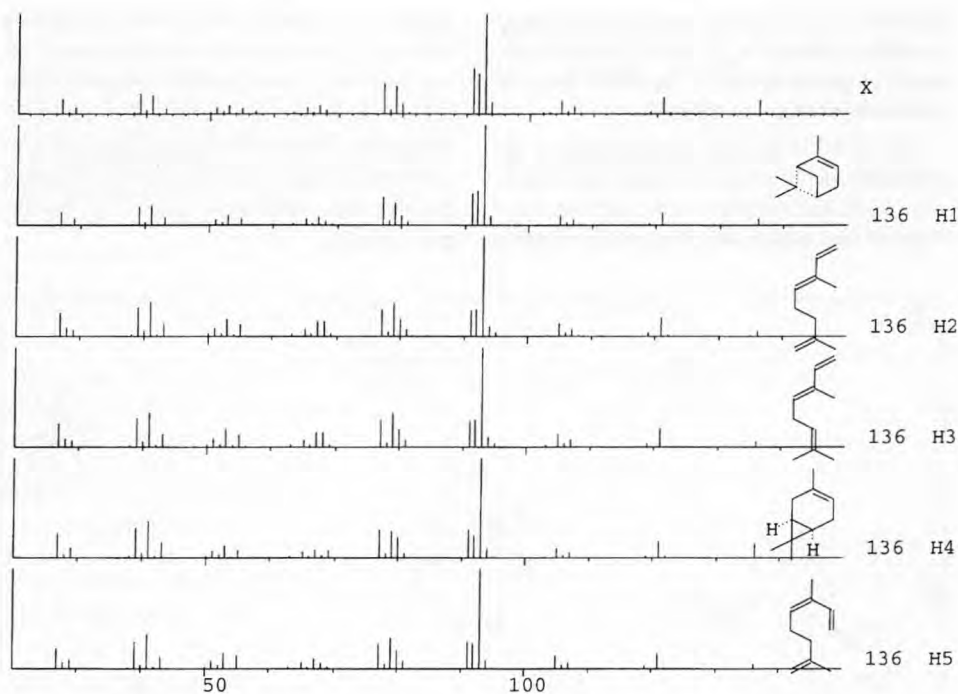


Fig 3. Reference spectra of an unknown compound (x), identified as α -pinene (H1). The other spectra (H2–H5) correspond to other structures (best matches) found in different libraries.

Spectres de référence d'un composé inconnu (x) identifié comme étant le α -pinène (H1). Les autres spectres (H2–H5) correspondent aux structures les plus voisines trouvées dans les différentes banques de référence.

mentioned. The SISCOM approach, featuring multiple rankings and also a neutral loss search, often allows the identification of the structural class of compounds, even if no reference spectrum is available. Both spectra and structures may be searched within MassLib[®], the software featuring SISCOM. It has become possible to identify not only similar spectra, but also to retrieve spectra of similar structures, revealing much of the complex relationship between spectra and structures (Henneberg et al, 1993). The structure search procedure also finds poor quality spectra: this is often a considerable advantage for the expert trying to interpret the spectrum of an unknown compound.

The use of statistics is also helpful for the identification of structural information. Besides

structure statistics on 'hit lists', MassLib[®] offers principal component analysis or PCA (Varmuza et al, 1989). For a group of spectra — for instance the members of a hit list — a number of n spectral features is chosen to represent the spectra as points in an n -dimensional feature space. The task is then to investigate the relative position of the objects in this n -dimensional space, eg, to find clusters of spectra which lie close together or to determine which spectra are the nearest neighbours of an unknown spectrum. If such clusters of references can be found by the proper selection of features, the position of an object, most often the unknown spectrum, can be used (after a PCA has substantially reduced the number of dimensions) for predictions about the class to which the object belongs. The pointers

obtained using the various methods finally make possible a stepwise approach to structural elements or partial structures, hopefully even the unknown structure itself (figs 3, 4).

MassLib[®] also offers a second tool for the automatic identification of compounds in routine work: the retention index option. User libraries may additionally contain the retention

indices for an apolar, polar and an user-defined column. A calibration file containing index values and their corresponding retention times makes possible the calibration of chromatograms. The two-dimensional approach overcomes the problems associated with substances having very similar mass spectra, eg, for terpenes (fig 5).

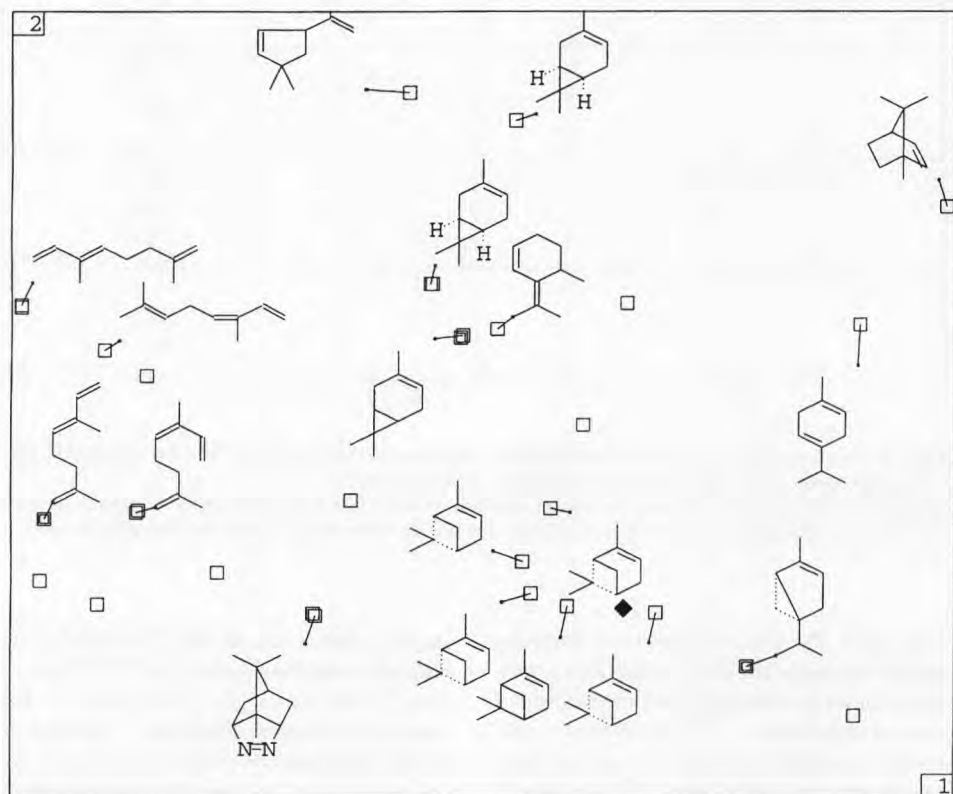


Fig 4. Principal component analysis showing the position of library spectra (\square) and thus their relative distances to the unknown (\blacklozenge). The black lozenge corresponds to the unknown compound. The libraries may contain more than one spectrum of a particular compound. Each square (with or without a line segment) represents a possible chemical structure within a cluster. The distance between the black lozenge and a given square is a measure of the similarity between these structures. For better readability of this figure, only the squares with a line segment show the corresponding chemical structure.

Analyse en composantes principales montrant la position des spectres de masse des banques de référence (\square) et leur distance relative au spectre inconnu (\blacklozenge). Le losange noir correspond au composé inconnu. Les banques de référence peuvent comporter plusieurs spectres d'un composé donné. Chaque carré (segmenté ou non) représente une structure chimique possible à l'intérieur d'un «cluster». La distance entre le losange noir et un carré donné est une mesure de la similitude existant entre ces structures. Pour améliorer la lisibilité de la figure, seuls les carrés segmentés indiquent la structure chimique correspondante.

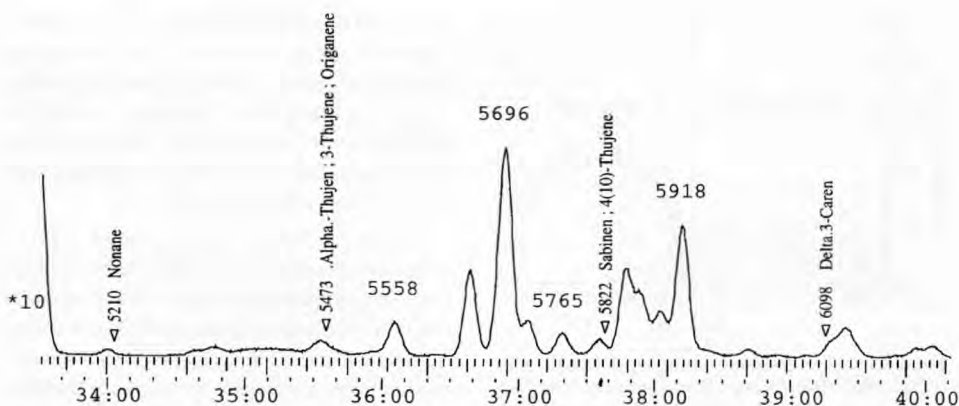


Fig 5. Combined search procedure (total ion current, TIC, and retention index) to identify terpenes. The presence of several possible names for a given mass spectrum and a given retention index indicates that it could be useful to use another stationary phase to identify the unknown compound (abscissa: retention time, min). *Procédure de recherche combinée (courant ionique total et indice de rétention) pour l'identification de terpènes. L'existence de plusieurs noms possibles pour un spectre de masse et un indice de rétention donnés indique qu'il pourrait être utile de recourir à d'autres phases stationnaires pour identifier le composé inconnu (abscisse: temps de rétention, min).*

ELECTRONIC OR ARTIFICIAL NOSES

The idea of an instrument for the analysis of volatile (aroma) compounds that mimics the human nose is not new. The goal has been to obtain a relatively quick response, as comparable as possible to that of the human nose. However, technological improvements have only recently provided 'sniffing' devices called 'electronic' or 'artificial' noses which claim to satisfy such requirements.

The principle of the functioning of such a nose is claimed to be similar to that of the human nose: several reactions take place between one or more volatile (flavour) components and an array of sensors (from four up to 32 according to the type of electronic nose). Their relatively rapid reaction involves the adsorption/desorption of volatiles on the surface of the sensors. These reactions are measured, and their results are expressed numerically. With the help of dedicated software, the numbers are then submitted to data analysis or signal processing and presented graphically.

Several types of gas sensors can be used for this task. Because of the specificity, selectivity and sensitivity of these sensors (more than 60 different polymers are available today), a final pattern ('fingerprint') of the sample can be obtained. The main types of sensors which have been developed for such analyses are the following:

- conducting organic polymers, such as polypyrrole and polyaniline, working at relatively low temperatures (usually < 80 °C). They require very little energy power. It is possible to prepare polymers which are highly specific for particular classes of volatiles;
- semiconductors such as metal oxides (SnO₂, ZnO, WO₃) working at high temperatures (up to 650 °C), which avoids interference from water and improves the response and regeneration time. In both cases, it is possible to measure the change in the electrical resistance (or conductance) during the reactions (fig 6) of the sensors with the volatile substances (Zannoni, 1995);

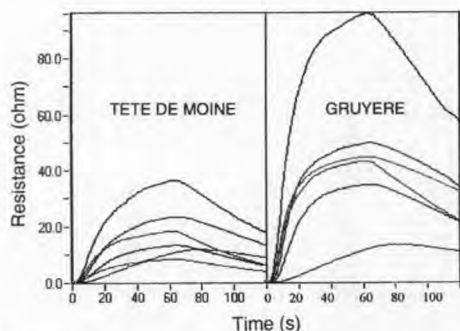


Fig 6. Change in resistance using an electronic nose (FOX 3000, Alpha MOS) with 12 sensors by analysing vapours (volatiles) of Tête de Moine and Swiss Gruyère cheese. The vapours are injected over the 12-sensor array for 60 s before switching off the automatic injection valve. The response of six sensors is plotted: adsorption for 60 s, desorption for 60 s.

Variation de la résistance d'un nez électronique (FOX 3000, Alpha MOS) à 12 capteurs lors de l'analyse des effluves de fromages de type Tête de Moine et Gruyère suisse. Les effluves sont injectées dans un réseau de 12 capteurs pendant 60 s avant la fermeture automatique de la valve d'injection. La réponse de six capteurs est représentée graphiquement : 60 s d'adsorption, 60 s de désorption.

- quartz resonators, which consist of a piezoelectric quartz oscillator coated with a sensing membrane. The adsorption of volatile (flavour) components results in a mass change that can be measured as a shift of the initial frequency (Zannoni, 1995);
- surface acoustic wave (SAW), amperometric and metal oxide semiconductor field effect transistor (MOSFET) devices can also be used as gas sensors for this purpose (Zannoni, 1995).

With conducting organic polymers, the change in conductance can be caused by two different mechanisms: direct interaction between the gas phase molecules (volatiles) and the polarons, which have n cationic sites, and bipolarons with $2n$ cationic sites in the polymer; interaction between the vapour molecules and the anionic counterions (primary dopants) and solvent matrix.

It is likely that both processes occur simultaneously in the polymer. The interaction between the analyte and the polymer (secondary doping) is rapid and reversible. The analyte molecules only interact with the surface layers of the polymer, which explains the relatively short recovery time after an exposure.

In porous metal oxide sensors (MOS), chemisorbed oxygen irreversibly oxidises volatile (flavour) compounds, a process which produces one or more conducting electrons. The free electrons tend to reduce the potential barrier between oxide grains, increasing the electron mobility and overall electrical conductivity of the material. This reaction depends on various physical and chemical parameters such as the microstructure of the sensor film and the operating temperature of the oxide (typically 250–650 °C). The material shows a sensitivity at the sub-ppm level for reducing combustible gases (Pearce et al, 1993; Tan et al, 1995). The mechanism can be summarised by the following reaction: $[R] + [O^-] \rightarrow [RO] + [e^-]$ (Tan et al, 1995).

After data generation and collection, the final stage of data processing uses pattern recognition algorithms, such as discriminant functions, K-nearest neighbour, template matching, cluster analysis, and partial least squares, in an artificial neural network (ANN) to distinguish between similar volatiles. The system is self-training; the more data are presented, the more discriminating the system becomes. These data sets are processed in a manner similar to that of the brain. The ANN's processing elements, or nodes, are akin to the neurones in the brain. Learning is accomplished by varying the emphasis placed on the output of one sensor in relation to another. It is derived from the mathematical or 'Euclidean' distance between data sets. Pattern recognition involves training and prediction. In the training phase, patterns from the measurements of standard reference samples are used to develop mathematical rules for assigning them to their respective classes (fig 7). In the prediction phase, the rules evolved are applied to assign unknown data sets to respective classes.

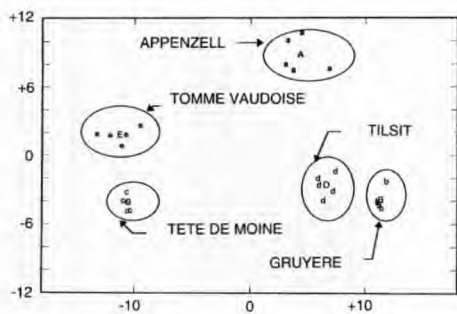


Fig 7. Clusters obtained by discriminant factorial analysis of five Swiss cheese varieties by using a 12-sensor electronic nose (FOX 3000, Alpha MOS). Refer to figure 6 for operating conditions.

Clusters obtenus par analyse factorielle discriminante de cinq sortes de fromages suisses en utilisant un «nez électronique» à 12 capteurs (FOX 3000, Alpha MOS). Voir figure 6 pour les conditions opératoires.

OTHER INSTRUMENTAL METHODS

In addition to the methods already described in this article for qualitative and quantitative analysis of volatile (flavour) compounds, photometry should be also mentioned. This technique may be applied to some classes of compounds bearing certain functional groups, such as carbonyls, carboxyls and alcohols, which can be derivatised by introducing specific chromophores with absorption in the UV or visible region (Imhof and Bosset, 1989). These methods have been used especially for quantifying carbonyl compounds (methyl ketones and aldehydes) with reagents such as 2,4 dinitrophenylhydrazine (DNPH) to form the corresponding coloured phenylhydrazones. Today, however, photometric methods are less frequently used, modern GC methods being much more sensitive, specific, reproducible and therefore very trustworthy. The former methods, however, can be useful in the exploratory stages and again later as confirmatory tests. Furthermore, photometric methods require frequent calibration and the elimination of background noise using a blank. In addition, the absorption bands used correspond more to chemical 'functions' (monocar-

bonyl, ketoglycerides, etc) than to particular substances. As a result, the photometrically determined absorbance corresponds to the absorbance of a mixture of compounds, even though each component has in fact its own specific molecular absorbance.

Several other methods have also been proposed for the analysis of volatile (flavour) compounds, such as high pressure liquid chromatography (HPLC) (Kubeczka, 1981; Dafflon et al, 1995), or NMR (Formáček and Kubeczka, 1982) which cannot be treated in the present article as they would be out of context.

CONCLUSION

This review updates the possibilities and limitations of modern instrumental analysis of volatile (flavour) compounds in milk and dairy products and describes some of the difficulties encountered in carrying it out. The harder it is to overcome an analytical challenge, the more creative the solution must be.

In general, GC is the method universally applied with the help of several highly efficient modes of extraction, (pre)concentration, injection, separation, and qualitative or quantitative detection of the volatile (flavour) compounds. All these techniques, however, have a common major drawback: the volatiles analysed are not entrapped or embedded in their original matrix, but have been converted into an extract. Consequently, they are present at concentrations that diverge greatly from those in the original food sample. Furthermore, the rheological properties of the matrix itself, which play a key role in flavour perception, are completely lost by this strategy.

In spite of the remarkable progress and development in this field, there is no ideal universal method for the simultaneous analysis of all volatile (flavour) compounds. Each method briefly described in this review presents both advantages and disadvantages (eg, table IV). This probably explains the numerous methods currently available. They may be easy and time-

Table IV. Summary of some main advantages and disadvantages of four techniques used in the authors' laboratory for the extraction and concentration of volatile (flavour) compounds from milk and dairy products as well as for their injection into a GC.

Résumé des principaux avantages et inconvénients de quatre techniques utilisées par les auteurs pour l'extraction et la concentration des composés volatils (de l'arôme) du lait et des produits laitiers et pour leur injection dans un GC.

<i>Method</i>	<i>High vacuum and low-temperature distillation</i>	<i>Steam distillation (stripping)</i>	<i>Dynamic headspace analysis (Rektorik equipment)</i>	<i>Dynamic headspace analysis (Tekmar equipment)</i>
Amount of sample (g)	2500	250	25	5–25
Duration of the analysis	3 days	3 h	2 h	1.5 h
Temperature (°C)	0–10	60	40	40
Pressure (mbar)	0.001	20	Ambient	Ambient
Application	Research	Research/routine	Research/routine	Research/routine
Advantages	Mild extraction Concentrated extracts Multiple injections possible Distillate can be used for other analyses	Suitable for components of medium to low volatility Multiple injections possible Distillate can be used for other analyses Internal standard possible	Small amount of sample No organic solvent needed Extraction and concentration in one step Internal standard possible	As for Rektorik and additionally: Suitable for components of high and medium volatility Several adsorbents available
Disadvantages	Large amount of sample ^a Time and labspace consuming Multiple processing steps High vacuum Organic solvent ^b Loss in concentration step Artefacts and impurities (eg, joint grease) ^c Suitable only for solid samples	Multiple processing steps Breakdown reactions of thermolabile components Organic solvent ^b Loss in concentration step Artefacts and impurities (eg, joint grease) ^c	Foam problems with liquid samples Multiple injection not possible Only two adsorbent materials available: graphite and charcoal Desorption temperature not measurable Production of artifacts in desorption step	Foam problems with liquid samples Multiple injection not possible Purity of water Purity of purge gas Cooling with liquid nitrogen Not possible to use split injection

^a Much smaller samples down to 10 g or even less can be used. ^b Aqueous distillates can be concentrated and injected directly into the GC. Liquid fat or even aqueous solutions can be used provided the water is first distilled off. ^c Joint grease can be replaced by Teflon sleeves.

^a Des quantités d'échantillons plus petites que 10 g peuvent être utilisées. ^b Les distillats aqueux peuvent être concentrés et injectés dans un GC. La graisse liquide et même des solutions aqueuses peuvent être utilisées, à condition d'éliminer l'eau auparavant. ^c La graisse pour haut vide peut être remplacée par des manchettes en téflon.

saving, or complicated and tedious. Some of them are specific for certain compounds, and suitable for routine assays, while others are more appropriate for research. The numerous methods and techniques proposed are complementary, but no one in particular seems to predominate today.

The other instrumental approach recently proposed is the so-called 'electronic' or 'artificial' nose, which allows one to investigate volatile (flavour) compounds directly in their original food matrix. Consequently, the electronic nose represents real progress. Such an analysis is very rapid compared to, for example, a GC analysis with its series of steps. However, in the perception of volatile flavours, an electronic nose, equipped with 4, 5, 6, 12, 18 or 32 sensors, cannot compete with the thousands of sensitive nerve endings active in the human nose and retronasal system.

Finally, in both strategies, ie, the sequential approach (GC analysis) and the direct global approach (assays with an electronic nose), highly efficient software is essential for the statistical treatment of data such as univariate and multivariate analyses.

To conclude, on the one hand, even the most efficient hardware and software currently available cannot compare with the extraordinary capacities of the human brain to analyse flavour stimuli with the help of memory in a context of long-term cultural and social education. On the other hand, it is well known that odour-detecting capacities vary greatly among individuals. The result of smelling can be influenced by the sniffing technique, the type of odorant mixtures, the adaptation to the environment, and the age and the psychophysical conditions of the subject. Many volatile molecules are barely detectable by the human nose. In addition, the response of our senses are difficult to quantify, even when using standard calibration procedures. For all these reasons, sensory analysis can be much more difficult and costly to apply in comparison with instrumental analysis, but, as far as flavour is concerned, the human nose is found to be the ultimate arbiter.

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