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Review

Capillary gas chromatography in quality assessment of flavours and fragrances

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ABSTRACT

The latest analytical advances in quality assessment of flavours and fragrances are reviewed, including capillary gas chromatography (cGC)–olfactometry for the identification and sensory evaluation of important odorants by means of odour activity values, aroma extract dilution analysis and stable isotope dilution assay (SIDA). Enantioselective cGC and comparative stable isotope ratio mass spectrometry (IRMS), coupled on-line with cGC, are reported as important tools in the authenticity control of flavours and fragrances. The literature on these topics is reviewed up to the beginning of 1992. The scope and limitations of chirospecific cGC and cGC–IRMS are discussed. The trends and perspectives in the origin control of flavours and fragrances are outlined.

CONTENTS

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1. INTRODUCTION

Flavours and fragrances are complex mixtures of volatile compounds and generally consist of hundreds of substances of different functionalities. They often occur in a great variety of concentrations, from trace levels in the sub-ppb range up to the amounts of the main constituents of essential oils.

Gas chromatography (GC) was introduced at an early stage of flavour research. Flavour chemists were among the pioneers of GC and also, more recently, of capillary GC(cGC) [1,2], as reviewed by Teranishi [3]. Whereas the first era of flavour research was directed to the comprehensive stock-taking of all the volatile constituents of complex flavour and fragrance extracts, the main efforts during the last decade have been focused on constitutional and stereochemical features, chemoreception and sensory relevance and biogenesis and biotechnological synthesis of sensorily active compounds, as reviewed by Schreier and Mosandl [4].

The state of the art in flavour research has been excellently reviewed in a series of papers on "Advances in aroma analysis" and "Progress in analysis of odorous substances" by Werkhoff *et al.* [5,6], including methods of isolation, concentration and separation of volatiles. New methods of chirospecific analysis, preparative techniques in GC and high-performance liquid chromatography (HPLC) and modern methods in structure elucidation were also summarized.

This review is directed to the analytical advances in the quality assessment of flavours and fragances, using capillary gas chromatography–olfactometry (cGC–O) for the identification of important odorants and combined GC techniques for the origin assignment of flavours and fragrances.

2. CAPILLARY GAS CHROMATOGRAPHY–OLFACTOMETRY (cGC–O)

2.1. Odour activity values (OAV)

In the early stages of flavour research, long lists of volatiles from complex matrices were presented [7] but with poor information about the influence on their sensoric relevance to the food investigated [8].

The first approach in determining the contribution of volatile compounds to bread flavour was made by Rothe and co-workers [9,10]. They calculated the ratio of the concentration (c) to the aroma threshold value (a) for some of the volatiles that had been detected in the crumb of wheat bread, and termed the result "aroma value", $A = c/a$. The odour thresholds (the lowest concentrations that can be sensorily recognized) were obtained by tasting the aroma compounds from aqueous solutions.

The "aroma values" (A) were replaced with the novel term "odour value" by Mulders [11] or "odour activity value" (OAV) as a more precise definition by Blank and Grosch [12]. Although the concepts of aroma and odour values have been criticized by Frijters [13], he stated that odour activity values may be used as a conclusive approximation to volatile compounds which significantly contribute to the flavour of a food.

Even if some synergistic and mutual enhancement effects of different aroma-active compounds are known, the contribution of flavour activity to the flavour impression as a whole normally depends on odour unit values of individual substances ($A \geqslant 1$).

2.2. Aroma extract dilution analysis (AEDA)

The human nose often recognizes aroma-active compounds from the GC effluent at the lowest levels that remain inaccessible even by highly sensitive detection techniques. Thus, "GC sniffing" has been developed as a simple and well appreciated method in locating positions of odorants in a gas chromatogram. However, one should realize that GC sniffing strongly depends on the amount of the food from which the flavour extract is isolated, the concentration of the compound analysed and the sample volume which is injected for GC analysis [8]. Therefore, a single GC sniffing run is completely insufficient in the sensory evaluation of flavour compounds from complex extracts. These difficulties had been overcome by sniffing of a dilution series of the original aroma extract from a particular food. Two variations of aroma extract dilution analysis (AEDA) have been developed as systematic approaches to evaluating potent odorants [14–18]. Acree and co-workers [14,15] calculated combined hedonic response measurements (CHARM) on the basis of the duration of the sensory responses which were maintained during GC sniffing of definitely

diluted flavour extracts. CHARM values correspond directly to odour activity values (OAV).

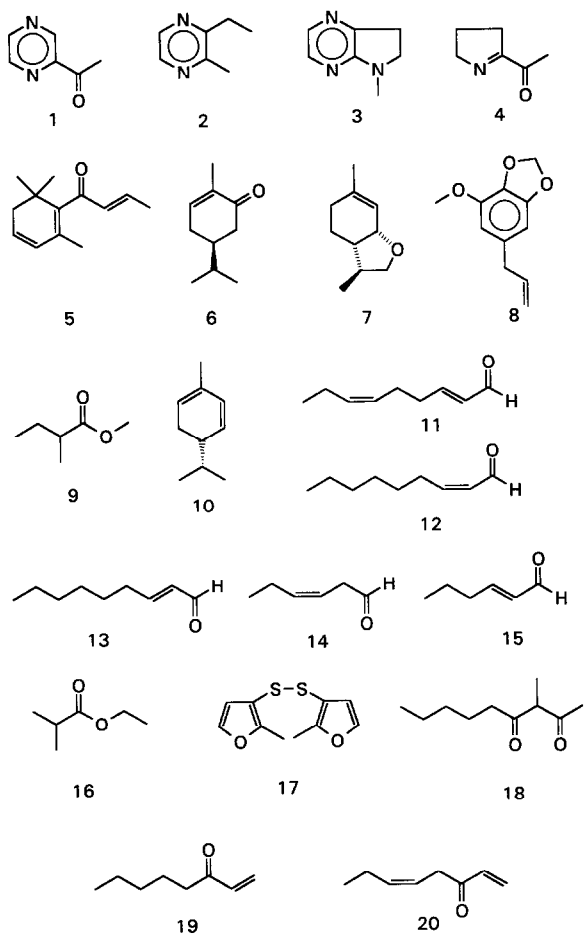
Using the method of Grosch and co-workers [16–18], the aroma extract is diluted stepwise with a solvent until the odour-active region in the GC effluent is no longer detectable. A flavour dilution factor (FD factor) has been defined as the highest dilution at which a compound is still sensorily recognized in the GC effluent. In this way, volatile compounds from an aroma extract are actually ranked according to their odour activity values (OAV).

3. STABLE ISOTOPE DILUTION ASSAY (SIDA)

Reliable quantification of character impact flavour compounds in foods can be a difficult task, because aroma-active compounds with high FD factors often occur in extremely low concentrations in foods, far below their direct analytical detection limit using cGC [19].

More or less laborious steps of enrichment by extraction and separation techniques are therefore essential before determination, and the losses caused by these manipulations have to be corrected with internal standards. However, accurate data can only be expected if the stability and the physical properties of both the compound to be analysed and the internal standards are comparable [20]. The use of stable isotope-labelled internal standards is known as the stable isotope dilution assay (SIDA) and has been widely applied for the determination of trace compounds. Apart from negligible isotope effects, the standards and odorants to be determined are also identical in chemical and physical properties. SIDA was introduced into flavour analysis by Schieberle and Grosch [20], who achieved the accurate determination of acetylpyrazine (**1**), 2-methyl-3-ethylpyrazine (**2**), 5-methyl-5*H*-cyclopenta[*b*]pyrazine (**3**) and 2-acetyl-1-pyrroline (**4**) in bread crust.

The mass spectra of **4** are shown in Fig. 1. Mass chromatography was performed with cGC, coupled to the ion trap detector, running in the chemical ionization (CI) mode. Quantitative analysis in conjunction with OAV values evaluated **4** as the most important and character impact odour compound of the wheat bread crust. It is interesting that the high level of **4** (96 μg/kg; OAV 4800) in the crusts of the wheat breads was the most striking difference between wheat and rye breads [21]. These investigations have been extended, indicating **4** as one of the characteristic roasty/malty flavour notes of fresh popcorn [22] and cooked rice [23].



With regard to the quality assessment of flavour and fragrances, the evaluation of both potent odorants [12,24,26–28] and off-flavour compounds is highly desirable [19,30,31].

SIDA measurements have been developed for the determination of β-damascenone (**5**) in foods and applied to roasted coffee, black tea, honey and beer [24]; (4*S*)-(+)-carvone (**6**) has been described as the character impact compound of dill seed aroma. Dill ether (**7**), myristicin (**8**), methyl 2-methylbutanoate (**9**) and (4*S*)-(+)-α-phellandrene (**10**) were established as the decisive compounds forming the typical odour notes of fresh dill herb [12]. The odour differences between cucumbers and musk melons have

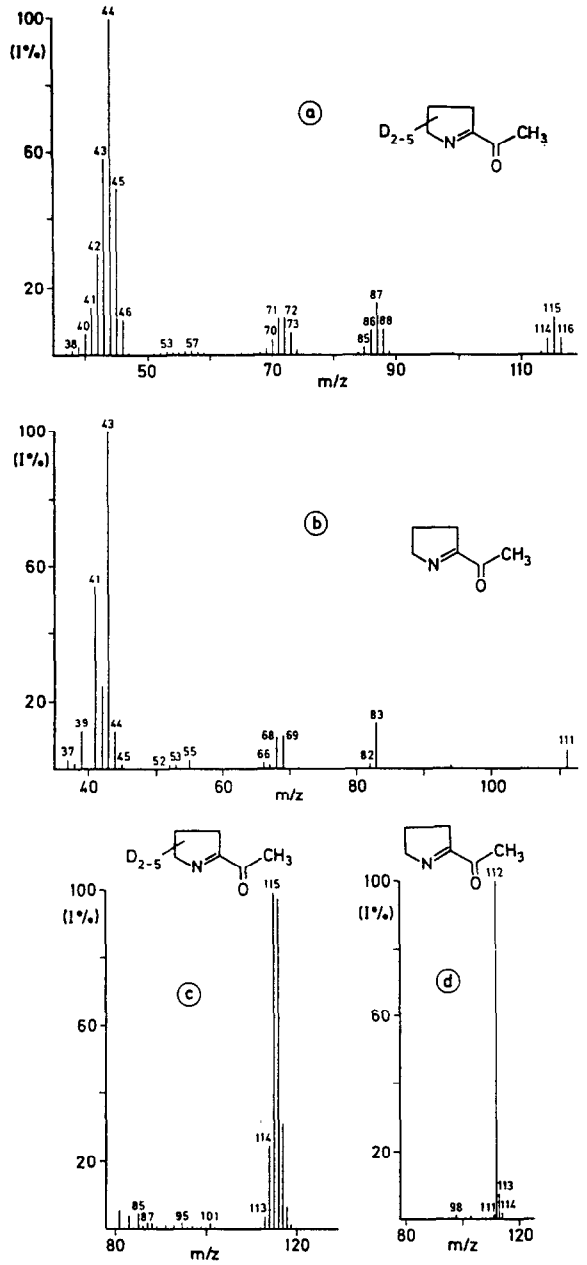


Fig. 1. Mass spectra of (a) [^{2}H]-2-acetyl-1-pyrroline (EI-MS), (b) 2-acetyl-1-pyrroline (EI-MS), (c) [^{2}H]-2-acetyl-1-pyrroline (CI-MS) and (d) 2-acetyl-1-pyrroline (CI-MS). From ref. 20.

been evaluated, identifying (*E,Z*)-2,6-nonadienal (**11**), (*Z*)-2-nonenal (**12**) and (*E*)-2-nonenal (**13**) as the most significant odorants of cucumber, whereas methyl 2-methylbutanoate (**9**), (*Z*)-3-hexenal (**14**), (*E*)-2-hexenal (**15**) and ethyl 2-methyl propanoate (**16**) were responsible for the fruity, green odour of musk melons [26]. Unsaturated alcohols and aldehydes also contribute significantly to the green odour notes of the well appreciated virgin olive oils [27] and the flavour of boiled meat is substantially influenced by sulphur-containing odorants [28].

Fig. 2 compares the EI-MS spectra of bis(2-methyl-3-furyl) disulphide (**17**) with the corresponding bis-2-[$^2H_3$]-derivative as internal standard for SIDA.

AEDA and SIDA techniques have been successfully applied to judge limiting changes in aroma quality during processing or storage of foods [30–32]. In contrast to other plant oils, soya-bean oil is known to be sensitive to daylight. In a daylight-stored sample of soya-bean oil a rapid increase in 3-methyl-2,4-nonadione (**18**) during the first 48 h has been detected [30]. Compound **18** clearly causes the light-induced off-flavour and it is generated by photooxidation of furanoid fatty acids of soya-bean oil [31].

1-Octen-3-one (**19**) and (*E*)-2-nonenal (**13**) are

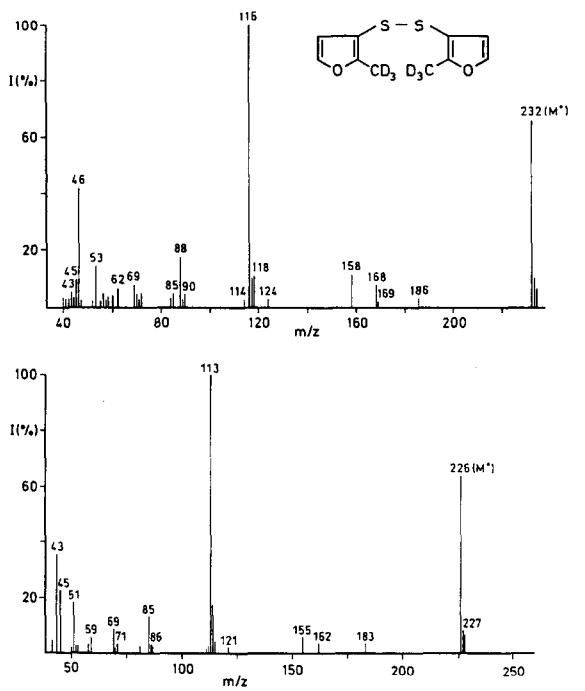


Fig. 2. EI-MS of bis(2-methyl-3-furyl) disulfide (**17**) and labelled derivative (top). From ref. 19.

known as autoxidation products of linoleic, and (*Z*)-1,5-octadien-3-one (**20**) of linolenic acid. As their concentrations may increase significantly during storage of butter oil, **19**, **13** and **20** are recommended as indicator substances for the assessment of the deterioration of butter oil caused by peroxidation [32].

4. GENUINENESS OF FLAVOURS AND FRAGRANCES

Comparative stable isotope ratio analysis (SIRA), also known as isotope ratio mass spectrometry (IRMS), has been described as an important method in the origin assessment of food compounds [49–54]. On the other hand, naturally occurring chiral flavour compounds of high optical purity should be expected, as enzymatic reactions are commonly characterized by a high degree of stereospecificity. Both phenomena, stable isotope discrimination and enantioselectivity, during biosynthesis may serve as the basis to judge the origin of flavour compounds if comprehensive data from authentic sources are available.

Chirospecific cGC methods coupled on-line with SIRA measurements have been reported as highly sophisticated methods in the authenticity control of flavours and fragrances. This is of considerable interest, as naturalness of foods and beverages is in high demand by the customer. Further, legal regulations order the differentiation of natural and non-natural flavouring substances and these methods have also been adopted into quality assurance by the flavour industry.

4.1. Chirality evaluation

Chiral discrimination has been recognized as one of the most important principles in biological activity and also odour perception [33–41]. Besides enantioselective biogenesis, the evaluation of chirality in the origin control of flavours and fragrances has to be discussed with regard to some fundamental conditions.

4.1.1. Chiral resolution and chromatographic behaviour of enantiomers

Resolution (R_s) is defined as the separation of two peaks in terms of their average peak width at half-height (eqn. 1) or at a base width of 4σ (eqn. 2) [57]:

$$R_s = 1.177\left(\frac{\Delta t_R}{w_{h1} + w_{h2}}\right) \quad (1)$$

$$R_s = 2\left(\frac{\Delta t_R}{w_{b1} + w_{b2}}\right) \quad (2)$$

where

Δt_R = absolute difference in retention time of the two peaks 1 and 2;

$w_{h1(2)}$ = width of peak 1 (2) at half-height;

$w_{b1(2)}$ = width of peak 1 (2) at base (4σ).

In the case of enantiomeric pairs, the term chiral resolution (cR_s) is used [55]. As outlined in Table 1, 100% separation occurs if chiral resolution cR_s = 2.50; in practice $cR_s \geqslant 1.50$ (99.73% separation) is defined as a baseline resolution [57]. Thus, optimum chiral resolution ($cR_s \geqslant 1.50$) should be achieved using a suitable chiral stationary phase in enantioselective cGC.

Although the separation factor α has the greatest impact on peak resolution, only in the case of highly resolved enantiomer separation is an accurate determination of high enantiomeric excess (ee values) possible, as shown in Fig. 3 [67].

With the present state of knowledge, the mechanisms of GC enantiomer separation have not been elucidated. Unusual chromatographic behaviour and reversal of the elution order of enantiomers have been observed. Consequently, the usefulness of a given chiral stationary phase and the order of elution of separated enantiomers cannot be predicted. References of definite chirality are essential to identify the separated isomers, no matter whether directly stereoanalysed with chiral stationary phases [61–66,162] or via derivatized stereoisomers [44,62, 66].

TABLE 1

SEPARATION AS A FUNCTION OF cR_s [57]

cR_s	Separation (%)	Δt_R (in σ)
0.5	68.28	2
0.9	92.82	3.6
1.0	95.44	4
1.1	97.22	4.4
1.25	98.76	5
1.50	99.73	6
2.0	99.99	8
2.50	100.00	10

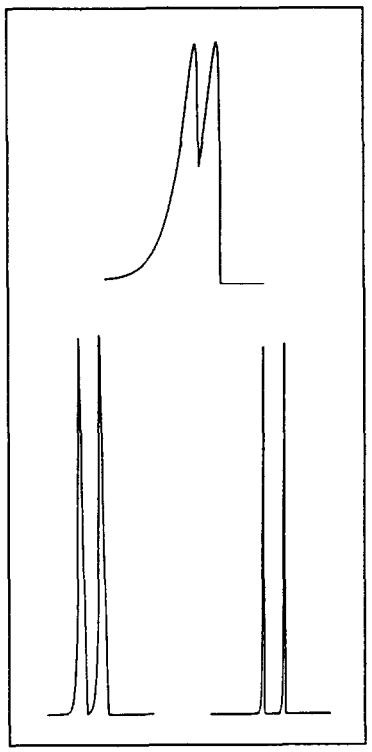


Fig. 3. Chiral separations with identical separation factors (α) and different chiral resolutions (cR_s). From ref. 67.

4.1.2. Sample clean-up

Optimum chiral resolution without any racemization and reliable interpretation of chromatographic behaviour of enantiomers have to be considered as the first targets in chirospecific analysis (section 4.1.1). Even if a universal recommendation on sample clean-up cannot be given, depending on the complexity of samples to be analysed, preseparation procedures of the highest efficiency are needed if a reliable stereodifferentiation is to be achieved.

The demanding challenge is to obtain chiral volatiles of the highest chemical purity, ready for the direct resolution into their mirror images by GC techniques. This ideal may be realized by preseparation techniques in non-chiral media (chiral discriminations excluded), using high-performance thin-layer chromatography (HPTLC) [80,81] or HPLC, off-line [69,71] or on-line [72,73] coupled with enantioselective cGC. In particular, enantioselective multi-dimensional GC (enantio-MDGC) with the combination of a non-chiral precolumn and a chiral main column [74,75] has been demonstrated as a powerful method for the direct stereoanalysis of chiral volatiles without any further clean-up or derivatization procedures [60,68,70,76–79,82–97].

A schematic diagram of enantio-MDGC, well proved in quality assurance and origin control of flavours and fragrances, is shown in Fig. 4. A double-oven system with two independent temperature controls, two flame ionization detectors (D_{M1}, D_{M2}) and a live switching coupling piece is used. With optimum pneumatic adjustment of the MDGC system, definite fractions, eluted from the precolumn, are selectively transferred on to the chiral main column (heart-cutting technique).

First the chromatographic conditions of the chiral main column must be optimized carefully. Capillary columns coated with chiral stationary phases of suitable enantioselectivities are used as main columns. Chiral resolutions are commonly achieved isothermally or by low temperature programming rates, starting at least 20°C below the precolumn temperature. Precolumns are chosen with respect to the versatility of application, to the direct injection of high sample volumes and with respect to the requisite time of analysis.

Under optimized operating conditions, uncoated and deactivated restriction capillaries are installed between the injector and precolumn by means of simple press-fit connectors to reduce the carrier gas velocity within the precolumn. By means of such a column combination, suitable pre- and main columns may be easily exchanged and adapted for optimum efficiency [76].

4.1.3. Detection systems

If optimum chiral resolution and high-efficiency sample clean-up, the first priorities in enantioselective analysis (sections 4.1.1 and 4.1.2), are realized, simple detection systems, such as flame ionization detection (FID) are suitably used. The ideal detector is universal yet selective, sensitive and structurally informative. Mass spectrometry (MS) currently provides the closest approach to this ideal [98]. The combination of multi-dimensional GC with high-resolution MS or mass-selective detectors in the single ion monitoring (SIM) mode is currently the most potent analytical tool in enantioselective analysis of chiral compounds in complex mixtures [77]. Nevertheless, it must be pointed out that the applica-

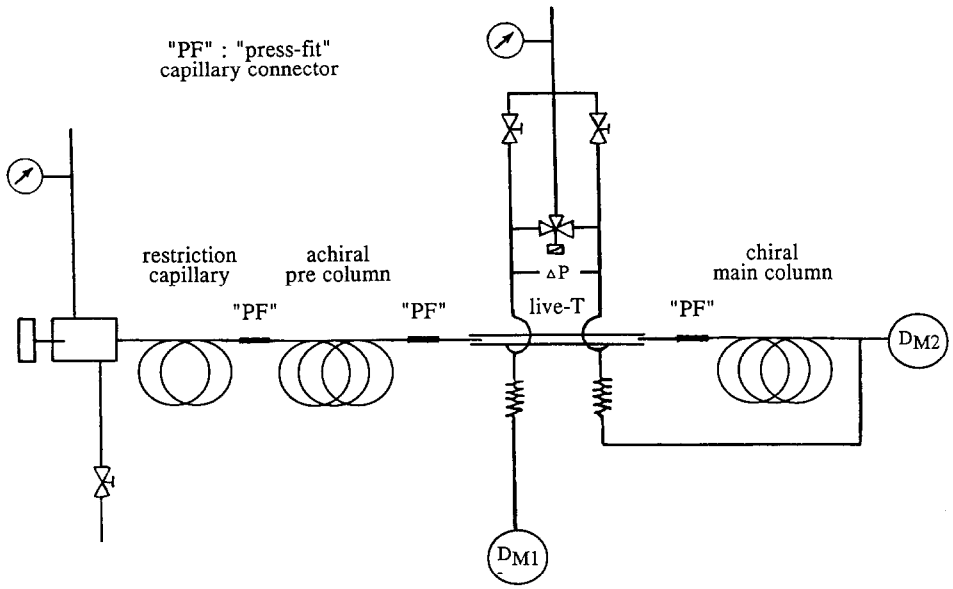


Fig. 4. Schematic diagram of enantio-MDGC according to ref. 76.

tion of structure-specific detection systems such as MS [99] or Fourier transform infrared (FT-IR) spectroscopy [100] cannot save the fundamental challenges to optimum (chiral) resolutions and effective sample clean-up.

The effectiveness of mass selective detection in the SIM (MIM) mode and selected wavelength chromatograms (SWC) in FT-IR detection depends on efficient sample clean-up. In raw extracts the risk of co-eluting substances which cannot be distinguished from chiral volatiles increases with the complexity of flavour and fragrance extracts to be analysed. Hence the direct use of MS (FT-IR) might be seriously limited and the use of such detection techniques in enantioselective cGC without effective preseparations cannot be recommended.

4.1.4. Stereodifferentiation and quantification

The total amount of characteristic flavour constituents and their relative distribution in aroma extracts have been included in official legal assessments for many years, using internal standards for quantification and referring to the merits of comprehensive stock-taking of volatile constituents from complex flavour and fragrance extracts during the first era of flavour research [7]. The measurements are precise and reproducible, but with respect to the validation of flavours and the origin of essential oils their utility is limited [68].

Chirospecific differentiations of optically and sensorially active compounds point out new possibilities in structure–function relationships and also the biogenesis of chiral volatiles [68]. Chiral fruit flavour compounds have been detected with characteristic enantiomeric ratios, defined as fruit-specific enantiomeric distributions [69].

Enantiomeric purity and enantiomeric excess (ee) are the usual terms used in the determination of enantiomers. Enantiomeric purity is defined as the measured ratio (expressed as a percentage) of the detected enantiomers, whereas ee values describe the relative difference of the separated enantiomers (expressed as a percentage). Usually quantifications are given in terms of ee values, but one should note that convincing results can be concluded only for baseline-resolved enantiomers ($cR_s \geqslant 1.50$). Exact calculations of partially resolved mirror images, as frequently occur in the current literature, remain unintelligible in view of significant differences in sensory qualities and odour thresholds of enantiomers [33–41,58–60]. Partially separated enantiomeric pairs ($cR_s = 0.9$–1.50) should be calculated approximatively as the ratio of enantiomers (expressed as a percentage). Approximative quantifica-

tions in terms of enantiomeric ratios are also more conclusive than ee values if concentrations are too low for precise calculations [70].

4.1.5. Limitations

Three types of limitations have to be accepted in chirospecific analysis: racemates of natural origin, generated by non-enzymatic reactions (autoxidation, photooxidation, etc.); racemization during processing or storage of foodstuffs, if structural features of chiral compounds are sensitive; and blending of natural and synthetic chiral flavour compounds. Nevertheless, the systematic evaluation of origin-specific enantiomeric ratios has proved to be a valuable criterion for differentiating natural flavour compounds from those of synthetic origin.

4.1.6. Analysis of individual classes of compounds

The first success in chirospecific flavour analysis was achieved by chromatographic separations of diastereomeric derivatives. In spite of limited sensitivity and frequently laborious work-up conditions, these methods revealed a reliable insight into the enantiomeric distribution of $\gamma(\delta)$-lactones and other chiral fruit flavour compounds [42–48], as reviewed previously [101].

A real breakthrough in chirospecific analysis occurred when enantioselective cGC became available. Three classes of chiral stationary phases are of special interest: amino acid derivatives, chemically bonded to polysiloxanes [102,103]; optically active metal chelates [104]; and modified cyclodextrins [63,67,105–110,162–165]. In particular, since 1988 selectively alkylated/acylated α-, β- and γ-cyclodextrins have been synthesized, serving as chiral stationary phases in enantioselective cGC.

Owing to the high melting points of pure cyclodextrins and many of their derivatives, the chromatographic efficiency of such chiral stationary phases was poor. Improved performance was achieved by König and co-workers [63] and Armstrong *et al.* [107], who introduced cyclodextrin derivatives which are liquid or waxy at room temperature. As an alternative approach, Schurig and co-workers [106,164,165] and Bicchi *et al.* [25] diluted high-melting cyclodextrin derivatives with polysiloxane stationary phases to obtain chiral selectivity below the melting point of the pure cyclodextrin. Dilution of high-melting cyclodextrin derivatives lowers the minimum GC working temperature, but chiral resolution decreases with increasing dilution of the cyclodextrin. In order to prepare columns with maximum chiral selectivity, dilution should be minimal, as demonstrated by Schmarr *et al.* [56].

Although the mechanisms of chiral recognition and molecular inclusion have not been elucidated, modified cyclodextrins have been reported as versatile chiral phases with a wide range of applications. The chiral resolution of many cyclic monoterpenes [67,107,124,127] and investigations of the enantiomeric distribution of α-pinene, β-pinene and limonene from foods, drugs and essential oils have been reported [68,83–85,87]. Also *cis–trans*-α-irones, highly responsible fragrance compounds of iris oil [114,162], filbertone [(*E*)-5-methyl-2-hepten-4-one], the character impact compound of hazel-nuts [163], damascone [151] and geosmin [114] have been separated into their mirror images. Even polar molecules such as 1,2-ketols, 1,2-glycols and free carboxylic acids were successfully stereoanalysed by Mosandl *et al.* [66].

1,3-Oxathiane derivatives. 3-Methylthiohexanol and *cis–trans*-2-methyl-4-propyl-1,3-oxathiane have been reported by Winter *et al.* [111] to play important roles in the delicate flavour of the yellow passion fruit, in spite of their occurrence at extreme trace levels. Using complexation GC, the chirospecific analysis of all four stereoisomers of 2-methyl-4-propyl-1,3-oxathiane was achieved. This was the first direct stereochemical analysis of an essential chiral trace compound from fruit flavours [61]. While first attempts at the chirality evaluation of this potent odorant from yellow passion fruits were described [112], the direct chirospecific analysis of natural 1,3-oxathianes is further under investigation [113]. Modified cyclodextrins [110,114], using enantioselective cGC, serve as promising alternatives (Fig. 5).

γ-Lactones. Chiral γ-lactones are important compounds of many fruits and give strawberries, peaches, apricots and many other fruits their characteristic and distinctive notes [116]. Recently Albrecht and Tressl [117] investigated the biogenetic sequence of γ-decalactone. These results indicate that (*E*)-3,4-epoxydecanoic acid, formed from (*E*)-3-decenoyl-CoA, an intermediate of the β-oxidation of linoleic acid, is the genuine precursor in the biosynthesis of γ-decalactone (Fig. 6.).

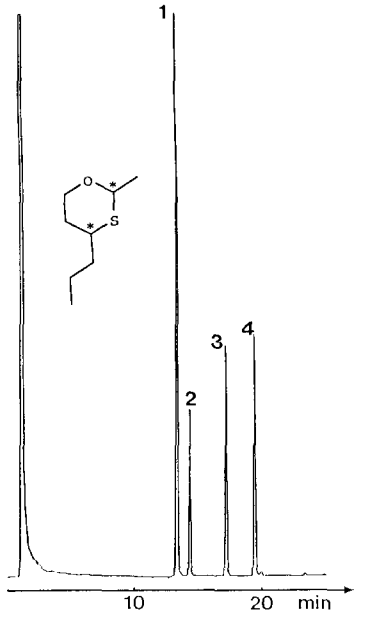


Fig. 5. Chirospecific differentiation of 2-methyl-4-propyl-1,3-oxathiane. Elution order: (1) 2*R*, 4*S* (enriched); (2) 2*S*, 4*R*; (3) 2*R*, 4*R*; (4) 2*S*, 4*S*. From ref. 110.

Owing to their common enzymatic pathways, chiral aroma compounds from fruits and other natural sources should be characterized by origin-specific enantiomeric ratios. Indeed, from freshly harvested strawberries γ-decalactone and γ-dodecalactone have been detected with high optical purity favouring the 4*R*-configured γ-lactones: γ-C_{10} (4*R*; >98% ee), γ-C_{12} (4*R*; >99% ee) [76]. On the other hand, racemic γ-decalactone in aroma-relevant amounts has not yet been observed in fruits. Thus, the detection of racemic γ-decalactone in fruit-containing food indicates the addition of nature-identical γ-C_{10}-lactone. Furthermore, enantioselective MDGC, employing heart-cutting techniques from DB-1701 as the preseparation column on to heptakis (3-O-acetyl-2,6-di-O-pentyl)-β-cyclodextrin as the chiral main column, was described by Mosandl *et al.* [76] as a powerful tool in the direct enantiomer separation of chiral γ-lactones from complex matrices without any further clean-up or derivatization procedures. Comprehensive data on natural γ-lactones from fruits have been reported by Nitz *et al.*

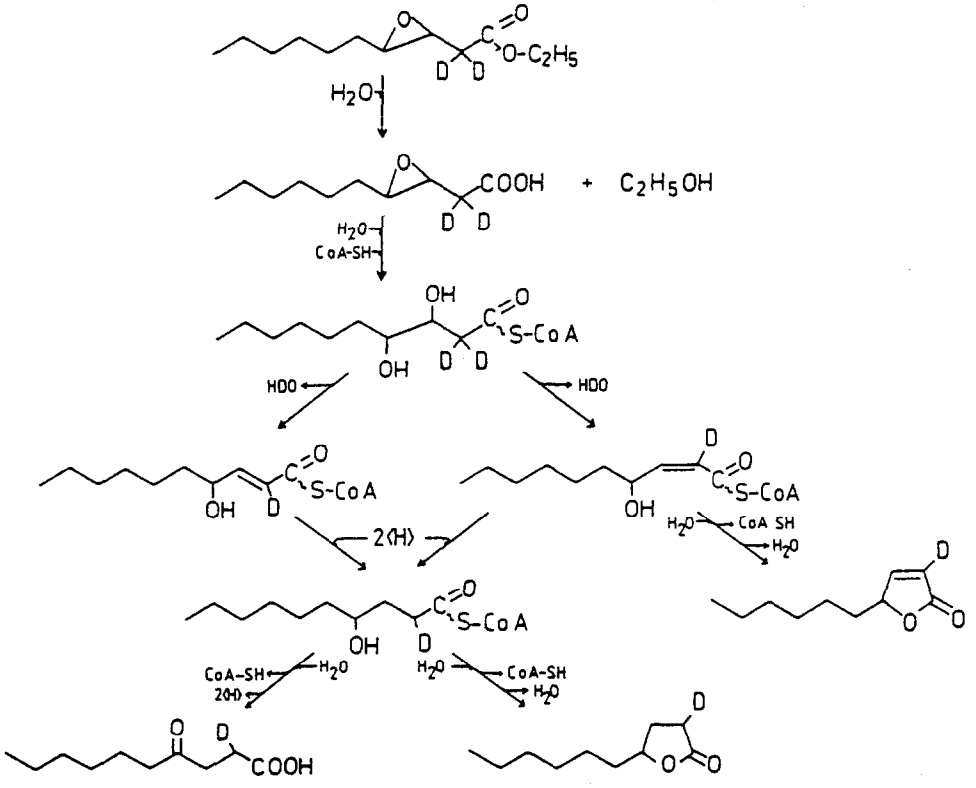


Fig. 6. Proposed transformation of ethyl (*E*)-3,4-epoxydecanoate by cells of *S. odorus*. From ref. 117.

[77], Bernreuther *et al.* [78] and Guichard *et al.* [79] (Fig. 7).

Comparative investigations of γ-lactones from apricots of different varieties and cultivars [118] in connection with corresponding enantioselective analysis [79] indicate some important conclusions: if present at all, odd numbered γ-lactones occur only in trace amounts; the most abundant γ-lactones are the even-numbered homologues γ-C_6, γ-C_8, γ-C_{10} and γ-C_{12}; and the enantiomeric distribution of chiral γ-lactone homologues in fruits has been demonstrated to increase in favour of the 4*R*-configured lactones with increasing length of the alkyl-side chain [77–79,96].

In spite of relatively large amounts of the lowest homologue, γ-C_6 is useless for the origin assessment of flavours, owing to the wide range of ee values detected. A high odour threshold ($a = 13\ 000$ μg/kg) [119] and unspecific odour quality [40] also revealed γ-hexalactone as insignificant to the odour impression of apricots [79]. It is also interesting to note the detection of dihydroactinidiolide as the first γ-lactone racemate of natural origin, generated by photooxidation in ripening apricots [79]. This surprising fact has also been confirmed for dihydroactinidiolide from raspberries [114].

With regard to chirality evaluation as an indicator for the genuineness of natural flavours and fragrances, only chiral volatiles of high optical purity and with characteristic and small ranges of ee values should be validated in relation to their total amounts. Under these conditions, the even-numbered γ-lactones C_8 ($a = 95$ μg/kg), C_{10} ($a = 88$ μg/kg) and C_{12} [119], in particular γ-decalactone, have proved to be useful indicators of naturalness for many fruit flavours and fragrances if their genuine amount occurs in the aroma relevant range (OAV > 1). Of course, chiral compounds occurring in trace amounts and far below their odour activity values in the foods investigated, *e.g.*, γ-decalactone in raspberries (2 μg/kg) and gooseberries [120], have to be neglected.

In this context, it should be mentioned that γ-lactones do not occur as genuine constituents of coconuts [121]. Thus, it seems to be clear that aroma-relevant amounts of γ-lactones in coconut products are of non-natural origin and there is no reason to look for their stereoanalysis in the low-ppb range [120]. On the other hand, chiral δ-lactones from coconuts, in particular δ-octalactone and δ-decalactone, have been evaluated as suitable indicators of naturalness [86].

δ-Lactones. Likewise, chiral δ-lactones are known as characteristic flavour compounds of fruits and dairy products [116]. Their stereodifferentiation was achieved with modified γ-cyclodextrin by König *et*

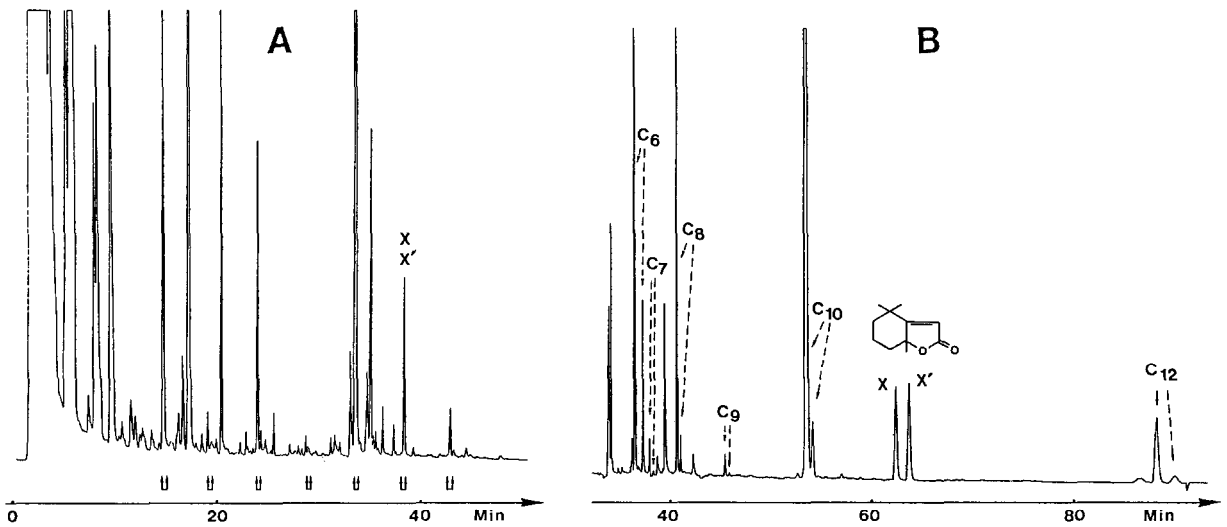


Fig. 7. (A) Raw apricot flavour extract, preseparated with DB-1701. (B) Chirality ealuation of γ-lactones from apricots, using modified β-cyclodextrin. From ref. 79.

al. [122] and their chromatographic behaviour interpreted by co-injection with optically pure references, as described by Palm *et al.* [86]. Using enantioselective MDGC and the column combination OV-1701–octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin, the simultaneous stereodifferentiation of all aroma relevant 4(5)-alkyl-substituted γ(δ)-lactones has been reported recently (Fig. 8) [92]. Only the less abundant lactone enantiomers (4*S*)-γ-heptalactone and (5*S*)-δ-hexalactone co-elute. If necessary, γ-heptalactone or δ-hexalactone can be analysed alternatively.

Peaches, apricots and greengages contain γ(δ)-lactones with characteristic enantiomeric ratios in favour of *R*-configured lactones. Whereas aroma-relevant amounts of γ-lactones from raspberries are not detectable, their δ-C_8, δ-C_{10}-lactones are optically pure *S*-enantiomers [92]. In cheddar cheese aroma the optical purity of (5*R*)-δ-lactones (C_{10}–C_{14}) increases with increasing side-chain length [114]. The enantioselective analysis of massoilactone (dec-2-en-5-olide), a coconut-typical δ-lactone of natural origin, has been reported by Bernreuther *et al.* [97] and Werkhoff *et al.* [114], revealing (−)-massoilactone of high optical purity (5*R*; >99% ee).

Alkan(alken)-2-yl acetates. Using enantioselective MDGC, the enantiomeric distribution of alkan (alken)-2-yl acetates from banana flavour was achieved. After simple acetylation their corresponding alcohols 2-pentanol (**1**), 2-hexanol (**2**), 2-heptanol (**3**) and (*Z*)-4-hepten-2-ol (**4**) are also exactly stereodifferentiated by this method. The main column chromatogram of these esters from a genuine banana flavour extract is shown in Fig. 9, indicating *S*-configured esters of high ee values [91].

The decreased optical purity of 2-pentanol and (*Z*)-4-hepten-2-ol from fully ripe bananas [91], also reported by Fröhlich *et al.* [123], is suggested to be caused by competitive enzyme activities during the latest stage of ripening but is not finally revealed as yet. Further studies, including enantio-MDGC and SIRA measurements, are in hand [124].

2-Alkylcarbonic acids (esters). 2-Alkylcarbonic acids have been separated into their enantiomers without any derivatization and their sequence of elution was assigned by co-injection with optically pure references [66]. Latest results on stereospecific flavour evaluation revealed characteristic sensory properties for all the enantiomers of 2-alkyl-branched acids, esters and corresponding alcohols. Tremendous differences between the mirror images of 2-methylbutanoic acid have been found. While

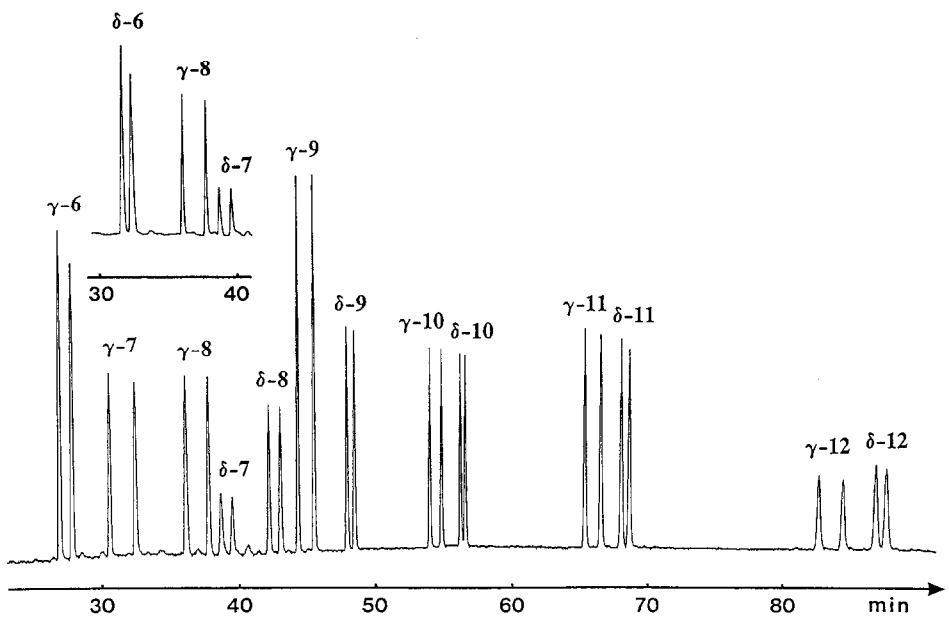


Fig. 8. Simultaneous stereodifferentiation of chiral γ(δ)-lactones using enantio-MDGC. From ref. 92.

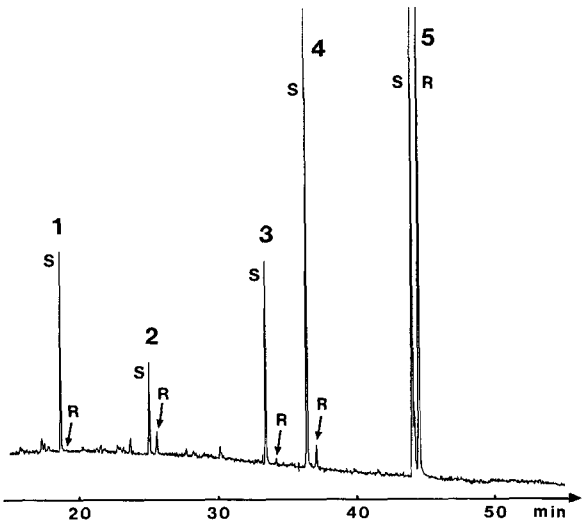


Fig. 9. *S*-Configured esters of (1) 2-pentanol, (2) 2-hexanol, (3) 2-heptanol and (4) (*Z*)-4-hepten-2-ol from a genuine banana flavour extract; (5) (*Z*)-4-hepten-2-yl-propionate, internal standard. From ref. 91.

the *R*-enantiomer exhibits a penetrating, cheesy-sweaty odour, the *S*-enantiomer emits a pleasant sweet and fine fruity note [125].

From the analytical point of view, it is worth noting the biogenetic pathway of 2-methylbutanoic acid starting from isoleucine [(2*S*)-amino-(3*S*)-methylpentanoic acid]. The *S*-configuration of the precursor is expected to remain, but also enzymatic racemization (by enolization of the intermediate 2-oxo-3-methylpentanoic acid) is known from the literature [126].

Appropriate analytical techniques without any racemization were developed and applied to apples and apple-containing foods. In all the investigated foods the (*S*)-enantiomer has been identified with high optical purity. Thus, the addition of nature-identical, synthetic 2-methylbutanoic acid racemate is easily detected [89,128].

The direct (but not baseline resolved) stereodifferentiation of ethyl 2-methylbutanoate, a well known impact flavour compound of the apple aroma, was reported simultaneously by Takeoka *et al.* [127] and Mosandl *et al.* [66]. Meanwhile further enantioselective procedures have been developed, indicating nearly optically pure (*S*)-ethyl 2-methylbutanoate as the unique antipode from natural flavours [89,128] and its impressive and pleasant apple note at extreme dilution has been recognized recently [125]. As the latest advance in this field the first simultaneous stereoanalysis of 2-methylbutanoic acid, its methyl (**1**) and ethyl (**2**) esters and its corresponding alcohol 2-methylbutanol (**3**) was realized, using selectivity-adjusted enantio-MDGC with perethylated β-cyclodextrin as the chiral stationary phase. All four chiral compounds were detected as optically pure *S*-enantiomers from apples. The application of this technique to a commercially available apple flavour concentrate reveals the adulteration by means of methyl (**1**) and ethyl ester (**2**) racemates (Fig. 10) [95].

Theaspirane stereoisomers. Theaspirane (2,6,10,10-tetramethyl-1-oxaspiro [4.5]dec-6-ene) has been found as a mixture of diastereomers in black tea [130], vanilla [131], raspberries [132,166], grapes and guavas [133,167] and the yellow passion fruit [134]. Using enantioselective analysis of synthetic theaspirane with permethylated β-cyclodextrin, both stereoisomers are resolved into racemic pairs of diastereomers [129]. Although the absolute configurations of theaspirane stereoisomers have not been elucidated so far, their optical activities were defined by polarimetric detection. It is interesting to note the preponderance of dextrorotatory diastereomers in osmanthus oil, whereas the levorotatory

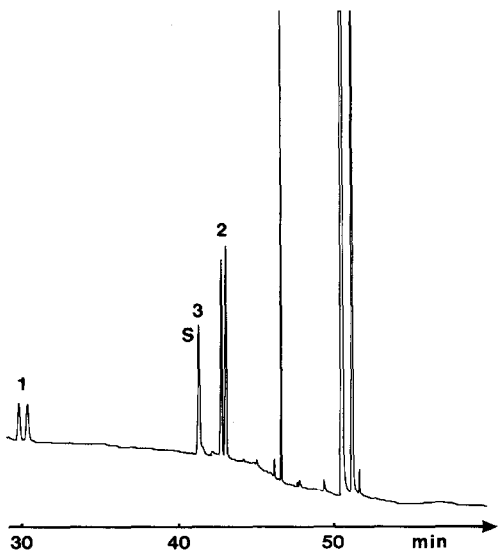


Fig. 10. Enantio-MDGC analysis of an apple flavour concentrate, adulterated by synthetic racemates of (1) methyl and (2) ethyl 2-methylbutanoate. From ref. 95.

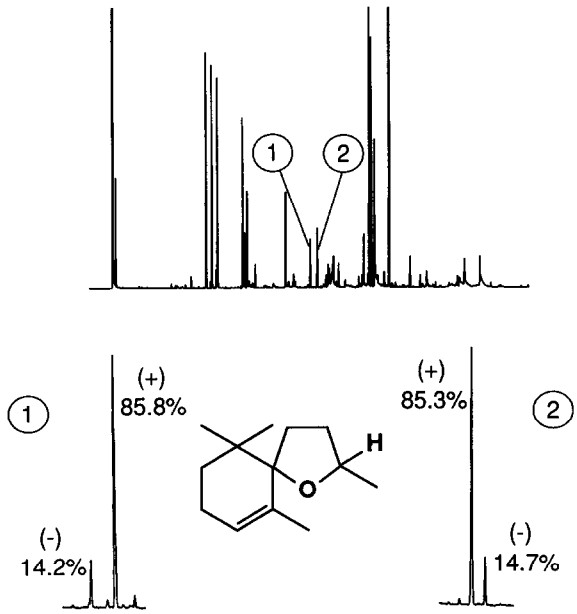


Fig. 11. Enantioselective analysis of theaspirane stereoisomers from *Osmanthus* oil. Top: achiral separation phase, 60 m × 0.32 mm I.D. DB-1 (0.25 μm); peak 1 separated on a chiral phase, see bottom left. Bottom right: the same for peak 2 from the achiral phase. Chiral separation phase: 25 m × 0.25 mm I.D. heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin. Bottom centre: structure of theaspirane. From ref. 114.

enantiomers predominate in authentic raspberry flavour (Fig. 11) [114].

Borneol, bornyl acetate, isoborneol. With respect to their natural origin, it seems to be reasonable to evaluate systematically chiral monoterpenes and derivatives. Borneol, bornyl acetate and isoborneol are well known and appreciated bicyclic monoterpenes that occur abundantly in nature, in particular in the essential oils of *Pinus* species.

The less expensive method of high-performance thin-layer chromatography (HPTLC) and subsequent extraction of the areas of interest has been successfully applied for the direct stereodifferentiation of borneol, bornyl acetate and isoborneol by one-dimensional cGC on permethylated β-cyclodextrin phase (Fig. 12). The method is recommended as a low-cost alternative and applicable with high accuracy and precision if definitive work-up conditions are observed, including (off-line) MS detection control [81].

The chromatographic behaviour of all the investigated 2-bornane derivatives was unambiguously assigned, referring to the enantiomers of camphor, which are preferably reduced by $LiAlH_4$ to the corresponding isoborneols (93%) of definite chirali-

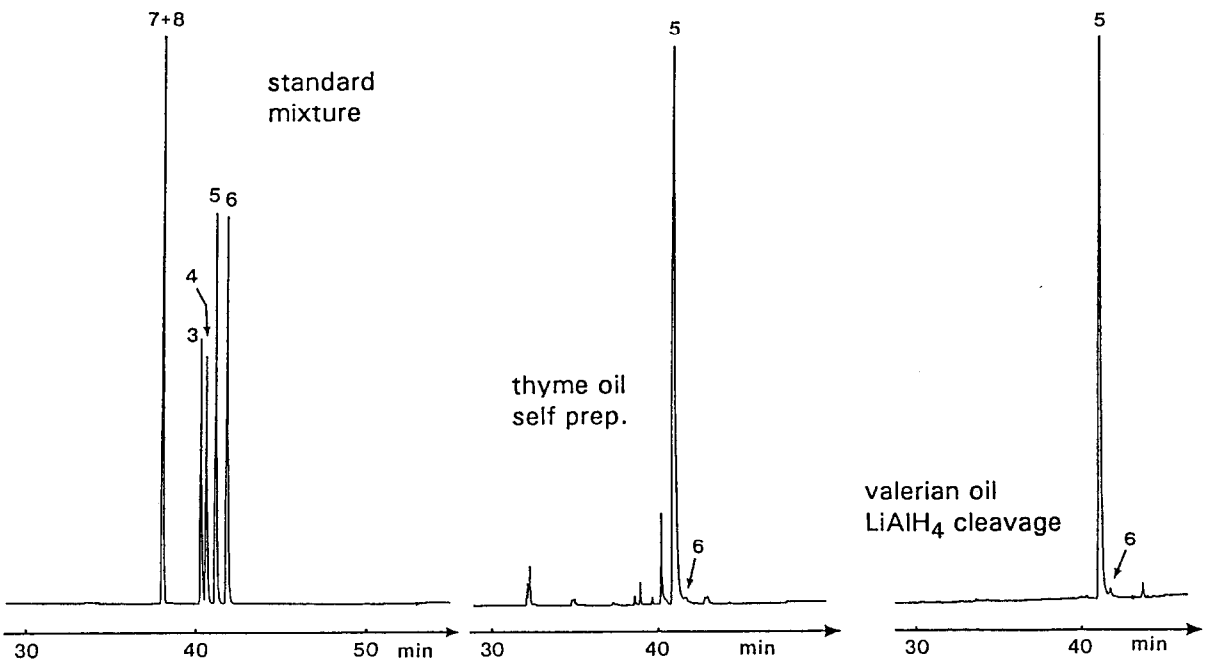


Fig. 12. Chirospecific analysis of (3,4) isoborneol, (5,6) borneol and (7,8) bornyl acetate [(−)-isomer first in each instance], with (−)-borneol (5) from thyme oil and valerian oil (after hydrolysis). From ref. 81.

ty (**3**,**4**). On the other hand, both bornyl acetates (**7**, **8**) are transferred to the corresponding borneols without any change in chirality by reductive ester cleavage.

Menthol, menthyl acetate, menthone, isomenthone. The essential oils of *Mentha* species are known to be valuable ingredients of pharmaceutical and cosmetic preparations. Menthone (**1**), isomenthone (**2**), menthol (**3**) and menthyl acetate (**4**), the most important *Mentha* oil constituents, are defined by their amounts as substantial parameters of *Mentha* oil quality. Racemic menthyl acetate was detected by Kreis *et al.* [80] as a frequently used adulterant of *Mentha* oils.

Two-dimensional GC in the direct enantiomer separation of **1**–**3** with $Ni(HFC)_2$ as the chiral main column was reported by Bicchi and Pisciotta [135]. Werkhoff *et al.* [114] isolated **1**–**4** from peppermint oils before stereoanalysis with permethylated β-cyclodextrin.

The simultaneous optical resolution of **1**–**4** has already been achieved using a combination of three different columns coated with modified cyclodextrins. All four enantiomeric pairs of **1**–**4** were directly stereoanalysed. Appropriate dilutions of mint and peppermint oils were analysed by a single chromatographic run and without any preseparation [145] (Fig. 13).

Linalyl acetate. Complexation GC with $Ni(HFC)_2$ as the chiral stationary phase [104] was found to be a convenient and effective method for stereoanalysing 1-octen-3-yl acetate in addition to linalyl acetate, the most important component of lavender oil [82, 135]. (*R*)-(−)-Linalyl acetate has to be considered as an indicator of the genuineness of lavender oil, as the optical purity of linalyl acetate is not influenced by the variety of lavender, and it is also independent from work-up and storage conditions [82].

Linalool. Linalool(3,7-dimethyl-1,6-octadien-3-ol) is widespread in plants and fruits and is one of the most frequently used compounds in flowery fragrance compositions. As linalool is an important intermediate in vitamin E synthesis, several large-scale processes for its production have been developed [136]. The stereo- and origin-specific analysis of linalool is of fundamental interest.

Perpentylated β-cyclodextrin [67,137] and the perethylated derivative [93] have been synthesized and applied successfully in the enantioselective MDGC of linalool from essential oils and fruits

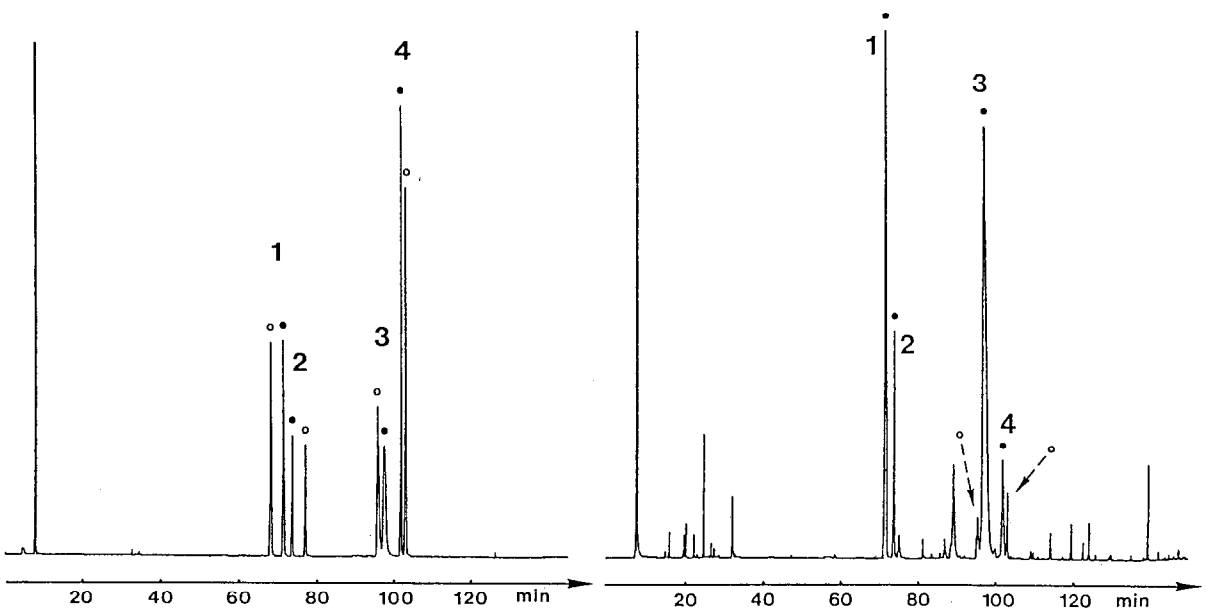


Fig. 13. Simultaneous optical resolution of (1) menthone, (2) isomenthone, (3) menthol and (4) menthyl acetate. Left, standard mixture; right, adulterated mint oil. (●) Genuine constituents of mentha oils; (○) enantiomers not detectable in mentha oils. From ref. 145.

[94,114,138]. However, referring to a proposal of Rapp and Mandery [139], the reversible hydration of linalool to geraniol or nerol in acidic media should result in an equilibration of linalool's optical activity to yield racemates. Indeed, linalool, α-terpineol and linalool oxides (fur.) were detected as racemates from a 3-year-old bottle of matured white wine [93]. On the other hand, comparing the enantiomer distributions of linalyl acetate and linalool from lavender species, high optical purities in favour of the (*R*)-(−)-enantiomers were found in laboratory-prepared diethyl ether extracts and in officinal steam distillates of lavender. However, in the case of prolonged times of hydrodistillation (≥1 h), increasing amounts of (*S*)-(+)-linalool (5–15%) are detectable. The genuine main component of lavender oil, (*R*)-(−)-linalyl acetate, is hydrolysed in considerable amounts during hydrodistillation, but nevertheless the absolute configuration of the ester remains unchanged [140,168]. Hence, enantioselective analysis of linalool, α-terpineol and linalool oxides (fur.) will not conclusively reflect their genuine enantiomeric ratios in any case, as the chiral stability of linalool (and its cyclic derivatives) obviously depends on the stage of fruit ripeness, pH value of plant materials and technological influences.

1-Octen-3-ol. 1-Octen-3-ol is one of the character-impact flavour compounds of mushrooms [141] and is formed by enzymic oxidative breakdown of linoleic acid [142]. (*R*)-(−)-1-octen-3-ol is responsible for the fruity soft odour, in accordance with the genuine mushroom note [38]. High ee values in favour of the (*R*)-(−)-antipode have been identified in mushrooms via diastereomeric esters with Mosher's acid [42], (*S*)-O-acetyllactic acid [143] and by enantiomeric resolution of 1-octen-3-yl-acetate [101].

As shown in Fig. 14, the direct and simultaneous stereodifferentiation of 1-octen-3-ol, 3-octanol and linalool is available as a valuable tool in the enantioselective analysis of many flavours and fragrances [110].

Nerolidol. Nerolidol, a sesquiterpenoic alcohol, analogous in structure to linalool, is a valuable base note in perfumery, owing to its long-lasting and moderate floral odour. Recently nerolidol was separated into its four stereoisomers via MPLC of the diastereomeric (1*S*,4*R*)-camphanoates and subsequent ester hydrolysis. Distinct sensory properties of all stereoisomers and a chirospecific cGC method to resolve the enantiomeric pairs of (*Z*)- and (*E*)-nerolidol were described (Fig. 15) [144].

8-Mercapto-p-menthan-3-one. Owing to their characteristic minty–fruity notes, reminiscent of blackcurrants, buchu leaf oils are well appreciated in the composition of flavours and fragrances. 8-Mercapto-*p*-menthan-3-one and its thiolacetate have been described by Sundt *et al.* [158] and independently by Lamparsky and Schudel [159] as impact flavour compounds of cassis flavours. They have not so far been identified in blackcurrant. All four stereoisomers of 8-mercapto-*p*-menthan-3-one exhibit distinct and characteristic sensory impressions and their first chirospecific analysis was described by Köpke and Mosandl [29] (Fig. 16).

Rose oxides. Rose and geranium oils are precious natural products in fine perfumery. The diastereomers of rose oxides are known to be characteristic chiral constituents and attributed to the distinct bloomy-green top notes of rose and geranium oils. The first enantiomeric separation of both *cis* and *trans* rose oxides using complexation GC was published by Schurig [160]. Werkhoff *et al.* [114] reported on the chirospecific analysis of *cis* and *trans* rose oxides from geranium oil after preseparation by preparative cGC and alternative chirospecific analysis of *cis–trans* diastereomers, using two different modified γ-cyclodextrins.

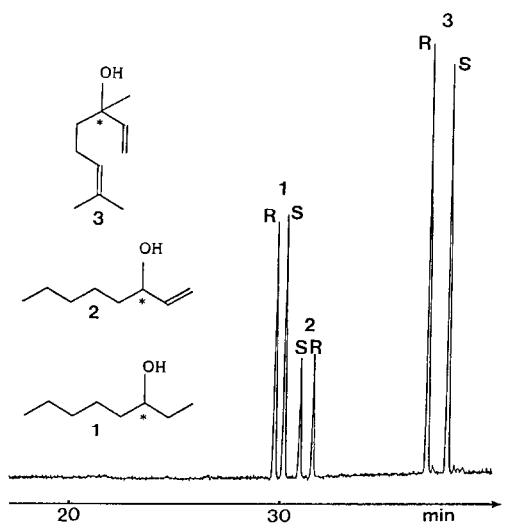


Fig. 14. Chirospecific differentiation of (1) octanol, (2) 1-octen-3-ol and (3) linalool. From ref. 110.

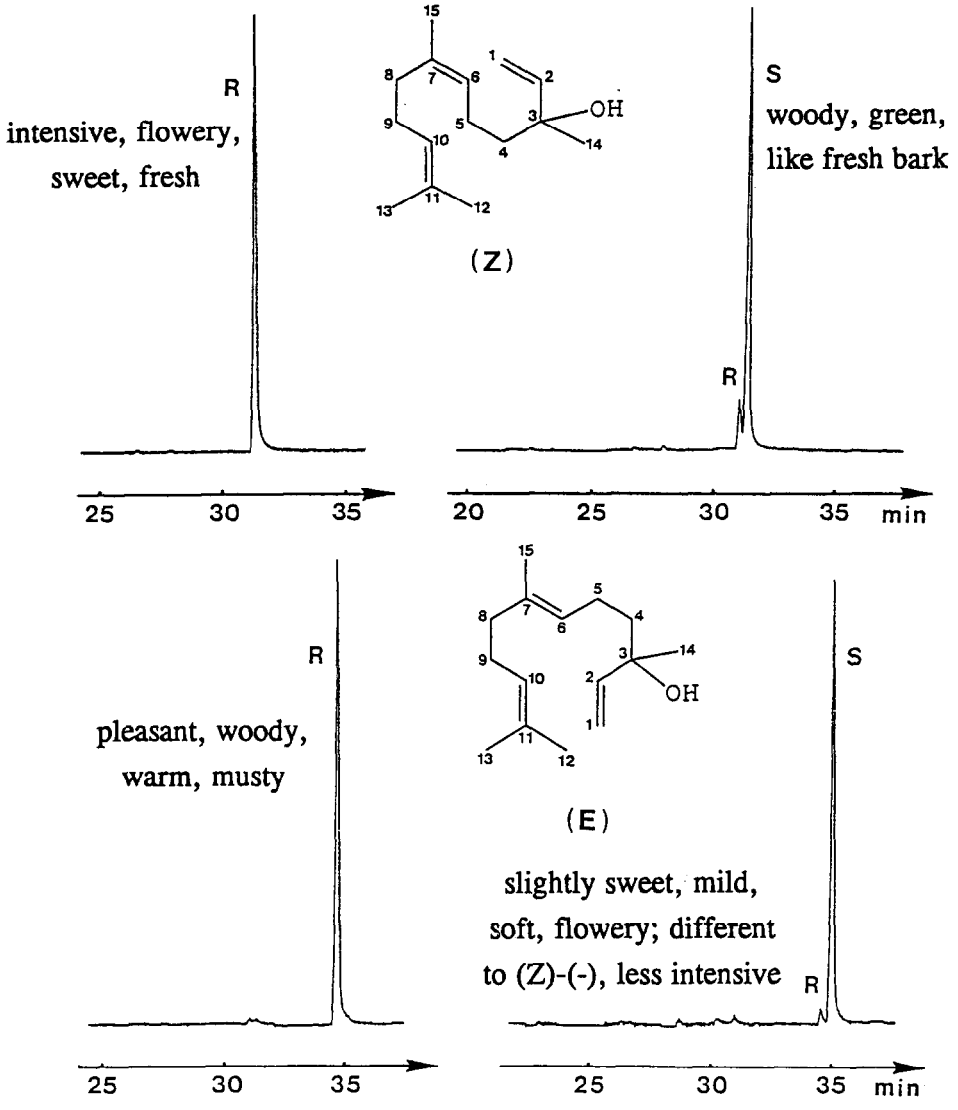


Fig. 15. Sensory description of nerolidol stereoisomers and enantiomeric purity control using heptakis(2,3,6-tri-O-methylhydroxypropyl)-β-cyclodextrin. From ref. 144.

2,3-Di-O-acylated cyclodextrin derivatives were introduced as a new type of chiral stationary phases in cGC [109]. Heptakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin is reported as a versatile stationary phase for the simultaneous stereodifferentiation of a wide range of chiral volatiles with different functionalities. The simultaneous chiral resolution of *cis–trans* rose oxides, citronellol and linalool, the most important chiral compounds of rose and geranium oils, including the complete interpretation of the chromatographic behaviour of all stereoisomers investigated, is the latest result in this field, reported by Dietrich *et al.* [110] (Fig. 17). Although there are tremendous difficulties in obtaining genuine rose or geranium oils, their authenticity control by enantio-MDGC has been achieved [140,168].

3-(2H)-Furanones. Cyclopentenolones with a

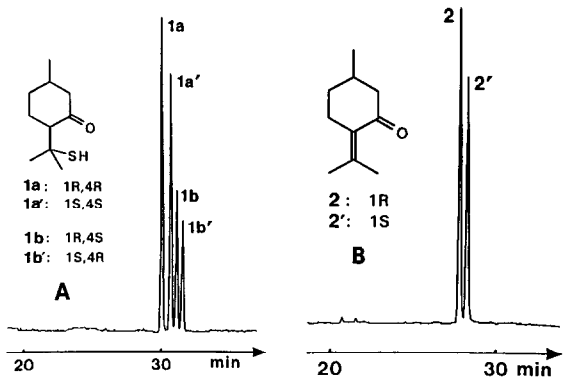


Fig. 16. Chirospecific analysis of (A) 8-mercapto-*p*-menthan-3-one and (B) pulegone on permethylated *β*-cyclodextrin phase. From ref. 29.

planar vicinal enol–oxo configuration are known to be powerful aroma-active substances with distinct caramel notes. By methylation of the enolic function, this flavour impression is changed drastically to a sweet, mildew and mouldy odour in the case of 2,5-dimethyl-4-methoxy-3-(2*H*)-furanone (**2**). This so-called "mesifurane" and also "pineapple ketone" (**1**) were stereodifferentiated with modified cyclodextrin [154]. Although (**1**) and (**2**) can be stereoanalysed without any racemization, both compounds were detected in strawberries, pineapples, grapes and wines as racemates (Fig. 18).

Obviously optically active furanones are equilibrated by keto–enol tautomerism in acidic media. Thus, enantioselective analysis cannot be applied to assign the origin of chiral 3-(2*H*)-furanones [108]. This can only be expected by stable isotope measurements [151].

4.2. Stable isotope ratio mass spectrometry (IRMS)

4.2.1. cGC–IRMS

The elucidation of stable isotope distributions is highly desirable with respect to fundamental studies in biochemistry, in nutrition and drug research and in the origin assignment and authenticity control of flavours and fragrances. cGC coupled on-line via a combustion interface with isotope ratio mass spectrometry (IRMS) has been realized successfully in the case of $\delta^{13}C$ determinations [146]. The substances eluting from the cGC column are converted into carbon dioxide in a combustion oven and then directly analysed in the isotope mass spectrometer, adjusted for the simultaneous recording of masses 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$; $^{12}C^{16}O^{17}O$) and 46 ($^{12}C^{16}O^{18}O$) in the nmol range and with high precision $\leqslant 0.3‰$).

Owing to the possible ion–molecule reaction between H_2O and CO_2, HCO_2^+ ions may be generated, simulating the existence of $^{13}CO_2$. Hence the

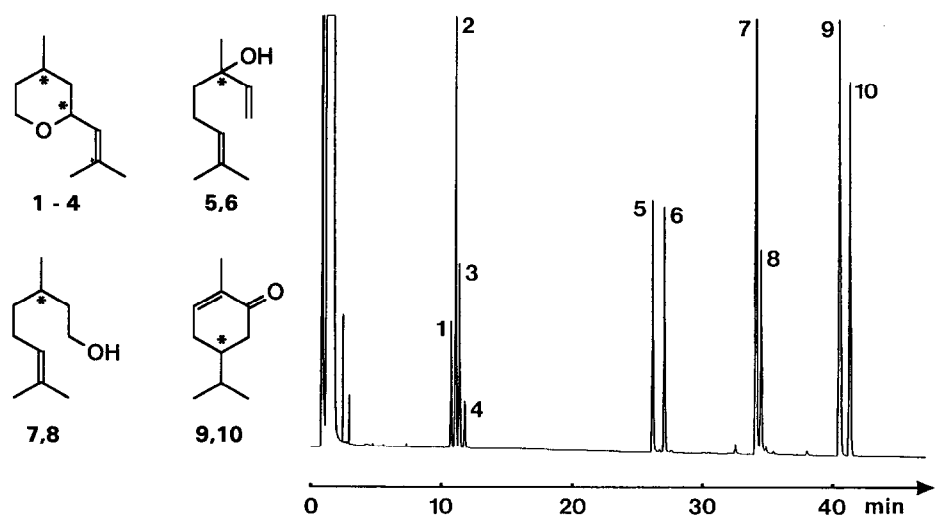


Fig. 17. Simultaneous stereodifferentiation of chiral rose oil compounds. Rose oxides: *cis* isomers (2*R*,4*S*)-(+)-**1**, (2*S*,4*R*)-(−)-**2**; *trans* isomers (2*R*,4*R*)-(−)-**3**, (2*S*,4*S*)-(+)-**4**. Linalool: (*R*)-(−)-**5**, (*S*)-(+)-**6**. Citronellol: (*S*)-(−)-**7**, (*R*)-(+)-**8**. Carvone: (*R*)-(−)-**9**, (*S*)-(+)-**10**. From ref. 110.

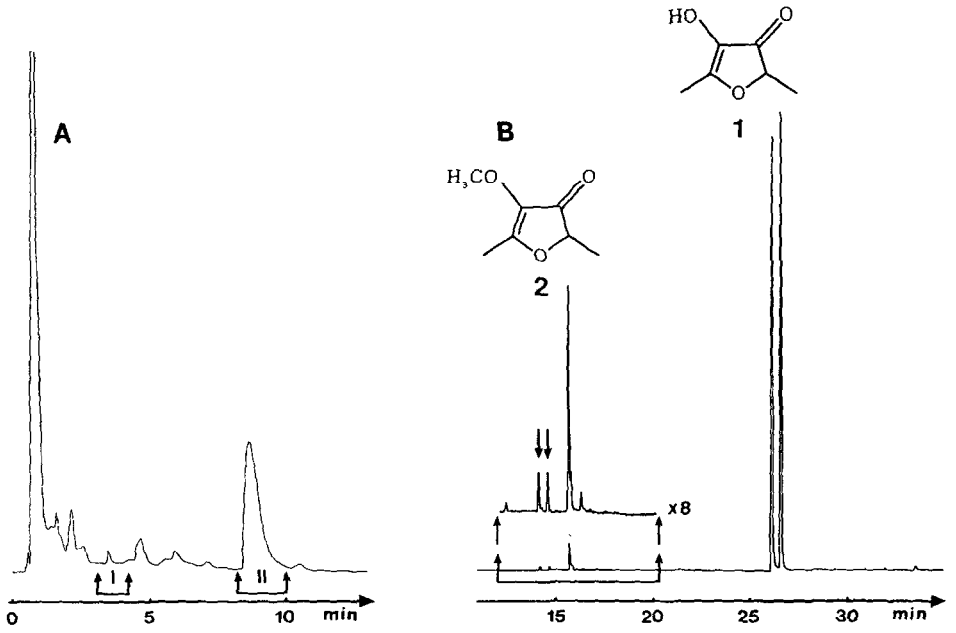


Fig. 18. (A) HPLC of a raw pineapple flavour extract. (B) Enantioselective analysis of 3-(2*H*)-furanones (**1** and **2**) from HPLC eluates I and II. From ref. 108.

combustion product, H_2O, must be removed just behind the furnace either by a capillary-shaped phase separator, made of water-permeable membrane materials [115,147], or by a capillary cryogenic trap [148].

In comparison with classical IRMS methods, the instrumental configuration of cGC–IRMS combines the precision of IRMS with the high purification effect of cGC separation, with large savings on laborious sample clean-up procedures. Isotope ratios are calculated relative to standard values, commonly defined as δ-values, where the δ-value is the relative difference in the isotope ratio of the sample (R_{sa}) to that of the standard (R_{st}), given in parts per thousand [146]:

$$\delta = \left(\frac{R_{sa}}{R_{st}} - 1\right) \cdot 1000\,(‰)$$

The isotope ratio traces of the GC peaks exhibit a typical S-shape, caused by a vapour pressure isotope effect, eluting the heavier isotopic species of a compound more rapidly from the cGC column than the light species. The actual ratio is computed from the ratio of the areas of the two isotopic peaks. Hence, care must be taken to integrate across the full width of the chromatographic peaks [147]. Of course, reliable results on isotope ratios from cGC–IRMS experiments can only be expected from real high-resolution cGC. Also, accurate sample clean-up procedures without any isotope fractionation must be guaranteed [120,148,149].

4.2.2. Applications

γ-Lactones. Alternative investigations of γ-decalactone from various sources were reported by Bernreuther *et al.* [96], confirming high ee values in favour of the 4*R*-enantiomer [76–79], whereas $\delta^{13}C_{PDB}$ values differ in relation to their origin (Table 2). The most striking deviations in $\delta^{13}C_{PDB}$ values were detected in the group of stone fruits. With the present state of knowledge final explanations cannot be given.

Blends of γ-decalactone from different origins were analysed in model experiments, using enantioselective cGC, coupled on-line with IRMS [enantio-SIRA(IRMS)], to demonstrate the advantages of enantio-SIRA, although some decrease in sensitivity has to be taken into account in comparison with

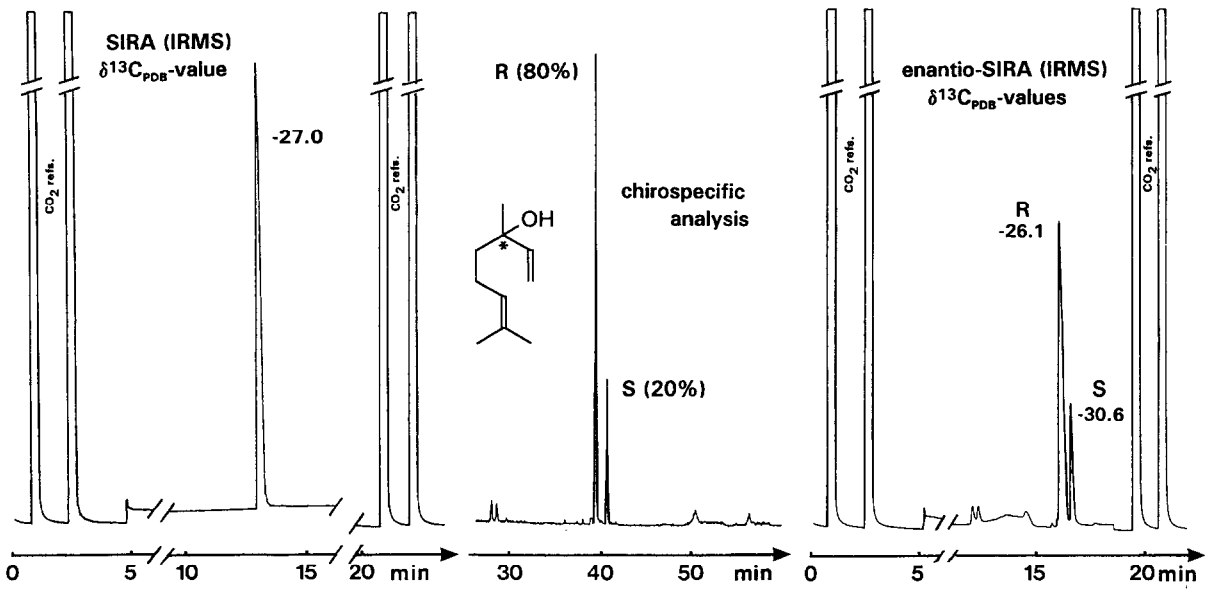


Fig. 19. cGC–IRMS, chirospecific analysis and enantio-SIRA measurements of linalool from a commercially available spike oil. $\delta^{13}C_{PDB}$ values (‰): linalool −27.0 (±0.2); *R*-enantiomer −26.1 (±0.2); *S*-enantiomer −30.6 (±0.6). From ref. 161.

cGC–IRMS using non-chiral stationary phases [150]. Enantio-IRMS detects enantiomers of the same source with identical $\delta^{13}C$ levels. Enantio-IRMS also offers a direct method to detect conclusively a blend of optically pure chiral flavour compound with synthetic racemate: a fruit-specific enantiomeric ratio may be imitated but is not yet detectable neither by chirospecific analysis nor by IRMS measurements. However, in the case of enantio-IRMS a simulated fruit-specific enantiomeric distribution is proved by different $\delta^{13}C$ levels of the detected enantiomers.

Furthermore, enantiomers with identical $\delta^{13}C$ levels are expected from genuine compounds, even if the structural features of chiral molecules to be analysed may be sensitive to (partial) racemization. On the other hand, it seems improbable that the same biological organism pursues different biochemical pathways to synthesize precursors of mirror images. Hence, enantio-SIRA measurements indicating significantly different $\delta^{13}C_{PDB}$ values of the investigated enantiomers should be explained as a blended compound of different origin (Fig. 19).

As outlined in Fig. 19, cGC–IRMS detects the $\delta^{13}C_{PDB}$ value of linalool from a commercially available spike oil in the presumed natural range of

TABLE 2

^{13}C CONTENT OF γ-DECALACTONE ORIGINATING FROM VARIOUS SOURCES [96]

Except for the value given for apricot, which showed a variation of ±2.0‰ due to interfering peaks, for the other HRGC–IRMS determinations variations of ±0.5‰ were found. For the conventional measurements ±0.2‰ was determined.

Origin	$\delta^{13}C_{PDB}$ (‰)	
	HRGC–IRMS	Conventional
Natural		
Strawberry		
Spadeka	−28.2	
Bogota	−28.9	
Bel Ruby	−30.5	
Peach		
Italy	−40.9	
Germany	−38.5	
Plum (Mirabelle)	−39.6	
Apricot	−38.0	
Microbial (A)	−31.2	−30.8
Microbial (B)	−30.3	−30.7
Synthetic		
Aldrich	−26.9	−27.3
Roth	−24.4	
Takasago	−26.0	

(−26.0 to −28.2‰), whereas chirospecific analysis and enantio-SIRA measurements will allow much more conclusions with respect to the authenticity control of this chiral compound. Systematic investigations are in hand to confirm these results [161].

Origin-specific analysis of (E)-α(β)-ionone. (*E*)-α-Ionone from raspberries and many other natural sources occurs as the optically pure (*R*)-(+)-enantiomer [151,152]. Thus, nature-identical (*E*)-α-ionone racemate from *Boronia* absolue and optically pure (*R*)-(+)-(*E*)-α-ionone from a genuine oil of *Osmanthus* are detected unambiguously [114] (Fig. 20).

The sensory quality of (*E*)-α-ionone racemates is not equivalent to the *R*-configured (*E*)-α-ionone [152], as concluded from the sensory characteristics of (*E*)-α-ionone enantiomers [151]. Using stable isotope mass spectrometry, (*E*)-α- and (*E*)-β-ionone from raspberries are well differentiated from their synthetic analogues and from a commercially available product, which was declared to be of natural origin [152] (Table 3).

New clean-up procedures. Nitz *et al.* [148] reported the first application of multi-dimensional GC coupled on-line to isotope mass spectrometry (MDGC–IRMS) (Fig. 21). This highly sophisticated and elegant method will be of considerable interest in the field of flavour analysis. However, major restrictions must also be taken into account if δ-values from sub-nanogram samples are to be evaluated from complex mixtures in the presence of extreme amounts of other substances. With respect to the minimum IRMS sensitivity level (about 1 nmol of substance to be analysed), direct MDGC–IRMS might be insufficient, in spite of a high sample loading provided by a thick-film precolumn capillary.

Preparative high-resolution segment chromatography (PHSC) has been applied successfully as an alternative to MDGC in sample clean-up for the cGC–IRMS analysis of orange oils [149].

Fig. 22 compares a genuine orange oil with the same oil after separating (*R*)-(+)-limonene (L) by

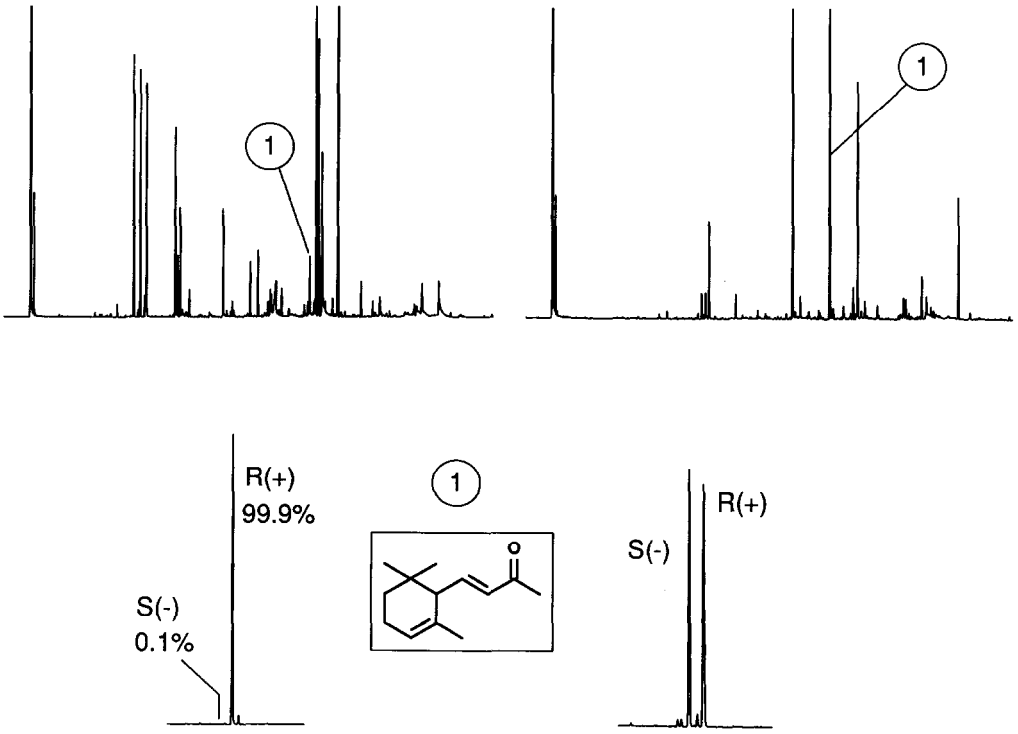


Fig. 20. Chirospecific analysis of (*E*)-α-ionone of *Osmanthus* oil (left) and *Boronia* absolue (right). Top: cGC analysis with achiral column, 60 m × 0.32 mm I.D. DB1 (0.25 μm). Bottom left: separation of naturally occurring enantiomers in *Osmanthus* oil. Bottom right: separation of enantiomers of commercially available *Boronia* absolue. Chiral column, 25 m × 0.25 mm I.D. heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin. Bottom centre: structure of (*E*)-α-ionone. From ref. 114.

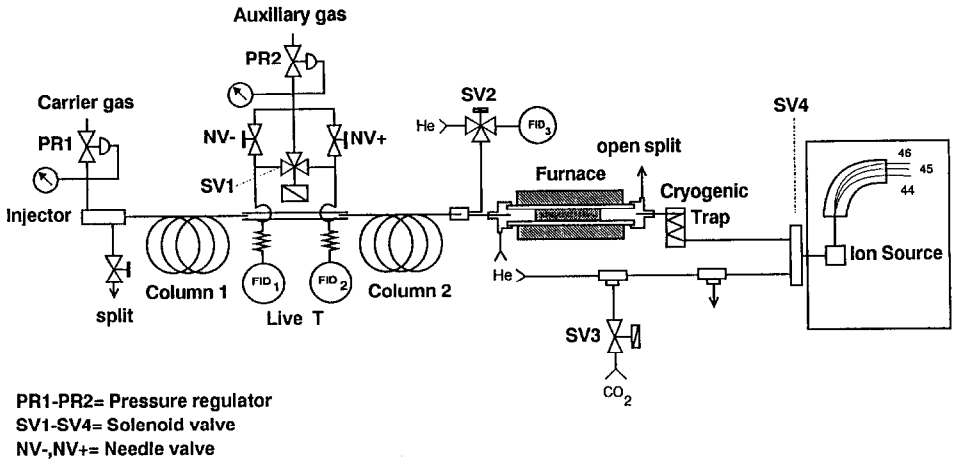


Fig. 21. Schematic diagram of MDGC–IRMS. From ref. 148.

PHSC on silica gel and ready for one-dimensional cGC–IRMS analysis. Every step during sample clean-up was achieved without any isotope fractionation. PHSC has proved to be an inexpensive preseparation and simple concentration step in the cGC–IRMS analysis of fatty aldehydes, in particular of octanal and decanal, two minor compounds which contribute substantially to the sensory quality of cold-pressed orange oils.

The results, shown in Table 4, are remarkable, in particular for decanal, one of the main aldehydes in orange oil. The $\delta^{13}C$ values vary in a small range (about 1‰), independent of variety, provenance and growing conditions [149].

4.3. *Trends and perspectives*

cGC coupled on-line with IRMS is one of the latest developments, useful in the origin control of flavour and fragrances. While genuine flavour compounds from C_4 plants differ significantly from those of C_3 plants, partial overlapping ranges of $\delta^{13}C$ values of substances from C_3 plants with those prepared from fossil sources and from microbial origin have to be accepted. Further, the incorporation of the isotope abundances, especially $\delta^{13}C$ and $\delta^{2}H$, has enhanced the capability to determine the authenticity of cassia and bitter almond essential oils [54,154].

Three principles in the future progress of stable

TABLE 3

$^{13}C_{PDB}$ VALUES OF (*E*)-α(β)-IONONE FROM DIFFERENT ORIGINS [152]

Origin	(*E*)-α-Ionone			(*E*)-β-ionone
	(*R*)-(+)- (%)	(*S*)-(−)- (%)	$\delta^{13}C_{PDB}$ (*s*)[a]	$\delta^{13}C_{PDB}$ (*s*)[a]
Synthetic A	50	50	−24.33 (0.16)	n.d.[b]
Synthetic B	50	50	−26.69 (0.10)	n.d.
Synthetic C	n.d.	n.d.	n.d.	−28.63 (0.22)
Synthetic D	50	50	−27.05 (0.15)	−25.59 (0.09)
Raspberry (fruit)	>99.9	<0.1	−32.86 (0.59)	−33.41 (0.37)
Raspberry (mash)	>99.0	<0.1	−33.43 (0.38)	−31.41 (0.38)
Fermentative	50	50	−9.12 (0.06)	−8.57 (0.16)

[a] Standard deviations (*s*) are given in parentheses ($n = 5$).
[b] n.d. = Not detectable.

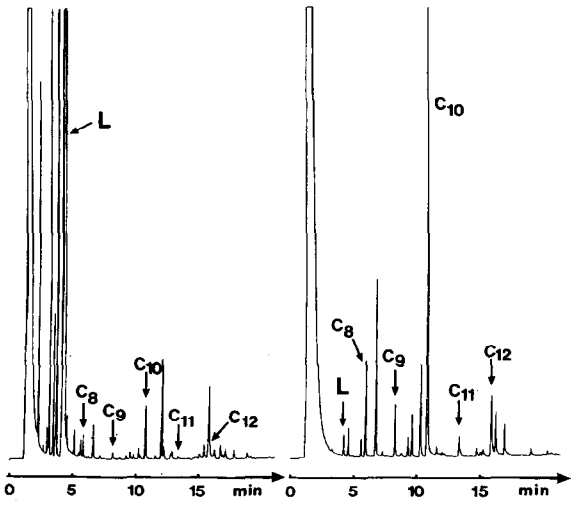


Fig. 22. cGC analysis of a genuine orange peel oil, (left) laboratory prepared and (right) after separating (*R*)-limonene using PHSC. L = limonene (L); C_8–C_{12} = aldehydes. From ref. 149.

isotope analysis were defined by Schmidt *et al.* [155]: multi-compound analysis, using cGC–IRMS (Section 4.2.1); multi-element analysis, replacing the combustion unit by a pyrolytic converter to produce CO, N_2 and H_2 from organic material and analysis by on-line IRMS; and automated positional isotope analysis from the combination of a controlled pyrolysis with IRMS analysis.

In particular, on-line hydrogen, nitrogen and oxygen stable isotope analysis in organic compounds and water by interfacing cGC with IRMS via a catalytic Pt–Rh capillary reactor is the latest progress in cGC–IRMS and may open up exciting perspectives in many biosciences [155]. In addition, chirality evaluation as an indicator of the naturalness of flavour and fragrance compounds has advanced tremendously.

While the direct analysis of single chiral compounds and their homologues [76–79] was reported in the first stage of enantioselective flavour analysis, simultaneous stereodifferentiation of chiral compounds with different functionalities has subsequently been achieved [92,95]. Developing tailor-made chiral columns for the direct and simultaneous enantioselective analysis of most chiral impact compounds of rose oil or lavender oil is the latest advance in this field of research [110,140] and indeed remains a challenge.

5. CONCLUSIONS

Aroma extract dilution analysis (AEDA) and stable isotope dilution assay (SIDA) have proved to be valuable tools in the quality assessment of flavours and fragrances. Concerning the genuineness of natural compounds, the importance of comparative stable isotope ratio mass spectrometry (IRMS) and chirality evaluation is increasing. Promising developments in both fields of research

TABLE 4

$^{13}C_{PDB}$ VALUES OF C_8–C_{12} ALDEHYDES FROM ORANGE PEEL OILS OF DIFFERENT ORIGINS [149]

Origin	$\sigma^{13}C_{PDB}$ (*s*)[a]			
	Octanal	Nonanal	Decanal	Dodecanal
Navel A[b]	−27.40 (0.31)	−28.31 (0.55)	−26.10 (0.36)	−27.13 (0.45)
Navel B[b]	−29.15 (0.28)	−28.23 (0.39)	−25.89 (0.16)	n.s.[d]
Navel C[b]	−28.25 (0.21)	−28.07 (0.23)	−26.11 (0.15)	−28.15 (0.17)
Valencia[b]	−28.02 (0.06)	−27.51 (0.25)	−25.75 (0.10)	n.s.
Siracusa[b]	−26.72 (0.24)	−26.80 (0.30)	−25.33 (0.16)	−27.10 (0.31)
Italy[c]	−27.67 (0.09)	−27.33 (0.25)	−26.60 (0.13)	n.s.
Florida[c]	−28.42 (0.23)	−28.60 (0.24)	−26.93 (0.19)	−27.60 (0.34)
California[c]	−27.96 (0.17)	−27.71 (0.08)	−26.34 (0.35)	−27.22 (0.40)

[a] Standard deviations (*s*) are given in parentheses ($n = 5$).
[b] Laboratory-prepared orange peel oils.
[c] Commercially available orange peel oils.
[d] No quantitative separation by GC as yet.

will strongly accelerate insights into the origin of flavours and fragrances. Continuous advances in analytical origin assignment will be adopted for quality assurance in the flavour industry and also should reflect legal regulations as a consequence.

6. ABBREVIATIONS

A	aroma value
a	odour threshold
AEDA	aroma extract dilution analysis
c	concentration
cR_s	chiral resolution
cGC	capillary gas chromatography
cGC–IRMS	capillary gas chromatography coupled on-line with isotope ratio mass spectrometry
CHARM	combined hedonic response measurements
CI	chemical ionization
ee	enantiomeric excess
EI	electron impact ionization
enantio-MDGC	enantioselective MDGC
enantio-SIRA(IRMS)	enantioselective cGC coupled on-line with SIRA(IRMS)
FD factor	flavour dilution factor
FID	flame ionization detection
FT-IR	Fourier transform infrared spectroscopy
fur.	furanoid
GC	gas chromatography
GC–O	gas chromatography–olfactometry
HPLC	high-performance liquid chromatography
HPTLC	high-performance thin-layer chromatography
IRMS	isotope ratio mass spectrometry
Lipodex D	glass capillary column, coated with heptakis(2,6-di-O-pentyl-3-O-acetyl)-β-cyclodextrin
MDGC	multi-dimensional gas chromatography
MDGC–IRMS	multi-dimensional gas chromatography coupled on-line with isotope ratio mass spectrometry
MIM	multiple ion monitoring
MS	mass spectrometry
OAV	odour activity value
PHSC	preparative high-resolution segment chromatography
s	standard deviation
SIDA	stable isotope dilution assay
SIM	single ion monitoring
SIRA	stable isotope ratio analysis
SWC	selected wavelength chromatogram
Δt	absolute difference in retention time of two peaks
w_h	peak width at half-height
w_b	peak width at base (4σ)
σ	peak variance

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