Authenticity Assessment: A Permanent Challenge in Food Flavor and Essential Oil Analysis

A. Mosandl
Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität,
Marie- Curie- Str. 9, D- 60439 Frankfurt am Main, Germany

Abstract

Both phenomena, enantioselectivity as well as isotope discrimination during biosynthesis, may serve as "endogenous" parameters, provided that suitable methods and comprehensive data from authentic sources are available. This review reports on enantioselective capillary gas chromatography and online methods of isotope-ratio mass spectrometry in the authentication of food flavor and essential oil compounds, referring to literature references published in the last decade.

Introduction

The acceptance of food strongly depends on their flavor impressions. Consequently, authentication of genuine flavors and spices is an important topic regarding to consumer protection and quality assurance in food production. In the context of authentication, one should first look for characteristic components of definite origin. Nowadays both phenomena, enantioselectivity as well as isotope discrimination during biosynthesis, have been recognized as important principles of authenticity. Highly sophisticated techniques are used in order to determine the authenticity of genuine aroma-active compounds:

(*a*) Site-specific natural isotope fractionation, detected by quantitative ^{2}H-NMR measurements (^{2}H-SNIF–NMR); (*b*) isotope-ratio mass spectrometry (IRMS), online coupled with elemental analyzer (EA) or capillary gas chromatography (cGC–IRMS); and (*c*) enantioselective cGC (enantio-cGC).

Even if enantio-cGC and online IRMS methods have been proven to be highly efficient tools in origin-specific analysis, analytical authentication remains a permanent challenge because of the complexity of natural product (food) matrices. So far, online coupling techniques are the methods of choice in the origin evaluation of flavor and fragrance samples.

Experimental

Enantio-cGC

Scope

In the early 1980s, stereoanalysis of chiral flavor compounds was rather difficult because of the lack of suitable stationary GC phases. A real break through in this field occurred when enantio-cGC became more and more available. In particular, since 1988 selectively modified cyclodextrins have been synthesized, serving as chiral stationary phases in enantio-cGC; first published by Schurig et al (1), König et al. (2,3), Armstrong et al. (4), Mosandl et al. (5,6), Tabacchi et al. (7), and Bicchi et al. (8). From our own experiences, we must point out that the enantioselectivity of modified cyclodextrin phases considerably depends on the polarity of the polysiloxane solvents used. 6-*O*-Silylated modified β(γ) cyclodextrin derivatives of well defined structure and purity were synthesized and have proven to be chiral stationary phases of unique selectivity and versatility and, therefore, are successfully used in simultaneous enantio-cGC analysis (5,6).

Enantioselective multidimensional GC

Because of high complexity of natural flavors, essential oils or spice extracts, reliable chirality evaluation needs highly efficient sample clean-up procedures. The online GC–GC coupling, the so called enantioselective multidimentional GC (enantio-MDGC) system, has proved to be the method of choice. A schematic diagram of enantio-MDGC (Siemens Sichromat) is shown in Figure 1.

The design has been well proven in quality assurance and origin control of flavors and fragrances. The figure shows a double-oven system with two independent temperature controls and two detectors (DM 1 and DM 2). A "live switching" coupling piece is used to switch the effluent flow to either the first detector or the chiral column. With optimum pneumatic adjustment of the MDGC system, certain fractions are selectively transferred onto the chiral main column as they elute from the precolumn (heart-cutting technique).

Detection systems

If optimum chiral separation conditions and high efficiency sample clean-up are properly employed, the first priorities in enantioselective analysis have been achieved. Under such conditions, simple detection systems, such as flame ionization detection (FID), can be used. However, the ideal detector is universal yet selective, sensitive, and structurally informative. Mass spectrometry (MS) currently provides the closest realization of this ideal. The combination of enantio-MDGC with

high resolution MS or mass selective detectors, used both in full scan or in the single ion monitoring (SIM) mode, is currently the most potent analytical tool in enantioselective analysis of chiral compounds from complex mixtures.

Limitations

Three types of limitations have to be accepted in enantio-cGC: (*i*) racemates of natural origin, generated in some special cases (10–14); (*ii*) racemization during processing or storage of foodstuffs, if structural features of chiral compounds are sensitive; and (*iii*) blending of natural and synthetic chiral compounds.

Nonetheless, the systematic evaluation of natural enantiomeric ratios has been proven to be a valuable criterion for differentiating natural compounds from those of synthetic origin.

Results and Discussion

Chiral γ (δ) lactones

Because of their pleasant odors, many γ (δ) lactones are known to be important flavor compounds of fruits and contribute essentially to the characteristic and distinctive notes of strawberries, peaches, apricots, and many other fruits (15). Chiral aroma compounds from fruits and other natural sources should be characterized by origin-specific enantiomeric ratios, because their biogenetic pathways are normally catalyzed by enzymes.

Using enantio-MDGC, the simultaneous stereoanalysis of γ (δ) lactones has been reported. This technique was applied to many fruits, proving that enantiomeric ratios of γ (δ) lactones can be used as indicators of authenticity because the genuine enantiomeric purities remain unaffected during fermentation and all other stages of fruit processing (16).

β-Methyl-γ-octalactone ("oak lactone, whisky lactone")

Through the years, it has been clearly demonstrated that factors such as quality of the starting material, the actual fermentation process, and storing and ageing conditions can make important contributions to the quality of the final product. Suomalainen and Nykänen (17,18) were the first to report on the natural occurrence of β-methyl-γ-octalactone in distilled alcoholic beverages matured in oak barrels. They named it whisky lactone (Figure 2).

The presence of oak (whisky) lactone in wine and spirits is attributed to the extraction from the oak wood barrel into the alcoholic beverage. Oak lactone is described as constituting a substantial part of the aroma of wood-aged spirits. From a theoretical point of view, four stereoisomers of oak lactone exist, owing to two stereogenic centers in the molecule. Earlier, all four stereoisomers were synthesized and evaluated regarding their sensorial properties. The data reviewed clearly demonstrate that oak lactones can make a significant sensory contribution to the overall flavor of alcoholic beverages (20). Concerning the natural occurrence of oak lactones, it is interesting to note the *cis* (3*S*,4*S*) and *trans* (3*S*,4*R*) stereoisomers as the genuine natural compounds (21).

Although the *cis/trans* ratio of oak lactone and absolute amounts may differ with respect to the genetic source of the wood (American or European), in any case the (3*S*)-configured diastereomers are exclusively detected (19). Thus, the presence of all four isomers in an alcoholic beverage is a good indicator of falsification with synthetic oak lactone.

2-Alkylbranched acids (esters)

From the analytical point of view, it is worth noting the bio-

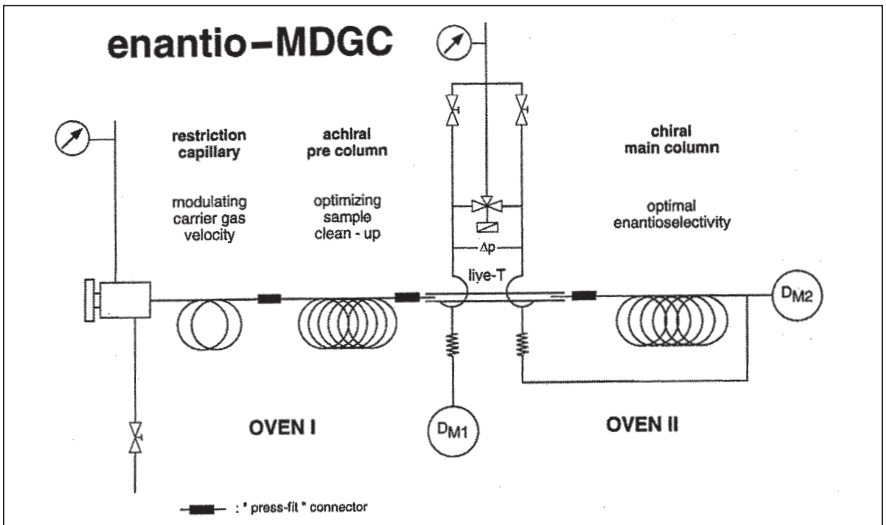

Figure 1. Enantio-MDGC, "Live-T" column switching, Siemens Sichromat, taken from (9).

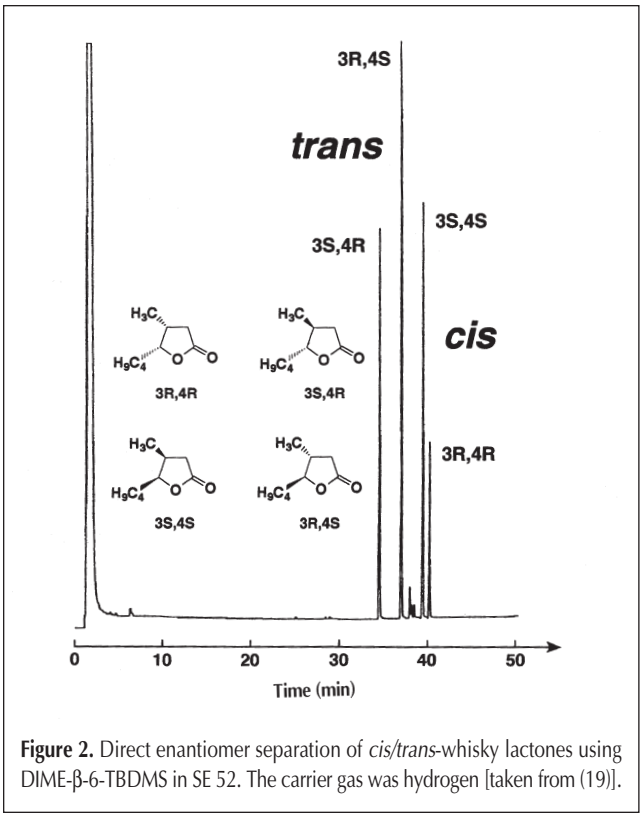

Figure 2. Direct enantiomer separation of *cis/trans*-whisky lactones using DIME-β-6-TBDMS in SE 52. The carrier gas was hydrogen [taken from (19)].

genetic 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. So far, what the case is for apples is of special interest.

In all food investigated, the (*S*)-enantiomer of ethyl 2-methylbutanoate (the impact flavor compound of apples) was identified with high enantiomeric purity, irrespective of apple variety investigated and unaffected by processing conditions (e.g., distillation, concentrating, or storage of apple juices).

Of course, during processing of fruit juices, hydrolysis effects may occur, leading to decreased amounts of ethyl 2-methylbutanoate. However, its enantiomeric purity remains unchanged, and the corresponding 2-methylbutanoic acid is found as the (*S*)-enantiomer (≥ 99.5%). Consequently, the detection of

Table I. Enantiomeric Distribution of Ethyl 2-Methylbutanoate in Apples and Apple Juices Using Enantio-MDGC–MS Analysis*

	Enantiomeric distribution	
Ethyl-2-methylbutanoate	***R*(%)**	***S*(%)**
Fresh apples (head space extracts)		
Melrose	0,2	99.8
Granny Smith	0.5	99.5
Fresh apple juice (liquid–liquid extract)		
Melrose	0.2	99.8
Granny smith	0.5	99.5
Apple juice		
Var. "Gloster"	0.2	99.8
Comm. sample 1†	0.3	99.7
Comm. sample 2†	0.3	99.7

* Taken from (22). High preference of the (*S*)-enantiomer is valid for apple fruit in general.
† Apples of different varieties.

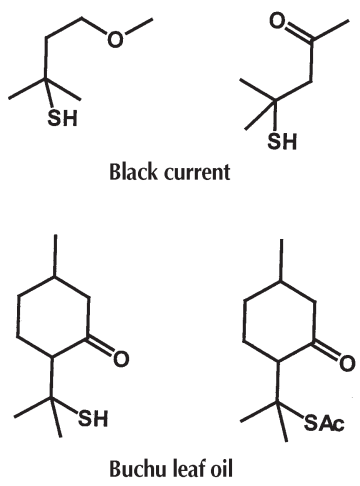

Figure 3. Characteristic black currant and buchu leaf oil components (19).

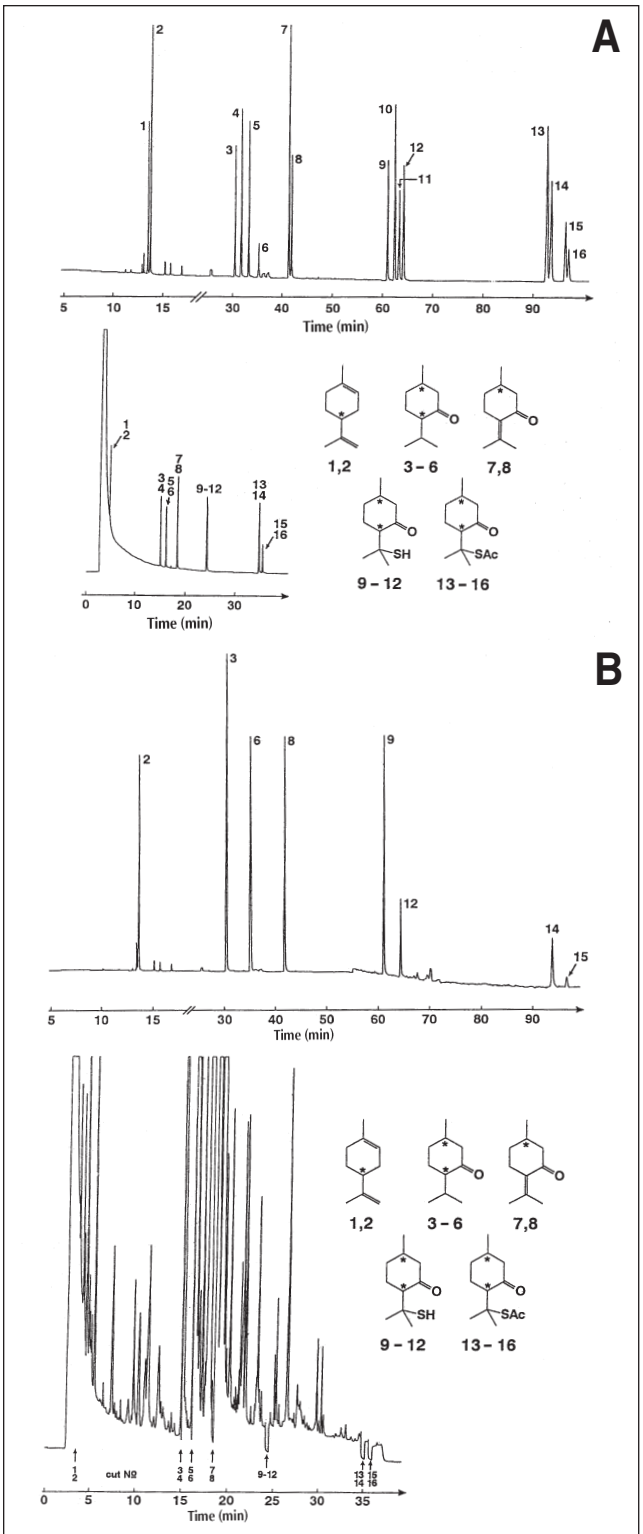

Figure 4. (A) Simultaneous stereodifferentiation of buchu leaf oil constituents using enantio-MDGC analysis (standard mixture). Limonene, **1** (*S*) and **2** (*R*); menthone, **3** (1*S*,4*R*) and **4** (1*R*,4*S*); isomenthone, **5** (1*R*,4*R*) and **6** (1*S*,4*S*); pulegone, **7** (*R*) and **8** (*S*); *cis*-3-oxo-*p*-menthane-8-thiol, **9** (1*S*,4*R*) and **11** (1*R*,4*S*); *trans*-3-oxo-*p*-menthane-8-thiol, **10** (1*R*,4*R*) and 12 (1*S*,4*S*); *trans*-3-oxo-*p*-menthane-8-thiol acetate, **13** (1*R*,4*R*) and **14** (1*S*,4*S*); and *cis*-3-oxo-*p*-menthane-8-thiol acetate, **15** (1*S*,4*R*) and **16** (1*R*,4*S*). Preseparation of racemic reference compounds and chiral resolution of enantiomeric pairs (top). (B) Enantio-MDGC analysis of laboratory prepared bucchu leaf oil, taken from (24).

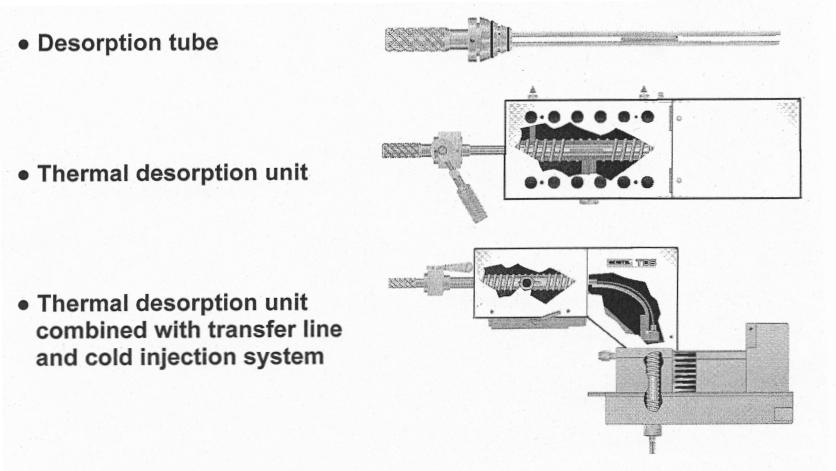

Figure 5. TDS from Gerstel, Mühlheim/Ruhr.

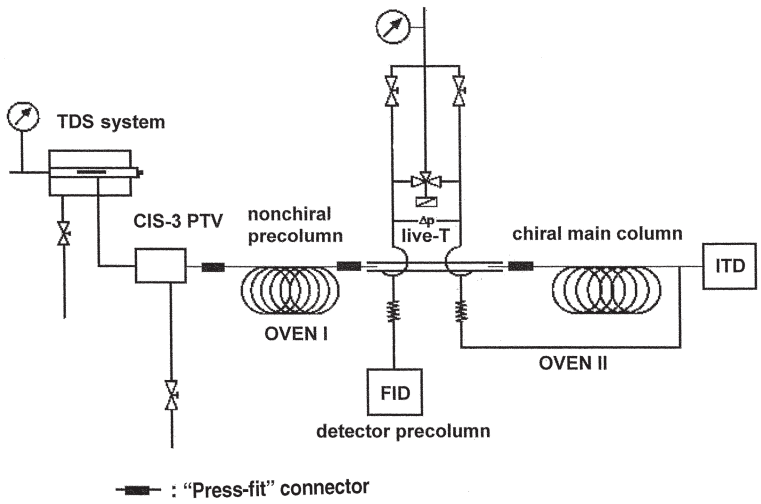

Figure 6. TDS system; schematic diagram of SBSE–enantio-MDGC–MS, taken from (29).

racemic 2-methybutanoic acid (or esters) definitely proves the addition of a synthetic (so called "nature-identical") flavor compound (Table I).

In the context of European Union (EU) food law fruit juices must be genuine; in view of their aroma, only aroma concentrates of the fruit concerned are suitable for fruit juices from concentrates. Other natural flavorings (from other fruits or biotechnology) are not allowed.

Black currant flavor

Minute traces of mercapto-ketones can contribute to both a desirable and off-flavor of several foods and beverages such as cheese, beer, fruit juice, and wine. They are among the most powerful odorants known. Because of some similar, characteristic odor of tomcat urine associated with mercapto-ketones, their odor qualities were evaluated with catty, sweaty and fruity notes and correlated with tertiary mercapto (amyl) substructures (19).

Black currants, the fruits of *Ribes nigrum* L. do not have a pleasant odor, per se, but after a slight fermentation, the aroma is modified and the flavor assumes a pleasing aroma profile, essentially characterized by 4-methoxy-2-methyl-2-butanethiol. In a similar manner, 4-mercapto-4-methylpentan-2-one is known to be a powerful aroma trace component, responsible for the boxwood-like aroma of Sauvignon wine and for the German "Scheurebe", and also for the black currant-like odor of virgin olive oil (Figure 3).

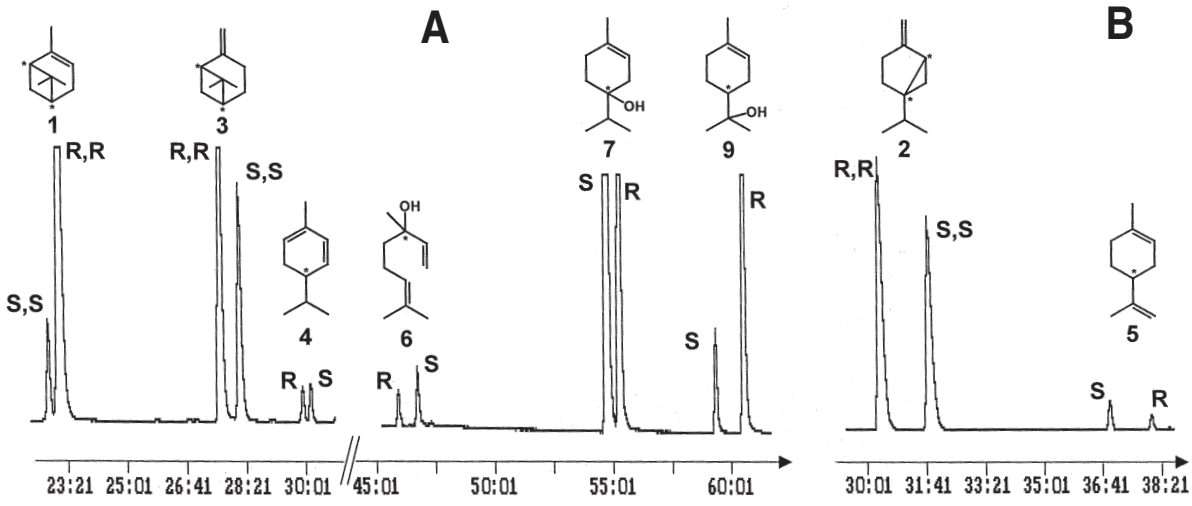

Figure 7. SBSE-enantio-MDGC–MS analysis of tea tree oil, main column separation, taken from (29).

Liqueur de cassis or *Crème de cassis*

These black currant products are very popular in Europe. Because of their characteristic mint, fruity notes (reminiscent of black currants), buchu leaf oils are also very much appreciated in the composition of cassis-like flavors. 8-Mercapto-*p*-menthan-3-one and its thiolacetate have been described as impact flavor compounds of cassis-like flavors. They have so far not been identified in black currant. All four stereoisomers of 8-mercapto-*p*-menthan-3-one exhibit distinct and chacteristic sensory impressions. The (1*R*)-configured stereoisomers emit a more intense odor than the (1*S*)-configured compounds, but only the latter isomers have been recognized as being peculiar to buchu leaf oil and reminiscent of black currant (23). An enantio-MDGC method was developed, detecting (1*S*)-configured menthone (**3**), isomenthone (**6**), (1*S*)-pulegone (**8**), (1*S*)-configured thiols (**9**,**12**), and (1*S*)-thiol-acetates (**14**,**15**) as enantiopure chiral sulfur compounds from genuine buchu leaf oil (24) (Figure 4).

This is why buchu leaf oil is used as an ingredient for WONF-type aromas (with other natural flavors). But one should keep in mind, when 8-mercapto-*p*-menthan-3-one is prepared by reacting pulegone with hydrogen sulfide, the synthesis yields racemic *cis*/*trans* diastereomers in the case of racemic pulegone or strange (1*R*)-configured diastereomers when (1*R*)-pulegone from Pennyroyal oil (*Mentha pulegium* L.) is used as starting material. In any case, these products can be differentiated exactly from genuine buchu leaf oil compounds by enantio-MDGC analysis (24).

From a legal point of view, it is interesting to note that up until now, (1*R*)-configured stereoisomers of 3-oxo-*p*-menthane-8-thiol have not been detected in natural products. Consequently, those stereoisomers have to be classified as "artificial" flavorings in the sense of order regulations by the EU.

Stir-bar sorptive extraction–enantio-MDGC–MS
SBSE–enantio-MDGC

A novel, solventless, and simple technique for extraction of organic analytes from aqueous samples [stir bar sorptive extraction (SBSE)] has been recently introduced by Sandra et al. (25). SBSE takes advantage of the high enrichment factors of sorptive beds but with the application range and simplicity of SPME (26). The stir bar is coated with a thick film of polydimethylsiloxane (PDMS), in which the aqueous sample extraction takes place during stirring for a predetermined time. After that time, it is removed and placed into a glass tube, which is transferred into a thermal desorption system (TDS) where the analytes are thermally recovered and evaluated online with a capillary MDGC–MS system.

In addition to the extraction of organic analytes from aqueous samples, the PDMS stir bars are also suitable for headspace and *in vivo* headspace sampling. Headspace sampling is a technique widely used to characterize the volatile fraction of several matrices, particularly aromatic and medicinal plants. SBSE has also been shown to be a successful technique for headspace sampling, because the PDMS stir bars enrich higher amounts of trapping material than SPME and, therefore, exhibit better extraction efficiency for analyzing minor com-

Table II. Monoterpene Compounds from Melaleuca and Eucalyptus Species*

Tea tree oil *Melaleuca alternifolia* Cheel *Melaleuca linariifolia* Sm. *Melaleuca dissitiflora* Mueller			**Eucalyptus oil** *Eucalyptus globulus* Labill. *Eucalyptus fructicetorum* F. v. Mueller ex Miquel *Eucalyptus smithii* R.T. Baker			
			Tea tree oil		**Eucalyptus oil**	
	Chiral A†	**Nonchiral B‡**	**C§**	**D****	**C§**	**D****
α-Pinene	x		R: 86–91	1.5–2.5	R: 93–99	2.0–8.0
β-Pinene	x		R: 58–65	0.1–1.0	S: 59–65	< 0.5
α-Phellandrene	x		–	< 0.1	–	< 1.5
Limonene	x		R: 62–68	1.0–6.0	R: 64–72	4.0–12.0
1,8-Cineole		x	–	< 15.0	–	>70.0
Camphor	x		–	–	–	< 0.1
Terpinen-4-ol	x		S: 65–70	>30.0	S: 53–58	< 1.0
α-Terpineol	x		R: 69–78	1.5–8.0	R: 66–72	< 4.0

* Taken from (29).
† Enantio-MDGC–MS
‡ GC–IRMS mutltielement analysis ($\delta^{13}C$, $\delta^{2}H$, and $\delta^{18}O$ values).
§ Enantiomeric purity (%).
** Total percentage (%).

Table III. IRMS–Online Coupling Techniques

GC–combustion–IRMS (GC–C–IRMS)	$\delta^{13}C$
GC–combustion/reduction–IRMS	$\delta^{15}N$
GC–pyrolysis–IRMS (GC–P–IRMS)	$\delta^{18}O$ $\delta^{2}H$
Thermochemical conversion/element analyzer (TC–EA)	$\delta^{18}O$ $\delta^{2}H$

Table IV. Specifications for cGC–IRMS Coupling Techniques Using DELTAplus XL, Thermo Electron, Bremen (Germany)

		On column		
Bioelement	**Analyzed gas**	**Need (mol)**	**Need (ng)**	**Precision**
Carbon	CO_2	0.8 nmol C	10 ng C	0.2 per mil
Nitrogen	N_2	1.5 nmol N_2	42 ng N	0.5 per mil
Hydrogen	H_2	15 nmol H_2	30 ng H	3 per mil
Oxygen	CO	5 nmol O	80 ng O	0.80/00

ponents (27) (Figure 5).

This connection allows the combination of the high extraction efficiency of the stir bar (coated as a thick film of PDMS) with the high selectivity of the enantio-MDGC–MS system (28) (Figure 6).

By the way, it is possible to determine the exact enantiomeric ratios of chiral compounds in complex natural materials such as food flavors or essential oils. Even headspace sampling and *in vivo* headspace sampling from living plants is successfully realized.

Tea tree oils

The essential oils from *Melaleuca alternifolia* (Myrtaceae) are recommended for many medicinal and cosmetic purposes. More than 100 varieties of *Melaleuca* are known and have considerable differences in their essential oil composition. In order to standardize the essential oil quality, minimum and maximum conditions are given by Deutscher Arzneimittel-Codex and ISO 4730 (1996).

Unfortunately, enantiomeric purities (C) and total percentages (D) of α(β)pinene, limonene, α-terpineol from tea tree oils are more or less overlapping with those of Eucalyptus oils. Only enantiomeric purities and total percentages of terpinen-4-ol and α-phellandrene are significantly different, when Melaleuca and Eucalyptus oils are compared in view of authenticity assessment (29) (Figure 7).

Enantio-cGC, however, fails in the case of nonchiral compounds, such as 1,8- cineol. In this special case 1,8-cineol may be attributed to high-level Melaleuca varieties or to the fraudulent addition of Eucalyptus oil. In order to get reliable results, enantio-selective MDGC–MS analysis or IRMS measurements (or both) (as far as possible) are necessary (Table II).

Isotope discrimination

The natural cycles of the bioelements carbon, oxygen, and hydrogen (nitrogen and sulfur) are subjected to various discrimination effects, such as thermodynamic isotope effects during water evaporation and condensation or isotope equilibration between water and CO_2. On the other hand, the processes of photosynthesis and secondary plant metabolism are characterized by kinetic isotope effects, caused by defined enzyme catalyzed reactions (30).

The highly precise measurement of isotope ratios has a long tradition in organic geochemistry. Nowadays, the elucidation of stable isotope distributions is highly desirable in view of fundamental studies in biochemistry, nutrition, drug research, and also in the authenticity assessment of food ingredients.

In 1981, G.J. Martin and M.L. Martin (31) showed that the ^2H distribution of organic molecules does not follow a statistic pattern, but is discriminated by isotopic effects that are measurable by ^2H-NMR and IRMS, respectively. Meanwhile, systematics of $^{18}O/^2H$ patterns in natural plant products are more and more understood and reported by Schmidt et al. (32–34) as new and reliable tools for the elucidation of biosynthetic pathways and helpful indicators in the authenticity assessment of natural compounds.

Stable isotope ratio analysis, measured by ^2H-site-specific NMR(^2H-SNIF-NMR) and $^{18}O/^{16}O$ IRMS, have been adopted as official methods by the Commission of the European Communities (EC). These methods play a key role in detecting adulterations, such as the addition of water and inadmissible wine sweetening or chaptalization with beet or cane sugar (35).

Global isotope ratios by MS

Fundamentals

IRMS has become more and more important in food authenticity assessment since cGC coupled online via a suitable combustion/pyrolysis interface with IRMS has been realized. The substances eluting from the cGC column are converted into the corresponding gas (carbon dioxide, nitrogen, hydrogen, and oxygen, respectively) and then directly analyzed in the isotope MS. The spectrometer is adjusted for the simultaneous recording of the reactand gas isotopomers. Incidentally, the components can be detected in the nanomole range with high precision (Tables III and IV).

The isotope ratio traces of the GC peaks exhibit a typical S-shape. The heavier isotopic species of a compound elute more rapidly than the light species. Similar effects can be observed for all chromatographic processes, whereas the size of isotope fractionation and the elution order of the isotopomers depends on: (*a*) the chromatographic system applied, (*b*) the temperature, and (*c*) the structural features of the compounds analyzed. In any case, care must be taken to integrate across the full width of the chromatographic peaks. Of course, reliable results on isotopic ratios from cGC–IRMS experiments can only be expected from very high-resolution cGC ($R_s \geq 1.5$). Also, accurate sample clean-up procedures without any isotope fractionation must be guaranteed. Under the conditions of validated procedures and calibrated instruments, IRMS-data are valuable indicators in the authenticity assessment of flavor and fragrance compounds (Figure 8).

As the latest development, MDGC is reported online coupled

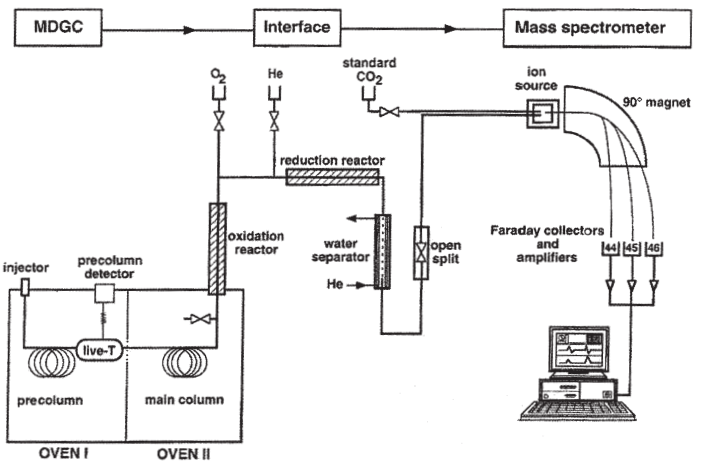

Figure 8. Schematic diagram: MDGC–IRMS, taken from (36).

with IRMS. This coupling technique combines the advantages of both highly sophisticated techniques to achieve the utmost accuracy of IRMS measurements (36).

Indeed MDGC–IRMS is the method of choice for precise and accurate measurements of compounds in complex matrices. Laborious clean-up procedures, entailing the risk of isotope discrimination, will be avoided.

Characteristic finger prints in the authenticity assessment of petitgrain oil compounds are shown in Figure 9. The average is the result of approximately 20 authentic samples. An authenticity range is defined, given by measured minimum/maximum values. By correlating isotopic data with concentrations and enantiomeric ratios of compounds analyzed, a comprehensive authenticity evaluation can be concluded (37).

Authenticity assessment of 2(3)-methylbutanol (38)

2-Methylbutanol is a well-known fermentation byproduct and a valuable natural starting material for the biotechnological generation of 2-methyl butyric acid and esters (Table V).

Authenticity assessment

Conclusions

The following conclusions were drawn: (*i*) 2-methylbutanol (**1**) is biosynthesized almost completely as the (*S*)-enantiomer (> 99%), regardless of which carbohydrate source used for fermentation; (*ii*) type of CO_2-fixation during photosynthesis influences principally the $^{13}C/^{12}C$ ratios of starting materials in alcoholic fermentation; (*iii*) $\delta^{13}C$ values of 2-methylbutanol (**1**) and 3-methylbutanol (**2**) differ significantly (in general, $\delta^{13}C$ values of were approximately 4–5‰ lower than those of produced in the same fermentation process); and (*iv*) 3-methylbutanol (derivatives) are no genuine apple flavor compounds—they are mostly generated by microbial degradation.

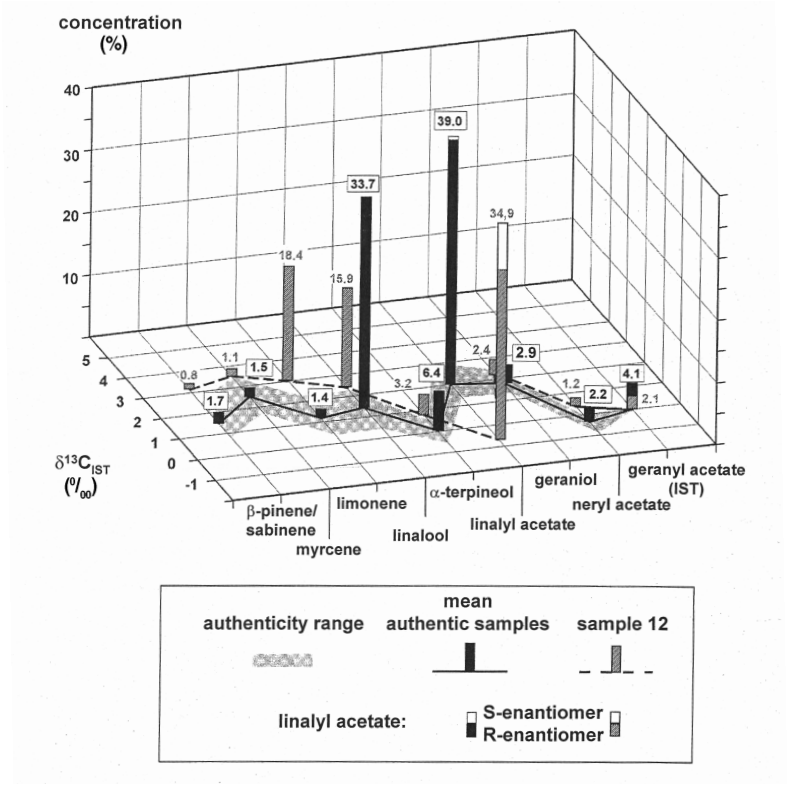

Figure 9. Authenticity profile of petitgrain oil, taken from (37).

Authenticity assessment of lavender oil

In accordance with the European Pharmacopoeia (Ph. Eur.), the official lavender oil is steam distilled from the fresh flowers or inflorescences (or both) of *Lavandula angustifolia* MILLER (*Lavandula off.* CHAIX). Related—but nonofficial products—are lavandin oil, *Lavandula hybrida* REVERCHON (*L. angustifolia* MILL. X *L. latifolia* VILL.) or spike oil, the nonofficial oil from *L. latifolia VILL.*

Lavender notes have been used and appreciated for hundreds of years, and lavender oils belong to the most frequently used natural raw materials for perfumery purposes. So far, authenticity control of this product is of fundamental interest. During the last decade, enantioselective analysis of chiral monoterpenoids has been introduced as a powerful tool in the authenticity control of lavender oils. In particular, linalyl acetate from genuine lavender oils has high enantiomeric purity, favoring the (*R*)-configuration, irrespective of *Lavandula* species and storage or work-up conditions (39). In this context, it should be emphasized that linalool is a typical example for scope and limitations in the analytical authenticity control of flavors and essential oils because of its 1-alken-3-ol structure, which is sensitive in acidic media. Nevertheless, under the conditions of good manufacturing practice (GMP) the chirality evaluation of linalool has been proven to be a reliable indicator in the authenticity assessment of bergamot, sweet orange, or lavender oils (40). Although these facts are reflected in the literature since 1989, it was finally decided that enantioselective analysis was to be integrated into the lavender monograph of the Ph. Eur. in 2003 (Figure 10 and Table VI). In the sense of Ph. Eur., authenticity assessment of lavender oil is now based on enantioselective analysis, in relation to the percentage of chiral compounds to be analyzed.

Latest developments

In recent years, enantioselective chromatography was extended to semipreparative separations, and now simulated moving-bed technology (SMB) has become available for the resolution of some synthetic racemates in preparative scale. In the pharmaceutical industry this progress is of considerable interest with respect to enantiopure drug application.

Under the condition of large amounts of chiral stationary phases with reproducible batch-to-batch properties and economical feasibility of the SMB process, this new

Table V. $^{13}C/^{12}C$ Ratios of 2-Methylbutanol and 3-Methylbutanol from Various Spirits and Apple Products*

	2-Methylbutanol	3-Methylbutanol
C4 plants		
Glucose syrup of maize	–7.7	–12.3
Maize starch	–7.7	–11.3
Cane sugar	–13.9	–14.5
Brown cane sugar	–13.4	–15.4
C3 plants		
Rye starch	–17.0	–22.6
Fructose from chicory	–21.2	–27.5
Wheat starch	–21.4	–25.5
Glucose from wheat	–21.7	–26.3
Beet sugar	–22.2	–27.3
Rice starch	–22.2	–26.8
Potato I	–25.4	–31.5
Potato II	–26.1	–32.7
Grape	–26.1	–32.8
Apple	–26.1	–33.3
Processed apples		
Calvados	–24.5	–29.3
British cider	–19.0	–23.3
French cider	–26.4	–30.2
Hessian cider	–26.2	–34.4
German apfelwein	–27.0	v33.8
Applie juice I	–38.9	
Applie juice II	–39.3	
Applie juice III	–38.1	

* Samples (n = 5–10) and standard deviation (< 0.3 per mil), taken from (38).

technique might also be promising for large-scale continuous separations of synthetic flavor and fragrance racemates (41), and perhaps in delivering enantiopure compounds. However, from the legal point of view, one should note these compounds are of synthetic origin and are judged as "nature-identical" but not as "natural" flavorings.

Consequently, in the case of enantiopure compounds generated by the SMB process, the expressiveness of enantioselective analysis and the conclusions on the authentication of the enantiomeric analytes might be seriously hampered or even impossible. Bringing together both online techniques (enantioselective analysis and IRMS) will be the key to overcoming the difficulties in the legal interpretation of enantiomers from chromatographic racemate separation.

Presently, online cGC–IRMS determination of $\delta^{13}C$ and $\delta^{2}H$ values of flavor compounds are established (42–47), and, furthermore, $\delta^{18}O$-measurements using online cGC–IRMS procedures will be available soon. Finally, enantio-MDGC combined with multielement- and multicomponent IRMS methods will open the door to comprehensive authenticity assessment of natural flavor and fragrance compounds in the near future.

So far, general conditions for the official monographs of essential oils are recommended as follows: (*i*) extraction by steam distillation, according to GMP conditions; (*ii*) evaluation of characteristic components by ring tests, using authentic drug material; (*iii)* quantitation by cGC–MS, using internal standards; and (*iv*) authenticity assessment, using enantio-MDGC–MS or enantio-MDGC-IRMS (or both), including multielement ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, $\delta^{15}N$) and multicomponent analysis.

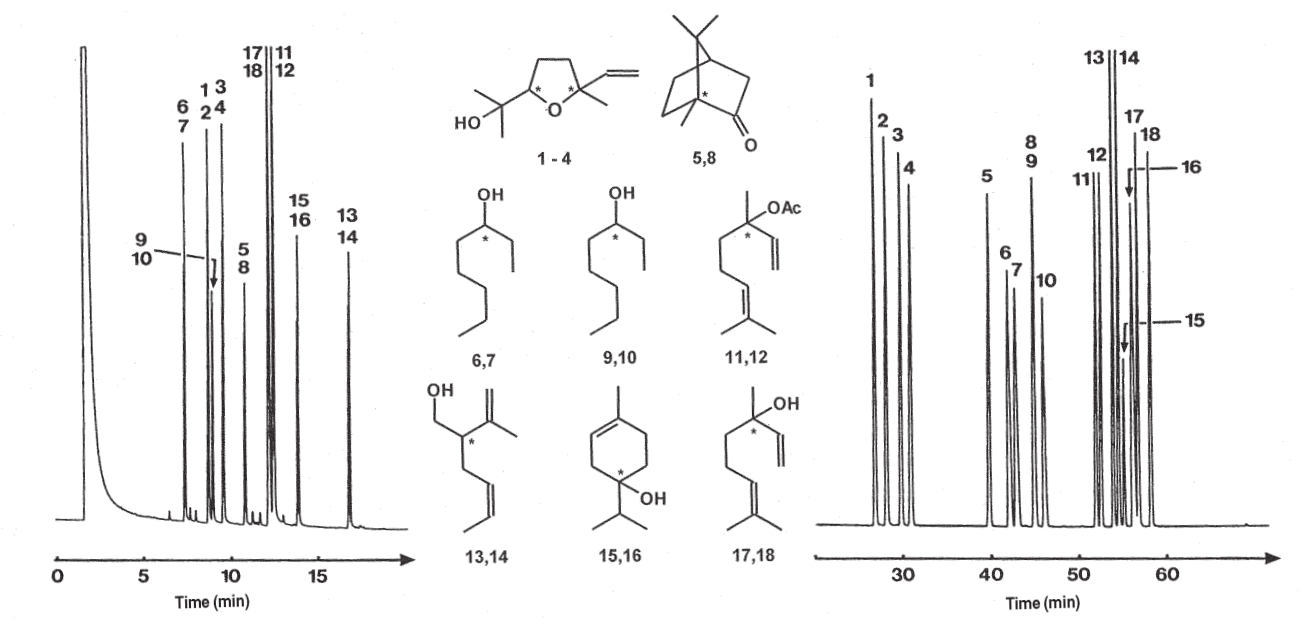

Figure 10. Simultaneous stereoanalysis of lavandula oil constituents using enantio-MDGC (standard mixture). (A) Preseparation of racemic compounds; unresolved enantiomeric pairs of octan-3-ol (**6,7**), *trans*-linalool oxide (**1,2**), oct-1-en-3-ol (**9,10**), *cis*-linalool oxide (**3,4**), camphor (**5,8**), linalool (**17,18**), linalyl acetate (**11,12**), terpinen-4-ol (**15,16**), and lavandulol (**13,14**). (B) Chiral resolution of enantiomeric pairs, transferred from the precolumn: *trans*-linalool oxide, **1** (2*S*,5*S*) and **2** (2*R*,5*R*); *cis*-linalool oxide, **3** (2*R*,5*S*) and **4** (2*S*,5*R*); camphor, **5** (1*S*) and **8** (1*R*); octan-3-ol, **6** (*R*) and **7** (*S*); oct-1-en-3-ol, **9** (*S*) and **10** (*R*); linalyl acetate, **11** (*R*) and **12** (*S*); lavandulol, **13** (*R*) and **14** (*S*); terpinen-4-ol, **15** (*R*) and **16** (*S*); and linalool, **17** (*R*) and **18** (*S*) [taken from (39)].

Table VI. Enantiomeric Distributions of Chiral Monoterpenoids from Authentic Samples of Lavandula Oils*

Sample no.	*trans*-Linalol oxide (2*R*,5*R*) 2	*cis*-Linalol oxide (2*R*,5*S*) 3	Linalyl acetate (*R*) 11	Lavandulol (*R*) 13	Terpinen-4-ol (*S*) 16	Linalol (*R*) 17
1	86.4	88.5	> 99	93.2	–	97.4
2	–	–	> 99	98.5	97.8	94.5
3	88.6	86.0	> 99	98.0	–	96.6
4	84.6	86.7	> 99	89.8	98.0	95.1
5	90.2	91.5	> 99	> 99	94.2	97.5
6	96.1	91.5	> 99	> 99	98.2	97.3
7	95.8	92.9	> 99	98.3	98.0	96.9
8	> 95	>95	> 99	98.7	98.3	98.2
9	85.4	90.0	> 99	> 99	98.1	96.1
10	87.5	95.8	> 99	> 99	98.4	97.1
11	–	–	> 99	> 99	98.1	95.2
12	76.7	82.9	98.8	> 99	98.1	97.2
13	86.2	89.3	> 99	96.2	89.1	95.1

* Taken from (39).

Conclusion

It must be emphasized that in the field of authenticity assessment of food flavor and essential oils some hundred papers were published during the last decade. This is why comprehensive citation of all these papers remains definitely impossible. Therefore, the following key words are recommended for the interested reader in a literature search: *authentication in conjunction with flavors, essential oils, enantioselective (multidimensional) capillary GC, and multielement-/multicomponent IRMS.*

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