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Chromatography of Aroma Compounds and Fragrances

Tibor Cserháti

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Preface

The quantity and composition of aroma and flavour compounds in foods and food products exert a marked influence on the consumer acceptance and, consequently, on the commercial value of the products. It has been established many times that one of the main properties employed for the evaluation of the product quality is the flavour, that is, an adequate flavour composition considerably enhances the marketability. Traditional analytical methods are generally unsuitable for the accurate determination of the quantity of this class of compounds. Moreover, they do not contain any useful information on the concentration of the individual substances and they are not suitable for their identification. As the stability of the aroma compounds and fragrances against hydrolysis, oxidation and other environmental and technological conditions shows marked differences, the exact determination of the flavour composition of a food or food product may help for the prediction of the shelf-life of products and the assessment of the influence of technological steps on the aroma compounds resulting in more consumer-friendly processing methods. Furthermore, the qualitative determination and identification of these substances may contribute to the establishment of the provenance of the product facilitating the authenticity test. Because of the considerable commercial importance of flavour composition, much effort has been devoted to the development of methods suitable for the separation and quantitative determination of flavour compounds and fragrances in foods and in other industrial products. The high separation capacity of gas chromatography (GC) technologies and the volatility of the majority of aroma compounds make it a method of preference for the analysis of flavour and aroma compounds and fragrances. Other separation technologies such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and electrically drived techniques have also found application in the separation and quantitative determination of aroma compounds. As the development of chromatographic separation techniques is very rapid, the number of new chromatographic methods employed for the analysis of flavour compounds is also rapidly increasing. The objectives of the book are the compilation of the newest results in this field of research, the critical evaluation of the results and the prediction of the future trends in the study of these compound classes. The book aims to be self-sufficient in terms of the need of the professional intending to work in this interesting field. I

am confident that the book will be useful as a valuable reference for researchers and advanced students interested in the topics covered.

The author is grateful to Ms. Eva Tarlós and Ms. Esther Bartha for their valuable technical assistance.

Budapest, Hungary Tibor Cserháti

Contents

Abbreviations

2-AAP	2-aminoacetophenone
ACN	acetonitrile
AD	air-drying
AED	atomic emission detection
APCI	atmospheric pressure chemical ionisation
BP-ANN	back-propagation feed-forward artificial network
CAR	carboxen
CE	capillary electrophoresis
CF	cold finger distillation
CGA	chlorogenic acid
CGE	capillary gel electrophoresis
CIEF	capllary isoelectric focusing
CITP	capillary isotachophoresis
CLND	chemiluminescence nitrogen detection
CLSA	closed-loop stripping analysis
CTAH	cetyltrimethylammonium hydroxide
CZE	capillary zone electrophoresis
DFA	detection frequency analysis
DGGE	denaturing gradient gel electrophoresis
DHS	dynamic headspace extraction
D-HS	dynamic headspace sampling
DI-SPME	direct-immersion solid-phase microextraction
DLLME	dispersive liquid–liquid microextraction
DTD	direct thermal desorption
DVB	divinylbenzene
ED	electrochemical detection
ECD	electron capture detector
EI-MS	electron impact mass spectrometry
ELSD	evaporative light-scattering detector
Enantio-MDGC-MS	enantioselective multidimensional gas chromatography-mass spectrometry
ESI-MS	electron spray ionisation mass spectrometry
FAA	free amino acids

FAB-MS	fast atom bombardment mass spectrometry
FDA	factorial discriminate analysis
FFA	free fatty acids
FID	flame ionisation detector
FPD	flame photometric detector
GC	gas chromatography
GCO, GC-O	gas chromatography-olfactometry
GCO-H	gas chromatography-olfactometry of headspace
GLC	gas–liquid chromatography
GSC	gas–solid chromatography
H	theoretical plate height
HCC-HS	high concentration capacity headspace sampling
HD	hydrodistillation
HMF	hydroxymethylfurfurol
HPGFC	high-performance gel filtration chromatography
HPLC	high-performance liquid chromatography
HPSEC	high-performance size exclusion chromatography
HRGC-IRMS	gas chromatography-isotope ratio mass spectrometry
HS-LPME	headspace liquid-phase microextraction
HP-SPME	headspace solid-phase microextraction
HSSE	headspace sorptive extraction
HVD	high-vacuum distillation
I	retention index
i.d.	internal diameter
IEC	ion-exchange chromatography
IGC	inverse gas chromatography
IS	internal standard
K_D	distribution constant
k'	capacity factor
LC	liquid chromatography
LD	limit of detection
LDA	linear discriminant analysis
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
MAD	microwave-accelerated distillation
MDGC-O	multidimensional GC-olfactometry
MEKC	micellar electrokinetic chromatography
MN	methyl nicotinate
MS	mass spectrometry
NCI	negative chemical ionisation
NMR	nuclear magnetic resonance
NPSD	nitrogen purge steam distillation
ODS	octadecyl silica
PAGE	polyacrylamide gel electrophoresis

PCA	principal component analysis
PDMS	polydimethylsiloxane
PDO	protected designation of origin
PFPD	pulsed flame photometric detection
PGC	porous graphitised carbon
PHW	pressurised hot water
PLS	partial least squares
PTV	programmable temperature vaporisation injector
Py-GC-MS	pyrolysis gas chromatography mass spectrometry
RP	reversed-phase
RP-HPLC	reversed-phase high-performance liquid chromatography
RP-TLC	reversed-phase thin-layer chromatography
RSD	relative standard deviation
SbCWE	subcritical water extraction
SBF	simulated beef flavour
SBSE	stir bar sorptive extraction
SD	steam distillation
SDE	simulataneous distillation extraction
SEC	size exclusion chromatography
SFE	supercritical fluid extraction
SIM	selected ion monitoring
SAFE	solvent-assisted flavour evaporation
SFSI	solvent-free solid injection
SPACE	solid-phase aroma concentrate extraction
SPE	solid-phase extraction
SPI	soy protein isolate
SPME	solid-phase microextraction
T_R	retention time
TDCT	thermal desorption cold-trap
TFA	trifluoroacetic acid
TLC	thin-layer chromatography
TOFS	time-of-flight mass spectrometer
USE	organic solvent extraction under ultrasonic irradiation
UV-VIS	ultraviolet-visible
V_N	net retention volume
VBD	vacuum belt drying
VOC	volatile organic compound

Chapter 1
Chromatography of Aroma Substances and Fragrances

1.1 Theory and Practice of Chromatographic Techniques

The base of any chromatographic separation methods is the partition of the analytes between a solid or semisolid stationary and a mobile phase consisting of gas or fluid. Because of the different physicochemical characteristics of the analytes, they differ in their capacity to bind to the stationary and mobile phases. Because of the differences between the binding energies, analytes show different mobility in the chromatographic system resulting in their separation.

1.1.1 Preconcentration and Prepurification of Analytes

The concentration of aroma substances, flavour compounds and fragrances is generally low in the samples; moreover, they are present in complicated matrices frequently containing both organic and inorganic components such as various foods and food products, cosmetics, pharmaceutical preparations. The aim of the sample preparation is the prepurification and/or preconcentration of the solutes to be analysed and the possible decrease of the disturbing accompanying components which can deteriorate the efficacy of the analysis, reduce the theoretical plate number, modify separation factor, peak symmetry, reproducibility and repeatability. Sample preparation often plays a decisive role in the success of any chromatographic analysis. The conventional method of sample preparation is the shake flash extraction and its modern variant, the Soxhlet extraction. These liquid extraction or liquid–liquid extraction (LLE) techniques are generally very efficient but time-consuming, and the considerable amount of organic solvent can endanger the health of the laboratory staff and can increase environmental pollution. The extracting solvent has to comply with some requirements. First of all, it cannot be toxic neither to humans nor to any living organism; it has to be selective as much as possible dissolving maximally the analytes and minimally the other components present in the sample. Because of the complexity of both the composition of aroma substances and that of other undesirable compounds in the sample, the objective to find and use the ideal solvent can be only approximately fulfilled.

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In order to overcome the disadvantages of the LLE method mentioned above, a considerable number of up-to-date extraction methods were developed and successfully applied for the preconcentration and prepurification of aroma substances and fragrances in various accompanying matrices.

Solid-phase extraction (SPE) can be applied for the preconcentration and prepurification of both liquid samples and for the liquid extract of solid samples. The liquid containing the compounds to be analysed is passed through a glass or plastic cartridge filled with a sorbent with high adsorption capacity. The analytes will remain in the cartridge adsorbed on the surface of the sorbent. After finishing the adsorption step, the sorbent is washed to remove the majority of coadsorbed components.

Then a strong sorbent is applied for the removal of the molecules of interest. The main requirements for an efficient SPE method are the highest possible selectivity and adsorption capacity of the sorbent.

The selection of the sorbent depends on theoretical consideration. Nonpolar analytes can be retained on nonpolar sorbents while analytes with polar substructures can adsorb on sorbent containing adsorption centres of opposite polarity. The volume of sorbing commercially varies between 1 and 50 ml.

Solid-phase microextraction (SPME) uses a short thin solid rod (generally 1 cm long and 0.1 μm outer diameter), sometimes coated with a polar or nonpolar polymer. The SPME fibre is attached to a metal rod. Before analysis the fibre is withdrawn into a protective sheath. The sample is placed in a vial and the vial is closed with a cap containing a septum. The sheath is pushed through the septum, the plunger is lowered forcing the adsorptive fibre into the vial. The fibre can be immersed in the liquid sample or let in contact with the headspace over the solid or liquid sample. Analytes are adsorbed into the fibre. After reaching equilibrium between the adsorbed and nonadsorbed fractions of analytes, the fibre is withdrawn into the sheath and the sheath is pulled out of the vial, and inserted in the septum of the injector of gas chromatograph (GC). After thermal desorption the analytes are separated in the GC column. The adsorption characteristics of polymers (polydimethylsiloxane, polyacrylate, carbowax/divinyl benzene, etc.) have been vigorously investigated and their application in solution of various separation problems was proposed. As the equilibrium between the adsorbed and nonabsorbed analytes may be different even for molecules of very similar chemical structure, the efficacy of SPME shows high differences depending on both the character of the adsorbent and that of the analytes.

A careful calibration of each components of the sample is a prerequisite for the precise quantitation of the data using SPME. The method has been extensively applied for the determination of furan in baby-food (Bianchi et al., 2006), volatile oak compounds (Carrillo et al., 2006) and volatile phenols in wine (Mejias et al., 2003), sorbic and benzoic acids in beverages (Dong and Wang, 2006), aroma active compounds in orange essence oil (Hognadottir and Rouseff, 2003), organophosphorus insecticides in strawberry and cherry juices (Lambropoulou and Albanis, 2002), headspace flavour compounds of banana (Liu and Yang, 2002), volatile compounds in fruit juices and nectars (Riu-Aumatell et al., 2004) and aroma volatiles from orange juices (Rouseff et al., 2001).

Supercritical fluid extraction (SFE) is a solvent-free alternative to the extraction methods discussed above. It applies inexpensive and environmental-friendly mobile phase (carbon dioxide or carbon dioxide mixed with organic solvents). The extraction time is shorter and the extract can be used directly for GC, TLC and HPLC analyses. The theoretical basis of the use of SFE is the fact that in supercritical state the physicochemical properties of an extracting agent are between those of gases and liquids. The mass transfer is rapid because the dynamic viscosity is near to those of gases under normal conditions. The increase of temperature increases the diffusivity at fixed pressure and decreases the viscosity.

Besides the extraction methods listed above, some other prepurification and preconcentration technologies were developed and their application in various fields of chromatographic separation was reported (microwave-assisted extraction, pressurised liquid extraction, continuous-flow liquid membrane extraction, simultaneous steam distillation extraction, etc). However, the application possibilities of these techniques are not entirely elucidated, and at the present state of our knowledge, the advantages and disadvantages of these method cannot be correctly evaluated.

1.1.2 Gas Chromatography (GC)

Gas chromatography (GC) includes separation technologies based on the difference between the adsorption of volatile analytes, when the mobile phase is gas and the stationary phase is solid (gas–solid chromatography = GSC) or semisolid liquid (gas–liquid chromatography = GLC). Because the overwhelming majority of aroma substances, flavour compounds and fragrances are volatile, GC techniques are the methods of preference for their separation and quantitative determination. However, the use of GC technologies is limited to a relative low number of compounds: the analyte has to have an appreciable vapour pressure below 350–400°C and it has to be stable at the temperature of separation.

A common GC instrument consists of a carrier gas delivery system (mobile phase), an injector port, separation column, detector and data-processing unit. A considerable number of injector systems were developed. Injectors have to deliver the vapourised sample to the beginning of the separation column with the initial bandwidth as small as possible. The two main classes of injectors are the vaporisation and on-column injectors. The temperature of vaporisation injectors is identical or higher than the temperature of GC column. In these systems the sample is rapidly evaporated. Samples can be introduced into the injector by a syringe. Sample components not volatile at the injector temperature remain bonded to the injector or at the beginning of the analytical column, causing the decrease of the efficacy of the entire GC system. On-column injectors deposit the sample directly in the column.

GC columns can be divided into two separate groups, packed and capillary columns. Packed columns are prepared by filling metal or glass columns with small particles generally coated with a thin layer of high molecular mass nonvolatile polymer. Solid supports are often diatomaceous earth, graphitised carbon black, glass beads, etc. Besides nonvolatility, the coating agent has to be chemically stable and of low visocity at the temperature of the measurements, good selectivity for the

components of the sample, and good wetting capacity for the surface of both the inert particles and the inner wall of the column. Because of the increasing pressure, the maximal length of packed columns is about 3 m while capillary columns can reach 60 m length. The advantage of the packed column is the greater sample capacity; however, the theoretical plate number of capillary columns is considerably higher.

The internal diameter of capillary columns is 0.2–0.53 mm, they are generally prepared from fused silica. The inner wall of the capillary column is coated with a thin layer of polymeric stationary phase varying between 0.1 and 5 μm. The stability of the column coating can be enhanced by cross-linking the polymer and binding it covalently to the surface of the inner wall. The reliable temperature control of the column plays a decisive role in the efficacy of the separation process. Measurements can be performed at constant temperature (isocratic separation mode) or the column temperature can be increased according to a predetermined program (temperature gradient). In the majority of cases the carried gas is a permanent gas without marked adsorption capacity such as helium, hydrogen or nitrogen. Because of high flammability, hydrogen is not frequently applied and the use of helium is limited by its price. GC practice generally employ nitrogen; however, other gases can also be used for the solution of special problems both in the practice and in the theory of GC. Detectors are placed at the end of the GC column. They interact with the solute molecules; the interaction is converted into a signal, which is sent to the recording and/or data-processing unit. The plot of the intensity of the signal vs analysis time is created (chromatogram). The main criteria of the selection of a detector are the sensitivity (lowest detectable amount of the analyte) and selectivity (differences between the detector responses for different analytes at the same concentration). A considerable number of detectors were developed for the solution of various detection problems. The detectors most frequently used are flame-ionisation, nitrogen-phosphorous, flame photometric, electron capture, thermal conductivity, chemiluminescence detectors. Past decade's various types of mass spectrometric (MS) techniques were developed and coupled to GC. Besides sensitivity, the main advantage of the MS systems is that they make possible the identification of analytes, which cannot be directly achieved by other detection methods.

The partition of analyte molecules between the solid stationary phase and the gaseous mobile phase can be characterised by the distribution constant (K_D), which can be defined by

K_D = analyte concentration in the stationary phase/analyte concentration in the mobile phase. (1.1)

The dependence of the distribution constant on the column temperature can be described by

$$\ln K_D = \frac{-\Delta G^0}{RT}, \tag{1.2}$$

where G^0 is the change in Gibbs free energy for the removal of an analyte molecule from the stationary phase, T is the temperature of the separation column and R is the ideal gas constant T. Equation (1.2) indicates that the differences in the Gibbs free energy of analytes result in different retention. Retention time of an analyte (t_R) is defined by the time difference between the beginning of the separation process and the maximum of its chromatographic peak. Capacity factor (k') is the time what an analyte spends in the stationary phase relative to the mobile phase:

$$k' = \frac{(t_R - t_0)}{t_0}, \tag{1.3}$$

where t_0 is the time required for a nonretained analyte to travel through the column.

Retention index (I) was introduced to increase the reproducibility and reliability of the determination of analyte retention:

$$I = 100c + 100\frac{(\log V_{Nx} - \log V_{Nc})}{(\log V_{Nc+1} - \log V_{Nc})}, \tag{1.4}$$

where x refers to the analyte, c refers to the number of carbon atoms of the n-hydrocarbon eluting before the analyte, $c + 1$ refers to the number of carbon atoms in the n-hydrocarbon eluting after the analyte and V_N is the net retention volume. The exact determination of the V_N has been vigorously discussed in the chromatographic practice. It is generally accepted that the net retention time is equal to the difference between the peaks of nonadsorbed and adsorbed analytes. The separation factor (α) characterising the efficacy of the GC system is defined by

$$\alpha = k'_2/k'_1, \tag{1.5}$$

where k'_1 is the partition ratio of the earlier eluting peak and k'_2 is the partition ratio of the later eluting peak. Another parameter used for the description of the quality of separation of two neighbouring peaks is the resolution number (R):

$$R = 1.18\frac{(t_{R2} - t_{R1})}{(w_{h1} + w_{h2})}, \tag{1.6}$$

$$R = 2\frac{(t_{R2} - t_{R1})}{(w_{b1} + w_{b2})}, \tag{1.7}$$

where t_{R1} and t_{R2} are the retention times of peaks 1 and 2, respectively, w_{h1} and w_{h2} are peak width at half widths of peaks 1 and 2, respectively, w_{b1} and w_{b2} are the peak widths at the base of peaks 1 and 2, respectively. The separation capacity of the GC column can be characterised by the theoretical plate number (N):

$$N = 5.545\,(t_R/w_h)^2\,. \tag{1.8}$$

Various theoretical and practical aspects of GC have been frequently discussed in detail in excellent reference books. Thus, the retention parameters and characterisation of stationary phases (Rotzsche, 1991), mixed stationary phases (Price, 1989), column-switching technologies (Willis, 1989), solvating GC using packed columns (Shen and Lee, 1998), theoretical aspects of capillary GC (Hill and McMinn, 1995), practical application (Grob, 1995), specially in analytical chemistry (Jennings, 1987), in laboratory analysis (Guiochon and Guillemen, 1998), applications in the analysis of air pollutants (Berezkin and Drugov, 1991) and of natural products (Coleman and Gordon, 1994) were evaluated.

1.1.3 Liquid Chromatography (LC)

Liquid chromatography (LC) includes chromatographic methods using a liquid mobile phase and a solid organic or inorganic stationary phase. LC methods differ in the shape of the stationary phase. Thin-layer chromatography applies planar stationary phases while high-performance liquid chromatography (HPLC) is carried out in column of different dimensions. Another method of classification is based on the polarity and apolarity differences between the mobile and stationary phases. Adsorption (normal or direct) phase LC uses a polar stationary and a nonpolar mobile phase, while reversed-phase (RP) LC applies apolar stationary and polar mobile phase.

1.1.3.1 Thin-Layer Chromatography (TLC)

Although TLC methods are easy to carry out, are relatively rapid, make possible the application of various detection methods and the simultaneous analysis of a considerable number of samples, its application in the chromatographic analysis of aroma compounds, flavours and fragrances is fairly limited. This fact can be explained by the high volatility of some analytes resulting in considerable loss during the separation process and detection, and by the low separation capacity compared to that of GC and HPLC. The theory of TLC and HPLC is fairly similar, and the equations describing the theoretical background of separation are quasi-identical. The practice and theory of various TLC technologies have been discussed many times in excellent reference books and reviews (Sherma and Fried, 2003; Hahn-Deinstrop, 2000; Sherma, 2004; Siouffi, 2002; Gocan, 2002).

1.1.3.2 High-Performance Liquid Chromatography

HPLC technologies apply liquid mobile phase and a solid stationary phase filled in columns of different dimensions. The average diameter of the particles of the stationary phase is between 2 and 10 μm with a narrow particle size distribution (except monolithic columns). The dimensions of columns show high variety and the column length is generally between 5 and 25 cm; the internal diameter is between 2

and 5 mm. The rapid development of miniaturisation results in columns with considerably smaller dimensions. Besides the traditional normal and RP stationary phases, a considerable number of other phases were developed showing different separation selectivity. Thus, ion-exchange, ion-pair, size exclusion, gel permeation and affinity chromatography have found application in the chromatographic practice. However, their importance in the analysis of aroma substances and related compounds is fairly low, and they are not frequently employed for the separation and quantitative determination of this class of analytes.

Separation capacity in HPLC can be characterised by the theoretical plate height (N):

$$H = A + \frac{B}{\mu} + C_s\mu + C_m, \tag{1.9}$$

or by

$$H = H_p + H_d + H_s + H_m, \tag{1.10}$$

where μ is the linear velocity of the mobile phase, H_p and A are the heights of the theoretical plate and H_d is the contribution of the molecular diffusion to H. H_p depends on the parameters of the stationary phase (particle sizes, particle diameter, mode of packing, etc.). H_d can be defined by

$$H_d = \frac{b}{\mu} = 2D_m, \tag{1.11}$$

where D_m is the diffusion coefficient of the mobile phase. H_s is the theoretical plate height related to the mass transfer in the stationary phase showing the peak broadening because of the resistance of mass transfer to the stationary phase. It is defined by

$$H_s = C_s\mu = \frac{2d_s^2\, k'\mu}{3D_s\left(1 + k'\right)^2}, \tag{1.12}$$

where d_s is the thickness of the mobile phase on the surface of stationary phase and D_s is the diffusion coefficient of the solute. The theoretical plate height (H_m) based on the mass transfer in the mobile phase is equal to

$$H_m = C_m\mu = \frac{wd_p^2\mu}{D_m}, \tag{1.13}$$

where w is a constant and d_p is the mean of the particle diameter.

The theoretical plate height can also be defined by

$$H = \frac{1}{\left[(1/H_p) + (1/H_{pm})\right]} + H_dH_sH_m, \tag{1.14}$$

where the values H_{pm} and H_p were corrected for the multipath effect.

Similar to GC, the capacity factor can be calculated by

$$k' = \frac{(t_R - t_0)}{t_0}. \tag{1.15}$$

The dependence of retention of an analyte on the polarity of the eluent system can be determined by

$$\frac{k'_2}{k'_1} = 10^{(P'1-P'2)2}, \tag{1.16}$$

where k'_2 and k'_1 are the capacity factors of the analyte measured in the second and first eluent system, and P'_1 and P'_2 are the polarities of the first and second mobile phases. In the case of binary mobile phase, the dependence of the retention on the volume fraction of the component with a higher elution strength (C) can be described by

$$\log k' = \log k'_0 + bC, \tag{1.17}$$

where k' is the capacity factor measured at a given volume fraction of the stronger component in the mobile phase, k'_0 is the capacity factor extrapolated to 100% concentration of the weaker component in the eluent, and b is the change of the $\log k'$ caused by unit change of C in the mobile phase.

Silica or surface-modified silica are preferentially employed in the HPLC practice. Silica sorbents are porous and noncrystalline and their polarity highly depends on the amount of silanol groups on the surface.

These surface silanols make possible the covalent binding of various organic ligands to the silica surface and display some ion-exchange properties which influence the retention of analytes with dissociable polar substructures. Unfortunately, silica and silica-based stationary phases are not stable in alkaline environment; they cannot be applied over pH 8.0. To overcome this disadvantage, a considerable number of other stationary phases were synthesised and used for the solution of various separation problems (polar and apolar polymers, porous graphitised carbon, zirconium oxide and its derivatives, alumina and its derivatives, etc.). The various aspects of theory and practice of HPLC methods have been discussed in exquisite book, such as the use of HPLC-MS in drug analysis (Rossi and Sinz, 2002), the basic theory (Cazes and Scott, 2002) and fundamentals of chromatography (Cazes, 2001; Pool, 2003), the application of ion chromatography (Fritz and Hahn-Deinstrop, 2000), the solution of frequent problems in HPLC (Kromidas, 2000), the separation and quantitative determination of foods and food products (Nollet, 2000), macromolecules (Gooding and Regnier, 2002), peptides (Aguilar, 2002), etc.

1.1.4 Electrically Drived Chromatographic Systems

The efficacy of capillary electrophoresis (CE) and related technologies is extremely high although the instrumentation is relatively simple. The name of these methods indicates that the separation is performed under the effect of electric field. The main advantages of the electrically drived systems are the low sample volume requirements, on-capillary detection and the easy automatisation of the system. CE measurements are carried out in a capillary tube filled with a buffer and the ends of the capillary are immersed into buffer reservoirs being at the same level. A high voltage is applied for the capillary. Charged analytes migrate according to their charge-to-mass ratio. Capillary gel electrophoresis uses a capillary tube filled with a gel. The pores of gel act as sieves and the analytes are eluted according to their charge and size. Micellar electrokinetic chromatography was developed for the separation of neutral analytes. One or more ionic surfactants over their critical micelle concentration are added to the running buffer. Analytes are partitioned between the apolar core of the surfactants and the hydrophilic buffer according to their lipophilicity. Capillary isotachophoresis employs a leading and a terminating buffer. Because of the two buffers, the electric field changes along the capillary resulting in the sharpening of the band of analytes. Capillary isoelectric focusing uses a pH gradient for the separation of amphoteric analytes. The main parameters of CE separation are the electroosmotic flow (μ_{EOF}) and the electrophoretic flow (μ_{EP}). Electroosmotic flow is the bulk flow of running buffer in the capillary under the effect of applied high voltage. Electrophoretic flow is the flow of ions due to their charge. Electroosmotic flow can be defined by

$$\mu_{\mathrm{EOF}} = (\varepsilon\sigma/\xi)\,, \tag{1.18}$$

where μ_{EOF} is EOF mobility, ε is the dielectric constant, σ is the zeta potential and ξ is the bulk viscosity. The parameters characterizing separation efficacy, resolution, etc. in electrically drived systems are the same as in GC and HPLC. Migration time (t) is equal to the time required for an analyte to migrate to the point of detection. The apparent mobility (μ_{a}) can be calculated by

$$\mu_{\mathrm{a}} = I/tE = IL/tV, \tag{1.19}$$

where I is the effective capillary length, L is the total length, E is the electric field and V is applied voltage. Moreover, the apparent mobility can be defined by

$$\mu_{\mathrm{a}} = \mu_{\mathrm{e}} + \mu_{\mathrm{EOF}}, \tag{1.20}$$

where μ_{e} is the effective mobility. Electroosmotic flow can be measured using a neutral marker moving with the same velocity as the electroosmotic flow.

The capacity factor of neutral analytes in MEKC (k') is equal to

$$k' = \frac{(t_r - t_0)}{\left[t_0\,(1 - t_r/t_m)\right]} = K\left(\frac{V_s}{V_m}\right), \tag{1.21}$$

where t_r is the retention time of the analyte, t_0 retention time of the unretained analyte, t_m is the micelle retention time, K is the partition coefficient, V_s is the volume of the micellar phase and V_m is the volume of the mobile phase. A considerable number of reviews and books were published dealing with the theoretical and practical problems of CE (Grossmann and Colburn, 1992; Li, 1992) and MEKC (Vindevogel and Sandra, 1992). The application of electrically drived systems in biological sciences (Karger et al., 1989: Jorgenson, 1986) and in the analysis of surfactants was also discussed (Kohr and Engelhardt, 1991).

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Chapter 2
Food and Food Products

The consumer acceptance and, consequently, the commercial value of foods and food products depend considerably not only on the quality and quantity of colour pigments but also on the composition and amount of aroma substances. As this class of compounds occurs generally at very low concentration, their separation and quantitation require the use of preconcentration techniques shortly discussed above. The method of preference for the preconcentration of flavour compounds is the solid phase microextraction (SPME). Its application in various extraction processes and in the preconcentration of flavour compounds in vegetables, fruits, juices and other soft dinks, alcoholic beverages, dairy products, etc. has been previously reviewed (Kataoka et al., 2000). The application of pressurised hot water (PHW) extraction for the analysis of aromatic compounds in essential oils, catechins, proanthocyanidins, eugenol and eugenol acetate, isoflavons and other volatile compounds has been also investigated in detail, and the impact of various experimental conditions such as temperature, pressure, extraction time and flow rate was investigated in detail (Kronholm et al., 2007). Because of the volatility of the majority of aroma compounds, flavour substances and fragrances, various GC technologies are the methods of preference for their analysis. GC combined with olfactometry (GC-O) can be successfully employed for the characterisation of odour-active compounds. Its application in the analysis of foods and food products such as milk, cheese, coffee, meat and fruits has been recently reviewed (Zellner et al., 2007), and the various aspects of the use of different GC-O methods have been discussed in detail (van Ruth, 2001).

2.1 Fruits

Similar to other food products, the aroma compounds in fruits exert a marked effect on the commercial value of the product. The quantity and composition of flavour components characterising the quality of the fruit can be determined by GC and can be used not only as a marker of quality but also may promote the determination of the origin and type of the products.

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2.1.1 Tropical Fruits

The aroma composition of the tropical fruits has also been extensively investigated. Thus, a GC-MS method was employed for the study of the aroma profile of the pulp of caja-umbu (*Spondias* sp.). The main components were β-caryophyllene, 2-methyl-butanal, 2-hexanol, ethyl butirate and α-caryophyllene (Narain et al., 2006).

The effectivtiy of pitanga fruit (*Eugenia uniflora*) against many diseases has been many times established. It contains antioxidant compounds such as anthocyanins, flavonols and carotenoids (Lima et al., 2002), shows hypotensive (Consolini and Sarubbio, 2002) and antiviral effects (Lee et al., 2000), inhibits the increase of plasma glucose and triglyceride levels (Matsumura et al., 2000), and shows antifungal activity (Souza et al., 2002). The composition of volatiles in pitanga fruit were separated and quantitatively determined by GC-MS. Measurements were carried out on a capillary column (25 m × 0.2 mm, film thickness, 0.33 μm). Oven starting temperature was 50°C for 2 min, then raised to 180°C at 4°C/min. A typical chromatogram is shown in Fig. 2.1. Analytes were well separated from each other as demonstrated in Fig. 2.1. The volatiles identified by MS are compiled in Table 2.1.

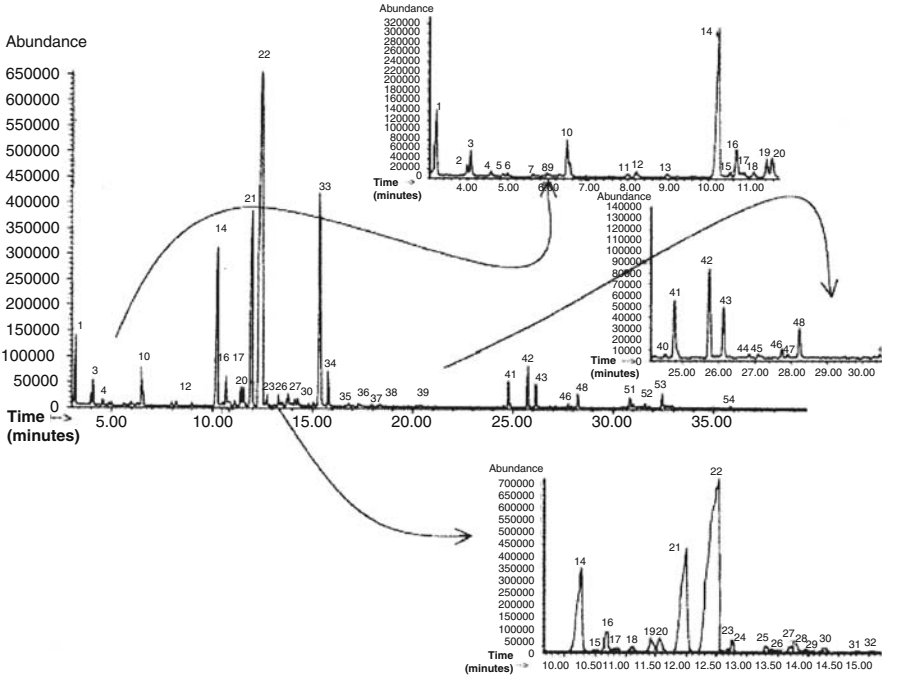

Fig. 2.1 Total ion chromatogram of the pitanga fruit extract trapped on Porapak-Q. The inserts show expansions of the chromatogram in which less abundant compounds are detected. Well-defined chromatographic peaks are sequentially numbered as a function of increasing retention time. See Table 2.1 for peak identification. Reprinted with permission from Oliveira et al. (2006)

Table 2.1 Volatile compounds of pitanga fruit trapped on Porapak-Q and identified by GC-MS analysis

Compound	Peak	Retention time (min)	Retention index[c]	Area (%)
Propyl acetate	1	3.22	–	1.8
Ethyl propionate	2	4.00	226	0.4
Isobutyl acetate	3	4.08	240	0.8
n-Butyl acetate	4	4.58	321	0.3
N.I.	5	4.89	367	0.1
N.I.	6	5.00	383	0.1
N.I.	7	5.63	466	0.1
N.I.	8	5.99	509	0.2
N.I	9	6.30	546	0.2
3-Methyl butyl acetate	10	6.49	565	2.0
α-Thujene[a]	11	8.00	712	0.2
α-Pinene[a]	12	8.21	730	0.3
N.I.	13	8.99	793	0.2
β-Pinene[a]	14	10.26	886	9.3
N.I.	15	10.54	903	0.3
1,5,8-*p*-Menthatriene	16	10.70	915	1.8
β-Mycerene[a,b]	17	10.88	927	0.4
α-Terpinene[b]	18	11.12	942	0.3
p-Cymene[b]	19	11.44	962	1.2
trans-Ocimene[b]	20	11.57	970	1.2
cis-Ocimene[b]	21	11.98	995	13.4
trans-β-Ocimene	22	12.46	1024	36.2
N.I.	23	12.68	135	0.2
λ-Terpinene[a,b]	24	12.75	1041	0.6
N.I.	25	13.31	1069	0.4
p-Mentha-1,5,8-triene	26	13.50	1084	0.3
N.I.	27	13.71	1090	0.4
Terpinolene[a,b]	28	13.79	1100	0.9
Rosefuran	29	14.13	1116	0.4
Linalool[b]	30	14.27	1122	0.4
N.I.	31	14.82	1144	0.2
N.I.	32	15.06	1155	0.2
β-Ocimene	33	15.38	1172	15.4
Allo-ocimene[a]	34	15.79	1189	1.5
N.I.	35	16.81	1232	0.2
N.I.	36	17.43	1258	0.1
N.I.	37	17.97	1279	0.1
N.I.	38	18.35	1294	0.2
Acetophenone	39	20.38	1288	0.1
N.I.	40	24.53	1497	0.1
β-Elemene[a,b]	41	24.78	1409	1.3
β-Caryophyllene[a,b]	42	25.75	1440	0.1
λ-Elemene[a,b]	43	26.14	1452	1.1
N.I.	44	26.86	1561	0.1
N.I.	45	27.11	1567	0.1
Germacrene-D	46	27.76	1502	0.2
N.I.	47	27.94	1588	0.1

Table 2.1 (continued)

Compound	Peak	Retention time (min)	Retention index[c]	Area (%)
Curzerene[b]	48	28.25	1576	0.7
N.I.	49	30.51	1650	0.1
N.I.	50	30.86	1658	0.5
Caryophyllene oxide	51	31.02	1729	0.2
β-Elemenone[a]	52	31.60	1,744	0.2
Selina-1,3,7(11)-trien-8-one[b]	53	32.45	1767	0.8
N.I.	54	35.85	1823	0.1

[a]These volatile components were also identified in Cuban pitanga fruit using steam distillation and solvent extraction.
[b]Volatile constituents were also found in pitanga leaf extracts.
[c]On HP Ultra II column.
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Because of the considerable amount of volatiles with beneficial biological activity, the consumption of the pitange fruit is advocated (Oliveira et al., 2006).

Similarly to pitanga fruit, the fruits of *Evodia* species also show beneficial pharmaceutical effects. Because of the marked biological efficacy, the volatile composition of *Evodia* species fruits has been investigated using HP-SPME coupled with GC-MS. Separation was carried out in a capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Initial oven temperature was 50°C for 2 min, then raised to 280°C at 8°C/min, final hold 2 min. The volatile compounds identified in the samples are compiled in Table 2.2. The data in Table 2.2 illustrate that the aroma profile of various species show marked differences (Pellati et al., 2005). The leaves, fruits and seeds of wampee [*Clausena lansium* (Lour.) Skeels] also show considerable biological activity. The antifungal activity and HIV reverse transcriptase-inhibitory activities of the extract have been demonstrated (Ng et al., 2003). The volatile composition of the leaves, fruits and seeds of wampee was investigated in a separate study employing HP-SPME followed by GC-MS. Measurements were performed in a capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Initial oven temperature was 40°C, raised to 100°C at 3°C/min, then to 230°C at 5°C, final hold 2 min. MS conditions were electron energy, 70 eV, ion source temperature, 230°C, mass range 35–400 *m/z*. The results are compiled in Table 2.3. The date demonstrated that the aroma profiles of leaves, fruits and seeds are markedly different. It was further established that the method is suitable for the separation and quantitative determination of the low-temperature volatile aromatic compounds (Chokeprasert et al., 2007).

A SPME-GC-TOFMS method was applied for the separation and quantitative determination of volatile flavour compounds in minimally processed durian (*Durio zibethinus* cv. D24 fruit). The change of the quality and quantity of flavour compounds during storage at 4°C was followed. GC analyses were performed on a capillary column (10 m × 0.10 mm, film thickness 0.10 μm), injector and detector temperature were 250°C. Initial column temperature was 40°C for 1.5 min, raised to 240°C at 50°C/min, final hold was 2 min. The flavour compounds are listed in Table 2.4. The data indicated that the composition and amount of flavor

Table 2.2 Volatile aroma components of *Evodia* spp. obtained by HS-SPME

Peak no.	Compound[a]	Kovats index (l)[b]	*E. rutaecarpa* % RA[c]	SD	*E. officinalis* % RA[c]	SD	Method of identification[d]
1	Myrcene	993	5.83	0.45	32.79	2.16	a,b,c,d
2	δ-3-Carene	1010	0.11	0.03	0.67	0.04	a,b,c,d
3	α-Terpinene	1022	–	–	0.12	0.04	a,b,c,d
4	*p*-Cymene	1029	0.52	0.06	0.10	0.04	a,b,c,d
5	Limonene	1034	33.79	5.10	18.36	0.30	a,b,c,d
6	*cis*-β-ocimene	1040	0.71	0.07	2.06	0.21	a,b,c,d
7	*trans*-β-ocimene	1052	1.08	0.10	6.04	0.84	a,b,c,d
8	γ-Terpinene	1063	0.56	0.06	–	–	a,b,c,d
9	*cis*-Linalool oxide	1079	0.20	0.05	0.06	0.01	b,d
10	Terpinolene	1091	–	–	0.12	0.03	b,d
11	*cis*-Linalool oxide	1095	0.17	0.02	–	–	b,d
12	Linalool	1104	8.15	0.47	5.88	0.53	a,b,c,d
13	Nonanal	1109	2.83	0.37	–	–	b,d
14	Borneol	1175	–	–	0.18	0.01	a,b,c,d
15	4-Terpineol	1185	0.51	0.06	0.40	0.03	a,b,c,d
16	α-Terpineol	1198	3.99	0.15	0.21	0.01	a,b,c,d
17	Citronellol	1234	–	–	0.34	0.01	b,d
18	Lynalyl acetate	1257	4.13	0.15	0.16	0.05	a,b,c,d
19	Geraniol	1261	–	–	0.25	0.01	b,d
20	Tridecane	1298	0.84	0.03	0.42	0.03	b,d
21	δ-Elemene	1343	0.47	0.04	0.24	0.02	b,d
22	α-Cubebene	1356	0.65	0.09	0.35	0.02	a,b,c,d
23	α-Copeane	1385	1.21	0.42	0.43	0.06	a,b,c,d
24	β-Elemene	1401	10.78	1.11	7.85	0.96	b,d
25	β-Caryophyllene	1431	4.62	0.83	9.92	0.83	a,b,c,d
26	γ-Elemene	1442	2.05	0.33	1.20	0.11	b,d
27	α-Guaiene	1447	0.07	0.03	–	–	b,d
28	α-Humulene	1465	1.77	0.13	0.99	0.09	a,b,c,d
29	Valencene	1499	4.73	0.40	4.62	0.45	a,b,c,d
30	2,6-Di-*tert*-butyl-4-hydroxy toluene	1519	7.34	0.78	5.02	0.51	a,b,c,d
31	δ-Cadinene	1535	2.86	0.28	1.82	0.01	b,d

(–): Compound not detected.
[a]Compounds are listed in order of elution.
[b]Retention index on RTX-5 column.
[c]Percent relative area.
[d]a:retention time, b:retention index, c:peak enrichment, d:mass spectrum.
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compounds show marked modifications during storage (Voon et al., 2007a). Similar results have been previously reported (Chin et al., 2007). The aroma composition in lulo (*Solanum quitoense*) leaves under the effect of enzymatic hydrolysis was investigated in detail. The volatile hydrolysis products were separated by GC and identified by GC-MS. GC analyses were performed in a fused capillary column (25 m × 0.2 mm, film thickness, 0.33 μm). Column temperature started at 50°C and raised to 300°C at 4°C/min. Injector and detector temperatures were set to 300°C. Analytes were detected by FID. GC-MS measurements used the same GC system as

Table 2.3 Volatile compounds identified in wampee using headspace sampler with HP-5MS nonpolar column

No	Compounds	RI	%Relative area				ID
			Leaf	Flesh	Skin	Seed	
1	Ethanol		t	2.46	t	t	MS
2	2-Propanone		3.02	t	t	t	MS
3	Propanal		1.63	t	t	t	MS
4	2-Methylfuran		1.10	t	t	t	MS
5	Butanal		8.61	t	t	t	MS
6	1-Pentene		1.89	t	t	t	MS
7	2-Ethylfuran		4.61	t	t	t	MS
8	Ethanone		0.20	t	t	t	MS
9	Acetic acid		0.94	2.65	0.08	0.03	MS
10	*cis*-2-pentenol		0.71	t	t	t	MS
11	Hexanal	802	1.55	0.47	0.04	t	MS, RI_1
12	2-hexanal	854	1.46	t	t	t	MS, RI_1
13	3-Hexen-1-ol	857	0.17	t	t	t	MS, RI_1
14	Styrene	921	0.13	t	t	t	MS, RI_1
15	Tricyclene	928	t	t	0.03	t	MS, RI_1
16	α-Thujene	931	t	t	0.02	0.59	MS, RI_1
17	α-Pinene	939	1.99	2.08	9.41	4.26	MS, RI_1
18	Camphene	945	0.98	t	0.47	0.04	MS, RI_1
19	Benzaldehyde	958	2.56	t	t	0.02	MS, RI_1
20	β-Piene	967	t	0.21	0.17	t	MS, RI_1
21	Sabinene	973	14.92	50.64	69.07	83.56	MS, RI_1
22	6-Methyl-5-hepten-2-one	976	2.26	t	t	t	MS, RI_1
23	Myrcene	993	1.10	1.70	3.15	2.94	MS, RI_1
24	α-Phellandrene	1,001	1.38	5.30	10.63	3.08	MS, RI_1
25	3-Carene	1,010	t	t	0.10	t	MS, RI_1
26	(+)4-Carene	1,018	t	3.98	0.40	1.13	MS, RI_1
27	Limonene	1,026	t	0.21	t	t	MS, RI_1
28	*trans*-Ocimene	1,035	t	t	t	0.02	MS, RI_1
29	Benzeneactaldehyde	1,037	0.30	t	t	t	MS, RI_1
30	1,3,6-Octatrine	1,039	1.96	t	0.06	t	MS, RI_1
31	1,4-Cyclohexadiene	1,043	t	6.19	0.32	t	MS, RI_1
32	γ-Terpinene	1,057	t	t	0.04	1.95	MS, RI_1
33	Cyclohexene	1,065	t	6.50	0.17	0.39	MS, RI_1
34	2-Nonanone	1,079	3.42	t	t	0.01	MS, RI_1
35	Linalool	1,086	2.25	t	0.16	t	MS, RI_1
36	E-4,8-Dimethyl-1,3,7-nonatriene	1,089	1.22	t	t	t	MS, RI_1
37	3-Methyl-4-brendene	1,095	t	0.13	t	t	MS, RI_1
38	3-Cyclohexen-1-ol	1,097	t	15.17	0.28	0.51	MS, RI_1
39	2-Cyclohexen-1-one	1,099	t	t	0.03	0.01	MS, RI_1
40	3-Cyclohexen-1-methanol	1,106	t	t	0.02	t	MS, RI_1
41	β-Fenchyl alcohol	1,109	t	0.54	t	0.06	MS, RI_1
42	Benzoic acid	1,163	0.16	t	t	t	MS, RI_1
43	*cis*-3-Hexenyl 2-methylbutanoate	1,218	0.19	t	0.01	t	MS, RI_1
44	Bornyl acetate	1,286	t	t	t	0.01	MS, RI_1
45	Geranyl acetate	1,357	t	t	0.02	t	MS, RI_1
46	Copaene	1,373	0.28	t	t	t	MS, RI_1

Table 2.3 (continued)

No	Compounds	RI	%Relative area				
			Leaf	Flesh	Skin	Seed	ID
47	β-Caryophllene	1,417	7.72	t	t	0.55	MS, RI_1
48	α-Bergamotene	1,427	0.71	t	0.20	0.03	MS, RI_1
49	(+)-Aromadendrene	1,436	0.08	t	t	t	MS, RI_1
50	Isosativene	1,441	0.38	t	0.07	0.01	MS, RI_1
51	β-Santalene	1,444	t	t	0.02	t	MS, RI_1
52	α-Humulene	1,447	0.39	t	0.02	0.03	MS, RI_1
53	ar-Curcumene	1,475	1.27	0.12	0.87	0.03	MS, RI_1
54	Allaromadendrene	1,478	t	t	0.10	t	MS, RI_1
55	α-Zingiberene	1,486	6.52	t	t	0.06	MS, RI_1
56	Bicyclogermacrene	1,490	0.37	t	t	0.01	MS, RI_1
57	α-Farnesene	1,494	t	t	0.95	t	MS, RI_1
58	β-Bisabolene	1,496	9.88	t	t	0.15	MS, RI_1
59	β-Sesquiphellandrene	1,512	0.70	t	0.30	t	MS, RI_1
60	δ-Cadinene	1,524	0.33	t	t	t	MS, RI_1
61	Total monoterpenes		22.34	76.54	94.05	97.96	
62	Total sesquiterpenes		27.69	0.12	2.22	0.85	
63	Total alcohols		2.77	17.53	0.28	0.51	
64	Total aldehydes		16.12	0.47	0.04	0.04	
65	Total esters		0.19	0.00	0.03	0.01	
66	Total ketones		8.90	0.00	0.03	0.02	
67	Heterocyclics		5.72	0.00	0.00	0.00	
68	Carboxylic acid		1.10	2.65	0.08	0.03	
69	Hydrocarbons		1.35	0.13	0.00	0.00	
70	Unidentified						
71	Total		86.18	97.44	96.73	99.42	

RI = programmed temperature retention indices relative to the homologous series of *n*-alkanes (C5-C25), RI_1 = retention data in literature, t = traces > 0.01%, ID = identification method. Reprinted with permission from Chokeprasert et al. (2007).

GC-FID the electron energy being 70 eV and the mass range 30–350 *m/z*. The investigation confirmed that the lulo leaf glycosides play a considerable role in the aroma composition of lulo fruits (Osorio et al., 2003).

The volatile composition of the durian fruit (*Durio zibethinus*), an important seasonal product in tropical Asia, has been investigated in detail. The measurements were carried out by an HP-SPME preconcentration step followed by GC The physicochemical characteristics and composition of volatile aroma compounds of five Malaysian cultivars were determined and the similarities and dissimilarities among the cultivars were elucidated by PCA. The volatile analytes collected by HS-SPME were separated by GC-TOFMS using a fused capillary column (10 m × 0.1 mm, film thickness, 0.10 μm). Column temperature started at 40°C (1.5 min hold), raised to 240°C at 50°C/min, final hold 2 min. Injector and detector temperatures were set to 250°C. The ionising voltage was 70 eV, the mass range 35–350 *m/z*. The results of GC analyses are compiled in Table 2.5. It was established that PCA classified adequately the cultivars demonstrating that the procedure can be successfully applied for the differentiation of durian cultivars (Voon et al., 2007).

Table 2.4 Relative concentration of aroma volatiles in fresh and stored minimally processed durian (ng/g)

Peak no.	Flavour compound	Storage period (days)*						
		0	7	14	21	28	35	42
Aldehyde								
1	Acetaldehyde	204.7^{a}	289.5^{a}	59.9^{b}	51.1^{b}	55.7^{b}	60.0^{b}	38.0^{b}
3	Propanal	53.7^{ab}	37.4^{ab}	105.3^{a}	n.d.	10.3^{b}	6.8^{b}	n.d.
18	2-Methylbut-2-enal	127.5^{a}	70.8^{b}	7.77^{c}	n.d.	n.d.	n.d.	n.d.
Total		385.8^{a}	397.7^{a}	172.9^{b}	51.1^{bc}	66.1^{bc}	66.8^{bc}	38.0^{c}
Ketone								
30	3-Hydroxybutan-2-one	48.4^{ab}	58.0^{ab}	10.31^{a}	41.7^{ab}	n.d.	14.6^{b}	19.0^{b}
Alcohol								
7	Ethanol	697.6^{a}	644.2^{b}	739.1^{a}	639.9^{a}	393.7^{a}	839.8^{a}	551.8^{a}
13	1-Propanol	n.d.	85.1^{b}	236.6^{ab}	138.0^{b}	157.1^{ab}	315.7^{a}	133.4^{b}
24	1-Butanol	20.0^{a}	11.9^{a}	53.6^{a}	61.2^{a}	58.2^{a}	22.8^{a}	22.2^{a}
27	2-Methylbutan-1-ol	106.4^{b}	134.0^{b}	585.1^{a}	188.7^{b}	162.3^{b}	106.4^{b}	187.4^{b}
28	3-Methylbutan-1-ol	55.3^{a}	17.2^{a}	49.3^{a}	21.1^{a}	8.4^{a}	49.0^{a}	56.4^{a}
31	1-Hexanol	n.d.	n.d.	n.d.	2.6^{a}	9.5^{a}	6.7^{a}	18.0^{a}
33	1-Heptanol	n.d.	6.9^{bc}	59.1^{a}	31.2^{b}	5.9^{c}	n.d.	n.d.
36	Butane-2,3-diol	n.d.	n.d.	30.5^{b}	54.2^{ab}	10.7^{b}	22.9^{b}	90.4^{a}
40	Benzyl alcohol	n.d.	n.d.	n.d.	n.d.	29.8^{c}	102.5^{b}	327.3^{a}
Total		883.4^{a}	899.2^{a}	1753.4^{a}	1137.0^{a}	835.4^{a}	1465.8^{a}	1386.9^{a}
Sulphur containing compound								
2	Ethanethiol	759.9^{a}	45.1^{b}	53.4^{b}	n.d.	n.d.	n.d.	n.d.
4	1-Propanethiol	330.9^{a}	69.9^{b}	60.1^{b}	n.d.	n.d.	n.d.	n.d.
20	Methyl ethyl disulfide	182.1^{a}	161.6^{a}	128.8^{a}	152.0^{a}	133.9^{a}	179.6^{a}	76.5^{a}
25	Diethyl disulfide	2130.6^{a}	1470.8^{a}	1228.3^{a}	1966.0^{a}	1494.4^{a}	2868.7^{a}	1158.4^{a}
26	Methyl propyl disulfide	n.d.	15.6^{ab}	55.0^{ab}	104.7^{a}	58.3^{ab}	103.5^{a}	46.2^{ab}

Table 2.4 (continued)

Peak no.	Flavour compound	Storage period (days)*						
		0	7	14	21	28	35	42
29	Ethyl propyl disulfide	447.4[a]	459.7[a]	707.2[a]	1002.5[a]	717.2[a]	1040.5[a]	709.7[a]
32	Dipropyl disulfide	n.d.	n.d.	116.8[b]	220.3[a]	115.7[b]	118.5[b]	66.5[bc]
34	Diethyl trisulfide	1183.1[a]	417.6[b]	1321.5[a]	332.2[b]	302.6[b]	382.1[b]	523.2[b]
35	3,5-Dimethyl-1,2,4-Trithiolane (isomer 1)	371.2[a]	86.2[b]	127.6[b]	114.4[b]	70.1[b]	163.4[ab]	54.3[b]
37	3,5-Dimethyl-1,2,4-Trithiolane (isomer 2)	398.7[a]	89.8[b]	125.4[b]	124.4[b]	70.0[b]	154.0[b]	89.0[b]
38	Dipropyl trisulfide	36.5[b]	29.0[b]	80.2[a]	32.4[b]	34.0[b]	52.6[ab]	23.6[b]
39	1,1-Bis(ethylthio)-ethane	64.27[b]	40.8[b]	117.7[ab]	205.9[a]	111.5[ab]	222.1[a]	129.9[ab]
Total		5904[a]	2886[a]	4122[a]	4255[a]	3108[a]	5285[a]	2877[a]
Esters								
5	Ethyl acetate	38.6[a]	23.8[ab]	9.43[b]	1.53[b]	n.d.	n.d.	n.d.
6	Methyl propionate	202.0[a]	42.0[b]	n.d.	n.d.	7.2[c]	11.1[bc]	n.d.
8	Ethyl propanoate	1570.5[a]	422.1[b]	n.d.	57.7[c]	3.8[c]	n.d.	n.d.
9	Ethyl 2-methylpropanoate	212.0[a]	30.9[b]	n.d.	n.d.	n.d.	n.d.	n.d.
10	Methyl butanoate	57.4[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11	Methyl 2-methylbutanoate	129.33[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12	Ethyl butanoate	76.6[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14	Propyl propanoate	213.2[a]	17.0[b]	n.d.	n.d.	n.d.	n.d.	n.d.
15	Ethyl 2-methyl butanoate	339.3[a]	31.9[b]	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2.4 (continued)

Peak no.	Flavour compound	Storage period (days)*						
		0	7	14	21	28	35	42
16	Propyl 2-methylpropanoate	136.3[a]	42.4[b]	12.8[bc]	n.d.	n.d.	n.d.	n.d.
17	Ethyl 3-methylbutanoate	76.4[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19	Propyl butanoate	50.7[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21	Propyl 2-methylbutanoate	327.9[a]	162.7[b]	65.5[bc]	55.6[bc]	29.8[c]	16.0[c]	n.d.
22	Propyl 3-methylbutanoate	107.5[a]	42.4[b]	36.5[b]	n.d.	n.d.	n.d.	n.d.
23	Ethyl but-2-enoate	53.1[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total		3590.8[a]	815.0[b]	124.3[c]	114.8[c]	40.8[c]	27.1[c]	n.d.

n.d.: not detected.

* Values in the same row with the same letters are not significantly different (level of significance 5%).

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Table 2.5 Relative amounts of volatile compounds in the headspace of five different durian cultivars

Peak no.	RT	Compound	Relative amount in headspace				
			Chuk	D101	D2	D24	MDUR 78
Aldehyde							
1	38.8	Acetaldehyde	88.3[a]	75.5[a]	271.2[a]	211.2[a]	34.2[a]
3	43.9	Propanal	57.0[a]	49.9[a]	n.d.[b]	55.6[a]	n.d.[b]
19	122.4	2-Methylbut-2-enal	54.4[b]	n.d.[b]	n.d.[b]	122.2[a]	n.d.[b]
		Total	199.7	125.4	271.2	389.0	34.2
Ketone							
34	182.5	3-Hydroxybutan-2-one	66.5[b,c]	115.9[b]	232.0[a]	n.d.[c]	n.d.[c]
Alcohol							
8	67.1	Ethanol	283.2[a]	612.8[a]	1204.2[a]	688.7[a]	722.2[a]
15	104.0	1-Propanol	291.0[a]	104.5[a]	163.8[a]	n.d.[a]	158.7[a]
24	143.3	1-Butanol	26.3[b]	39.1[b]	219.6[a]	24.9[b]	15.8[b]
28	157.3	2-Methylbutan-1-ol	30.7[a]	98.2[a]	40.5[a]	n.d.[a]	173.9[a]
30	158.1	3-Methylbutan-1-ol	47.3[a]	38.5[a]	184.8[a]	n.d.[a]	35.7[a]
36	187.6	1-Hexanol	n.d.[d]	28.5[a]	16.7[b]	n.d.[d]	7.9[c]
43	231.5	Butane-2,3-diol	n.d.[c]	27.2[b]	77.4[a]	n.d.[c]	n.d.[c]
		Total	678.5	948.8	1907.0	713.6	1114.2
Sulphur-containing compound							
2	40.1	Ethanethiol	405.3[a]	42.4[a]	313.9[a]	625.5[a]	40.9[a]
4	46.8	1-Propanethiol	275.9[a,b]	n.d.[b]	73.9[b]	334.4[a]	50.5[b]
7	60.1	Methyl propyl sulphide	30.8[a]	n.d.[a]	n.d.[a]	39.7[a]	n.d.[a]
21	131.9	Methyl ethyl disulphide	83.7[b]	43.5[b]	99.9[b]	179.4[a]	62.7[b]
26	149.7	Diethyl disulphide	1139.1[a]	840.1[a]	989.1[a]	1796.6[a]	1217.4[a]
27	154.0	Methyl propyl disulphide	81.0[b]	12.2[b]	553.0[a]	n.d.[b]	34.0[b]
31	170.3	Ethyl propyl disulphide	674.7[a]	337.9[a]	188.0[a]	450.5[a]	507.0[a]
35	185.6	Dipropyl disulphide	121.6[a]	n.d.[b]	16.7[b]	n.d.[b]	43.6[b]

Table 2.5 (continued)

Peak no.	RT	Compound	Relative amount in headspace				
			Chuk	D101	D2	D24	MDUR 78
37	189.2	1-Methylethyl propyl disulphide	n.d.[b]	41.9[a]	n.d.[b]	n.d.[b]	n.d.[b]
40	213.1	Diethyl trisulphide	583.0[a]	331.0[a]	842.2[a]	1016.5[a]	298.7[a]
42	229.3	3,5-Dimethyl-1,2,4-Trithiolane (isomer 1)	118.9[a,b]	56.8[b]	198.2[a,b]	353.0[a]	269.7[a,b]
44	232.5	3,5-Dimethyl-1,2,4-Trithiolane (isomer 2)	128.9[a,b]	65.1[b]	230.2[a,b]	395.3[a]	288.5[a,b]
46	239.0	Dipropyl trisulphide	28.8[a,b]	n.d.[b]	n.d.[b]	31.2[a]	n.d.[b]
47	251.9	1,1-Bis(ethylthio)-ethane	66.8[b,c]	27.8[c]	114.5[b]	95.4[b,c]	221.8[a]
		Total	3129.0	1798.7	3619.7	5317.5	3034.8
Esters							
5	55.1	Ethyl acetate	4.47[a]	141.0[a]	96.7[a]	23.9[a]	77.7[a]
6	58.4	Methyl propionate	284.3[a]	26.3[a]	67.0[a]	203.1[a]	206.6[a]
9	70.9	Ethyl propanoate	507.2[b]	96.8[b]	1091.3[a,b]	1528.6[a]	264.7[b]
10	74.2	Ethyl 2-methylpropanoate	25.6[a]	n.d.[a]	71.0[a]	205.8[a]	n.d.[a]
11	80.6	Methyl buatanoate	n.d.[b]	n.d.[b]	64.10[a]	53.7[a]	n.d[b]
12	89.1	Methyl 2-methylbutanoate	222.2[a,b]	n.d.[b]	195.2[a]	140.4[a,b]	n.d.[b]
13	100.0	Ethyl butanoate	n.d.[a]	n.d.[a]	257.2[a]	68.7[a]	n.d.[a]
14	103.9	Propyl propanoate	131.7[a]	n.d.[a]	63.5[a]	196.6[a]	n.d.[a]
16	107.4	Propyl 2-methylpropanoate	n.d.[b]	n.d.[b]	n.d.[b]	132.8[a]	n.d.[b]
17	108.9	Ethyl 2-methyl butanoate	103.4[b]	82.4[b]	2030.5[a]	348.8[b]	42.5[b]
18	112.8	Ethyl 3-methylbutanoate	n.d.[b]	n.d.[b]	n.d.[b]	71.0[a]	n.d.[b]
20	130.0	Propyl butanoate	n.d.[a]	n.d.[a]	273.4[a]	45.9[a]	n.d.[a]
22	136.4	Propyl 2-methylbutanoate	121.8[a]	259.4[a]	338.8[a]	235.5[a]	35.9[a]
23	140.8	Ethyl but-2-enoate	19.5[a,b]	n.d.[b]	187.1[a]	n.d.[b]	n.d.[b]

Table 2.5 (continued)

Peak no.	RT	Compound	Relative amount in headspace				
			Chuk	D101	D2	D24	MDUR 78
25	146.3	Methyl hexanoate	n.d.^{b}	n.d.^{b}	98.3^{a}	n.d.^{b}	n.d.^{b}
29	157.9	Ethyl hexanoate	n.d.^{b}	n.d.^{b}	587.5^{a}	n.d.^{b}	n.d.^{b}
32	178.1	Propyl hexanoate	n.d.^{a}	n.d.^{a}	446.8^{a}	n.d.^{a}	n.d.^{a}
33	181.0	Ethyl heptanoate	n.d.^{b}	n.d.^{b}	101.3^{a}	n.d.^{b}	n.d.^{b}
38	196.2	Methyl octanoate	n.d.^{b}	4.3^{a,b}	56.3^{a}	n.d.^{b}	n.d.^{b}
39	203.9	Ethyl octanoate	14.2^{b}	21.3^{b}	419.0^{a}	n.d.^{b}	58.1^{b}
41	217.7	Ethyl 3-hydroxybutanoate	n.d.^{b}	n.d.^{b}	58.3^{a}	n.d.^{b}	n.d.^{b}
45	235.4	Ethyl decanoate	n.d.^{c}	n.d.^{c}	34.0^{a}	n.d.^{c}	15.3^{b}
		Total	1434.4	631.5	6537.3	3254.8	700.8

RT, retention time on a Supelcowax-10 capillary column.
ID: A, GC retention and MS data in agreement with that of authentic reference; B, tentatively identified by MS matching with library spectra only. Results are the means of triplicate analyses.
Letters$^{a–b}$ indicate there is no significant difference ($P < 0.05$) with the same letter using Fisher's least significance difference among the samples.
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Because of its marked commercial value, the composition of the aroma compounds in bananas has been extensively investigated (Jordán et al., 2001; Mirande Eduardo et al., 2001; Nogueira et al., 2003). The influence of the various technological steps on the aroma profile of banana products has also been studied in detail. The influence of ripening (Liu and Yang, 2002), air-drying (Boudhrioua et al., 2003), vacuum and microwave processing (Mui et al., 2002) was determined. The effect of vacuum belt drying (VBD), freeze-drying (FD) and air-drying (AD) on the aroma composition of banana powders was investigated using SPME and GC-MS. Aroma compounds were separated on a fused silica capillary column (30 m × 0.25. mm). Initial column temperature was 40°C (5 min hold), then raised to 60°C at 2°C/min (2 min hold), to 100°C (2 min hold) at 5°C, and to 230°C at the same rate (30 min hold). Ms conditions were eV, 70; mass range 35–335 *m/z*. The chromatograms of the aroma compounds in banana powders dried by different technologies are shown in Fig. 2.2 and the concentrations of volatiles are compiled in Table 2.6. The data in Fig. 2.2 and Table 2.6 illustrate that the aroma profiles of the banana powders are similar but not identical, demonstrating that the drying processes do not change drastically but slightly modify the aroma composition (Wang et al., 2007).

SPME followed by GC-MS was employed for the study of the effect of various microwave processing conditions on the aroma composition of avocado puree. Volatiles were preconcentrated on a DVB-CAR-PDMS fibre for 24 h at ambient temperature. Analytes were separated on a capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Initial oven temperature was 40°C (5 min hold), raised to 120°C at 5°C/min (final hold 3 min). Injector and detector temperatures were 180 and 230°C, respectively. The aroma compounds identified in the samples are compiled in Table 2.7. It was concluded from the results that the aroma composition markedly depended on the microwave time and pH (López et al., 2004).

2.1.2 Non-tropical Fruits

The effect of modified atmosphere packing on the quality of Honeoye and Korona strawberries was investiated in detail. Besides the measurement of sugar, acid, pH, colour and mould, the change of the aroma composition was followed by GC-MS and GC-O. GC-MS was performed on a capillary column (30 m × 0.32 mm, film thickness, 1.0 μm). Thermal gradient started at 25°C for 5 min, then increased to 180°C at 4°C/min and to 220°C at 50°C/min, final hold 15 min. Some data are compiled in Table 2.8. The results suggested that modified atmosphere enhances the shelf life of strawberries (Nielsen and Leufvén, 2008).

Another study analysed the fragrance composition of the leaves of 77 individual trees (*Cerasus, Padus, Laurocerasus, Prunus*). Volatiles were separated and quantitatively determined by GC. It was concluded from the data that the samples show marked differences according to their aroma profile (Takahashi et al., 2006).

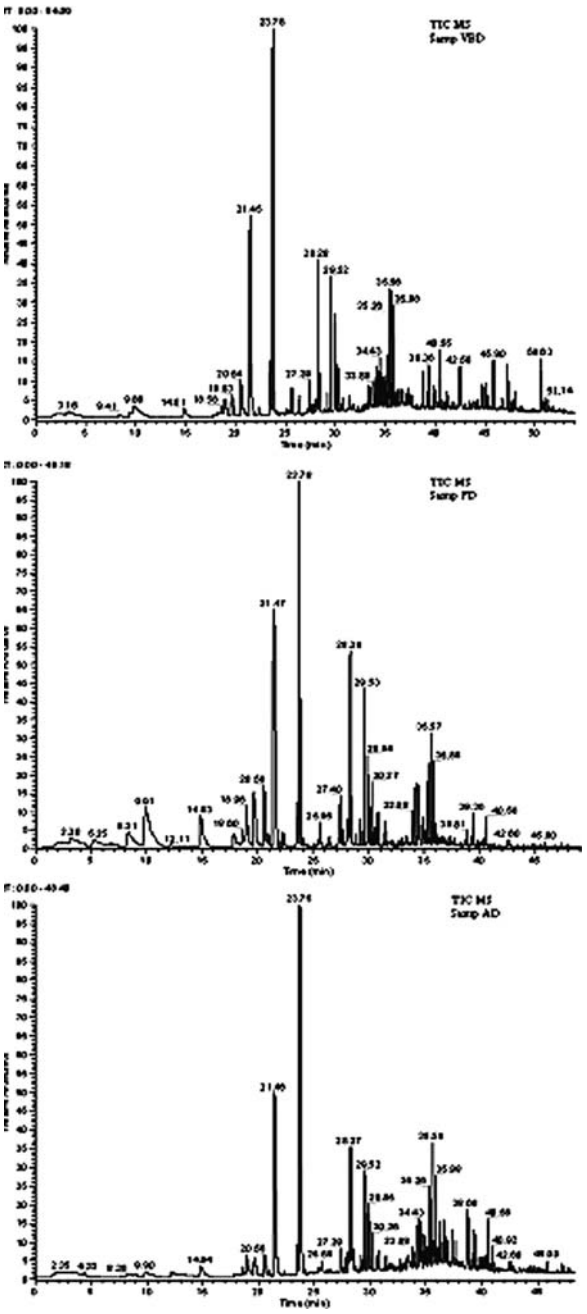

Fig. 2.2 Chromatograms of banana powder dried by VBD, FD and AD. For peak identification see Table 2.6 (VBD = vacum belt drying, FD = freeze-drying, AD = air-drying). Reprinted with permission from Wang et al. (2007)

Table 2.6 Volatile compounds of banana powder dried by FD, VBD and AD

Retention time (min)	Components	Fraction[a] (%)		
		FD	VBD	AD
Esters				
5.22	Acetic acid 2-methylpropyl ester	0.61		
8.31	2-Pentanol acetate	2.40		
9.89	3-Methylbutyl acetate	7.32	3.12	0.74
14.83	Butanoic acid 2-methylpropyl ester	4.13	1.12	1.67
17.85	Butanoic acid butyl ester	1.26		
18.96	Isobutyl isoval ester	4.51	1.60	3.23
19.65	Propanoic acid 1,2-dimethylbutyl ester	3.76	1.33	1.69
20.57	2-Heptanol acetate	3.54	2.34	1.67
20.96	Butanoic acid 3-methylbutyl ester	0.59	0.31	0.28
21.47	Isoamyl butyrate	15.83	13.69	14.75
22.33	Isoamyl isovalerate	0.55	0.41	0.21
23.45	Isoamyl-2-methyl butyrate	1.65	2.43	1.66
23.78	3-Methylbutanoic acid 3-methylbutyl ester	16.11	19.30	22.39
24.16	Butanoic acid 1-methyl octyl ester	0.73	0.63	0.59
25.54	Hexanoic acid 2-methyl propyl ester	0.20	0.14	0.22
27.40	Butanoic acid hexyl ester	1.74		0.98
27.77	Butanoic acid 3-hexenyl ester	0.27	0.32	
28.28	Butanoic acid 1-methylhexyl ester	7.45	6.85	5.87
29.53	Hexyl isovalerate	3.23	3.29	3.20
29.85	Octanoic acid 3-methylbutyl ester	2.01	2.07	2.31
34.10	Butanoic acid 1-ethenylhexyl ester	0.87	0.85	0.85
34.43	*E*7-Decenyl acetate	1.02	0.98	1.22
34.55	2-Methyl-5-(1-methylethenyl)-cyclohexanol acetate	1.21	1.28	1.66

Table 2.6 (continued)

Retention time (min)	Components	Fraction[a] (%)		
		FD	VBD	AD
34.97	Butanoic acid 2-methyl octyl ester			0.38
39.81	1,2-Benzene dicarboxylic acid diethyl ester		0.54	
39.85	*E*5-Dodecenyl acetate	0.14		
41.22	3-Methylbutyl decanoate			0.25
45.21	Isopropyl myristate		0.49	
45.90	1,2-Benzene dicarboxylic acid bis(2-methyl propyl) ester	0.08	0.95	0.27
Alcohols				
27.59	(*E*)-3-Octen-2-ol	0.44	0.28	
35.11	2-Methyl-5-(1-methylethenyl) cyclohexanol	0.21	0.19	0.51
37.34	3-Methyl-2-propyl-1-pentanol	0.17	0.49	1.12
39.26	(*Z*)-4-Decen-1-ol	0.15	0.28	0.34
39.36	1-Cyclohexyl-2-buten-1-ol (*cis* and *trans*)	0.55	0.80	
40.45	*cis*-9-Tetradecen-1-ol			0.37
Acids				
48.02	*n*-Hexadecanoic acid		0.45	
51.46	(*Z*)-9-Octadecenoic acid		0.16	
Ketones				
26.42	3-Octen-2-one	0.29	0.66	
31.39	2-Undecanone	0.62		0.37
37.49	2-Tridecanone	0.08	0.25	
38.72	1,6-Dioxacyclododecane-7,12-dione	0.37		

Table 2.6 (continued)

Retention time (min)	Components	Fraction[a] (%)		
		FD	VBD	AD
Benzenes				
33.40	Eugenol	0.45	1.23	0.76
38.81	Elemicin		0.67	1.26
Others				
35.36	(*Z*,*Z*)-1,4-Cyclooctadiene	1.92	2.20	2.25
35.56	2-Octyne	1.90	2.46	2.78
35.88	*trans*-Bicyclo[4.2.0]octane	1.59	1.78	2.00
40.18	Hexadecane	0.14	0.98	0.54
40.56	7-Propylidene-bicyclo[4.1.0]heptane	0.44	1.00	1.22
44.71	Octadecane		0.48	0.13

[a] Fractions of components in banana powder (%) = peak area of a component in banana powder/total peak area of all components in banana powder. FD = freeze drying, VBD = vacum belt drying, AD = air drying.
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Table 2.7 Volatile compounds of fresh avocado puree at 30 s heating time, pH 5.5 and 1% of avocado leaves

I[a]	Compounds	Fresh avocado	Avocado leaves	Microwaved avocado	Microwaved avocado added with avocado leaves
913	Ethanol	+	+	+	+
934	Pentanal			+	+
949	α-Pinene		+		+
960	1-penten-3-one			+	+
1104	Hexanal			+	+
1108	β-Pinene		+		+
1160	β-Myrcene		+		
1191	Limonene		+		
1195	Heptanal			+	+
1203	Eucalyptol		+		+
1220	3-Methyl-butanol	+		+	+
1228	2-Hexanal E		+	+	+
1262	Pentanol	+		+	+
1291	3-Hydroxy-2-butanone	+			
1295	Octanal			+	+
1309	1-Octen-3-one			+	+
1328	2-Heptenal (*E*)			+	+
1363	Hexanol	+	+	+	+
1434	2-Octenal (*E*)			+	+
1460	1-Octen-3-ol			+	+
1463	Acetic acid	+	+	+	+
1471	Copaene		+		
1483	Furfural			+	+
1542	2-Nonenal (*E*)			+	+
1572	Octanol			+	+
1580	Caryophyllene		+		
1660	2-Decenal (*E*)			+	+
1691	Estragole		+		+

[a]Kovats retention index.
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The occurrence of the aroma compound methyl nicotinate (MN) in various fruits has been vigorously investigated (Franco and Janzantti, 2005). Its presence in mammee apple has been established (Morales and Duque, 2002). Besides flavour-enhancing effect, NM induces skin vasodilation (Caselli et al., 2003) and it can be used in case of respiratory, vascular and rheumatoid disorders (Koivukangas et al., 2000).

The impact of the technological procedures on the apple aroma was investigated by enantio-MDGC-MS and HRGC-IRMS. Aroma compounds were extracted from industrial raw materials by simultaneous distillation extraction (SDE). GC-MS separations were performed on a fused silica capillary column (30 m × 0.25. mm, film thickness, 0.25 μm). Oven temperature started at 50°C (3 min hold), increased to 220°C at 4°C/min. Enantio-MDGC-MS was carried out in a dual column system. Preseparation of aroma compounds was achieved on a fused silica

Table 2.8

		Day 3			Day 7		
		Packaging condition			Packaging condition		
Aroma substance	Day 0	A	C	E	A	C	E
Amount of selected volatiles (expressed as μg/l headspace over 100 g sample) in Honeoye starawberries							
Acetaldehyde	0.40	0.76	0.20	0.43	0.61	0.60	1.2
Acetone	7.5	7.2	9.4	8.6	11.0	11.0	8.1
Ethyl acetate	0.26	0.78	0.60	0.65	1.4	1.1	0.67
Methyl butyrate	0.46	0.99	1.3	1.0	0.67	1.6	0.35
Dimethyl disulphide	0.02	0.04	0.09	0.07	0.12	0.06	0.02
Ethyl butyrate	0.18	0.43	0.57	0.32	0.25	0.18	0.28
Butyl acetate	1.6	4.7	6.5	5.8	5.0	4.6	5.3
2-hexenal	0.12	0.16	0.09	0.10	0.08	0.07	0.09
Heptanone	0.31	0.85	0.95	0.74	1.2	0.88	0.71
Butyl butyrate	0.19	0.16	0.32	0.24	0.07	0.07	0.08
Ethyl hexanoate	0.25	0.22	0.32	0.28	0.23	0.11	0.26
Hexyl acetate	13	14	14	19	15	14	14

Table 2.8 (continued)

		Day 3					Day 7				
		Packaging condition									
Aroma substance	Day 0	A	B	C	D	E	A	B	C	D	E
Amount of selected volatiles (expressed as μg/l headspace over 100 g sample) in Korona starawberries											
Acetaldehyde	0.29	0.89	0.88	0.99	0.53	0.67	0.39	0.50	0.50	0.19	1.6
Acetone	6.6	12	9.7	11	8.9	9.3	10	14	8.0	10	8.1
Ethyl acetate	2.8	15	57	3.1	45	3.0	68	251	168	175	13
Methyl butyrate	55	70	56	62	66	63	50	21	31	35	72
Dimethyl disulphide	0.41	0.36	0.35	0.47	0.48	0.57	0.61	0.20	0.22	0.28	0.58
Ethyl butyrate	8.6	56	51	30	67	45	35	160	220	131	138
Butyl acetate	2.1	6.6	4.5	5.5	11	5.3	3.3	8.0	4.8	9.2	6.2
1-Methyl-ethyl-butyrate	5.0	11	5.8	12	8.3	14	8.4	44	22	8.8	14
Heptanone	0.41	0.53	0.25	0.59	0.42	1.0	0.26	0	0	0	0.82
Methyl hexanoate	3.0	4.8	3.2	3.4	4.9	4.9	3.7	2.2	4.7	3.4	9.5
Butyl butyrate	1.2	8.0	6.3	6.5	12	14	3.3	20	30	15	54
Ethyl hexanoate	1.1	5.0	3.8	4.1	7.1	7.1	1.6	10	15	7.7	27
Hexyl acetate	7.7	7.3	7.5	6.7	8.7	5.0	9.0	14	13	12	9.8

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capillary column (30 m × 0.25. mm, film thickness 0.25 μm), column temperature increasing from 50°C to 240°C at 10°C/min. Enantiomers were separated on a cyclodextrin column (25 m × 0.25 mm, film thickness, 0.15 μm). Oven temperature started at 50°C (20 min hold), then raised to 200°C at 2°C/min. HRGC-IRMS measurements were performed in a fused silica capillary column (60 m × 0.32. mm, film thickness, 0.25 μm). Temperature gradient initiated at 50°C and was raised to 220°C at 5°C. Typical chromatograms showing the good separation capacity of the GC system are shown in Fig. 2.3. Some aroma compounds found in single strength apple juices and apple juice aromas are compiled in Table 2.9. It was stated that the GC methods are suitable for the determination of the aroma profile of apples and they can be employed for the quality control of apple juices (Elss et al., 2006).

Another study also using GC-MS established that the lower drying temperature and freeze-drying are the best procedures for the reduction of the loss of aroma compounds (Krokida and Philippopoulos, 2006).

The efficacy tin-oxide gas sensor and GC-MS was compared in the separation of aroma compounds in various apple varieties. Analytes were preconcentrated by HP-SPME and separated and quantitated by GC-MS. The dimensions of the fused silica capillary column were 30 m × 0.1 mm, film thickness, 0.33 μm. Initial oven temperature was 40°C for 2.5 min, then raised to 200°C at 10°C/min (final hold 5 min). The results are compiled in Table 2.10. The data were evaluated by PCA, PLS and

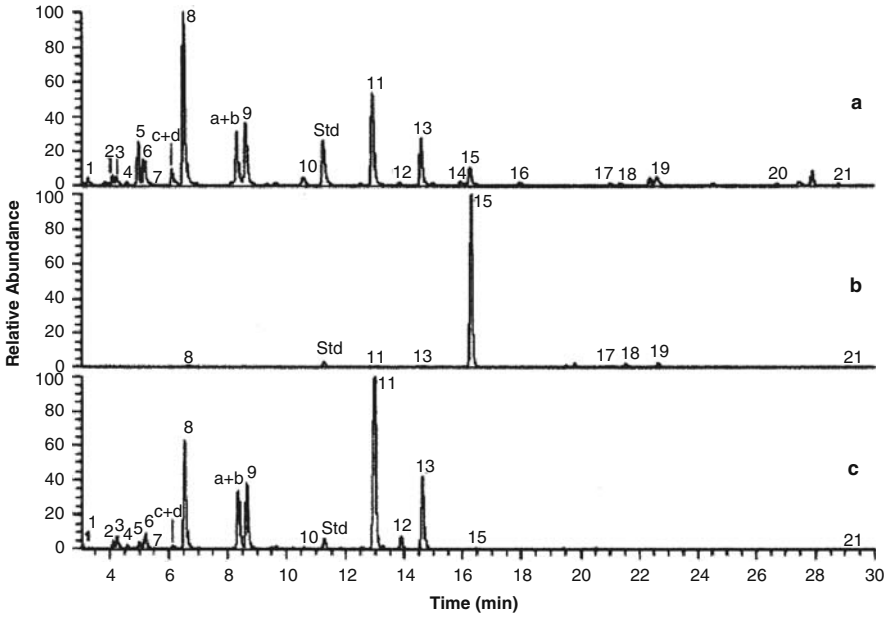

Fig. 2.3 Representative total ion chromatograms (TIC) of apple volatiles in the production line of (**a**) single strength juice, (**b**) apple concentrate and (**c**) apple juice aroma. For peak identification see Table 2.9. Reprinted with permission from Eiss et al. (2006)

Table 2.9 Aroma cmpounds determined by HRGC-MS in industrial single strength apple juices (a) and apple juice aromas (b) (each $n = 31$)

		(a)		(b)	
Peak No.	Aroma compound	Range (mg/l)	Mean (mg/l)	Range (mg/l)	Mean (mg/l)
1	Propyl acetate	0–0.1	0.03	0–27	4.1
2	1-Propanol	0–0.3	0.1	0–43	10.7
3	Ethyl butanoate	0–0.3	0.1	0–37	13.0
4	Ethyl 2-methylbutanoate	0–0.1	0.05	0–25	4.1
5	Butyl acetate	0–1.7	0.4	0–165	29
6	Hexanal	0–0.6	0.2	0–95	20
7	2-Methylpropanol	0–0.5	0.2	0–175	21
c+d	2/3-Methylbutyl acetate	n.s.	n.s.	n.s.	n.s.
8	1-Butanol	0.1–4.7	2.5	17–370	154
a+b	2/3-Methyl-1-butanol	n.s.	n.s.	n.s.	n.s.
9	*E*-2-hexenal	0–0.3	0.9	0–470	107
10	Hexyl acetate	0–0.7	0.1	0–28	9.5
11	1-Hexanol	0.006–5.9	3.0	47–685	270
12	*Z*-3-Hexanol	0–0.9	0.2	0–79	15
13	*E*-2-Hexanol	0.01–3.4	1.2	12–300	100
14	Acetic acid	0–5.3	0.4	0–2.0	0.4
15	Furfural	0–25	1.6	0–21	1.1
16	Benzaldehyde	0–0.3	0.06	0.02–45	4.6
17	Butanoic acid	0–1.2	0.2	0–0.2	0.01
18	Phenylacetaldehyde	0–1.1	0.05	0–4.2	0.5
19	2-Methylbutanoic acid	0–5.9	0.7	0–5.2	0.4
20	β-Damascenont	0–0.1	0.03	0–5.1	0.8
21	2-Phenylethanol	0–2.3	0.2	0–2.6	0.4
	1,3-Octanediol	0–5.4	0.2	n.d.	n.d.
	4-Vinylguaiacol	0–0.8	0.04	n.d.	n.d.

For each component, ranges of amounts and mean values are given.
n.s. = not separated on DB-Wax, n.d.= not detected.
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back-propagation feed-forward artificial neural network (BP-ANN). It was established that the multivariate mathematical statistical methods increased the classification power of the measurements (Xiaobo and Jiewen, 2008).

Gas chromatography-olfactometry with headspace gas dilution analysis was applied for the study of the composition and quantity of aroma compounds in Fuji apple. The measurement indicated that the type of the GC column exerts a considerable influence on the number of analytes separated (Komthong et al., 2006).

The quantity and quality of aroma compounds in Xinjiang wild apple (*Malus sieversii*) was used for the investigation of genetic diversity. Samples were preconcentrated by a PDMS fibre at 40°C for 35 min. Analytes were separated on a fused silica capillary column using temperature program as follows: initial oven temperature 34°C for 3 min, raised to 50°C at 3°C/min, to 140°C at 6°C/min, to 230°C at 10°C/min, final hold 4 min. The characteristic odour compounds are compiled in Table 2.11. The application of the aroma profiles for the study of the genetic diversity was proposed (Chen et al., 2007).

Table 2.10 The mean values of the 22 most abundant volatile compounds in "Jina", "Fuji", "Huaniu" apples identified by SPME-GC-MS (μg/L) together with their retention times and correlation coefficients *R* between the specific volatiles and the first two principal components of the principal component analysis of all compounds

		Content of different volatiles $\chi \pm \sigma$ (μg/l)			*R*
Volatile compounds	Retention time (min)	"Jina"	"Fuji"	"Huaniu"	PC1
Ethanol	1.57		416±26		0.001
1-Butanol	2.67	875.2±83	350±18	1205±24	0.001
Ethyl propionate	3.34			8211±35	0.089
1-Butanol,2-methyl	3.81	54340±306	19800±503	3070±32	−0.67
Hexenal	5.14	5430±167	9090±95	688±24	−0.04
Butylacetate	5.56	533±23	9700±78		0.023
2-Hexenal	6.85	41931±133	6063±143	3404±54	−0.53
1-Hexanol	7.10	407±14	816±76	7035±485	0.073
1-Butanol,2-methanyl acetate	7.39	1961±12	648±106		−0.02
Propyl butyrate	7.95	17913±132			−0.25
Propyl acetate	8.28	20673±185			−0.29
Pentyl acetate	8.46	9748±121	21280±212		−0.06
5-Hepten-2-1,6-methyl	10.92	820±45	695±36	7781±434	0.075
Butyl isobutoanoate	11.01		684±25		0.002
Hexyl acetate	11.056	399,8±45	695±54		−0.00
2-Methyl-1-hexanol	12.04	697±63			−0.00
2-Methyl butyl butyrate	12.44	838.8±72	3063±87		−0.00
Ethyl hexyrate	14.04		1769±132		0.005
Hexyl butyrate	16.94	265,3±67	3312±271		0.006
2-Methyl hexyl butyrate	18.24	354±34	3955±154	466.3±34	0.012
Butyl butyrate	19.35		1975±131	1663±44	0.024
Hexyl hexanoate	22.27	27604±534	26580±567	823.9±45	−0.29

χ = mean value.
σ = standard deviation.
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Table 2.11 Character impact odours in *Malus sieversii* and *Malus pumila* cultivars

No.	RT (min)	Compounds	Formula	Odour thresholds (μg/L)	Odour units				
					Malus sieversii*	"Golden Delicious"	"Starking"	"Ralls"	"Fuji"
1	9.40	Hexanal	$C_6H_{12}O$	64	3.57 (0.24–8.21)	5.34	6.28	7.35	7.47
2	10.43	2-Methyl-ethyl-butanoate	$C_7H_{14}O_2$	18	0.41 (0–1.26)	0	0.04	0	3.39
3	12.34	(*E*)-2-hexanal	$C_6H_{10}O$	17	21.37 (3.35–38.98)	81.13	51.68	39.78	28.81
4	16.16	Hexyl acetate	$C_8H_{16}O_2$	2	1.10 (0–11.,28)	17.97	54.98	0.55	39.83
5	5.50	1-Butanol	C_4H_9O	80	1.35 (0–7.22)	0	0	0.87	0.13
6	8.67	Ethyl butanoate	$C_6H_{12}O_2$	20	3.12 (0.08–9.88)	0	0.35	0.31	0.88
7	13.14	1-Hexanol	$C_6H_{14}O$	500	0.32 (0 –1.44)	0.25	0.27	0.35	0.13
8	15.61	Ethyl hexanoate	$C_8H_{16}O_2$	14	1.37 (0–23.04)	0.19	0.54	0.17	0
9	18.52	3-Octen-1-ol	$C_8H_{16}O$	1.4	0.26 (0 –2.29)	0	0	0	0
10	20.83	Ethyl octanoate	$C_{10}H_{20}O_2$	5	2.59 (0 –9.45)	0	0	0	0
11	25.77	Damascenone	$C_{13}H_{18}O$	0.05	9.58 (0 –46.98)	0	0	0	0
12	5.55	Propyl acetate	$C_5H_{10}O_2$	48	0	0	1.27	0	1.31
13	11.34	(*Z*)-3-Hexenal	$C_6H_{10}O$	0.25	0	0	0	177.20	37.29
14	11.85	1-Futanol,methyl-acetate	$C_7H_{14}O_2$	30	0.01 (0–0.18)	0	9.43	0	0
15	12.20	Pentyl acetate	$C_7H_{14}O_2$	7.5	0	0	0	0	1.40
16	14.37	3-Furanmenthanol	$C_5H_6O_2$	5	0.02 (0–0.51)	2.13	0	0	0
17	18.77	Benzene acetaldehyde	C_8H_8O	4	0	0	1.87	0	0

*Variation range in brackets.

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Various analytical methods were employed for the discrimination of eight different apricot varieties (*Prunus armeniaca*). The procedures included the application of electronic nose, LLE and SPME followed by GC-MS. LLE was performed by using CH_2Cl_2 as extracting agent; the organic phase was concentrated by microdistillation. GC-FID investigations were carried out in a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Column temperature was raised from 40°C to 200°C at 3°C/min, then to 250°C at 5°C/min, final hold 5 min. Hydrogen was the carrier gas. Injector and detector temperatures were 250°C and 300°C, respectively. MS detection was performed by scanning at 70 eV in the *m/z* range of 60–600 units. The data were evaluated by principal component analysis (PCA) and factorial discriminate analysis (FDA). GC methods separated and identified 27 aroma compounds in the extract of apricots. It was established that the extraction methods combined by GC and multivariate mathematical statistical computations can be applied for the classification of apricot varieties (Solis-Solis et al., 2007).

HP-SPME coupled to GC-MS and GCO was employed for the comparison of the aroma profile of various appricot varieties. The adsorption capacity of PDMS, PDMS/DVB, CAR/DMS were compared for the preconcentration of the aroma compounds of apricots. Measurements were performed on a fused silica capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Column temperature started at 60°C and raised to 200°C at 5°C/min, then to 250°C at 6°C/min, final hold 5 min. A characteristic chromatogram is shown in Fig. 2.4, indicating the complexity of the aroma profile of apricot varieties. The aroma compounds indentified are compiled in Table 2.12. It was found that 10 volatiles are the most important for the determination of the aroma of apricot (ethyl acetate, hexyl acetate, β-cyclocitral, γ-decalactone, limonene, 6-methyl-5-hepten-2-one, linalool, β-ionone, methone, (*E*)-hexen-2-al). They can be applied for the classification of apriocts according to their aroma profiles (Guillot et al., 2006).

Direct thermal desorption-GC-TOF/MS was employed for the elucidation of the effect of various drying techniques on the aroma composition of apricot. Samples were desorbed at 150°C for 5 min and then cryofocused at –30°C on graphitised carbon black and molecular sieve trap. Analytes were desorbed by heating the sample to 325°C and held for 30 min. GC separation was performed on a capillary column (60 m × 0.32 mm, film thickness, 1 μm). Initial oven temperature was set to 37°C

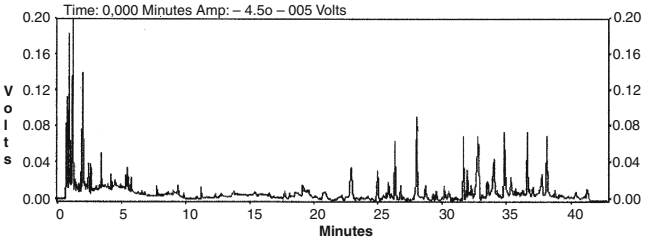

Fig. 2.4 HS–SPME–GC chromatogram of Rouge du Roussillon apricot. Reprinted with permission from Guillot et al. (2005)

Table 2.12 Amount[a] of common volatile compounds identified in the six apricot cultivars studied (peak area × 10^4)

Compounds	RI[b]	Orange-red	Iranian	Goldrich	Hal-grand	R. Roussillon	A 4025
Ethyl acetate	872	8.5 ± 0.4	10.5 ± 0.6	16.2 ± 0.5	13.5 ± 0.5	23.7 ± 1.1	12.4 ± 0.5
Butyl acetate	990	2.2 ± 0.08	6.5 ± 0.1	6.5 ± 0.08	26.7 ± 1.2	25.3 ± 0.9	5.5 ± 0.07
Hexanal	1022	19.1 ± 0.6	17.1 ± 0.5	13.7 ± 0.6	36.1 ± 0.6	25.0 ± 0.6	15.4 ± 0.5
Limonene	1178	20.2 ± 0.7	21.5 ± 0.8	17.5 ± 0.6	24.5 ± 0.6	8.5 ± 0.2	22.8 ± 0.8
E-Hexen-2-al	1223	9.5 ± 0.3	11.5 ± 0.2	40.1 ± 1.8	30.1 ± 0.8	21.9 ± 0.8	7.3 ± 0.1
p-Cymene	1246	6.0 ± 0.1	13.0 ± 0.3	17.0 ± 0.5	18.3 ± 0.5	30.5 ± 0.5	21.1 ± 0.3
Hexyl acetate	1308	6.2 ± 0.2	11.5 ± 0.2	0.1 ± 0.05	45.7 ± 0.9	14.2 ± 0.5	15.0 ± 0.2
6-Methyl-5-hepten-2-one	1336	11.0 ± 0.3	7.1 ± 0.1	19.2 ± 0.3	30.2 ± 0.4	13.8 ± 0.3	13.7 ± 0.1
1-Hexanol	1345	5.1 ± 0.1	17.3 ± 0.1	3.5 ± 0.1	12.0 ± 0.1	14.5 ± 0.3	7.1 ± 0.1
Acetic acid	1446	Trace	1.5 ± 0.01	3.8 ± 0.02	5.1 ± 0.01	3.0 ± 0.01	1:5 ± 0.01
1-Octen-3-ol	1448	2.5 ± 0.05	1.5 ± 0.03	3.1 ± 0.05	Trace	1.5 ± 0.05	1.5 ± 0.02
Menthone	1486	1.0 ± 0.02	0.9 ± 0.01	1.5 ± 0.02	1.5 ± 0.02	1.5 ± 0.02	Trace
2-Ethyl-1-hexanol	1492	3.0 ± 0.01	4.2 ± 0.01	3.0 ± 0.01	4.3 ± 0.01	3.8 ± 0.01	1.8 ± 0.01
Benzaldehyde	1500	2.1 ± 0.04	0.9 ± 0.01	4.4 ± 0.04	25.1 ± 0.6	15.3 ± 0.4	2.2 ± 0.01
Linalool	1540	9.4 ± 0.06	1.9 ± 0.01	4.0 ± 0.02	29.8 ± 0.2	6.1 ± 0.02	4.0 ± 0.05
β-Cyclocitral	1598	10.3 ± 0.2	0.5 ± 0.2	9.9 ± 0.2	15.1 ± 0.2	2.5 ± 0.2	7.3 ± 0.2
Pulegone	1600	2.0 ± 0.01	1.5 ± 0.01	2.4 ± 0.01	2.9 ± 0.01	2.0 ± 0.01	1.5 ± 0.01
Z-Citral	1666	5.5 ± 0.05	4.3 ± 0.05	5.1 ± 0.05	9.1 ± 0.4	4.5 ± 0.05	2.7 ± 0.05
E-Citral	1718	8.0 ± 0.1	6.1 ± 0.01	9.6 ± 0.1	15.0 ± 0.3	3.8 ± 0.1	5.4 ± 0.01
Geranyl acetone	1798	1.5 ± 0.01	Trace	Trace	8.1 ± 0.2	3.1 ± 0.5	2.1 ± 0.01
Benzyl alcohol	1866	0.5 ± 0.0	1.0 ± 0.2	Trace	0.8 ± 0.02	2.1 ± 0.2	1.4 ± 0.2
β-Ionone	1914	3.1 ± 0.02	0.1 ± 0.01	3.5 ± 0.02	8.0 ± 0.3	0.5 ± 0.02	0.5 ± 0.02
g-Decalactone	2106	2.5 ± 0.01	3.4 ± 0.02	2.0 ± 0.01	3.8 ± 0.01	1.9 ± 0.01	4.7 ± 0.03
Total		139.2 ± 3.4	143.8 ± 3.5	186.1 ± 5.1	365.7 ± 7.9	229.0 ± 6.8	156.9 ± 3.3

[a] Means of three analysis ± standard deviation.
[b] Retention index on D-WAX column.
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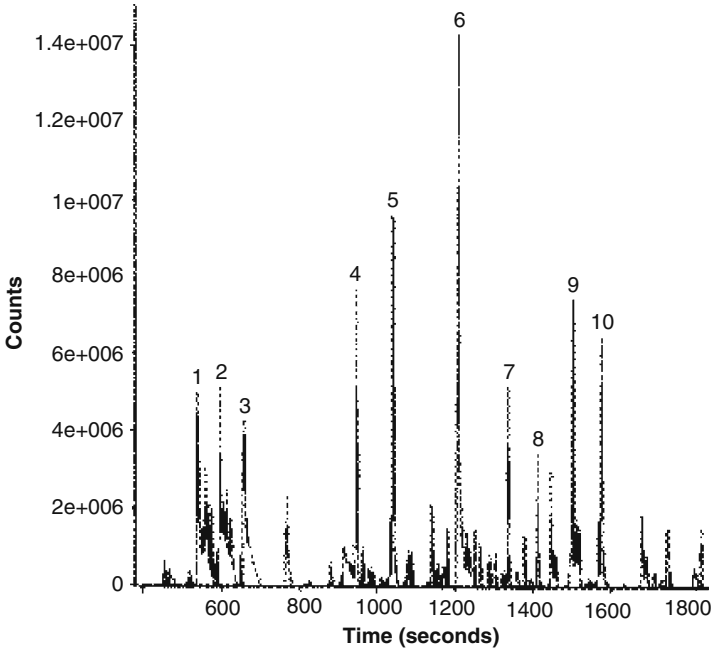

Fig. 2.5 GC-TOF/MS chromatogram of Sekerpare apricot volatile components at 150°C using the DTD technique (1: isobutanal; 2: acetic acid; 3: ethyl acetate; 4: butyl acetate; 5: (*E*)-2-hexenal; 6: limonene; 7: decanol; 8: butyrolactone; 9: γ-decalactone; 10: β-Ionone). Reprinted with permission from Gőgűs et al. (2007)

(7 min hold), then raised to 240°C at 15°C/min (final hold 10 min). A characteristic chromatogram illustrating the good separation capacity of the system in shown in Fig. 2.5. The retention parameters and concentration of the analytes are compiled in Table 2.13. It was established that the method of drying (desiccator, sun, hot air, microwave) exerts a marked influence on the aroma profile of the samples. It was further found that direct thermal desoprtion (DTD) technique is rapid, reliable and easy to carry out and can be applied for the investigation of the quality of drying processes (Gőgűs et al., 2007).

SPME followed by GC-MS, GC-O and HPLC were simultaneously applied for the investigation of the changes in the aroma profile, chemical and physical properties of Yali pear (*Pyrus bertschneideri* Reld) during storage. The influence of the concentration of ethyl butanoate, ethyl hexanoate, α-farnesene, hexanal, ethyl acetate, hexyl acetate and ethanol on the aroma of the Yali pear was demonstrated (Chen et al., 2006).

SPME coupled with GC-MS has also been employed for the investigation of the aroma composition of cantaloupe, Galia and honeydew muskmelons. It was found that cantaloupe melons contain mainly sulphur-containing esters and analytes with straight six-carbon chain. Molecules with straight nine-carbon chain were

Table 2.13 Compounds, retention indices, percentage compositions of Sekerpare-type apricot volatile constituents for various techniques

Compound[a]	RI[b]	Sun (%)[c]	Microwave (%)[c]	Hot air (%)[c]	Desiccator (%)[c]
Isobutanal	540	0.37 ± 0.03[d]	–[e]	–	4.78 ± 0.36
Acetic acid	600	2.68 ± 0.22	2.87 ± 0.19	1.77 ± 0.22	4.83 ± 0.51
Ethyl acetate	628	–	–	–	3.37 ± 0.30
Methylbutanal	641	0.61 ± 0.05	1.32 ± 0.12	–	–
Pentanal	732	0.09 ± 0.02	0.14 ± 0.02	0.65 ± 0.09	–
Hexanal	801	0.70 ± 0.06	0.17 ± 0.03	1.02 ± 0.08	1.27 ± 0.23
Hydroxyacetone	803	–	–	–	0.47 ± 0.09
2,3-Butanediol	806	–	–	0.65 ± 0.08	–
Butyl acetate	816	1.71 ± 0.15	–	0.13 ± 0.03	6.94 ± 0.77
Furfural	829	3.05 ± 0.22	4.27 ± 0.25	4.15 ± 0.51	1.11 ± 0.12
(*E*)-2-Hexenal	854	3.43 ± 0.31	3.18 ± 0.31	3.78 ± 0.44	9.32 ± 0.67
1-Nonene	891	–	0.13 ± 0.02	0.15 ± 0.03	0.17 ± 0.03
α-Pinene	939	0.05 ± 0.01	0.05 ± 0.01	–	–
Benzaldehyde	960	0.68 ± 0.08	0.54 ± 0.04	0.45 ± 0.06	0.82 ± 0.09
5-Methylfurfural	978	–	0.96 ± 0.07	1.38 ± 0.11	–
1-Octen-3-ol	982	–	–	0.22 ± 0.04	0.28 ± 0.04
6-Methyl-5-heptenone	985	1.65 ± 0.12	1.57 ± 0.11	1.12 ± 0.15	0.87 ± 0.06
Pentylfuran	993	0.32 ± 0.04	0.23 ± 0.04	0.47 ± 0.08	–
Decane	1000	0.32 ± 0.05	0.18 ± 0.03	0.47 ± 0.06	2.71 ± 0.21
Hexyl acetate	1014	0.82 ± 0.07	0.52 ± 0.06	0.36 ± 0.04	0.21 ± 0.03
2-Ethylhexanol	1032	1.83 ± 0.09	1.56 ± 0.21	0.27 ± 0.05	1.37 ± 0.15
Limonene	1033	7.71 ± 0.69	5.41 ± 0.49	4.34 ± 0.52	16.33 ± 2.03
Phenylacetaldehyde	1048	–	0.52 ± 0.05	1.72 ± 0.24	–
Furaneol	1064	–	0.85 ± 0.09	1.41 ± 0.15	–
2-Decen-1-ol	1110	–	–	–	0.71 ± 0.09
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	1140	13.15 ± 1.28	12.18 ± 1.13	17.54 ± 1.54	2.11 ± 0.30
Nonanol	1169	1.97 ± 0.22	–	–	2.78 ± 0.23
α-Terpineol	1195	0.87 ± 0.06	1.12 ± 0.20	2.18 ± 0.23	1.51 ± 0.20
(*E*,*Z*)-2,4-Nonadienal	1196	–	–	–	1.06 ± 0.12
Dodecane	1200	0.84 ± 0.07	0.37 ± 0.04	0.45 ± 0.09	0.40 ± 0.08
Decanal	1209	1.63 ± 0.11	0.96 ± 0.07	0.49 ± 0.06	2.34 ± 0.22
5-HMF	1241	38.68 ± 2.59	43.75 ± 4.11	39.88 ± 3.81	1.78 ± 0.20
Decanol	1263	–	–	1.43 ± 0.11	3.83 ± 0.45
Butyrolactone	1299	1.31 ± 0.14	1.64 ± 0.15	1.43 ± 0.24	3.27 ± 0.29
Tetradecane	1400	1.23 ± 0.09	1.76 ± 0.23	1.15 ± 0.26	1.12 ± 0.15
Geranyl acetone	1448	–	–	0.61 ± 0.09	–
γ-Decalactone	1472	3.79 ± 0.32	3.52 ± 0.21	3.64 ± 0.44	7.89 ± 0.92
β-Ionone	1493	2.67 ± 0.36	2.11 ± 0.25	1.18 ± 0.20	5.96 ± 0.59
Tridecanol	1593	1.49 ± 0.11	0.53 ± 0.08	0.38 ± 0.09	2.22 ± 0.29
Hexadecane	1600	0.38 ± 0.05	1.06 ± 0.09	0.59 ± 0.09	1.46 ± 0.12

Table 2.13 (continued)

Compound[a]	RI[b]	Sun (%)[c]	Microwave (%)[c]	Hot air (%)[c]	Desiccator (%)[c]
1-Pentadecanol	1787	0.59 ± 0.06	0.55 ± 0.07	–	1.38 ± 0.14
Unknown		5.38 ± 0.45	6.01 ± 0.64	4.54 ± 0.71	5.33 ± 0.68

RI: retention index. [a]As identified by GC–TOF/MS software, names according to NIST mass spectral library, and by comparing their Kovats retention indices.
[b] Kovats retention indices of each component was collected from the literature for column DB5.
[c] Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100 (in the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte).
[d] The standard deviations for four ($n = 4$) experiments.
[e] Not detected or percentage of the component is lower than 0.05%.
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characteristics for honeydew melon. Methyl esters were present mainly in Galia melons (Kourkoutas et al., 2006).

A complex chromatographic system consisting of HPLC, GC-MS and GC-O was employed for the investigation of the volatile compounds released from mild hydrolysates of odourless precursors in Tempranillo and Grenache grapes (*Vitis vinifera* cv. Tempranillo and Grenache). The prefractionation of the extracts was performed by HPLC. Separation was carried out on a C18 RP column (25 cm length, 1 cm i.d., particle size, 5 μm). Thirty fractions were collected and analysed by GC-MS and GC-O. GC-MS investigations were performed on a capillary column (60 m × 0.25 mm, film thickness, 0.25 μm); the temperature program initiated at 40°C (5 min hold) then increased to 220°C at 3°C/min. The mass range of the detection was set to 35–250 *m/z*. The main odorants identified in juice and skin hydrolysates are compiled in Table 2.14. It was concluded from the data that the main aroma precursors are volatile phenols, unsaturated fatty acid derivatives, β-damascenone, vanillin and ethyl dihydrocinnamate (López et al., 2004).

2.2 Legumes and Vegetables

Because of their importance in human nutrition, aroma compounds in legumes and vegetables have also been investigated. The various headspace sampling technologies employed in the analysis of volatiles in vegetable matrices have been recently reviewed with special emphasis on in-tube sorptive extraction (INCAT, HS-SPDE), headspace sorptive extraction (HSSE), solid-phase aroma concentrate extraction (SPACE), large surface area HCC-HS sampling (MESI, MME, HS-STE), high concentration capacity headspace (HCC-HS) sampling, headspace liquid-phase microextraction and dynamic headspace extraction (D-HS) (Bicchi et al., 2008).

The aroma composition of various plant materials such as common tomato, cherry tomato, durian, longan, mango and allium was analysed by using simultaneous distillation extraction (SDE) and steam distillation (SD) followed by GC-MS. GC separations were performed on a capillary column (60 m × 0.32. mm, film

Table 2.14 Main odourants found in juice and skin hydrolysates

RI	Odour description	Identity	Temprallino juce	Temprallino skins	Grenache juice	Grenache skins	Abundance
	Lipid derivatives						
1094	Fruity, crushed grapes	Hexanal[a]	4	2	4	4	++
1221	Green	*E*-2-Hexenal[a]			1		+
899	Fruity, sweet	Ethyl acetate[a]	4	4	1		++
1248	Flowery, fruity	Ethyl hexanoate[a]			2		++
1309	Damp, humid	1-Hecten-3-one[d]	4	4	4	1	Trace
1336	Beer	*E*-2-Heptenal[b]		2	1	1	+
1382	Green, cypress	*Z*-3-Hexen-1-ol[a]	1	1	1		++
1573	Pleasant, sweet	1-Octanol[a]	2	2	4	1	++
1646	Green	*E*-2-Decenal[a]			1		+
1743	Fatty, sweet	Pentanoic acid[a]		4			+
1825	Fatty	*E,E*-2,4-Decadienal[a]	1		4	2	++
1860	Fatty, cheese	Hexanoic acid[a]	4	4	4	4	+
2056	Lactone-like, peach	γ-Nonalactone[a]	1		1		+
2075	Fatty	Octanoic-acid[a]		4			+
2177	Coconut	γ-Decalactone[a]	1	4	1		Trace
2220	Coconut, lactone-like	δ-Decalactone[d]	4	4		2	Trace
2247	Spice, lactone-like, coconut	γ-Undecalactone[a]	1	1	4	1	Trace
2264	Lactone-like, coconut	ε-Decalactone[b]	4	4	4	4	Trace
2276	Aromatic herbs	Ethyl hexadecanoate[a]		1			+++
2371	Lactone-like	γ-Decalactone[d]				1	Trace
2398	Dry wood, oak	(*Z*)-6-Dodecene-7-lactone[d]	2	2	1		Trace
	Shikimic derivatives						
1550	Dry plastic synthetic	Benzaldehyde[a]	2	2			+
1879	Phenolic, wood	Guaiacol[a]	4	2	4	4	+
1900	Pleasant, faint	Ethyl dihydrocinnamate[a]	4	1			+

Table 2.14 (continued)

RI	Odour description	Identity	Temprallino juce	Temprallino skins	Grenache juice	Grenache skins	Abundance
1932	Flowery, dry fruits	2-Phenylethanol[a]			4	2	++
1969	Phenolic	Phenol			1		+
2112	Unpleasant, machine	*m*-Cresol			1	1	+
2156	White flowers	Ethyl cinnamate[a]	1	1	4	2	Trace
2183	Alcoholic	2-Phenoxyethanol[a]	1				++
2192	Phenolic, flowery	Eugenol[a]	2				+
2209	Shoe, polish	4-Ethylphenol[a]		1	1		Trace
2213	Phenolic, synthetic	4-Vinylguaiacol[a]	1	1	2	2	+++
2303	Phenolic, synthetic	2,6-Dimethoxyphenol[a]	4	4	4	4	+
2376	Tangerine, wood	Isoeugenol[a]			4	1	Trace
2425	Almond shell	4-Vinylphenol[a]	4	2	1	1	+
2457	Aromatic flowery	Benzoic acid[a]			2	1	+
2575	Honey, flowery	Phenylacetic acid[a]	4	4	1	1	+
2589	Vanilla	Vanillin[a]	4	4	4	4	++
2653	Dry herbs	Methyl vanillate[a]				1	+
2668	Flowery	Ethyl vanillate[a]			1	1	++
2685	Tangerine, flowery	Acetovanillone[a]				1	+
	Norisoprenoids						
1751	Pleasant, flowery	TDN[b]		4			+
1834	Dry plum	β-Damascenone[a]	4	1	4	4	++
1891	Flowery	Unknown norisoprenoid[d,e]	1	1	4	2	Trace
	Terpenes						
1660	Sweet, cookie, bun	Citronellyl acetate[b]	1		1		+
1721	Pleasant	α-Terpineol[a]			1		++
1983	Aromatic flowery	3,7-Dimethyloct-1-ene-3,7-diol[c]	2	2	4	4	+
2382	Flowery	Farnesol[a]	2	4	1		++

Table 2.14 (continued)

RI	Odour description	Identity	Temprallino juce	Temprallino skins	Grenache juice	Grenache skins	Abundance
	Thiols						
1741	Mango, anise	3-Mercaptohexyl acetate[d]			1		Trace
1866	Lemon, green	3-Mercaptohexanol[a]	1	1		1	Trace
	Miscellaneous						
1074	Fruity	Ethyl 2-methylbutyrate[a]			4		+
1466	Vinegar	Acetic acid[a]	4	4	4	4	++
1499	Pleasant, soap	2-Ethyl-1-hexanol[a]	1	4			++
1535	Sweet, fruity	2,5-Dimethyl-3(2H)-furanone[d]			2	2	Trace
1556	Blue cheese	Propanoic acid[a]	1				+
1629	Toasty burnt	2-Acetylpyrazine[d]	2		2	2	Trace
2225	Liquorice, celery	Sotolon[d]	2	2	4	4	Trace
2257	Sweet, noney	Methyl anthranilate[d]	2	2	2		Trace
	Unknown odorants						
977	Lactic	n.i.		4			
981	Butter, strawberry	n.i.	2	2			
983	Orange, sweet	n.i.	1				
1060	Fruity	n.i.	4		2		
1086	Fruity, ester	n.i.				1	
1113	Fruity	n.i.			4		
1116	Rancid	n.i.	1				
1164	Apple	n.i.		1			
1241	Gas	n.i.		2			
1298	Perfume	n.i.		1			
1388	Pleasant sweet	n.i.			1	1	
1390	Synthetic, metallic	n.i.	1				
1395	Flowery	n.i.			1		

Table 2.14 (continued)

RI	Odour description	Identity	Temprallino juce	Temprallino skins	Grenache juice	Grenache skins	Abundance
1407	Dust, pollen, jasmine	n.i.			1		
1432	Flowery, pleasant	n.i.	1				
1443	Toast bread, ashen	n.i.	1	1			
1571	Chlorine damp	n.i.	4	2	4	4	
1600	Green, flowery	n.i.			1	1	
1625	Chlorine	n.i.	4	2	4		
1694	Rubber	n.i.	2	2			
1734	Strange	n.i.	4				
1783	Rubber	n.i.	1				
1872	Rubber	n.i.	2				
1896	Sour, green tomatoes	n.i.		1			
2015	Citric	n.i.			1		
2026	Dry fruit, violets	n.i.		4	4	4	
2064	Vanilla	n.i.			1		
2086	Dry fruit	n.i.			1		
2137	Phenolic	n.i.		1			
2240	Synthetic chemical	n.i.		1			
2315	Wine fruity	n.i.			1		
2334	Cherry candy, liquor	n.i.	1		1		
2423	Fatty, rancid	n.i.				1	
2517	Fresh	n.i.				1	
2527	Machine, unpleasant	n.i.					
2531	Clove	n.i.		1			
2556	Toasty	n.i.		1			

Table 2.14 (continued)

RI	Odour description	Identity	Temprallino juce	Temprallino skins	Grenache juice	Grenache skins	Abundance
2607	Phenolic	n.i.				1	
2765	Dry herbs	n.i.				1	
2810	Flowery	n.i.				1	

[a]Reliability: Identification based on coincidence of gas chromatographic retention and mass spectrometric data with those of the pure compound available in the lab.
[b]Reliability: The pure compound was not available, but gas chromatographic retention and mass spectrometric data were coincident with those reported in the literature.
[d]Reliability: Identification based in gas chromatographic retention data and odour quality. The compound did not produce any clear signal in the mass spectrometer because of its low concentration; n.i.: not detected.
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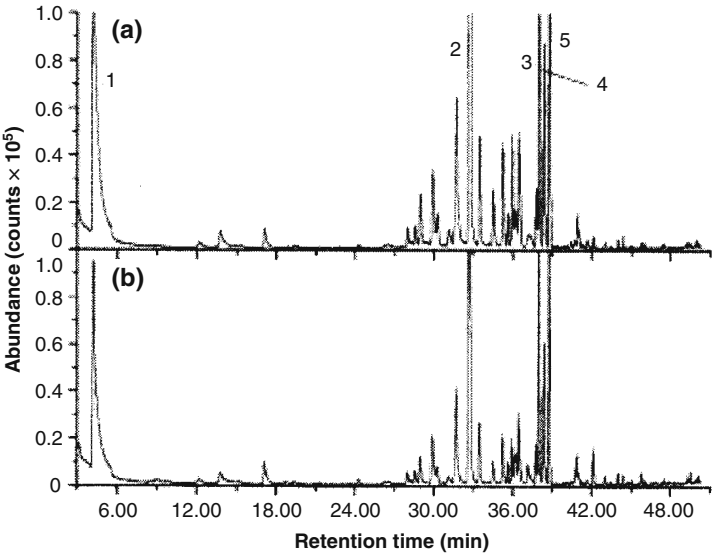

Fig. 2.6 The typical aroma profile chromatograms of the fresh (**a**) and stale (**b**) longan by HSSPME. The peak numbers corresponded to the main aroma volatiles. 1, ethyl acetate; 2, β-ocimene; 3, *allo*-ocimene; 4, 4-ethyl-1,2-dimethyl-benzene; 5, 3,4-dimethyl-2,4,6-octatriene. Reprinted with permission from Zhang and Li (2007)

thickness, 1.8 μm) using various temperature programs for the different extracts. Carrier gas was helium, and the injector temperature was set to 250°C. MS conditions were: transfer line temperature 280°C; energy of electron 70 eV, ion mass/charge ratio, 20–500 *m/z*. Typical chromatograms of fresh and stale longan are depicted in Fig. 2.6. The chromatograms illustrate the good separation capacity of the analytical system and indicate that storage exerts a marked influence on the aroma profile. Some compounds contributing to the aroma profile of samples are compiled in Table 2.15. The application of the method was proposed for the investigation of the secondary metabolism in plants and for quality control purposes (Zhang and Li, 2007).

Capillary electrophoresis was employed for the measurement of non-drivatised methiin and alliin in vegetables (garlic, Chinese chive, *Allium* and *Brassica*). Analytes were extracted from the samples by distilled water, filtered and used for CE measurements. Capillary was of 100 cm length (effective length 91.5 cm) and 50 μm i.d. Running buffer consisted of 20 mM sodium benzoate and 0.5. mM TTAB (tetradecyltrimethyl-ammonium bromide) pH 12.0. Sample was injected hydrodynamically (50 mbar for 5.0 s). Voltage was –30 kV, and capillary temperature was set to 25°C. Indirect UV detection was applied (350 nm signal wavelength and 225 nm reference wavelength). The baseline separation of alliin and methiin is illustrated in Fig. 2.7. The concentrations of the analytes in *Allium* and *Brassica* vegetables are compiled in Table 2.16, demonstrating the considerable differences between plant

Table 2.15 Top five compounds contributing to the difference of aroma profile characteristics at different storage stages

Common tomato		Cherry tomato		Longan		Durian	
Aroma volatile	Percentage of contribution	Aroma volatile	Percentage of contribution	Aroma volatile	Percentage of contribution	Aroma volatile	Percentage of contribution
Hexanal	65.17	Hexanal	73.19	β-Ocimene	39.50	2-Methyl butanoic acid ethyl ester	48.20
(*E*)-2-Hexenal	4.07	3-Hexenol	2.26	3,4-Dimethyl-2,4,octa-triene	14.00	Propanoic acid ethyl ester	11.70
6-Methyl-5-hepten-2-one	3.28	(*E*)-2-Octenal	1.98	Ethyl acetate	9.96	2-Methyl butanoic acid methyl ester	7.97
(*E*)-2-Octenal	1.32	1-Hexanol	1.76	Allo-ocimene	8.54	2-Methyl butanoic acid	6.39
(*E,E*)-2,4-Hexadienal	0.57	(*E*)-2-Hexenal	1.03	1-Ethyl-6-ethylidene-cyclohexene	3.21	Hexanoic acid ethyl ester	6.31

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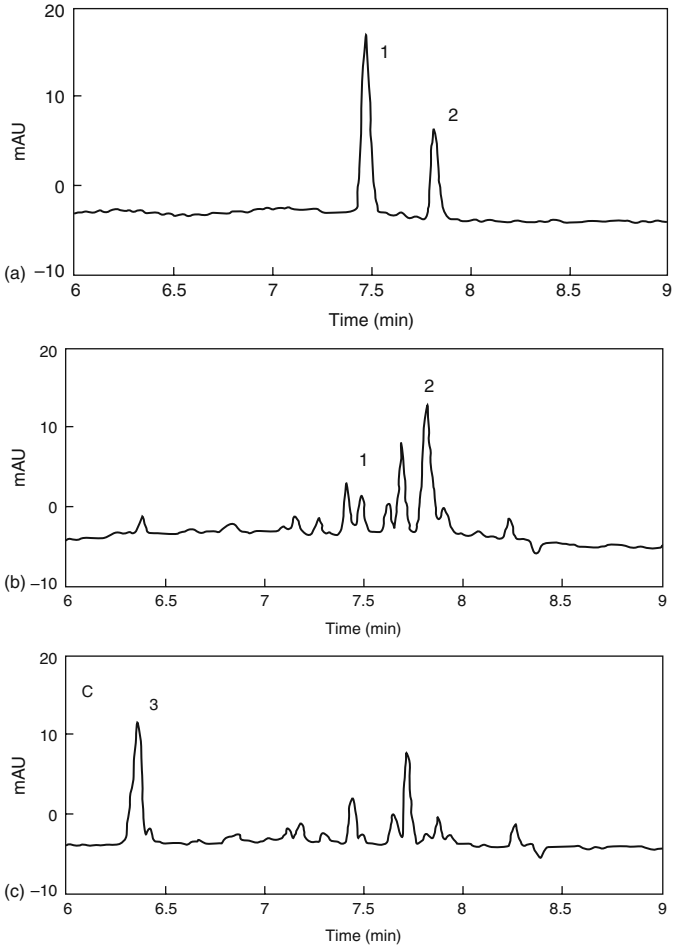

Fig. 2.7 Electropherograms of alliin and methiin. (**a**) Standard alliin and methiin, the concentrations were 200 mg/l, (**b**) the extract of garlic clove (blanched, 50 times diluted), (**c**) the extract of garlic clove (unblanched, 50 times diluted), 1, methiin; 2, alliin; 3, pyruvate. Reprinted with permission from Horie and Yamashita (2006)

species. It was stated that the method is simple and rapid (25 min analysis time) and it can be employed for quality control (Horie and Yamashita, 2006).

2.3 Cereals

Besides fruits, the aroma compound methyl nicotinate (MN) has been found also in rice. Samples were grounded and extracted with methanol using sonification. The optimal extraction conditions were 50°C and 120 min. GC measurements were performed on a capillary column (30 m length, 0.25 mm internal diameter and 1.4 μm film thickness). Separation started at 40°C for 2 min, 10°C/min to 240°C, final hold

Table 2.16 The contents of alliin and methiin in Allium and Brassca vegetables

Academic name			Country production	Dilution	g/kg[a]	
					Alliin	Methiin
Alliium						
Garlic	*Alliium sativun*	Cloves	Japan	50	12.67(1.57)	1.18(0.12)
			China	50	16.93(0.44)	1.71(0.04)
		Stems	China	25	5.26(0.29)	0.72(0.07)
Chinese chive		Leaves	Japan	20	1.01(0.03)	4.11(0.13)
Brassica						
Cabbage		Leaves	Japan	25		1.29(0.04)
Broccoli		Buds	Japan	25		1.35(0.06)
			USA	25		1.83(0.01)

[a] Average of three separate extractions. ( ): Standard deviation ($n = 3$).
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8 min. It was found that the method is economic, rapid and suitable for the fast screening of rice samples (Mualidhara Rao et al., 2007).

The composition and amount of phenolic compounds in cereals has been many times investigated. These measurements were motivated by the fact that this class of compounds shows beneficial effects against chronic diseases (Liu et al., 2003) such as diabetes (Liu et al., 2000; Montonen et al., 2003), cardiovascular disease (Anderson et al., 2003). This effect may be due to their antioxidant activity (Adom and Liu, 2002; Adom et al., 2003). Besides their biological activity, phenolic compounds influence markedly the flavour of cereals (Heiniö et al., 2003).

Phenolic compounds and their influence on the sensory characteristics of rye were studied in detail. Water-soluble and water-insoluble phenol fractions were separated and analysed by HPLC (phenolic acids and alkylresorcinols) while lignans were separated and quantitated by GC-MS. The concentrations of non-bound and bound phenolic acids, alkylresorcinols and lignans are compiled in Table 2.17. The various flavour characteristics (intensive flavour, aftertaste, bitterness, germ-like flavour) were successfully related to the analytes separated by HPLC and GC (Heiniö et al., 2008).

The impact of high pressure on the interaction of aroma compounds with various maize starches has also been investigated. The adsorption of aroma compounds was determined by GC. It was established that the sorption depends on the hydrophobicity and other molecular parameters of the volatiles, for example, hydrocarbons and aliphatic esters were the most strongly adsorbed (Blaszczak et al., 2007).

The changes in the composition and quantity of volatiles during baking has also been extensively investigated. The analysis of aroma compounds released during baking was performed by SPME coupled to GC-MS and with GC-O (Rega et al., 2006).

Table 2.17 Amounts of non-bound (free, esterified and glycosidic phenolic acids together) and bound phenolic acids in the rye fractions (mg/100 g)

	Yield (%)	Sinapic acid	Syringic acid	Vanillic acid	Ferulic acid	Caffeic acid	*p*-OH-benzoic acid	Veratric acid	Total non-bound phenolic acids	Total phenolic acids (non-bound+bound)
B flour	19	0.4	–	0.1	0.4	–	–	–	0.9	3.6
C flour	16	0.5	–	–	0.8	–	–	–	1.3	4.6
Shorts	16	2.8	0.1	0.5	1.3	–	0.2	0.3	5.2	49.4
Bran	49	5.3	–	1.0	2.5	–	0.5	0.6	9.9	127.2
Enriched bran	*	15	1.1	0.9	4.8	0.5	0.6	0.5	23.4	131.5
Wholemeal rye	100	0.2	–	0.3	0.6	–	0.3	–	1.4	65.3

– Below the detection limit. * "The enriched bran" is a subfraction of "the bran". Its yield was 19% of original rye grain, 40% of total bran.

Table 2.17 (continued)

	Yield (%)	Alkr C17:0	Alkr C19:1	Alkr C19:0	Alkr C21:0	Alkr unknown	Alkr C23:0	Alkr C25:0	Total alkylresorcinols
Amounts Alkybresorcinols in the rye fractions (mg/100 g)									
B flour	19	0.3	0.6	0.5	0.3	0.4	0.4	0.1	2.6
C flour	16	0.6	0.6	0.7	0.4	0.4	0.5	0.2	3.4
Shorts	16	20.1	3.5	20.1	14.8	4.3	8.8	5.6	77.2
Bran	49	51	7.2	51.3	35.9	5.4	33.3	12.5	196.6
Enriched bran	*	71.9	10.9	72	47.6	7.2	26.5	16	252.1
Wholemeal rye	100	35.3	4.5	38.1	29.3	3.6	17.4	14.8	143.0

* "The enriched bran" is a subfraction of "the bran". Its yield was 19% of original rye grain, 40% of total bran.

Table 2.17 (continued)

	Yield (%)	Secoi-solariciresinol	Matairesinol	Isolariciresinol	Lariciresinol	Pinoresinol	Syringaresinol	Total lignans
Amounts of lignans in the rye fractions (μg/100 g)								
B flour	19	1.8	0.0	0.0	0.0	0.0	22.2	24.0
C flour	16	1.3	0.0	0.0	0.0	0.0	28.1	29.4
Shorts	16	28.9	20.9	89.2	16.4	10.2	1457.1	1622.7
Bran	49	55.1	64.5	207.4	66.6	165.1	2723.0	3281.7
Enriched bran	*	87.7	100.2	312.5	74.4	241.0	3271.6	4087.3
Wholemeal rye	100	36.2	32.4	108.3	146.8	179.1	1770.6	2273.3

* "The enriched bran" is a subfraction of "the bran". Its yield was 19% of original rye grain, 40% of total bran.
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The influence of various microorganisms such as *Kluyveromyces marxianus, Lactobacillus delbrueckii* ssp. Bulgaricus and *Lactobacillus helveticus* on the quality and quantity of volatiles formed during sourdough bread-making has been investigated in detail. Volatiles were preconcentrated by SPME and separated and identified by GC-MS. SPME extraction was carried out at 60°C for 60 min. The analytical column was 60 m × 0.32 mm i.d., film thickness 0.25 μm. Initial oven temperature was 35°C (5 min hold), raised to 50°C at 5°C/min (5 min hold), then to 230°C at 5.5°C/min (5 min final hold). Injector temperature was 280°C. Analytes were detected in the range 33–200 *m/z*. The volatiles identified by GC-MS in various bread samples are compiled in Table 2.18. It was established that the addition of various cultures considerably improve the sensorial quality of bread (Plessas et al., 2008a).

A similar SPME and GC-MS method was applied for the assessment of the evolution of aroma compounds during storage of sourdough breads made by the addition of the cultures employed by Ref. (Plessas et al., 2008a). The concentration of volatiles during the storage of breads is compiled in Table 2.19. It was found that breads prepared by cultures had a more complex aroma profile, longer shelf-life and higher sensory qualification (Plessas et al., 2008b).

The changes in the aroma profile of palm sap (*Arenga pinnata*) during the production of palm sugar was followed by HS-SPME and GC-MS. The main components of the volatile fraction were 5-methyl-6,7-dihydro-5*H*-cyclopenta pyrazine and 4-hydroxy-2,5-dimethyl-3(2*H*) furanone. It was found that the concentration of pyrazine compounds and furan derivatives increased during the heating process (Ho et al., 2007).

The aroma profiles of a soy protein isolate (SPI) and acid-hydrolysed vegetable protein (aHVP) were compared using GC-MS and GC-O. It was found that aliphatic aldehydes and ketones are characteristics for SPI, whereas pyrazines and sulphur-containing compounds were dominant in aHVP. (Solina et al., 2005).

2.4 Edible Oils

The commercial value of olive oil markedly depends on the aroma, taste and colour characteristics of the product. The importance of the volatile components and the development of flavour compounds during processing and storage have been previously reviewed (Kalua et al., 2007).

The composition of edible oils was investigated by various chromatographic technologies. Thus, SPME followed by GC-MS and GC-FID was applied for the separation, identification and quantitative determination of the volatile compounds in olive oils. The performance of PDMS (100 μm), CAR-PDMS (75 μm), PDMS-DVB (65 μm) and DVB-CAR-PDMS (50 and 20 μm) fibres was compared for the preconcentration and prepurification of volatiles. The same column (30 m × 0.25 mm, film thickness, 0.25 μm) was employed for both GC-FID and

Table 2.18 Volatile by-products identified in bread produced using 50% w/w sourdough containing 1% *K. marxianus* and 4% LAB (samples 4 and 9) and bread made with traditional, wild microflora sourdough (sample 13)

Kovats index	Compound	*K. marxianus and L. bulgaricus*	*K. marxianus and L. helveticus*	Wild microflora
Alcohols				
832	Ethanol	a	a	A
1012	Isobutyl alcohol	–	a	–
1120	1-Butanol, 3-methyl	–	a	–
1257	1-Hexanol	a		A
1395	1-Decanol, 2-ethyl	a	a	–
1434	2-Nonen-1-ol	a	–	–
1452	3-Pentanol,2,4-dimethyl	–	–	A
1466	1-Octanol	a	–	–
1502	Non-2-en-1-ol	a	–	A
1512	2-Undecanol	a	–	A
1600	3-Nonen-1-ol	a	–	–
1670	Benzyl alcohol	a	a	–
1812	Phenyl ethanol	a	a	A
Esters				
<800	Ethyl acetate	a	a	–
1107	Butyl acetate	a	–	–
1590	Isobutyl acetate	a	–	A
1682	3-Hydroxy butyl acetate	a	–	–
1925	Ethyl pentadecanoate	a	–	–
Carbonyls				
1002	Hexanal	a	a	A
1067	Heptanal	a		–
1091	Butanal, 3-methyl	a		–
1324	Nonanal	–		A
1334	Furfural	a	a	–
1365	2-Nonenal	–	a	A
1448	Butyrolactone	a	a	A
1458	Benzaldehyde	a	a	A
1484	Hexadecanal	–	a	–
Organic acids				
1260	Lactic acid	a	a	A
1615	Acetic acid	a	a	A
1900	Hexanoic acid	a	a	–
1934	Octanoic acid	–	a	A
Miscellaneous compounds				
1452	2*H*-Pyran-2-one, tetrahydro-4,6 dimethyl	–	a	–

a = Positive identification from MS data and retention times.
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Table 2.19 Characteristics and shelf-life of breads made with sourdough containing *K. marxianus* and *L. bulgaricus* or *L. helveticus* and with traditional, wild microflora sourdough

Bread sample	Amount of microorganism in sourdough (% w/w on flour basis)	Fermentation temperature (°C)	Amount of sourdough (% on flour basis)	Final pH	TTA (ml)	Moisture loss (g)	Specific loaf volume (ml/g)	Mould spoilage (days)	Bread sample	Amount of microorganism in sourdough (% w/w on flour basis)	Fermentation temperature (°C)
	K. marxianus	*L. bulgaricus*									
1	1	4	40	30	4.5	8.1	121	2.1	11	1.95	0.21
2	1	2	40	30	4.6	8.1	119	2.2	10	1.53	0.35
3	1	1	40	30	4.6	7.5	120	2.0	9	1.11	0.12
4	1	4	40	50	4.3	9.2	113	2.1	12	2.88	0.25
5	1	4	30	30	4.6	6.6	110	2.3	11	1.55	0.24
	K. marxianus	*L. helveticus*									
6	1	4	40	30	4.5	7.1	125	2.0	10	1.25	0.35
7	1	2	40	30	4.5	6.9	130	1.9	8	1.11	0.22
8	1	1	40	30	4.6	6.6	145	1.6	8	1.10	0.19
9	1	4	40	50	4.4	8.2	105	2.2	12	3.41	0.38
10	1	4	30	30	4.6	6.3	122	1.9	10	1.28	0.25
11	1	–	40	30	4.6	5.9	135	1.7	8	0.82	0.27
12	–	–	40	30	5.2	3.6	129	1.8	8	1.05	0.17
13	–	–	40	50	5.0	5.1	121	1.9	8	1.10	0.15

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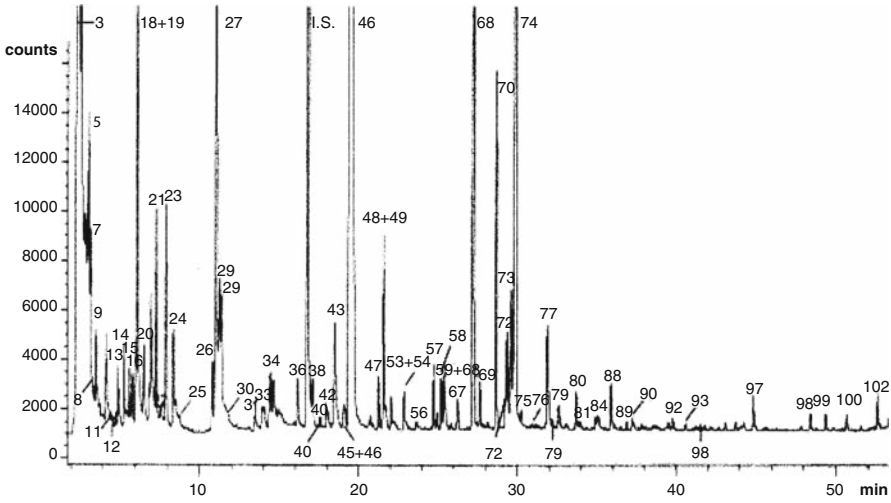

Fig. 2.8 HS-SPME–GC–FID chromatogram of sample 3, sampling being performed by DVB–CAR–PDMS and chromatographic separation being carried out on a Supelcowax-10 capillary column. Peak identification: 2-methylpentane (1), 3-methylpentane (2), hexane (3), heptane (4), octane (5), *E*-2-octane (6), 2-propanone (7), methyl acetate (8), 2-propenal (9), ethyl acetate (10), 2-methylbutanal (11), isovaleraldehyde (12), ethanol (13), 1-methoxyhexane (14), 1,5-hexadiene, 3,4-diethyl (*R,S+S,R*)(15), *meso*-1,5-hexadiene,3,4-diethyl (16), ethyl propanoate (17), pentalan (18), 3-pentanone (19), trichloroethene (20), 1,5-octadiene, 3-ethyl (*E* or *Z*) (21), 1-penten-3-one (22), 1,5-octadiene, 3-ethyl (*E* or *Z*) (23), toluene (24), (*E*)-2-butenal (25), 3,7-decadiene (*EE* or *ZZ* or *EZ*) (26), hexanal (27), 3,7-decadiene (*EE* or *ZZ* or *EZ*) (28, 29), isobutylalcohol (30), ethylbenzene (31), isoamylacetate (32), (*E*)-2-pentanal (33), *m*- or *p*-xylene (34), (*Z*)-3-hexenal (35), 1-penten-3-ol (36), 4-methyl-pentanol (I.S.) (37), *o*-xylene (38), 2-heptanone (39), heptanal (40), 3-octen-2-one (41), limonene (42), 1-methyl-3-(hydroxyethyl) propadiene (43), 3-methylbutanol (44), 2-methylbutanol (45), (*E*)-2-hexenal (46), n.i.[d](hydrocarbon) (47), β-ocimene (48), 1-pentanol (49), 1-acetylcyclohexene (50), methyl benzoate (51), styrene (52), hexyl acetate (53), 1,2,4-trimethylbenzene (54), octanal (55), ethyl hexanoate (56), (*E*)-4,8-dimethyl-1,3,7-nonatriene (57), (*Z*)-3-hexenyl acetate (58), (*E*)-2-heptenal (59), α-pinene (60), hexyl formate (61), (*Z*)-2-pentenom (62), *m*-ethyltoluene (63), *o*-ethyltoluene (64), 1,3,5-trimethylbenzene (65), 2-octanone (66), 6-methyl-5-hepten-2-one (67), 1-hexanol (68), (*E*)-3-hexen-1-ol (69), (*Z*)-3-hexen-1-ol (70), nonanal (71), 2,4-hexadienal 1 (72), 2,4-hexadienal 2 (73), (*E*)-2-hexen-1-ol (74), (*Z*)-2-hexen-1-ol (75), (*E*)-2-octenal (76), acetic acid (77), (*E*)-1-octen-3-ol (78), 2,4-heptadienal 1 (79), α-copaene (80), 2,4-heptadienal 2 (81), methyl nonanoate (82), decanal (83), formic acid (84), 3,5-octadien-2-one (85), (*E*)-2-nonenal (86), ethyl nonanoate (87), propanoic acid (88), 1-octanol (89), isobutylic acid (90), methyl decanoate (91), butanoic acid (92), (*E*)-2-decanal (93) 2,4-decadienal (94), 1-nonanol (95), pentanoic acid (96), (*E*,*E*)-α-farnesene (97), hexanoic acid (98), benzyl alcohol (99), phenylethyl alcohol (100), heptanoic acid (101), (*E*)-2-hexanoic acid (102). Reprinted with permission from Vichi et al. (2003)

Table 2.20 Concentrations (expressed in μg/g) of the compounds detected in the headspace of the virgin olive oil samples, calculated from SPME-GC-FID data

Compound	Sample							Ref.
	1	2	3	4	5	6	7	
2-Methylpentane[a]	0.26	0.14	0.05	0.15	0.10	0.03	0.38	
3-Methylpentane[a]	0.41	0.22	0.57	0.20	0.18	0.04	0.49	
Hexane[a]	12.57	7.20	2.45	11.55	4.44	2.10	2.08	
Heptane[a]	0.12	0.11	0.15	0.54	0.07	0.07	1.59	
Octane[a]	0.26	0.35	0.03	0.36	0.20	0.14	2.38	
(*E*)-2-Octane[a]	0.03	0.04	0.01	0.02	0.02	0.01	0.11	
2-Propanone[b]	2.00	0.28	0.23	0.18	0.19	0.16	1.24	
Methyl acetate[b]	0.16	0.13	0.08	0.41	0.08	0.09	0.00	
2-Propenal[b]	0.22	0.22	0.12	0.13	0.12	0.14	1.05	
Ethyl acetate[b]	0.17	0.11	0.02	0.05	0.02	0.02	0.68	
2-Methylbutanal[b]	0.06	0.04	0.02	0.00	0.08	0.00	0.00	
Isovaleraldehyde[b,c]	0.41	0.21	0.07	0.00	0.62	0.00	0.00	62–106 μg/kg (29) 1.5–7.9 μg/g (21)
Ethanol[b]	3.67	1.26	0.10	0.31	0.56	0.28	5.42	
1-Methoxyhexane[b]	0.00	0.04	0.09	0.06	0.00	0.00	0.75	
1,5-Hexadiene, 3,4-diethyl[b]	0.16	0.10	0.08	0.03	0.14	0.03	0.00	
Meso-1,5-Hexadiene, 3,4-diethyl[b]	0.13	0.09	0.07	0.03	0.13	0.03	0.00	
Ethyl propanoate[a,c]	0.00	0.00	0.00	0.00	0.00	0.09	0.00	
Pentanal[b] + 3 pentanone[b,c]	1.21	1.54	0.55	1.69	1.13	0.59	4.64	62–409 μg/kg (29)
Trichloroethane[b]	0.10	0.00	0.15	0.00	0.00	0.00	0.00	
1,5-Octadiene, 3-ethyl (E or Z)[b]	0.20	0.29	0.27	0.08	0.40	0.10	0.04	
1-Penten-3-one[b,c]	0.30	0.19	0.04	0.05	0.21	0.04	0.16	
1,5-Octadiene, 3-ethyl (E or Z)[b]	0.31	0.31	0.26	0.10	0.53	0.07	0.08	
Toluene[b]	0.13	0.14	0.14	0.12	0.12	0.19	0.25	
(*E*)-2-Butenal[b]	0.07	0.14	0.06	0.07	0.05	0.12	0.11	

Table 2.20 (continued)

Compound	Sample 1	2	3	4	5	6	7	Ref.
3,7-Decadiene(*EE* or *ZZ* or *EZ*)[b]	0.10	0.11	0.11	0.02	0.16	0.03	0.00	
Hexanal[b,c]	3.63	3.16	1.78	0.48	1.53	0.35	38.10	137–1770 μg/kg (29) 338–1574 μg/kg (22) 26.8–38 μg/g (21) 40–60 μg/L (30)
3,7-Decadiene (*EE* or *ZZ* or *EZ*)[b]	0.30	0.35	0.30	0.05	0.38	0.07	0.79	
3,7-Decadiene (*EE* or *ZZ* or *EZ*)[b]	0.43	0.27	0.24	0.09	0.34	0.05	0.73	
Isobutylalcohol[b]	0.11	0.14	0.08	0.21	0.05	0.01	1.05	
Ethylbenzene[b]	0.02	0.03	0.04	0.01	0.02	0.03	0.10	
Isoamylacetate[b]	0.02	0.03	0.00	0.05	0.01	0.01	0.16	
(*E*)-2-Pentenal[b]	0.15	0.22	0.03	0.03	0.17	0.03	2.17	
m- or *p*-Xylene[b]	0.06	0.10	0.12	0.06	0.06	0.07	0.43	
(*Z*)-3-Hexenal[b]	0.20	0.11	0.14	0.00	0.22	0.03	0.00	
1-Penten-3-ol[b]	0.21	0.22	0.09	0.04	0.21	0.08	0.72	
4-Methyl-2-pentanol[b]	I.S.	I.S.	I.S.	I.S.	I.S.	I.S.	I.S.	
o-Xylene[b]	0.07	0.09	0.09	0.06	0.06	0.06	0.17	
2-Heptanone[b]	0.01	0.03	0.01	0.01	0.02	0.01	0.32	
Heptanal[b,c]	0.07	0.14	0.04	0.02	0.12	0.02	0.80	
3-Octen-2-one[a]	0.04	0.04	0.02	0.02	0.02	0.02	0.00	
Limonene[b,c]	0.08	0.12	0.05	0.08	0.12	0.04	1.30	
1-Methyl-3-(hydroxyethyl)propane[b]	0.42	0.19	0.22	0.02	0.40	0.03	1.08	
3-Methylbutanol[a]	0.14	0.09	0.03	1.36	0.05	0.10	0.00	
2-Methylbutanol[a,c]	0.69	0.33	0.18	1.59	0.23	0.12	10.26	

Table 2.20 (continued)

Compound	Sample							Ref.
	1	2	3	4	5	6	7	
(*E*)-2-Hexenal[b,c]	31.62	10.85	16.75	0.95	29.17	2.03	1.50	6770 μg/kg (29) 365–4296 μg/kg (22) 121–438.5 μg/g (21) 560–1600 μg/L (30)
n.i. (hydrocarbon)[b]	0.08	0.28	0.07	0.01	0.03	0.01	0.05	
β-Ocimene[a]	0.15	0.05	0.12	0.03	0.09	0.02	0.08	
1-Pentanol[a]	0.01	0.06	0.01	0.13	0.24	0.58	1.18	
1-Acetylcyclohexene[a]	0.12	0.19	0.05	0.07	0.02	0.12	0.68	
Methyl benzoate[a]	0.04	0.03	0.01	0.01	0.01	0.01	0.02	
Styrene[b]	0.04	0.05	0.04	0.04	0.03	0.00	0.19	
Hexyl acetate[a,c]	0.26	0.49	0.04	0.17	0.09	0.03	0.87	
1,2,4-Trimethylbenzene[a]	0.07	0.05	0.04	0.03	0.04	0.03	0.37	
Octanal[b,c]	0.10	0.16	0.05	0.02	0.18	0.05	1.57	99–382 μg/kg(29)
Ethyl hexanoate[a]	0.00	0.00	0.00	0.02	0.00	0.00	0.29	
(*E*)-4,8-Dimethyl-1,3,7-nonatriene[b]	0.13	0.13	0.08	0.08	0.14	0.14	0.09	
(*Z*)-3-Hexenyl acetate[b,c]	0.15	1.32	0.19	0.01	0.06	0.01	0.55	2250 μg/kg(29) 3212–3383 μg/kg (22)
(*E*)-2-Heptenal[a,b]	0.15	0.18	0.02	0.00	0.12	0.00	4.61	
α-Pinene[a]	0.06	0.05	0.00	0.02	0.02	0.02	0.05	
Hexyl formate[a]	0.01	0.00	0.00	0.00	0.00	0.00	0.29	
(*Z*)-2-Pentenol[a]	0.70	0.05	0.03	0.34	0.26	0.27	0.58	
m-Ethyltoluene[a]	0.05	0.04	0.03	0.02	0.03	0.02	0.10	
o-Ethyltoluene[a]	0.02	0.02	0.02	0.01	0.01	0.01	0.06	
1,3,5-Trimethylbenzene[b]	0.02	0.01	0.01	0.01	0.01	0.01	0.08	
2-Octanone[a]	0.02	0.03	0.00	0.01	0.01	0.02	0.00	
6-Methyl-5-hepten-2-one[b]	0.05	0.13	0.05	0.03	0.04	0.05	0.44	

Table 2.20 (continued)

Compound	Sample 1	2	3	4	5	6	7	Ref.
1-Hexanol[b,c]	1.98	1.11	2.39	10.26	0.68	6.05	6.76	10–48.8 μg/g (21) 100–440 μg/L (30)
(*E*)-3-Hexen-1-ol[b,c]	0.09	0.08	0.10	0.08	0.06	0.13	0.16	
(*Z*)-3-Hexen-1-ol[b,c]	0.69	0.87	0.72	0.65	0.46	0.59	0.76	684 μg/kg(29) 662–796 μg/kg (22) 4.7–77.5 μg/g (21) 130–200 μg/L (30)
Nonanal[a,c]	3.74	1.99	1.02	0.93	1.39	0.85	14.98	
2,4-Hexadienal 1[b]	0.35	0.17	0.21	0.02	0.26	0.02	0.05	
2,4-Hexadienal 2[b]	0.45	0.18	0.23	0.03	0.34	0.04	0.10	
(*E*)-2-Hexen-1-ol[b,c]	6.83	2.23	9.27	1.24	2.26	10.40	8.79	26.6–48 μg/g (21) 310–880 μg/L (30)
(*Z*)-2-Hexen-1-ol[b,c]	0.11	0.06	0.14	0.08	0.09	1.12	0.17	
(*E*)-2-Octenal[b]	0.02	0.03	0.02	0.01	0.02	0.01	1.70	
Acetic acid[b]	1.33	1.58	0.26	0.72	0.44	0.07	3.84	
(*E*)-1-Octen-3-ol[b]	0.03	0.04	0.02	0.03	0.02	0.03	0.71	
2,4-Heptadienal 1[b]	0.08	0.17	0.05	0.03	0.03	0.02	0.45	
α-Copaene[b]	0.05	0.04	0.05	0.01	0.00	0.00	0.00	
2,4-Heptadienel 2[b]	0.02	0.04	0.02	0.01	0.01	0.01	0.29	
Methyl nonanoate[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Decanal[a,c]	0.19	0.10	0.06	0.21	0.14	0.10	3.44	
Formic acid[b]	0.15	0.45	0.08	0.33	0.07	0.00	2.65	
(*E*)-2-Nonenal[a,c]	0.45	0.22	0.08	0.07	0.21	0.09	2.98	24–91 μg/kg (29) 10–14 μg/kg (22)
Ethyl nonanoate[a]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	
3,5-Octadien-2-one[a]	0.02	0.09	0.01	0.00	0.00	0.01	0.19	

Table 2.20 (continued)

Compound	Sample 1	2	3	4	5	6	7	Ref.
Propanoic acid[b]	0.17	0.23	0.31	0.67	0.05	0.04	0.72	
1-Octanol[b,c]	0.13	0.22	0.10	0.14	0.10	0.18	1.07	3.6–5.6 μg/g (21)
Isobutyric acid[b]	0.06	0.03	0.02	0.37	0.03	0.01	0.05	
Methyl decanoate[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Butanoic acid[b]	0.06	0.07	0.02	0.05	0.02	0.01	0.17	
(*E*)-2-Decenal	0.01	0.03	0.01	0.01	0.02	0.00	0.16	
(*E,E*)-2,4-Decadienal[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.05	
1-Nonanol[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Pentanoic acid[b]	0.03	0.02	0.01	0.53	0.05	0.01	0.04	
(*E,E*)-α-Farnesene[b]	0.02	0.01	0.04	0.00	0.00	0.00	0.00	
Hexanoic acid[b,c]	0.97	1.17	0.31	4.77	0.78	0.10	20.19	
Benzyl alcohol[b]	0.03	0.02	0.02	0.03	0.02	0.01	0.05	
Phenyl ethyl alcohol[b]	0.05	0.03	0.02	0.07	0.03	0.01	0.10	
Heptanoic acid[b,c]	0.42	0.31	0.00	0.30	0.45	0.10	1.31	
(*E*)-2-Hexanoic acid[b]	0.08	0.04	0.04	0.35	0.04	0.02	0.11	

[a]Determined after separation on an apolar chromatographic column (SPB-1).
[b]Determined after separation on a polar chromatographic column (Supelcowax-10).
[c]Quantitatively determined applying the calculated relative response factor. Where not specified, the response factor was considered to be 1.
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GC-MS. The temperature of FID, ion source and transfer line were 280, 175 and 280°C respectively. Ionisation energy was 70 eV, the mass range varied between 15 and 250 *m/z*. A typical chromatogram illustrating the good separation capacity of the system is shown in Fig. 2.8. The concentrations of the compounds identified in the samples are compiled in Table 2.20. It was established that the method is suitable for the separation and quantitative determination of volatile compounds in olive oil samples (Vichi et al., 2003).

Because of the decisive role of the efficacy of extraction in the results of any chromatographic analyses, the performance of the various extraction techniques used for the analysis of volatiles in olive oils has been many times compared. Thus, HS-SPME, SDE and closed-loop stripping analysis (CLSA) have been simultaneously applied for the GC-MS analysis of the aroma profile of virgin olive oil. The extracts were analysed by GC-MS using a capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Initial oven temperature was 40°C for 3 min, raised to 75°C at 4°C/min, then to 250°C at 8°C/min (final hold 5 min). MS conditions were: temperature of ion source 175°C; transfer line, 280°C; ionisation energy, 70 eV; mass range, 40–300 *m/z*. The chromatograms of the SPME, SDE and CLSA extracts are depicted in Fig. 2.9. The comparison of the aroma profiles indicates that the aroma profile obtained markedly depended on the type of extraction. It was concluded from the data that the selection of optimal extraction technique has to be dependent on the class of volatiles to be investigated (Vichi et al., 2007).

The efficacy of electronic nose, sensory analysis and HS-SPME/GC/MS methods was compared for the analysis of single cultivar extra virgin oils. The similarity and dissimilarity of the aroma profiles were assessed by PCA. HS-SPME was carried out at 30°C for 30 min and then the analytes were separated on a capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Initial oven temperature was 40°C for

Fig. 2.9 (continued) (18), phenol (19), ethylphenol (20), hexanal (21), (*Z*)-3-hexenal (22), (*E*)-2-pentenal (23), heptanal (24), (*E*)-2-hexenal (25), octanal (27), (*E*)-2-heptenal (28), nonanal (29), (*E*,*Z*)- or (*E*,*E*)-2,4-hexadienal (30), (*E*,*Z*)-2,4-heptadienal (31), (*E*,*E*)-2,4-heptadienal (32), decanal (33), benzaldehyde (34), (*E*)-2-nonenal (35), (*E*)-2-decenal (36), undecenal (37), (*E*,*Z*)-2,4-decadienal (38), (*E*,*E*)-2,4-decadienal (39), vinylbenzaldehyde (40), 3-pentanone+pentanal (41), 2-octanone (42), 4-octanone (43), 6-methyl-5-hepten-2-one (44), phenylethanone (45), decane (46), 1,5-octadiene, 3-ethyl (*E* or *Z*) (47), 1,5-octadiene, 3-ethyl (*E* or *Z*) (48), toluene (49), n.i. hydrocarbon (*m/z* 41, 57, 76, 113) (50), 3,7-decadiene (*EE* or *ZZ* or *EZ*) (51), 3,7-decadiene (*EE* or *ZZ* or *EZ*) (52), 3,7-decadiene (*EE* or *ZZ* or *EZ*) (53), undecane (54), ethylbenzene (55), *m*-xylene (56), *p*-xylene (57), *o*-xylene (58), dodecane (59), propylbenzene (60), 3-ethyltoluene (61), n.i. hydrocarbon (*m/z* 55, 69, 97, 126) (62), 1,3,5-trimethylbenzene (63), styrene (64), 2-ethyltoluene (65), 1,2,4-trimethylbenzene (66), tridecane (67), n.i. hydrocarbon (*m/z* 55, 70, 83, 119 (68), (*E*)-4,8-dimethyl-1,3,7-nonatriene (69), butylbenzene (70), 1,2,3-trimethylbenzene (71), unsaturated C4-alkylbenzene (72), biphenyl (73), methyl pentanoate (74), methyl hexanoate (75), hexylacetate (76), (*Z*)-3-hexenylacetate (77), methylbenzoate (78), methylbenzoate (79), α-pinene (80), δ-3-carene (81), limonene (82), *p*-mentha-1,5,8-triene (83), (*E*)-β-ocimene (84), *p*-cymene (85), cyclosativene (86), α-copaene (87), α-cedrene (88), linalool (89), α-bergamotene (90), (*Z*)-β-farnesene (91), β-acoradiene (92), eremophyllene (93), α-zingiberene (94), α-muurolene (95), (*E*,*E*)-α-farnesene (96), oxygenated sesquiterpene (*m/z* 189, 207, 222) (97), pulegone (98), oxygenated sesquiterpene (*m/z* 189, 207, 220) (99), geranylacetone (100), farnesol (101). Reprinted with permission from Vichi et al. (2007)

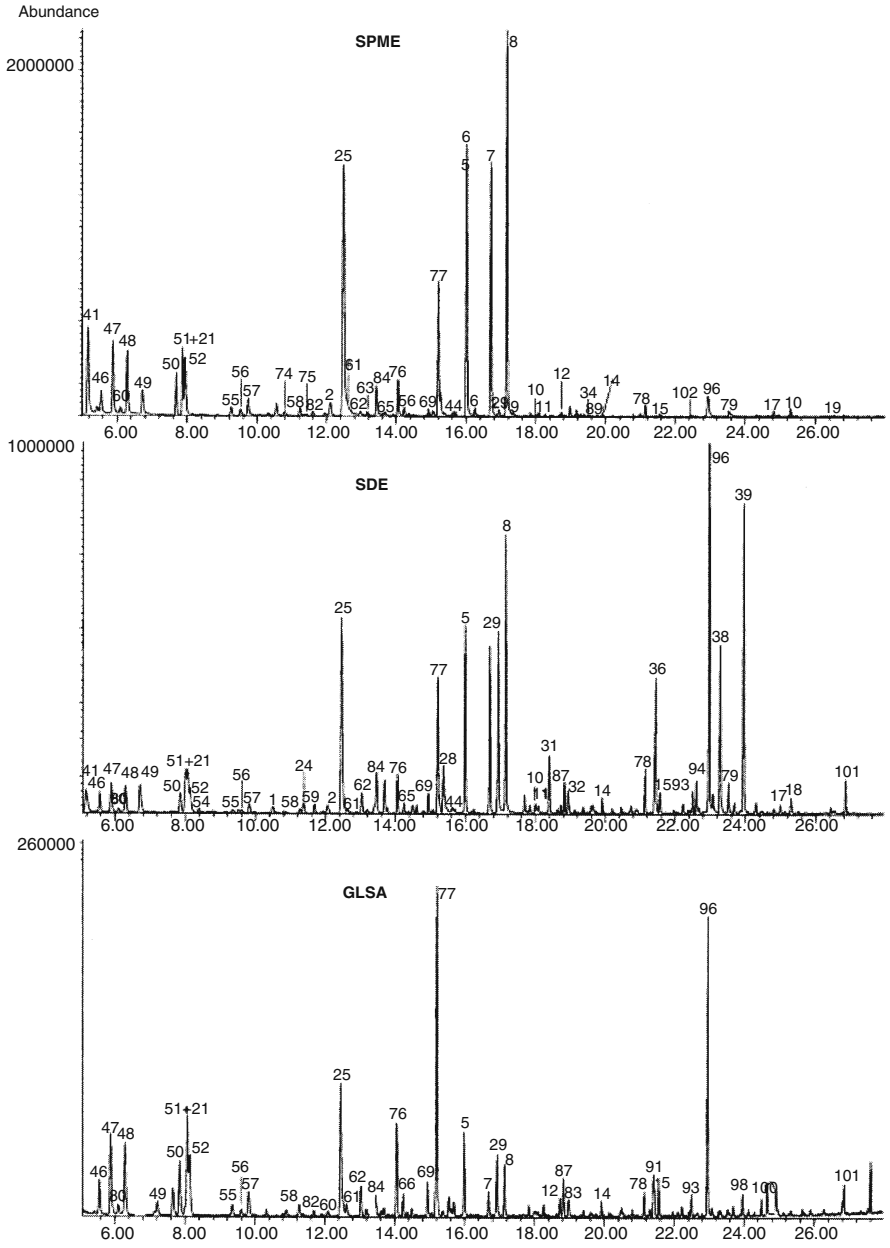

Fig. 2.9 Chromatographic profiles of virgin olive oil volatile fraction obtained by SPME, SDE and CLSA extraction followed by GC/MS analysis. The separation was carried out on a Supelcowax-10 capillary column. *Peak identification:* 1-penten-3-ol (1), isoamyl alcohol (2), (*E*)-2-pentenol (3), (*Z*)-2-pentenol (4), 1-hexanol (5), (*E*)-3-hexenol (6), (*Z*)-3-hexenol (7), (*E*)-2-Hexenol (8), (*Z*)-2-hexenol (9), 1-octen-3-ol (10), 1-heptanol (11), 2-ethyl-1-hexanol (12), (*Z*)-hepten-2-ol (13), 1-octanol (14), 1-nonanol (15), (*Z*)-6- or 4-nonenol (16), benzenemethanol (17), benzeneethanol

5 min, raised to 280°C at 8°C/min (final hold 5 min). The temperature of ion source was 230°C; mass range was set to 30–350 *m/z*. It was found that the information content of the methods is different and, therefore, their simultaneous application for the classification of extra virgin olive oils is advocated (Cimato et al., 2006).

SPME followed by GC-MS has also found application in the analysis of the volatile compounds of five new cultivars of virgin olive oils. The measurements illustrated that the main components of the volatile fractions were (*E*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, tricosane and β-selinene. As the aroma profiles of the samples showed considerable differences, the procedure was proposed for the differentiation of various olive oil cultivars (Baccouri et al., 2007).

HPLC (tocopherol analysis), GC (steroid analysis) and GC-MS (determination of aroma profiles) were simultaneously employed for the study of the influence of ultrasound bleaching of the composition of olive oils. SPME was performed at 40°C for 30 min. GC separations were carried out in a capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Initial oven temperature was 40°C, raised to 140°C at 3°C/min, then to 220°C at 10°C (final hold 220°C). The peak areas of identified volatile compounds found in treated and untreated samples are compiled in Table 2.21. It was established that the SPME/GC/MS method is sensitive and suitable for the detection of the changes in the oxidative state of olive oils (Jahouac-Rabai et al., 2008).

The volatiles of the pumpkin seed oil have also been analysed by SPME/GC/MS. SPME was performed at 50°C for 10 min. GC separations were carried out in a capillary column (30 m × 0.25 mm, film thickness, 1 μm). Initial oven temperature was –30°C for 1 min, raised to 250°C at 10°C/min (final hold 5 min). Injector and detector temperatures were 280°C. Mass range was 20–250 *m/z*. The volatiles identified in crushed and roasted pumpkin seeds are compiled in Table 2.22. It was established that alkylated pyrazines and 2-acetylpyrrole are responsive for the aroma of roasted pumpkin seeds (Siegmund and Murkovic, 2004).

2.5 Meat and Meat Products

The effect of volatile and non-volatile components on the flavour characteristics of various meats and meat products has been extensively investigated. Thus, the influence of dietary manipulation on grilled (Elmore et al., 2005) and cooked lamb (Elmore et al., 2000a) was elucidated. Similar to other food and food products, the majority of measurements were performed by GC-MS (Elmore et al., 2000b) or by GC-O (Machiels et al., 2004). GC-MS was employed for the investigation of the volatile compounds produced by spoilage bacteria (Jorgensen et al., 2001), SPME for the analysis of simulated beef flavour (Moon and Li-Chan, 2004), GC-MS, GC-O and SPME for the study of the composition of the volatile compounds in simulated beef flavour (Moon and Li-Chan, 2004; Moon et al., 2006). Inverse GC has also been applied for the investigation of the binding of flavour compounds to soy protein isolate (Zhou and Cadwallader, 2004).

Table 2.21 Peak area of identified volatile compounds[a] in treated and untreated olive oils

Numbers	Volatile compounds	Odour description	A (crude)	A_0 (%)[b,c]	A_I[c]	A_{II}[c]	A_{III}[c]	A_{IV}[c]
			Bleaching time (min)					
			–	13	20	20	30	45
			Temperature (°C)					
			–	30	40	70	40	40
1	Butanal		4.27	–	–	–	3.2	4.22
2	Toluene		7.16	4.42	1.75	2.62	0.89	0.80
3	Hexanal	Fatty, pungent, grassy	54.89	29.19	34.87	34.94	23.57	29.16
4	1,3-Octadiene		–	1.36	–	2.20	–	1.89
5	Styrene		1.17	1.57	–	0.93	0.73	0.41
6	1-Nonene		–	2.86	3.99	3.85	2.59	2.65
7	Heptanal		1.14	3.89	6.04	5.44	4.27	3.2
8	1,3-Nonadiene		–	0.79	1.83	1.40	–	0.64
9	2(*Z*)-Heptenal	Fishy, sweet	5.45	5.89	5.43	4.49	8.65	5.50
10	6-Methyl-5-hepten-2-one		1.57	2.41	–	2.81	1.93	2.52
11	1-Decene		–	3.48	5.99	3.77	4.16	4.18
12	Octanal	Citruslike	2.56	4.98	9.67	5.80	6.12	4.73
13	*d*-Limonène	Fresh, sweet	1.97	1.75	2.05	1.74	1.24	1.42
14	1,3-Hexadiene		0.50	–	–	0.59	1.23	0.79
15	1,3,7-Octatriene		–	0.39	–	0.32	–	–
16	2(*E*)-Octenal	Fatty-nutty	1.55	3.48	2.74	1.44	2.51	2.04
17	1-Undecene		–	1.64	2.98	1.61	2.59	1.95
18	Undecane		0.33	0.41	–	0.66	0.43	0.36
19	Nonanal	Fatty, waxy, citrus	3.99	7.85	9.58	9.94	15.67	13.67
20	5-Undecene		–	–	–	0.15	0.18	0.14

Table 2.21 (continued)

Numbers	Volatile compounds	Odour description	A (crude)	A_0 (%)[b,c]	A_I[c]	A_{II}[c]	A_{III}[c]	A_{IV}[c]
21	Ethyl cyclohexane carboxylate		0.64	0.75	–	0.36	0.33	0.36
22	2(*E*)-Nonenal	Cucumber-tallowy	0.47	1.30	2.70	3.09	4.57	4.41
23	4-Ethylphenol		10.63	17.42	6.58	8.09	9.06	9.51
24	1-Dodecene		–	1.12	–	0.87	1.09	1.19
25	Decanal		–	–	–	–	2.32	1.86
26	4-Ethy-l,2-methoxyphenol		1.65	2.81	2.26	1.59	1.63	1.69
27	1-Tridecene		–	0.17	–	0.42	0.75	0.58
28	2(*E*),4(*E*)-Decadienal	Fatty, deep, fried, citrus	–	–	0.54	0.25	0.28	–
29	*n*-Pentadecane		–	–	–	0.17	–	–
30	*n*-Hexadecane		–	–	0.61	0.21	–	–
31	*n*-Heptadecane		–	–	0.30	0.11	–	–
	Total peak area by SPME (E + 9)		1.76	1.31	3.00	2.60	1.95	2.05

[a] Data are means of triplicates.
[b] A_i (0, I, II, III, IV) bleached olive oils with ultrasound.
[c] Percentage of total peak area (%).
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Table 2.22 Compounds identified in the headspace of crushed and roasted pumpkin seeds using HS-SPME for sample preparation and GC–MS for the identification and determination of the relative concentrations

			Relative concentration (TCP equiv.×100)[e]						
			Roasting time (min)						
Compound	m/z[e]	RI(HP5)	0	10	20	30	40	50	60
Aldehydes									
2-Methylpropanal[a,b]	72	552[f,g]	7.8	7.7	3.7	5.3	8.4	25.0	38
2-Butenal [a,b]	70	645	12	116	4.0	2.9	2.2	3.0	4.3
3-Methylbutanal[a,b]	58	648	26	20	12	17	33	81	214
2-Methylbutanal	57	658	28	25	15	19	31	86	253
Pentanal	44	697	11	14	15	21	24	34	71
Hexanal	56	800	49	86	103	123	101	122	221
2-Hexanal (*E*)	83	850	0.7	2.3	–	–	–	–	–
2-Heptenal (*E*)	83	954	–	0.4	0.6	1.6	6.5	12	46
Nonanal	57	1105	2.4	3.4	4.3	5.8	6.3	12	18
Benzaldehyde	106	958	26	200	55	78	83	108	237
Phenylacetaldehyde	91	1043	7.4	12	11	27	53	123	537
Ketones									
2,3-Butandione	86	586	7.5	3.5	2.8	3.0	122	5.6	8.0
2-Butanone	43	597	35	21	15	18	20	37	43
2-Pentanone	57	685	6.0	3.8	4.9	7.5	5.1	0.4	0.6
2-Heptanone	58	890	3.9	5.9	5.3	5.2	8.3	29	42
Alcohols									
1-Penten-3-ol (*E*)	57	678	49	29	17	11	3.6	1.6	2.3
3-Methyl-1-butanol	55	730	68	170	62	39	8.3	2.4	3.4
2-Methyl-1-butanol	57	733	28	56	22	14	2.5	0.6	0.9
1-Pentanol	42	763	34	35	24	26	18	24	37
1-Hexanol	56	867	160	154	108	111	40	20	29

Table 2.22 (continued)

Compound	m/z[e]	RI(HP5)	Relative concentration (TCP equiv. ×100)[e]						
			Roasting time (min)						
			0	10	20	30	40	50	60
Phenylmethanol	108	1041	18	65	25	31	31	27	38
Phenylethanol	122	1113	6.6	41	19	24	24	23	34
Furan derivatives									
2-Methylfuran	82	600	18	59	8.7	3.2	2.0	1.7	2.4
2-Pentylfuran	81	991	–	7.2	9.0	13	17	30	76
2-Furancarboxaldehyde	96	830	3.1	1.7	2.5	3.1	19	25	36
2-Furanmethanol	98	852	–	–	–	–	–	5.9	8.5
Sulphur compounds									
Dimethylsulphide	62	<500	16	2.1	1.3	2.2	6.3	14	6.4
3-(Methylthio)-propanal	104	905	–	–	–	0.7	3.3	11	21
N-heterocyclic compounds									
2-Methylpyrazine	94	825	–	–	–	–	3.7	9.5	28
2,5-Dimethylpyrazine	108	914	5.1	3.9	3.1	5.9	12	38	245
2-Ethylpyrazine[t]	107	915	0.7	0.6	0.4	0.7	2.2	9.7	14

Table 2.22 (continued)

			Relative concentration (TCP equiv. × 100)[e]						
			Roasting time (min)						
Compound	m/z[e]	RI(HP5)	0	10	20	30	40	50	60
2-Ethyl-5(6)-methyl-pyrazine	121	997	1.6	1.4	1.5	2.2	3.6	8.5	117
2-Acetylpyrrole	109	1060	1.0	0.9	0.7	1.0	4.4	11	16
2-Ethyl-3,6-dimethyl-pyrazine	135	1079	0.9	0.8	0.9	1.2	1.7	4.4	39

[a]Retention indices (RI) were compared to those from reference compounds respectively with data from our retention index database.
[b]The compounds were identified by comparison of the measured mass spectra with mass spectra obtained from reference compounds if available as well as by comparison of the mass spectra from a mass spectra library.
[c]Retention indices are compared with data obtained from literature.
[d]The compounds are tentatively identified. Identification is only based on the comparison of the mass spectra with mass spectra from a mass spectra library.
[e]Integration of the peak area was performed by using characteristic ions (m/z) for the respective compounds to avoid possible interference by other compounds. As no response ratios between the different ions were taken into account, the concentrations are given in terms of equivalents to the internal standard (TCP-equiv.; concentration of the IS 2.32 mg/kg pumpkin seeds).
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Table 2.23 Mean concentrations of volatile aromas of cooked longissimus muscle from different breeds in headspace by SPME

Compound [*m/z* (relative intensity)]	Mean concentration in headspace (ng/100 g)[A]						SEM	P^B	LRI[C]	Method of identification[D]
	DLW	LW	TC	HB	LT	RC				
Alkanes	313	1707	590	2472	723	1398				
Heptane	24	66	5	37	11	25	22	NS	700	ms + lri
4-Methylheptane	0	86bc	29c	243a	47bc	128b	28	***	761	ms
Toluene	41	160	103	160	100	57	41	NS	771	ms + lri
Octane	85	409	119	101	101	127	86	NS	800	ms + lri
2,4-Dimethylheptane	0	0	0	37	0	138	58	NS	810	ms
1,3-Octadiene	0	9	5	13	0	4	5	NS	828	ms
2,4-Dimethyl-1-heptene	4c	324bc	176bc	901a	263bc	571ab	120	**	841	ms
2,3-Dimethylheptane	0	11bc	0	44a	5bc	26ab	7	**	856	ms
4-Methyloctane	0	156b	46b	330a	85b	94b	46	**	864	ms
1,3-Dimethylbenzene	13	37	0	32	15	10	9	NS	876	ms + lri
Styrene	0	20	0	9	0	3	8	NS	898	ms + lri
Nonane	9	22	5	3	0	6	7	NS	900	ms + lri
Decane	9	114	0	30	26	0	25	NS	1000	ms + lri
2,6-Dimethylnonane	0	172b	64b	521a	67b	206b	62	**	1016	se
Undecane	17	13	3	0	0	3	4	NS	1100	ms + lri
Dodecane	23ab	29a	8bc	11 abc	0	0	6	*	1200	ms + lri
Tridecane	22	16	3	0	0	0	6	NS	1300	ms + lri
Pentadecane	66	63	24	0	3	0	18	NS	1500	ms + lri
Terpenoid	51	197	105	286	45	75				
Limonene	51b	197ab	105b	286a	45b	75b	50	*	1034	ms + lri
Aldehydes	2410	4244	2906	1920	1750	1468				
2-Methylpropanal	0	0	33	10	0	44	21	NS	533	ms + lri
3-Methylbutanal	0	0	5	14	29	0	9	NS	656	ms + lri
2-Methylbutanal	0	0	0	13	10	8	6	NS	665	ms + lri
Pentanal	20	84	49	9	8	0	19	NS	709	ms + lri

Table 2.23 (continued)

Compound [*m/z* (relative intensity)]	Mean concentration in headspace (ng/100 g)[A]						SEM	P^B	LRI[C]	Method of identification[D]
	DLW	LW	TC	HB	LT	RC				
(*E*)-2-Pentenal	0	0	0	5	0	0	2	NS	774	ms + lri
3-Methyl-2-butenal	0	0	2	2	8	9	4	NS	791	ms + lri
Hexanal	518	1111	1229	537	417	497	248	NS	804	ms + lri
(*E*)-2-Hexenal	4	0	0	0	0	0	2	NS	860	ms + lri
Heptanal	90	217	107	19	50	78	54	NS	900	ms + lri
3-(Pthio)propanal	7	12	8	7	0	3	6	NS	914	ms
(*E*)-2-Heptenal	55	27	22	7	6	12	12	NS	962	ms + lri
Benzaldehyde	707	1302	839	1022	808	380	350	NS	971	ms + lri
Octanal	184	345	137	64	113	114	96	NS	1006	ms + lri
Benzeneacetaldehyde	31	30	20	21	19	50	19	NS	1054	ms + lri
(*E*)-2-Octenal	61	47	25	6	8	0	16	NS	1062	ms + lri
Nonanal	409	809	341	147	220	203	168	NS	1100	ms + lri
(*E*)-2-Nonenal	31	32	4	0	0	9	11	NS	1163	ms + lri
Decanal	75	81	39	37	46	23	16	NS	1209	ms + lri
(*E*)-2-Decenal	5	63	10	0	0	22	24	NS	1265	ms + lri
(*E,E*)-2,4-Decadienal	15	20	0	0	0	16	12	NS	1319	ms + lri
2-Undecenal	29	30	0	0	0	0	17	NS	1367	ms + lri
Dodecanal	12	0	0	0	0	0	3	NS	1400	ms + lri
Hexadecanal	157	34	36	0	8	0	51	NS	1825	ms + lri
Alcohols	237	1214	346	716	231	393				
1-Pentanol	40	41	58	0	32	39	21	NS	773	ms + lri
1-Hexanol	0	18	10	11	14	5	5	NS	876	ms + lri
1-Heptanol	29ab	40a	20b	0	3c	0	5	***	971	ms + lri
2-Ethyl-1-hexanol	80c	1001a	167c	504b	145c	342bc	81	***	1032	Ms
1-Octanol	58a	58a	31ab	0	0	7b	15	*	1071	ms + lri
(*E*)-2-Octen-1-ol	11	2	0	0	2	0	4	NS	1071	ms + lri
Butylated hydroxytoluene	19b	54b	60b	201a	35b	0	16	***	1510	ms

Table 2.23 (continued)

Compound [*m/z* (relative intensity)]	Mean concentration in headspace (ng/100 g)[A]						SEM	*P*[B]	LRI[C]	Method of identification[D]
	DLW	LW	TC	HB	LT	RC				
Ketones	372	709	685	885	973	583				
2-Butanone	8c	122bc	106bc	209ab	322a	108bc	50	*	602	ms + lri
2,3-Pentanedione	82	67	81	84	56	50	36	NS	703	ms + lri
2-Pentanone	0	0	0	13	0	4	6	NS	704	ms + lri
2-Methyl-3-pentanone	0	0	0	0	0	4	2	NS	705	ms
1-Hydroxy-2-propanone	71b	124b	195b	214b	417a	217b	47	**	714	ms + lri
3-Hydroxy-2-butanone	0	65	73	84	27	71	25	NS	742	ms + lri
2,4-Pentanedione	0	0	0	0	13a	0	3	*	788	ms
2-Methyl-2-hepten-4-one	0	0	0	95	0	0	20	*	830	ms
1-Acetyloxy-2-propanone	20	35	63	24	51	42	18	NS	879	ms
2-Cyclopentene-1,4-dione	0	0	0	18	6	12	8	NS	890	ms
2-Heptanone	20	78	19	35	19	33	16	NS	891	ms + lri
2,3-Octanedione	4	51	65	0	0	0	18	NS	983	ms + lri
1-Octen-3-ol	123	112	69	46	30	42	27	NS	983	ms + lri
6-Methyl-5-hepten-2-one	0	0	9	0	21	0	7	NS	989	ms
3-Octanone	7	6	0	7	0	0	5	NS	990	ms + lri
Acetophenone	9	0	5	0	0	0	4	NS	1072	ms + lri
2-Nonanone	4	20	0	0	0	0	6	NS	1092	ms + lri
2-Decanone	0	25	0	0	0	0	10	NS	1194	ms + lri
2,5-Cyclohexadiene-1,4-dione	4b	4b	0	56a	11b	0	7	**	1470	ms
2-Pentadecanone	20a	0	0	0	0	0	2b	***	1702	ms + lri
Furans	340	706	225	288	388	495				
2-Methylfuran	0	17	0	104	0	0	43	NS	602	ms + lri
2-Ethylfuran	6	0	0	0	0	0	2	NS	702	ms + lri

Table 2.23 (continued)

Compound [*m/z* (relative intensity)]	Mean concentration in headspace (ng/100 g)[A]						SEM	*P*[B]	LRI[C]	Method of identification[D]
	DLW	LW	TC	HB	LT	RC				
Dihydro-2-methyl-3(2*H*)-furanone	5c	12bc	28bc	39abc	70a	42ab	11	*	812	ms + lri
2-Methoxymethylfuran	0	0	14c	0	43b	69a	5	***	835	ms
Furfural	54	126	70	51	82	86	37	NS	837	ms + lri
2-Furanmethanol	13	110	34	38	95	218	53	NS	867	ms + lri
1-(2-Furanyl)ethanone	0	0	0	0	12	33	8	NS	915	ms
2-Pentylfuran	251	441	79	56	86	47	134	NS	993	ms + lri
2-Heptylfuran	11	0	0	0	0	0	3	NS	1194	ms + lri
Nitrogen-containing	49	314	126	297	141	145				
Pyrazine	0	25	13	17	12	24	9	NS	739	ms + lri
Methylpyrazine	30	177	46	111	85	63	58	NS	829	ms + lri
2,5 and 2,6-Dimethylpyrazine	11	112	50	67	25	21	49	NS	917	ms + lri
Ethylpyrazine	0	0	9	7	5	11	4	NS	921	ms + lri
2-Ethyl-6-methylpyrazine	8	0	3	20	6	26	9	NS	1002	ms + lri
2-Butylpyrroline 83, 55 (33), 69 (24), 41 (13), 168 (9), 111 (7) 125 (4), 97 (4), 29 (4)	0	0	5b	75a	8b	0	6	***	1068	se
Sulphur-containing	44	152	30	277	19	49				
Dimethyl disulphide	0	0	0	11	0	0	4	NS	740	ms + lri
2-Methylthiophene	5	52	0	88	0	26	33	NS	775	ms + lri
2-Furanmethanethiol	2	0	0	0	0	0	1	NS	914	ms
Dimethyl trisulphide	0	10bc	23b	46a	19b	19b	5	**	982	ms + lri
2-Thiophenecarboxaldehyde	0	70	0	128	0	0	42	NS	1011	ms + lri
2-Acetylthiazole	32	0	0	0	0	0	3	***	1024	ms

Table 2.23 (continued)

Compound [*m/z* (relative intensity)]	Mean concentration in headspace (ng/100 g)[A]						SEM	P^{B}	LRI[C]	Method of identification[D]
	DLW	LW	TC	HB	LT	RC				
3-Methyl-2-thiophenecarboxaldehyde	5	20	7	4	0	4	5	NS	1089	ms
Unknown										
Unknown A 116, 46 (62), 74 (30), 41 (24), 42 (22), 39 (14), 88 (7), 59 (6)	9	24	18	49	29	31	12	NS	988	
Unknown B 43, 56 (90), 70(69), 85(56), 111(20), 29(16), 154(9), 127(7), 99(6)	0	0	0	84	74	0	48	NS	1024	
Unknown C 69, 43(78), 57(58), 83(55), 111(44), 97(16), 125(15), 29(15), 154(7)	62b	93b	0	337a	32b	49b	52	**	1037	
Unknown D 69, 43(83), 57(62), 85(59), 111(50), 29(16), 97(15), 154(10), 125(7)	10	184	24	238	17	39	108	NS	1317	

[A] Data are means of six replicates; values in the same row with different letters were significantly different ($P < 0.05$ or $P < 0.01$). DLW, Duroc × Landrace × Large White; LW, Laiwu breed; TC, Tongcheng breed; HB, Dahuabai breed; LT, Lantang breed; RC, Rongchang breed.

[B] NS, not significantly different ($P > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level.

[C] Linear retention index on a HP-5 low bleed/MS column.

[D] ms + lri, mass spectrum identified using NIST/EPA/NIH Mass Spectral Database and LRI agrees with literature value; ms, mass spectrum agrees with spectrum in NIST/EPA/NIH Mass Spectral Database; se, tentative identification from structure elucidation of mass spectra.

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Various analytical technologies were applied for the study of the flavour differences of cooked longissimus muscle from Chinese indigenous pig breeds and hybrid pig breed (Duroc × Landrace × Large White). Neutral lipids and phospholipids were methylated and analysed by GC. Amino acids were determined by an automatic amino acid analyser. Volatile aroma compounds were preconcentrated by SPMA and separated and quantitated by GC-MS. Measurements were carried out in a capillary column (60 mm × 0.25 mm i.d., film thickness, 0.25 μm). Starting oven temperature was 40°C for 2 min, then raised to 280°C at 4°C/min. The results indicated that 23 volatile compounds were significantly influenced by breed, and the other characteristics of the cooked muscle also differs according to the breed as demonstrated in the data of Table 2.23 (Lu et al., 2008).

Not only the composition of meats and meat products but also that of soy protein isolates (SPI) with simulated beef flavour (SBF) was investigated. The characteristics of the analytes were measured by GC and GC-O. SPME was performed for 60 min at 60°C. The selected indicator peaks for beefy attribute in simulated beef flavour are compiled in Table 2.24. It was proposed that the method can be employed

Table 2.24 The 15 selected indicator peaks for beefy attribute in simulated beef flavour

Indicator peak	RT	Peak identification (ID No.)[a]	Detection frequency[b] (%)	Pearson correlation coefficient for beefy attribute
IP1	1.717	3-Methyl furan (P1)	50	0.710*
IP2	7.832	2-Acetyl furan (P7)	75	0.658*
IP3	12.046	delta-3-Carene (P17)	88	0.872***
IP4	15.772	2-Ethyl-3,6-dimethyl pyrazine (P24)	88	0.601*
IP5	16.133	Unknown	100	0.852***
IP6	16.864	Unknown	100	0.827**
IP7	18.199	Unknown	75	0.605*
IP8	19.744	2,3-Diethyl-5-methylpyrazine (P30)	50	0.590*
IP9	22.062	Decanal (P35)	75	0.715**
IP10	24.103	2-Isoamyl-6-methylpyrazine (P38)	50	0.599*
IP11	25.374	Unknown	75	0.598*
IP12	25.703	Unknown	75	0.794**
IP13	28.204	delta-Elemene (P44)	50	0.931***
IP14	30.291	beta-Cubebene (P49)	63	0.850***
IP15	35.680	Calamenene (P71)	75	0.883***

*, **, and ***, significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

[a] Peak numbers (*P#*) and tentative identification by GC–MS analysis as reported previously.

[b] Frequency of detection by eight panelists at a sniffing port in GC–O as reported previously.

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for the further research in the study of SPI-SBF interactions (Moon and Li-Chan, 2007a).

The effect of various ingredients (glucosamine, sucrose, ascorbic acid and/or ployethylene glycol) on the binding of SBF to SPI has also been studied in detail. Differential scanning calorimetry (DSC), FS-SPME coupled with GC, sulphydryl and disulphide content, surface hydrophobicity, FR-Raman spectroscopy and sensory evaluation were applied for the elucidation of the character of SBF–SPI interaction. The GC results obtained after incubation at 23°C or 60°C under the adsorption condition at 60°C are compiled in Table 2.25. It was suggested that the results can be applied for the elucidation of the optimal composition of SBF in soy-based products (Moon and Li-Chan, 2007b).

The effect of the addition of fatty acids to minced pork was investigated in detail using GCO-MS. It was established that the addition of omega-3-fatty acids results in fish-like odour, while the addition of fatty acid C18:$2_{(9,12)}$ caused an oily odour (Schäfer and Aaslyng, 2006).

The influence of brined onion extract on the sensory quality of refrigerated turkey breast rolls has also been investigated. The experiments were motivated by the fact that onion (*Allium* cepa L) contains a considerable amount of the flavonol quercetin, which can reduce cancer risk (Yang et al., 2001; Neuhouser, 2004). The HPLC determination of quercetin in onion juice and meat was performed on an ODS column (100 mm × 3 mm i.d.). Mobile phase consisted of 50% methanol in 0.5% aqueous orthophosphoric acid, the flow rate was 0.4 ml/min. The measurements indicated that the addition of onion juice exerts a positive effect on the quality of turkey breast roles (Tang and Cronin, 2007).

The composition of aroma compounds of the shellfish sea fig (*Microcosmus sulcatus*) was also elucidated by GC-O and GC-MS. The measurements indicated that trimethylamine is the key compound of sea fig aroma (Senger-Emonnot et al., 2006).

The compounds responsible for the off-flavour in farm-raised channel catfish was also separated and quantitatively determined by GC-MS. For the qualitative and quantitative analyses of geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB = 1-*R*-exo-1,2,7, 7-tetramethyl bicyclo-[2-2-1]-heptan-2-ol), two different temperature programs were applied. Qualitative analysis was performed by the following program: initial column temperature 40°C for 3 min, raised to 200°C at 5°C, then to 250°C at 50°C. Temperature program for quantitative analysis started at 80°C (1 min hold), raised to 100°C at 20°C/min, to 152°C at 7.5°C/min, to 250°C at 65°C/min. A typical chromatogram is depicted in Fig. 2.10. It was established that the results achieved by a sensory and instrumental analysis were highly similar (Grimm et al., 2004).

The influence of soybean and linseed oil on the chemical and sensory characteristics of fillets of freshwater fish tench (*Tinca tinca* L) was investigated using traditional wet methods (moisture, protein, lipid and ash).

The separation of fatty acid methylesters and volatile compounds were performed by GC. Volatile compounds were analysed on a capillary column (30 m × 0.25 mm,

Table 2.25 Effect of added ingredient in SBF–SPI mixtures[a] incubated at room temperature (RT, 23°C) or 60°C on the peak areas[b] of volatile compounds captured by HS-SPME under the adsorption condition at RT

IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation–RT adsorption	Peak characteristics	IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation –RT adsorption	Peak characteristics	IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation –RT adsorption	Peak characteristics	IP#[c]
Relative % area																		
		Area	%	SPIF	GF	SF	PF	AF	APF	SPIF	GF	SF	PF	AF	APF	Peak identification	DF[e] (%)	Beefy[f]
IP1	1.734	6953	100	106	91	113	94	**163**	**158**	80	83	85	78	**131**	**146**	3-Methylfuran	50	0.710*
IP2	7.893	5537	100	122	83	141	89	83	74	77	62	75	65	59	63	2-Acetylfuran	75	0.658*
IP3	12.132	3360	100	66	58	67	54	62	49	20	31	32	17	**43**	**45**	delta-3-Carene	88	0.872**
	13.271	1929	100	24	0	30	42	0	0	18	0	0	0	0	20	Limonene		0.713**
	13.915	1352	100	33	34	39	31	**240**	**174**	0	**82**	25	0	**133**	**148**	Unknown		0.648*
	15.014	6302	100	84	63	62	27	89	73	67	73	68	67	60	67	Unknown		0.773**
IP4	15.877	1761	100	54	51	88	80	87	47	64	50	53	52	48	51	2-Ethyl-3,6-dimethylpyrazine	88	0.601*
IP5	16.247	2543	100	49	23	68	75	82	49	45	35	36	32	43	49	Unknown	100	0.852**
IP6	16.953	1935	100	20	0	49	39	38	0	20	18	0	0	18	18	Unknown	100	0.827**
IP8	19.917	2881	100	0	23	35	28	62	0	46	0	53	0	0	37	2,3-Diethyl-5-Methylpyrazine	50	0.590*
	20.608	3328	100	47	44	61	59	**99**	**81**	49	46	45	48	**75**	71	4-Terpeneol		0.678*
IP9	22.151	3951	100	57	52	59	58	77	64	54	55	50	47	64	**75**	Decanal	75	0.715**
IP10	24.232	468	100	0	0	0	0	0	0	0	0	0	0	0	0	2-Isoamyl-6-Methylpyrazine	50	0.599*
IP12	25.794	8033	100	41	47	46	**51**	**50**	40	38	42	45	50	**71**	43	Unknown	75	0.794**
	26.319	3792	100	19	18	40	41	38	34	0	0	17	0	16	19	Unknown		0.684*
	26.954	2156	100	0	19	21	**99**	**63**	19	24	24	0	0	0	43	Unknown		0.752**
	27.841	3710	100	16	35	38	56	22	95	40	22	35	21	0	0	Unknown		0.887***
IP13	28.288	19545	100	48	36	55	55	62	42	50	42	47	42	46	46	delta-Elemene	50	0.931***
	28.830	8259	100	44	41	53	61	35	28	43	41	47	50	25	26	Unknown		0.678*
	29.122	7556	100	131	130	130	128	158	109	116	149	117	151	115	123	Unknown		0.713**
	29.971	25200	100	46	39	52	53	62	54	51	45	49	44	50	53	Unknown		0.928***
	30.220	1260	100	0	0	0	0	0	0	0	0	0	0	0	0	Unknown		0.617*
IP14	30.377	3031	100	16	14	49	**50**	**62**	**60**	40	17	15	19	36	36	beta-Cubebene	63	0.850***
	30.615	1639	100	0	0	0	0	0	0	0	0	0	0	0	0	Unknown		0.645*

Table 2.25 (continued)

IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation–RT adsorption	Peak characteristics	IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation –RT adsorption	Peak characteristics	IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation –RT adsorption	Peak characteristics	IP#[c]
Relative % area																		
		Area	%	SPIF	GF	SF	PF	AF	APF	SPIF	GF	SF	PF	AF	APF	Peak identification	DF[e] (%)	Beefy[f]
	30.729	9478	100	34	29	42	39	46	42	40	37	40	35	37	43	Unknown		0.929***
	30.950	721	100	0	0	0	0	0	0	0	0	0	0	0	0	Unknown		0.868***
	31.271	1849	100	88	0	106	102	0	0	34	0	46	41	0	0	Unknown		0.638*
	31.317	11730	100	54	71	60	66	**158**	**131**	69	77	67	57	94	**104**	Unknown		0.914***
	31.530	4843	100	44	50	39	56	98	76	50	74	72	82	74	73	Unknown		0.750**
	31.881	262026	100	50	43	57	57	55	50	55	47	54	49	48	53	Unknown		0.924***
	32.231	7117	100	41	25	43	44	53	47	42	38	40	33	54	48	Unknown		0.879***
	32.679	4244	100	42	29	48	44	30	30	42	39	40	36	40	48	Unknown		0.863***
	33.166	6537	100	43	38	44	45	56	53	51	45	45	38	56	57	Unknown		0.826**
	33.306	24572	100	50	45	57	57	55	51	54	48	53	49	48	52	Unknown		0.894***
	33.620	4026	100	9	13	12	25	25	14	29	9	11	0	24	34	Unknown		0.779**
	33.813	4478	100	0	0	0	0	0	0	0	0	0	0	0	0	Unknown		0.689*
	34.211	7156	100	31	17	31	49	26	30	32	26	33	37	15	24	Unknown		0.775**
	34.346	11082	100	30	26	31	35	31	28	36	32	35	32	22	26	Unknown		0.787**
	34.481	2104	100	0	0	0	0	0	0	0	0	62	47	0	0	Unknown		0.600*
	34.691	9569	100	40	34	44	46	45	43	43	38	29	26	39	41	Unknown		0.881***
	34.871	4778	100	33	9	11	10	11	9	20	18	18	19	8	0	Unknown		0.781**
	35.097	9752	100	27	35	43	45	40	38	38	36	38	36	38	42	Unknown		0.872***
	35.365	15997	100	34	31	34	37	37	36	36	33	33	31	36	40	Unknown		0.823**
IP15	35.759	16917	100	39	35	41	38	47	39	38	37	39	32	41	41	Calamenene	75	0.883***
	35.887	20361	100	39	42	40	**404**	55	**407**	43	48	40	140	46	**379**	Unknown		0.685*
	36.322	49502	100	36	33	40	40	46	43	38	35	37	34	41	43	Unknown		0.822**
	36.646	11216	100	37	26	40	40	30	28	41	38	41	37	29	32	Unknown		0.780**

Table 2.25 (continued)

IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation–RT adsorption	Peak characteristics	IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation –RT adsorption	Peak characteristics	IP#[c]	Retention time[d]	SBF	RT incubation–RT adsorption	60°C incubation –RT adsorption	Peak characteristics	IP#[c]
Relative % area																		
		Area	%	SPIF	GF	SF	PF	AF	APF	SPIF	GF	SF	PF	AF	APF	Peak identification	DF[e] (%)	Beefy[f]
	37.033	3176	100	0	61	26	54	64	54	59	27	69	23	41	43	Unknown		0.800**
	37.347	12581	100	32	33	38	40	38	30	38	33	39	37	27	31	Unknown		0.778**
	37.674	2119	100	0	0	0	0	0	0	0	0	0	0	0	0	Unknown		0.815**
	38.001	12714	100	45	39	47	47	31	26	38	31	51	53	35	37	Unknown		0.746**
	38.425	6531	100	44	36	43	48	42	41	40	43	46	42	37	33	Unknown		0.634*
	38.658	13153	100	40	36	43	52	39	38	39	36	44	44	34	30	Unknown		0.767**

[a] SPIF: SBF in the presence of SPI; GF, SF, PF, AF, and APF: SBF with SPI containing glucosamine, sucrose, polyethylene glycol, ascorbic acid, and ascorbic acid with polyethylene glycol, respectively.

[b] Peak areas are the average values from three replicate GC analyses. Relative % area of SBF–SPI mixtures are based on the peak area for SBF alone, designated as 100%. Bold numbers indicate significant (P 0.05) difference by Fisher's LSD test in GC peak area of SBF–SPI mixtures with added ingredient compared to SBF with only SPI (SPIF).

[c] Indicator peak number described in the previous study.

[d] Retention time in minutes on GC chromatogram in the present study.

[e] Detection frequency (%) of eight panelists who perceived the aroma compound in GC–olfactometry.

[f] Pearson correlation coefficient for beefy notes in descriptive sensory analysis.

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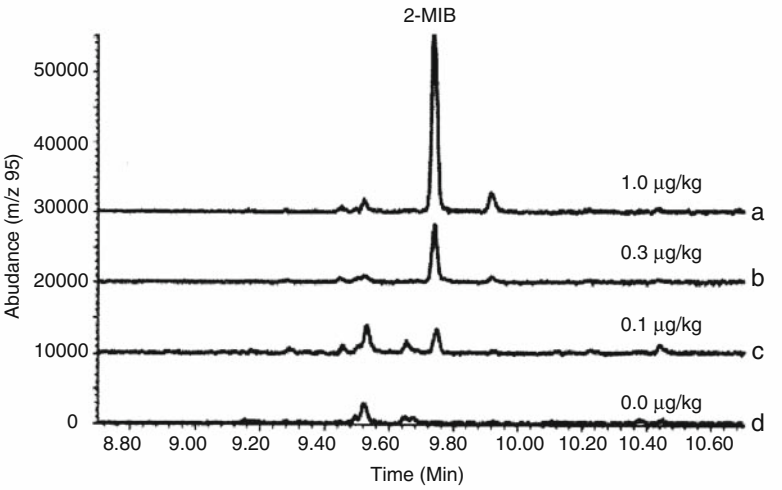

Fig. 2.10 Reconstructed ion chromatogram of *m/z* 95 for a blank, and spiked solutions of MIB at a 0.1, 0.3 and 1 µg/kg. (MIB = (1-*R*-exo-1,2,7,7-tetramethyl bicyclo[2-2-1]-heptan-2-ol). Reprinted with permission from Grimm et al. (2004)

film thickness, 0.25 µm). Initial oven temperature was 35°C (1 min hold), raised to 60°C at 120°C, to 280°C at 3°C/min. Ms conditions were 70 eV, detection range 35–300 *m/z*. The concentrations of volatile compounds in tench fed by different diets are listed in Table 2.26. The measurements demonstrated that the composition of diet exerts a marked influence on the chemical and sensory parameters of tench (Turchini et al., 2007).

The amount of volatile aldehyde in smoked fish, the analytical methods used for their separation and quantitative determination, their role in the sensorial characterisation of the product and their toxicity have been previously reviewed (Varlet et al., 2007).

Simultaneous steam distillation and solvent extraction coupled to GC-MS was employed for the investigation of the composition and amount of volatile compounds in cooked bullfrog (*Rana catesbeiana*) legs. Analytes were separated on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 µm). The starting temperature was 50°C for 2 min, then raised to 280°C at 4°C/min. The ionisation energy of MS detection was 70 eV, the mass range was set to 29–400 *m/z*. It was established that the main odour-active compounds in cooked bullfrog legs were (*E,E*)-2,4-decadienal, (*E,Z*)-2,4-decadienal, (*E,Z*)-2,6-nonadienal, 1-octanol and (*E*)-2-nonenal (Nóbrega et al., 2007).

Table 2.26 The fillet flavour volatile compounds (w/w%) of tench fed the different dietary treatments for 12 weeks (means ± SEM; $N = 3$tanks/treatment)

		Diet				
RI[a]		100SO	25LO	50LO	75LO	100LO
143	3-Hydroxy-2-butanone	3.56 ± 1.76	2.94 ± 0.67	3.41 ± 1.12	4.25 ± 0.53	7.83 ± 0.93
167	2-Ethoxy-2methyl-butane	1.93 ± 0.77	2.01 ± 0.32	1.94 ± 0.15	1.90 ± 0.30	1.87 ± 0.22
226	2,3,4-Trimethyl-pentane	1.14 ± 0.18	2.10 ± 0.36	2.22 ± 0.20	2.07 ± 0.80	2.06 ± 0.46
247	2-Pentenal	4.10 ± 0.47	4.65 ± 0.54	4.34 ± 0.18	4.10 ± 0.26	4.85 ± 0.35
271	2-Penten-1-ol	2.17 ± 0.67	3.54 ± 0.71	2.99 ± 0.57	2.23 ± 0.58	2.59 ± 0.55
353	Hexanal	11.7 ± 1.53	7.57 ± 0.37	9.64 ± 2.23	8.49 ± 0.85	6.09 ± 1.19
491	2-Hexenal	1.70 ± 0.03	2.03 ± 0.22	2.15 ± 0.45	2.14 ± 0.35	1.85 ± 0.18
514	Ethylbenzene	2.42 ± 0.40	2.76 ± 0.15	2.83 ± 0.07	2.74 ± 0.44	3.21 ± 0.71
523	3-Methyl-2-hexanol	5.84 ± 1.17	5.91 ± 0.93	5.29 ± 0.13	5.52 ± 0.68	5.07 ± 0.52
602	1,3-Dimethyl-benzene	0.86 ± 0.16	1.15 ± 0.20	1.62 ± 0.08	1.13 ± 0.18	1.62 ± 0.63
612	4-Heptenal	0.94 ± 0.14	0.85 ± 0.05	0.99 ± 0.01	0.90 ± 0.02	0.88 ± 0.01
621	2-Butoxy-ethanol	6.19 ± 1.58	3.24 ± 0.47	5.62 ± 2.35	4.46 ± 0.87	3.92 ± 0.52
635	3-Methylthio-propanol	1.03 ± 0.34	1.31 ± 0.24	1.22 ± 0.16	1.21 ± 0.11	1.20 ± 0.23
765	2-Heptenal	1.41 ± 0.13	2.08 ± 0.66	1.33 ± 0.04	1.11 ± 0.38	0.44 ± 0.36
786	Benzaldheyde	4.97 ± 0.70	9.44 ± 2.11	6.99 ± 0.09	6.47 ± 0.49	5.71 ± 0.75
805	3,5,5-Trimethyl-2-hexene	5.02 ± 2.42	3.98 ± 0.31	3.67 ± 1.48	5.55 ± 2.57	4.02 ± 1.57
821	1-Octen-3-ol	2.24 ± 1.01	3.07 ± 0.23	3.31 ± 0.46	2.56 ± 0.26	2.46 ± 0.39
849	2-Penthyl-furan	1.26 ± 0.20	1.18 ± 0.08	1.10 ± 0.11	1.07 ± 0.02	0.96 ± 0.08
865	2tr,4c-Heptadienal	1.44 ± 0.25	2.40 ± 0.55	2.00 ± 0.45	2.16 ± 0.21	2.21 ± 0.47
881	Octanal	2.24 ± 0.47	1.46 ± 0.06	1.64 ± 0.13	1.71 ± 0.19	1.35 ± 0.27
902	2tr,4tr-Heptadienal	3.04 ± 0.54	4.67 ± 0.46	4.28 ± 0.38	4.81 ± 0.12	5.66 ± 1.21
982	Benzeneacetaldehyde	1.13 ± 0.52	0.81 ± 0.08	0.65 ± 0.09	0.80 ± 0.05	0.85 ± 0.08
1010	2-Octenal	2.70 ± 0.60	2.47 ± 0.32	2.12 ± 0.09	2.38 ± 0.17	1.97 ± 0.17
1023	2-Methyl-decane	1.36 ± 0.03	1.02 ± 0.19	1.37 ± 0.25	1.30 ± 0.14	1.60 ± 0.20
1032	1,3-Cyclooctadiene	5.75 ± 1.11	3.51 ± 0.41	4.80 ± 0.30	5.06 ± 0.58	4.97 ± 1.24
1109	Nonanal	6.18 ± 0.83	3.23 ± 0.52	3.59 ± 0.32	4.97 ± 0.87	3.84 ± 0.94
1121	2,4-Octadienal	0.95 ± 0.07	1.43 ± 0.29	0.85 ± 0.12	0.98 ± 0.01	1.27 ± 0.31

Table 2.26 (continued)

RI[a]		Diet				
		100SO	25LO	50LO	75LO	100LO
1201	2,6-Nonadienal	1.03 ± 0.15	1.25 ± 0.30	0.92 ± 0.18	0.94 ± 0.05	1.17 ± 0.02
1215	2-Nonenal	1.55 ± 0.43	1.25 ± 0.12	1.08 ± 0.17	1.27 ± 0.06	0.90 ± 0.12
1257	1-(2-Butixyethoxy)-ethanol	3.69 ± 1.78	3.29 ± 1.64	3.11 ± 0.46	2.07 ± 0.43	2.76 ± 0.41
1367	2-Decenal	1.79 ± 0.80	0.96 ± 0.19	0.95 ± 0.21	1.37 ± 0.16	0.89 ± 0.19
1374	1,4-Octadiene	0.74 ± 0.08	0.65 ± 0.13	0.55 ± 0.06	0.77 ± 0.01	0.76 ± 0.13
1406	2tr,4c-Decadienal	1.07 ± 0.29	0.91 ± 0.18	0.82 ± 0.25	1.33 ± 0.10	1.05 ± 0.11
1432	2tr,4tr-Decadienal	2.28 ± 0.49	2.69 ± 0.42	2.66 ± 0.55	2.73 ± 0.58	2.36 ± 0.53
1476	2-Undecenal	1.14 ± 0.31	1.24 ± 0.12	0.95 ± 0.22	1.50 ± 0.04	1.35 ± 0.35
1700	Heptadecane	0.66 ± 0.23	0.84 ± 0.04	0.81 ± 0.15	2.81 ± 2.05	1.72 ± 0.78
1702	Pristane	2.09 ± 0.86	2.05 ± 0.40	1.81 ± 0.65	2.80 ± 0.88	4.02 ± 1.10
1755	Tetradecanal	3.83 ± 0.56	3.16 ± 0.89	2.25 ± 0.96	3.84 ± 0.35	4.37 ± 1.40
1828	9-Octadecanal	2.19 ± 0.33	2.82 ± 1.46	3.37 ± 0.65	4.49 ± 0.26	3.25 ± 0.42
	$\Sigma n-3$ Derived aldehydes[b]	11.0 ± 1.40	15.0 ± 1.43	13.7 ± 0.27	13.8 ± 0.45	15.4 ± 2.00
	$\Sigma n-6$ Derived aldehydes[c]	20.6 ± 2.06[b]	16.4 ± 0.43[a,b]	17.5 ± 2.17[a,b]	16.1 ± 1.15[a,b]	12.0 ± 0.47[a]

Means within rows without Superscript or with the same Superscript are not significantly ($P > 0.05$) different from each other by one-way ANOVA and S–N–K comparison test.

[a] RI: Kovàts retention indices.

[b] $\Sigma n-3$-derived aldehydes: sum of 2-pentenal, 2-hexenal, 2tr,4c-heptadienal, 2tr,4tr-heptadienal and 2,6-nonadienal.

[c] $\Sigma n-6$-derived aldehydes: sum of hexanal, 2-octenal, 2-decenal, 2tr,4c-decadienal and 2tr,4tr-decadienal.

SO = soybean oil; LO = linseed oil.

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The effect of total iron, myoglobin, haemoglobin and lipid oxidation on the livery flavour formation and on the concentration and composition of volatiles of cooked beef steaks have been investigated in detail. Samples were analysed by traditional wet methods, by RP-HPLC (quantification of myoglobin and hemoglobin, by sensory evaluation, by GC-FID (fatty acid composition) and by GC-MS (volatiles). Analytes were extracted by HS-SPME and separated on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The starting temperature was 60°C for 30 sec, then raised to 200°C at 5°C/min. A characteristic GC-MS profile is depicted in Fig. 2.11. The chromatogram illustrates that the volatile profile of the beef steak samples contains a considerable number of identified and not identified compounds. The volatiles found in livery and non-livery samples are compiled in Table 2.27. The data demonstrate that livery samples contain more volatile compounds than the non-livery ones. It was concluded from the data that the total iron content and myoglobin concentration may influence livery flavour (Yancey et al., 2006).

The quantity and quality of volatile sulphur compounds in roast beef have also been investigated. Volatiles were separated by GC-atomic emission detection

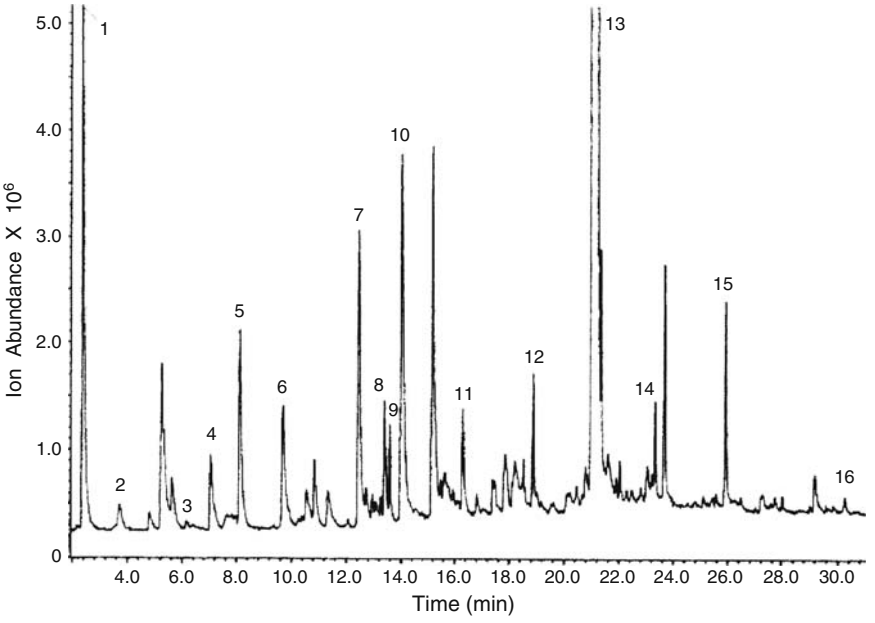

Fig. 2.11 An example for gas chromatogram from a sample rated as livery by the sensory panelists. This chromatogram corresponds to Table 2.27. The large number of peaks illustrates that many volatile compounds were found. Reprinted with permission from Yancey et al. (2006)

Table 2.27 Volatile compounds found to be higher in livery samples versus non-livery samples

Retention time (min)	ID # from GC/MS	Compound name
Compounds with higher concentrations in livery samples		
2.34	1	Hexanal
3.89	2	Butane, 1-(ethenylthio)
6.15	3	*dl*-Limonene
7.04	4	2-Octenal
8.10	5	Nonanal
9.68	6	2-Nonenal, (*E*)-
12.43	7	2-Decenal-(*E*)-
13.38	8	2,4-Decadienal, (*E*,*E*)-
13.99	9	2,4-Decadienal
15.17	10	2-Undecenal
18.88	12	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-
21.03	13	Propanoic acid, 2-methy-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
23.66	14	Tetradecanal
Compounds with higher concentrations in livery samples		
16.28	11	*trans*-2-Undecen-1-ol; or dodecanol
25.90	15	Octadecanal or hexadecanal
30.20	16	Octadecanal
16.28	11	*trans*-2-Undecen-1-ol; or dodecanol
25.90	15	Octadecanal or hexadecanal

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and by GC × GC/TOFMS. The dimensions of the first and second columns in the two-dimensional GC were 30 m × 0.25 mm i.d., film thickness, 0.25 μm and 1 m × 0.10 mm, film thickness, 0.10 μm. The initial oven temperature of the first column was 60°C for 3 min, raised to 220°C at 8°C/min (final hold, 5 min) The temperature of the second column was always held at a temperature 20°C higher than the first one. The ionisation energy was 70 eV, the mass range was 35–320 *m/z*. It was established that the separation capacity of the GC-AED was lower than that of the two-dimensional GC. A typical GC × GC/TOFMS chromatogram is depicted in Fig. 2.12. The chromatogram illustrates the high number of volatiles found in roast beef vapours. The two-dimensional GC system found more than 70 volatiles (thiophene, thiazole, thiol, sulphide and isothiocyanate derivatives), proving again the high separation capacity of the procedure (Rochat et al., 2007).

The influence of various processing steps on the composition and quantity of nonvolatile taste compounds in Nanjing cooked duck was investigated using RP-HPLC. Free amino acids (FAAs) were determined with an amino acid auto-analyser. Peptides were separated on an ODS column (25 cm × 4.6 mm i.d., particle

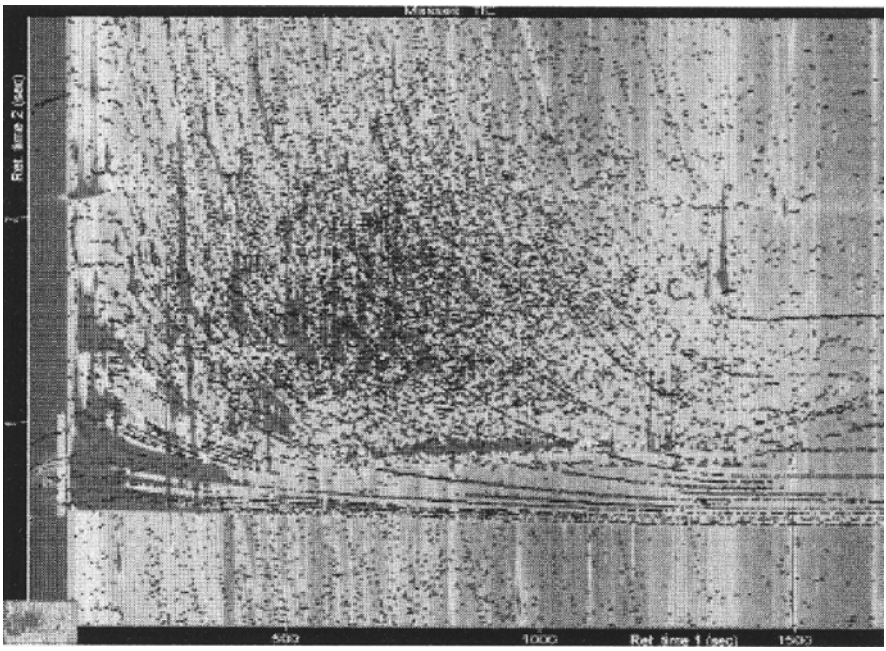

Fig. 2.12 GC × GC/TOF-MS chromatogram of roast beef vapours trapped with a SPME (black points represent the peak apexes). Reprinted with permission from Rochat et al. (2007)

size, 5 μm) using gradient elution. Solvents A and B were water and acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA), respectively. Initial mobile-phase composition was 3.2% B; 0.5 min linear change to 4.5% B; 5 min linear change to 8.5% B; 10 min linear change to 11.5% B; 22 min linear change to 99% B, kept for 12 min. Analytes were detected at 214 nm. Nucleotides were also separated by RP-HPLC using different mobile-phase gradient. Solvents A and B were 0.72% (v/v) triethylamine containing 0.35% (v/v) of phosphate buffer at pH 6.5 and methanol, respectively. Gradient started at 0% B; 15 min linear change to 5% B, kept for 20 min. The concentrations of FAAs in the various samples are compiled in Table 2.28. The data demonstrated that FFAs decrease during boiling while brining and roasting enhanced the amount of FAAs. The nucleotide concentrations are compiled in Table 2.29. The data in Table 2.29 indicate that the concentration of nucleotides also depends on the type of processing (Liu et al., 2007).

GC and electron microscopy were applied for the investigation of the chemical and structural changes in lipids during the ripening on Teruel dry-cured ham. The measurements indicated that the changes in the adipose tissue during the curing process contribute to the characteristic flavour and taste of the product (Larrea et al., 2007). The influence of fungal populations on the volatile composition of dry-cured

Table 2.28 Free amino acid concentrations in breast meat at different processing stages of Nanjing cooked duck[A]

	Raw duck	Dry-cured duck	Brined duck	Roasted duck	Nanjing cooked duck	Control duck
Asp	75.7 ± 1.30^{a}	98.0 ± 3.93^{b}	86.7 ± 4.07^{c}	103 ± 3.10^{b}	37.4 ± 0.23^{d}	22.3 ± 0.05^{e}
Thr	151 ± 2.61^{a}	196 ± 7.86^{b}	173 ± 8.13^{c}	205 ± 6.20^{b}	74.6 ± 0.47^{d}	44.7 ± 0.11^{e}
Ser	76.9 ± 3.80^{a}	97.9 ± 5.03^{b}	93.7 ± 1.26^{b}	109 ± 7.17^{c}	57.6 ± 1.63^{d}	42.8 ± 0.13^{e}
Glu	156 ± 9.09^{a}	178 ± 9.47^{a}	171 ± 2.88^{a}	211 ± 15.42^{b}	109 ± 3.61^{c}	78.3 ± 0.30^{d}
Gly	76.1 ± 3.38^{a}	104 ± 3.98^{b}	87.8 ± 1.82^{c}	104 ± 5.76^{b}	53.3 ± 1.29^{d}	39.9 ± 0.19^{e}
Ala	158 ± 8.56^{a}	172 ± 8.57^{a}	162 ± 1.97^{a}	203 ± 13.31^{b}	119 ± 3.38^{c}	93.9 ± 0.62^{d}
Cys	15.4 ± 1.02^{a}	9.82 ± 1.79^{b}	18.5 ± 0.32^{a}	17.0 ± 1.97^{a}	$6.99 \pm 1.54^{b,c}$	$6.70 \pm 0.14^{b,c}$
Val	47.3 ± 2.17^{a}	$50.1 \pm 2.21^{a,b}$	63.1 ± 0.69^{c}	66.4 ± 4.15^{c}	$42.5 \pm 0.78^{a,d}$	27.3 ± 0.22^{e}
Met	21.9 ± 0.92^{a}	18.8 ± 0.78^{b}	31.7 ± 0.45^{c}	30.4 ± 1.90^{c}	$21.1 \pm 0.20^{a,b}$	15.8 ± 0.56^{d}
Ile	23.0 ± 1.46^{a}	$18.0 \pm 0.75^{b,c}$	29.2 ± 0.26^{d}	35.2 ± 2.14^{e}	22.3 ± 0.39^{a}	16.0 ± 0.16^{c}
Leu	38.2 ± 1.94^{a}	29.6 ± 1.64^{b}	55.1 ± 0.63^{c}	64.1 ± 4.85^{d}	29.9 ± 0.96^{b}	20.0 ± 0.23^{e}
Tyr	37.9 ± 2.08^{a}	34.4 ± 2.15^{a}	53.5 ± 1.17^{b}	59.6 ± 4.62^{b}	25.0 ± 1.99^{c}	19.1 ± 0.43^{c}
Phe	28.4 ± 2.02^{a}	21.2 ± 1.56^{b}	39.3 ± 0.48^{c}	42.5 ± 3.19^{c}	18.7 ± 0.52^{b}	13.7 ± 0.49^{d}
Lys	28.9 ± 1.72^{a}	27.9 ± 1.35^{a}	44.8 ± 0.80^{b}	59.7 ± 4.06^{c}	32.9 ± 0.88^{a}	16.6 ± 0.07^{d}
His	12.0 ± 1.59^{a}	10.7 ± 0.75^{a}	18.3 ± 0.27^{b}	23.1 ± 1.89^{c}	11.7 ± 0.43^{a}	7.11 ± 0.19^{d}
Arg	63.5 ± 2.88^{a}	61.7 ± 3.38^{a}	82.5 ± 1.93^{b}	105 ± 8.04^{c}	60.2 ± 2.03^{a}	40.4 ± 0.67^{d}
Total	1011 ± 44.7^{a}	$1128 \pm 52.0^{a,b}$	1211 ± 25.0^{b}	1438 ± 86.4^{c}	723 ± 19.1^{d}	505 ± 0.45^{e}

[A] Contents of free amino acids were in mg/100 g^{-1} on the basis of duck meat dry matter and expressed as means ± standard error ($n = 4$). Means with different superscripts in the same row indicate significant difference ($P < 0.05$).
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Table 2.29 Nucleotide concentrations in breast meat at different processing stages of Nanjing cooked duck[A]

Nucleotides[B]	Raw duck	Dry-cured duck	Brined duck	Roasted duck	Nanjing cooked duck	Control duck
5′-IMP	470 ± 3.87^{a}	251 ± 1.66^{b}	197 ± 10.60^{c}	164 ± 2.80^{d}	241 ± 14.9^{b}	$182 \pm 0.99^{c,d}$
5′-GMP	18.9 ± 0.88^{a}	6.98 ± 0.95^{b}	$6.04 \pm 0.68^{b,c}$	3.67 ± 0.26^{c}	10.4 ± 1.64^{d}	$4.99 \pm 0.16^{b,c}$
5′-ADP	73.4 ± 1.35^{a}	33.3 ± 0.42^{b}	37.7 ± 0.66^{c}	39.5 ± 0.40^{c}	13.1 ± 1.11^{d}	11.0 ± 0.34^{d}
5′-AMP	5.34 ± 0.33^{a}	$2.50 \pm 0.25^{b,c}$	$3.41 \pm 0.20^{a,b}$	0.27 ± 0.05^{c}	24.6 ± 2.10^{d}	17.0 ± 0.19^{e}
Inosine	223 ± 1.34^{a}	249 ± 2.04^{b}	297 ± 2.14^{c}	319 ± 1.68^{d}	166 ± 8.97^{e}	105 ± 0.54^{f}
Hx	40.6 ± 0.51^{a}	25.1 ± 0.37^{b}	44.5 ± 1.10^{c}	49.2 ± 0.72^{d}	32.0 ± 1.51^{e}	26.0 ± 0.32^{b}
Flavour nucleotides[C]	489 ± 3.62^{a}	258 ± 2.15^{b}	203 ± 10.65^{c}	167 ± 2.91^{d}	251 ± 15.5^{b}	187 ± 1.06^{c}
5′-IMP	470 ± 3.87^{a}	251 ± 1.66^{b}	197 ± 10.60^{c}	164 ± 2.80^{d}	241 ± 14.9^{b}	$182 \pm 0.99^{c,d}$
5′-GMP	18.9 ± 0.88^{a}	6.98 ± 0.95^{b}	$6.04 \pm 0.68^{b,c}$	3.67 ± 0.26^{c}	10.4 ± 1.64^{d}	$4.99 \pm 0.16^{b,c}$
5′-ADP	73.4 ± 1.35^{a}	33.3 ± 0.42^{b}	37.7 ± 0.66^{c}	39.5 ± 0.40^{c}	13.1 ± 1.11^{d}	11.0 ± 0.34^{d}
5′-AMP	5.34 ± 0.33^{a}	$2.50 \pm 0.25^{b,c}$	$3.41 \pm 0.20^{a,b}$	0.27 ± 0.05^{c}	24.6 ± 2.10^{d}	17.0 ± 0.19^{e}
Inosine	223 ± 1.34^{a}	249 ± 2.04^{b}	297 ± 2.14^{c}	319 ± 1.68^{d}	166 ± 8.97^{e}	105 ± 0.54^{f}

[A] Contents of nucleotide were in mg/100 g^{-1} on the basis of dry matter and expressed as mean ± standard error ($n = 6$). Means with different superscripts in the same row indicate significant difference ($P < 0.05$).

[B] 5′-IMP, 5′-inosinic acid; 5′-GMP, 5′-guanosine monophosphate; 5′-ADP, 5′-adenosine diphosphate; Hx, Hypoxanthine.

[C] Flavour nucleotides: 5′-IMP+5′-GMP.

Means with different superscript in the same row indicate significant difference ($P < 0.05$).

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ham has also been studied applying SPME coupled to GC-MS and sensory evaluation. The experiments indicated that the presence of wild fungal population results in higher levels of short-chain aliphatic carboxylic acids and their ethers, branched carbonyls and alcohols and sulphur compounds. *Penicillium chrysogenum* and *Debaryomyces hansenii* increased the amount of long-chain aliphatic and branched hydrocarbons, furanones, long-chain carboxylic acids and their esters (Martin et al., 2006).

The volatile compounds present in the headspace of salted and occasionally smoked dried meats (cecines) were separated and quantitated by GC-MS. Analyses were performed on a fused-silica capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). The initial oven temperature was 40°C for 2 min, then raised to 280°C at 4°C/min, final hold 5 min. The concentrations of volatile compounds found in the headspace of cecinea are compiled in Table 2.30. The investigations indicated that the volatile compounds are derived from lipid oxidation, amino acid catabolism, carbohydrate fermentation, microbial esterification, smoke and spices (Hierro et al., 2004).

The volatile profile in Dalmatian traditional smoked ham and the influence of dry-curing and frying of its composition were investigated in detail. Analytes were extracted by solvent extraction (SE), SDE and nitrogen purge and steam distillation (NPSD) followed by GC and GC-MS. SE and SDE extracted 46 compounds (fatty acids, aldehydes, phenols, esters, ketones, etc.) while NPSD isolated 81 compounds (phenols, aldehydes, hydrocarbons, ketones, alcohols, esters and heterocyclic compounds). It was established that the amount of volatiles increases during the ripening process (Jerkovic et al., 2007). Another study investigated the flavour compounds in Chinese traditional smoke-cured bacon using NPSD extraction method coupled to GC-FID and GC-MS. It was found that the majority of volatiles are phenolic derivatives and they can be easily extracted by NPSD (AI-Nong and Bao-Guo, 2005).

Besides the volatile composition of ham and bacon, the flavour substances in various sausages have also been extensively investigated. Thus, the effect of salt concentration, curing ingredients (nitrate, nitrite, nitrite/ascorbate) and start culture (*Staphylococcus xylosus, S. carnosus*) on the volatile profile of the sausages was investigated by HS-SPME and GC-MS. The measurements demonstrated that the concentration of flavour substances increased during the fermentation process and depended on the concentration of NaCl, the type of curing ingredients and the starter culture (Olesen et al., 2004). D-HS followed by GC-MS was employed for the determination of the aroma profile of two types of typical Italian dry-sausages (Salame Mantovano. Salame Cremonese). GC measurements were carried out in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The initial oven temperature was 35°C for 8 min, then ramped to 60°C at 4°C/min, to 160°C at 6°C/min, to 220°C at 20°C/min, final hold 1 min. MS detection conditions were: 70 eV ionisation energy, mass range *m/z* 35–350. DHS-GC-MS total ion chromatograms of the two different sausages are depicted in Fig. 2.13. It was found that terpenes, aldehydes, ketones and alcohols were present at relatively high concentrations. PCA and linear discriminant analyses indicated that the differential power

Table 2.30 Volatile compounds (ng/100g) identified in the headspace of cecinas

LRI[a]	Compound	Mean concentration (ng/100g)				Method of identification[b]
		Venison	Beef	Horse	Goat	
	Lipid oxidation	**3996**	**2418**	**2784**	**2979**	
	Alcohols	394	562	1594	356	
560	1-Propanol	30	115	529	30	MS+LRI
653	1-Butanol	96	104	78	nd	MS+LRI
672	1-Penten-3-ol	94	60	656	227	ms+lri
705	2-Pentanol	nd	86	100	nd	MS+LRI
765	1-Pentanol	86	61	99	83	MS+LRI
862	1-Hexanol	46	65	109	16	MS+LRI
904	2-Heptanol	4	19	nd	nd	MS+LRI
980	1-Octen-3-ol	38	35	19	nd	MS+LRI
1075	1-Octanol	nd	17	4	nd	MS+LRI
	Aldehydes	1415	579	574	1255	
705	Pentanal	205	28	1	197	MS+LRI
802	Hexanal	961	144	428	806	MS+LRI
848	2-Hexanal	nd	nd	1	nd	MS+LRI
902	Heptanal	142	113	40	136	MS+LRI
1005	Octanal	37	87	26	49	MS+LRI
1065	*E,E*-2,4-Heptadienal	nd	nd	3	nd	MS+LRI
1105	Nonanal	54	189	64	66	MS+LRI
1217	Decanal	16	18	11	1	MS+LRI
	Ketones	459	745	152	178	
683	2-Pentanone	197	571	148	99	MS+LRI
789	2-Hexanone	95	nd	nd	nd	MS+LRI
898	2-Heptanone	129	134	4	29	MS+LRI
980	2,3-Octanedione	nd	nd	nd	50	
1099	2-Nonanone	38	40	nd	nd	MS+LRI
	Hydrocarbons	1703	472	396	1178	

Table 2.30 (continued)

LRI[a]	Compound	Mean concentration (ng/100g)				Method of identification[b]
		Venison	Beef	Horse	Goat	
600	Hexane	852	125	119	747	MS+LRI
700	Heptane	203	57	97	110	MS+LRI
792	1-Octene	nd	28	13	77	MS+LRI
800	Octane	183	147	124	114	MS+LRI
812	2-Octene	358	37	nd	11	MS+LRI
900	Nonane	62	26	19	60	MS+LRI
1000	Decane	31	40	22	27	MS+LRI
1092	1-Undecene	nd	12	nd	nd	MS+LRI
1100	Undecene	14	nd	2	32	MS+LRI
	Furans	25	60	68	12	
604	2-Methylfuran	nd	37	4	12	MS+LRI
701	2-Ethylfuran	12	12	64		MS+LRI
994	2-Penthylfuran	13	11	nd	nd	MS+LRI
	Amino acid degradation	**1859**	**2385**	**4174**	**1380**	
551	2-Methylpropanal	72	102	97	113	MS+LRI
629	2-Methylpropanol	170	392	970		ms+lri
654	3-Methylbutanal	818	1029	304	443	MS+LRI
662	2-Methylbutanal	186	419	96	141	MS+LRI
727	Dimethyl disulphide	53	42	nd	5	MS+LRI
730	4-Methyl-2-pentanone	nd	nd	nd	18	MS+LRI
740	3-Methylbutanol	412	682	2080	204	MS+LRI
744	2-Methylbutanol	72	105	194	nd	MS+LRI
752	3-Methyl-2-pentanone	56	47	346	101	ms+lri
857	3-Methylbutanoic acid	nd	nd	nd	223	ms+lri
868	2-Methylbutanoic acid	nd	nd	nd	47	ms+lri
972	Benzaldehyde	15	10	21	85	MS+LRI
984	Dimethyl trisulphide	5	7	nd	nd	ms+lri

Table 2.30 (continued)

LRI[a]	Compound	Mean concentration (ng/100g)				Method of identification[b]
		Venison	Beef	Horse	Goat	
1065	Benzeneacetaldehyde	nd	nd	66	nd	MS+LRI
	Carbohydrate fermentation	**7825**	**1749**	**3294**	**4806**	
649	Acetic acid	160	15	nd	493	MS+LRI
587	2,3-Butanedione (diacetyl)	2809	251	92	1205	ms+lri
604	2-Butanone	446	383	310	nd	MS+LRI
666	1-Hydroxy-2-propanone	32	78		48	ms+lri
711	3-Hydroxy-2-butanone (acetoin)	4205	539	173	2993	MS+LRI
503	Ethanol	80	391	2296	52	ms+lri
591	2-Butanol	93	92	423	15	MS+LRI
	Microbial esterification	**72**	**122**	**2790**	**104**	
531	Methyl acetate	10	8	13	3	ms+lri
615	Ethyl acetate	45	85	1285	13	MS+LRI
685	Methyl-2-methyl-propanoate	nd	nd	8	nd	ms+lri
709	Ethyl propanoate	7	9	119	nd	MS+LRI
716	Propyl acetate	nd		15	nd	MS+LRI
724	Methyl butanoate	4	6	3	nd	ms+lri
756	Ethyl-2-methyl-propanoate	nd	6	268	nd	ms+lri
	Methyl-3-methyl-butanoate	nd	nd	17	nd	ms
782	2-Methyl propanoate	nd	nd	nd	nd	ms+lri
805	Ethyl butanoate	nd	nd	115	88	MS+LRI
846	Ethyl-2-methyl-butanoate	nd	nd	211	nd	MS+LRI
849	3-Methylethyl butanoate	6	8	669	nd	MS+LRI
877	3-Methylbutyl acetate	nd	nd	17	nd	ms+lri
879	2-Methylbutyl acetate	nd	nd	2	nd	ms+lri
901	Ethyl pentanoate	nd	nd	6	nd	MS+LRI
	3-Methylpropyl butanoate	nd	nd	5	nd	ms

Table 2.30 (continued)

LRI[a]	Compound	Mean concentration (ng/100g)				Method of identification[b]
		Venison	Beef	Horse	Goat	
997	Ethyl hexanoate	nd	nd	27	nd	MS+LRI
	3-Ethylbutyl-3-methyl butanoate	nd	nd	6	nd	ms
1196	Ethyl octanoate	nd	nd	4	nd	MS+LRI
	Smoke	**1995**	**3565**	**271**	**2526**	
	Phenolic compounds	108	662	26	427	
992	Phenol	30	128	8	151	MS+LRI
1071	2-Methyl phenol (*o*-cresol)	9	55	3	53	ms
1086	4-Methyl-phenol (*p*-cresol)	nd	44	8	49	ms+lri
1091	2-Metoxyphenol (guaiacol)	60	370	7	154	ms
	4-Methyl-2-methoxyphenol (4-methylguaiacol)	9	65		20	ms
	Cyclopentanones/enones	121	1199	8	273	
795	Cyclopentanone	nd	571	nd	75	ms
847	2-Methylcyclopentanone	62	331	nd	19	MS+LRI
858	3-Methylcyclopentanone	17	35	8	10	ms
915	2-Methyl-2-cyclopenten-1-one	14	76	nd	39	ms
973	3-Methyl-2-cyclopenten-1-one	28	102	nd	86	ms
	2,3-Dimethyl-2-cyclopenten-1-one	nd	73	nd	44	ms
1076	Trimethyl-2-cyclopenten-1-one[a]	nd	11	nd	nd	se
	Aromatic hydrocarbons	1568	503	231	1546	
663	Benzene	28	56	49	62	MS+LRI
769	Methylbenzene (toluene)	1387	192	118	1260	MS+LRI
864	Ethylbenzene	36	23	12	59	MS+LRI
865	Dymethylbenzene[a]	75	43	28	134	ms
893	Vinylbenzene (styrene)	nd	109	1	nd	MS+LRI
971	1-Ethyl-2-benzene	15	35	11	nd	ms
1012	1,2,3-Trimethylbenzene	27	45	12	31	ms
	Furans	133	871	2	158	

Table 2.30 (continued)

LRI[a]	Compound	Mean concentration (ng/100g)				Method of identification[b]
		Venison	Beef	Horse	Goat	
893	2-Furancarboxaldehyde (furfural)	14	58	nd	12	ms+lri
856	2-Furanmethanol (furfuryl alcohol)	119	813	2	146	MS+LRI
	Pyridines	52	275	nd	28	
751	Pyridine	46	182	nd	16	MS+LRI
818	2-Methylpyridine	6	51	nd	8	ms
869	3-Methylpyridine	nd	42	nd	4	ms
	Pyrazines	13	55	4	94	
833	Methylpyrazine	5	23	1	16	MS+LRI
912	2,6-Dimethylpyrazine	5	12	3	36	MS+LRI
924	Ethylpyrazine	nd	12	nd	nd	MS+LRI
1014	Trimethylpyrazine	3	8	nd	42	MS+LRI
	Spices	**1683**	**234**	**191**	**206**	
934	α-Pinene	nd	24	8	51	ms+lri
946	Camphene	nd	13	3	20	ms+lri
956	2-Ethyl-hexanal	31	nd	nd	nd	ms
1031	Limonene	14	197	33	135	MS+LRI
1037	2-Ethyl-hexanol	1638		147	nd	ms
	Unknown origin	**1284**	**1865**	**574**	**307**	
	2-Propanone	1072	813	300	186	MS+LRI
524	2.Propanol	212	1052	274	22	MS+LRI
818	Butanoic acid	nd	nd	nd	99	MS+LRI
		nd	nd	nd	nd	
	Total volatiles	**18714**	**12788**	**14078**	**12308**	

[a]Linear retention index on a CP-Sil 8 CB low bleed/MS column.
[b]MS+LRI, mass spectrum and LRI agree with those of authentic compounds; ms+lri, mass spectrum and LRI in agreement with the literature; ms, mass spectrum agrees with spectrum int he HP Wiley 1378 Mass Spectral Database; se, tentative identificaton by mass spectrum.
nd: not detected.
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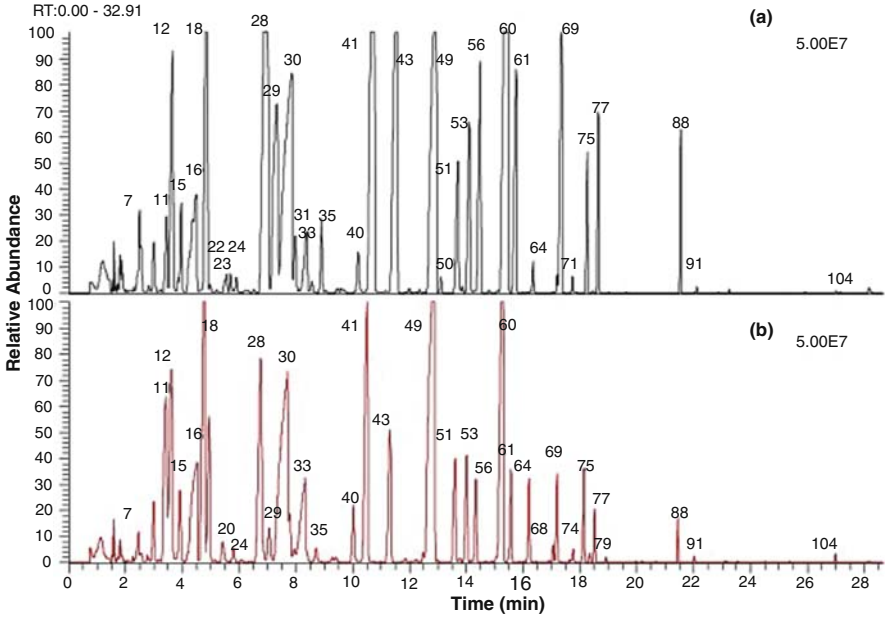

Fig. 2.13 DHS-GC–MS total ion chromatograms of (**a**) a "Salame Mantovano" sample (MN7) (**b**) a "Salame Cremonese" (CR5). Peak identification: acetone (7), butanal (11), ethyl acetate (12), 3-methylbutanal (15), ethanol (16), allyl methyl sulphide (18), ethyl isobutanoate (20), pentanal (22), 2,3-butanedione (23), 1-propene-1-methylthio (24), α-pinene (28), α-thujene (29), 2-butanol (30), toluene (31), 1-propanol (33), camphene (35), hexanal (40), β-pinene (41), 1,4-*p*-menthadiene (43), 3-carene (49), α-phellandrene (51), β-myrcene (53), α-terpinene (56), limonene (60), β-phellandrene (61), 3-methyl-1-butanol (64), β-(*Z*)-ocimene (68), γ-terpinene (69), β-(*E*)-ocimene (71), styrene (74), *p*- cymene (75), terpene (not identified) (77), octanal (79), terpene (not identified) (88), terpene (not identified) (91), β-caryophyllene (104). Reprinted with permission from Bianchi et al. (2007)

of 2-methylbutanal, 6-camphenol, dimethyl disulphide, 1-propene-3,3′-thiobis ethyl propanoate, 1,4-*p*-menthadiene and 2,6-dimethyl-1,3,5,7-octatetraene was the highest (Bianchi et al., 2007). A complex analytical program was employed for the elucidation of the differences among three Italian fermented sausages (Varzi, Brianza, Piacentino). Measurements included compositional, microbiological, biochemical and chromatographic technologies. The separation and quantification of volatiles were performed by HS-SPME coupled to GC-FID and GC-MS. Volatiles were extracted by a CAR/PDMS fibre at 35°C for 30 min). GC analyses were performed in a capillary column (50 m × 0.32 mm i.d., film thickness, 1.2 μm). The starting oven temperature was 40°C for 2 min, then raised to 200°C at 10°C/min, then to 250°C at 15°C/min, final hold 5 min. The main volatile compounds were alcohols, aldehydes and terpenes. The individual aroma substances are listed in Table 2.31. It was established that the volatile profiles of sausage show marked differences (Di Cagno et al., 2008).

Table 2.31 Volatile components (relative area percentages) as estimated in the headspace of the three Italian PDO sausages

Chemical class	Varzi	Brianza	Piacentino
Alcohols			
Ethanol	31.48 ± 20.38^{a}	27.64 ± 11.53^{a}	20.53 ± 14.02^{b}
1-Propanol	0.46 ± 0.33^{a}	0.93 ± 0.24^{a}	0.17 ± 0.05^{c}
1-Pentanol	0.42 ± 0.96^{b}	0.53 ± 0.21^{b}	1.47 ± 0.61^{a}
1-Hexanol	0.24 ± 0.12^{b}	0.70 ± 0.09^{a}	0.54 ± 0.25^{a}
Phenyl-ethyl-alcohol	ND	ND	0.59 ± 0.37
1-Octen-3-ol	0.59 ± 0.30^{a}	0.05 ± 0.03^{a}	0.04 ± 0.07^{b}
Isoamylic alcohol	1.93 ± 0.99^{a}	0.12 ± 0.03^{b}	0.18 ± 0.06^{b}
Total alcohols	35.12	29.97	23.52
Aldehydes			
Butanal	0.31 ± 0.15^{b}	1.14 ± 0.26^{a}	0.58 ± 0.25^{b}
2-Methyl-butanal	2.37 ± 1.29^{a}	0.90 ± 0.33^{c}	1.27 ± 0.96^{b}
3-Methyl-butanal	0.10 ± 0.05^{a}	0.14 ± 0.03^{a}	0.08 ± 0.04^{a}
Pentanal	1.77 ± 0.78^{b}	3.69 ± 0.61^{a}	1.72 ± 1.92^{b}
Hexanal	15.55 ± 8.92^{b}	21.10 ± 1.39^{a}	15.18 ± 3.60^{b}
Heptanal	0.02 ± 0.01^{a}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}
2-Heptenal	1.16 ± 0.15^{a}	1.42 ± 0.06^{a}	1.17 ± 0.46^{a}
2-Octanal (*E*)	1.72 ± 1.26^{a}	0.67 ± 0.21^{a}	0.03 ± 0.02^{c}
Nonanal	0.15 ± 0.08^{a}	0.15 ± 0.05^{a}	0.11 ± 0.07^{a}
2-Nonenal	0.22 ± 0.31^{a}	0.16 ± 0.02^{a}	0.11 ± 0.07^{a}
2,4-Nonadienal	0.28 ± 0.27^{a}	0.17 ± 0.01^{a}	0.14 ± 0.08^{a}
2-Decenal €	0.08 ± 0.02^{b}	0.37 ± 0.09^{a}	0.03 ± 0.02^{b}
Dodecanal	0.13 ± 0.31^{b}	0.10 ± 0.02^{b}	0.55 ± 0.25^{a}
Total aldehydes	23.86	30.05	21.00
Ketones			
2-Propanone	0.37 ± 0.53^{c}	1.92 ± 0.55^{a}	0.79 ± 0.23^{b}
2-Butanone	0.08 ± 0.04^{b}	1.33 ± 0.06^{a}	1.36 ± 0.04^{a}
2,3-Butandione	0.24 ± 0.24^{a}	0.46 ± 0.07^{a}	0.37 ± 0.20^{a}
2-Pentanone	2.32 ± 1.41^{a}	3.15 ± 1.29^{a}	2.94 ± 0.98^{a}
2-Heptanone	0.13 ± 0.12^{b}	1.53 ± 0.19^{a}	1.40 ± 0.34^{a}
2-Octanone	0.13 ± 0.11^{a}	0.18 ± 0.01^{a}	0.11 ± 0.04^{a}
2-Nonanone	0.15 ± 0.02^{a}	0.21 ± 0.08^{a}	0.19 ± 0.10^{a}
Total ketones	3.42	8.78	7.16
Acids			
Acetic	0.56 ± 0.22^{a}	0.40 ± 0.12^{b}	0.35 ± 0.05^{b}
2-Methylpropanoic	6.80 ± 0.59^{a}	2.59 ± 0.47^{b}	2.55 ± 2.47^{b}
Pentanoic	0.24 ± 0.15^{a}	ND	0.01 ± 0.01^{b}
Hexanoic	0.22 ± 0.19^{a}	0.07 ± 0.02^{b}	0.07 ± 0.10^{b}
Octanoic	0.50 ± 0.12^{a}	0.05 ± 0.05^{b}	0.08 ± 0.06^{b}
Decanoic	0.48 ± 0.13^{a}	0.20 ± 0.05^{b}	0.22 ± 0.01^{b}
Total acids	8.80	3.31	3.28
Esters			
Ethyl acetate	3.16 ± 1.90^{a}	ND	3.14 ± 1.08^{a}
Ethyl butanoate	0.71 ± 0.12^{a}	ND	0.82 ± 0.22^{a}
Ethyl 2-hydroxy-propanoate	1.17 ± 1.30^{b}	2.62 ± 0.25^{a}	0.47 ± 0.30^{c}
Ethyl 3-methyl-butanoate	0.22 ± 0.17^{a}	0.34 ± 0.05^{a}	0.17 ± 0.02^{a}

Table 2.31 (continued)

Chemical class	Varzi	Brianza	Piacentino
Ethyl pentanoate	0.10 ± 0.08^{a}	0.25 ± 0.04^{a}	0.08 ± 0.10^{a}
Ethyl decanoate	0.52 ± 0.01^{a}	0.01 ± 0.10^{b}	0.64 ± 0.02^{a}
Total esters	5.88	3.22	5.32
Terpenes			
Limonene	0.45 ± 0.29^{b}	1.28 ± 0.22^{b}	2.99 ± 0.65^{a}
Phellandrene	0.43 ± 0.32^{a}	0.52 ± 0.04^{a}	0.15 ± 0.01^{b}
A-Thujene	ND	1.41 ± 0.17^{a}	0.06 ± 0.03^{b}
Δ^3-Carene	0.04 ± 0.04^{b}	0.04 ± 0.03^{b}	0.28 ± 0.08^{a}
1*R*-α-Pinene	9.52 ± 3.98^{b}	10.84 ± 4.76^{b}	25.06 ± 5.75^{a}
1*S*-α-Pinene	0.05 ± 0.02^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
p-Cimene	0.81 ± 0.41^{a}	0.05 ± 0.06^{b}	0.09 ± 0.03^{b}
p-Elemene	0.27 ± 0.14^{a}	0.20 ± 0.05^{a}	0.01 ± 0.01^{b}
Total terpenes	11.57	14.35	28.65
Thio-compounds			
Dimethyldisulphide	0.24 ± 0.22^{b}	0.32 ± 0.05^{b}	0.59 ± 0.22^{a}
Hydrocarbons			
Pentane	0.89 ± 0.07^{a}	0.82 ± 0.39^{a}	0.24 ± 0.10^{b}
Hexane	1.49 ± 0.74^{a}	1.03 ± 0.27^{b}	0.63 ± 0.26^{b}
Heptane	0.44 ± 0.04^{a}	0.52 ± 0.31^{a}	0.41 ± 0.26^{a}
Undecane	1.46 ± 1.00^{a}	0.08 ± 0.03^{b}	0.14 ± 0.08^{b}
Total hydrocarbons	4.28	2.45	1.42

Results are expressed as means of three replicates for each batch (total of six analyses for each type of sausage) ± standard deviations.
ND: not detected.
[a–c] Means within a row with different letters are significantly different ($P < 0.05$).
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The concentration and composition of volatile aroma substances have also been studied in various fats.

Thus, DHS followed by GC-MS was employed for the measurement of aroma substances in pig back fat. Analyses were carried out in a capillary column (50 m × 0.2 mm i.d., film thickness, 0.33 μm). GC separation started at 50°C for 1 min, then ramped to 270°C at 5°C/min, final hold 5 min. Scatole and indole were determined by normal-phase HPLC. Indolic compounds were separated on an aminopropylsilica column (250 mm × 4.6 mm, particle size, 5 μm), mobile phase being hexane:2-propanol (92:8 v/v). Analytes were detected by fluorescence (excitation 280 nm, emission 360 nm). Androsterone and androsteronols were measured by RP-HPLC using an ODS column. Fractions separated by HPLC were further analysed by GC-MS employing capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The initial column temperature was 70°C for 1 min, then raised to 190°C at 10°C/min, then to 270C at 5°C/min, final hold 5 min. Typical chromatograms are shown in Figs. 2.14 and 2.15. It was concluded from the results that other volatile compounds can increase the off-flavours caused by skatole and indole in the pig back fat (Rius et al., 2005). The amount of the boar taint compounds indole

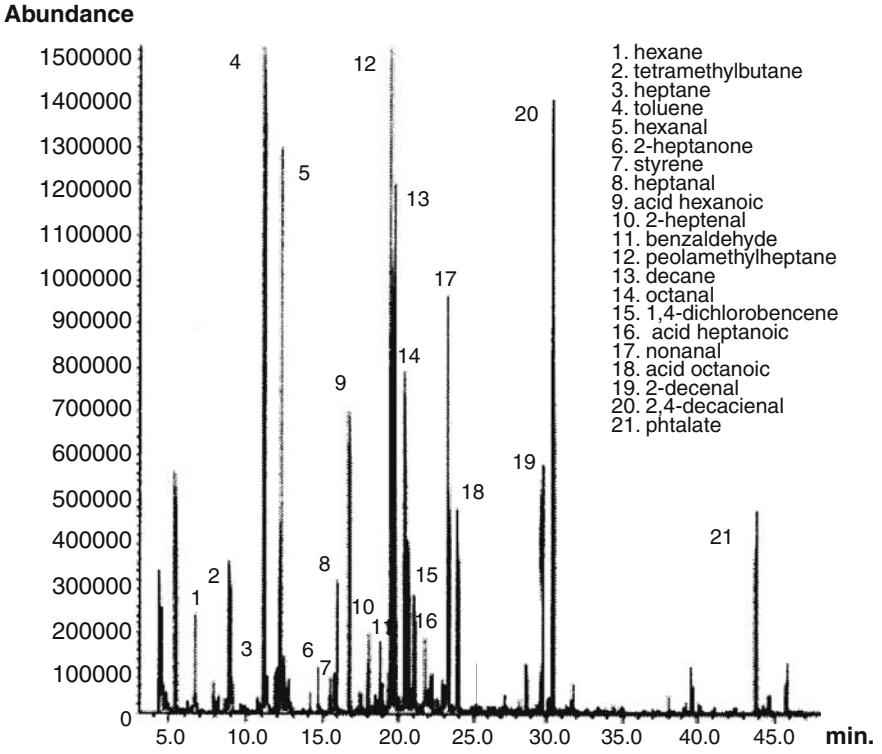

Fig. 2.14 Chromatographic profile of the volatile compounds identified in pig back fat samples classified with low concentrations of skatole and androstenone. Analysis by GC–MS. Reprinted with permission from Rius et al. (2005)

(2,3-benzopyrrole, ID), skatole (3-methylindole, SK) and androsterone (5α-androst-16-en-3-one, AEON) were determined in fat of male pigs. Analytes were extracted by methanol and separated by liquid chromatography-multiple mass spectrometry (LC-MS^n) using an ODS column. The recovery of the method varied between 96.91% and 104%, and good linear correlations were found between the detector response and the concentration of indole, skatol and androsterone (Verheyden et al., 2007).

The dependence of the concentration of indole and skatole on the grazing conditions of lambs were investigated by RP-HPLC employing a C18 column (150 × 4.6 mm) and an isocratic mobile phase consisting of 0.02 M acetic acid-2-propanol (70:30, v/v). The flow rate was 1 ml/min; analytes were detected by fluorescence detection (excitation, 285 nm; emission 350 nm). Lambs were grazed on condensed tannin-containing *Lotus corniculatus* L. (cv. Gassland Goldie) or on perennial ryegrass clover pasture (PRG/WC, *Lolium perenne/Trifolium repens*). The

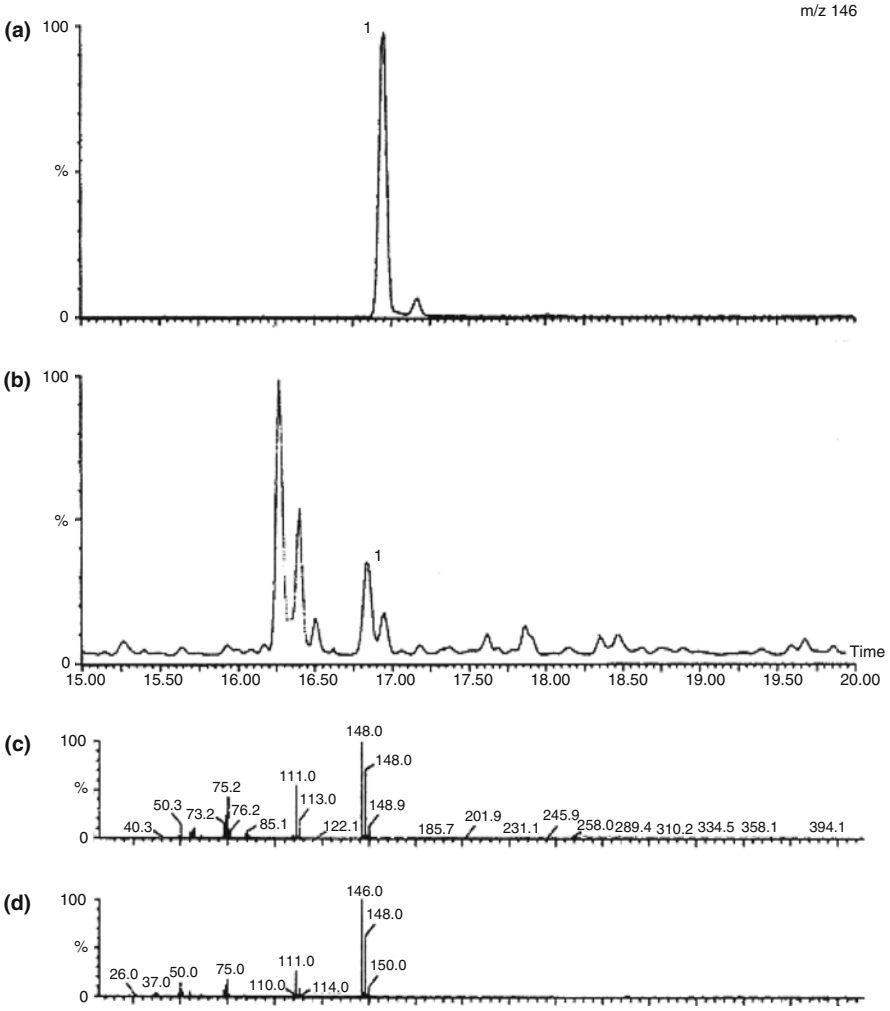

Fig. 2.15 GC–MS chromatogram in mode SCAN of back fat sample classified with low concentrations of skatole and androstenone: (**a**) selection of the molecular ion fragment 146; (**b**) total ion chromatogram; (**c**) and (**d**) comparison between the El mass spectrum of the compound identified as 1,4-dichlorobenzene in the evaluated fat samples with those obtained in the NBS library. Reprinted with permission from Rius et al. (2005)

amount of analytes was measured in the rumen fluid, blood plasma and body fat, and the odour of the fat was evaluated by a sensory panel. The chromatograms obtained from the rumen fluid and from jugular blood of lambs are depicted in Figs. 2.16 and 2.17. The data demonstrated that the condensed tannins in the forages can reduce the concentration of indole and skatole in the fat (Schreurs et al., 2007).

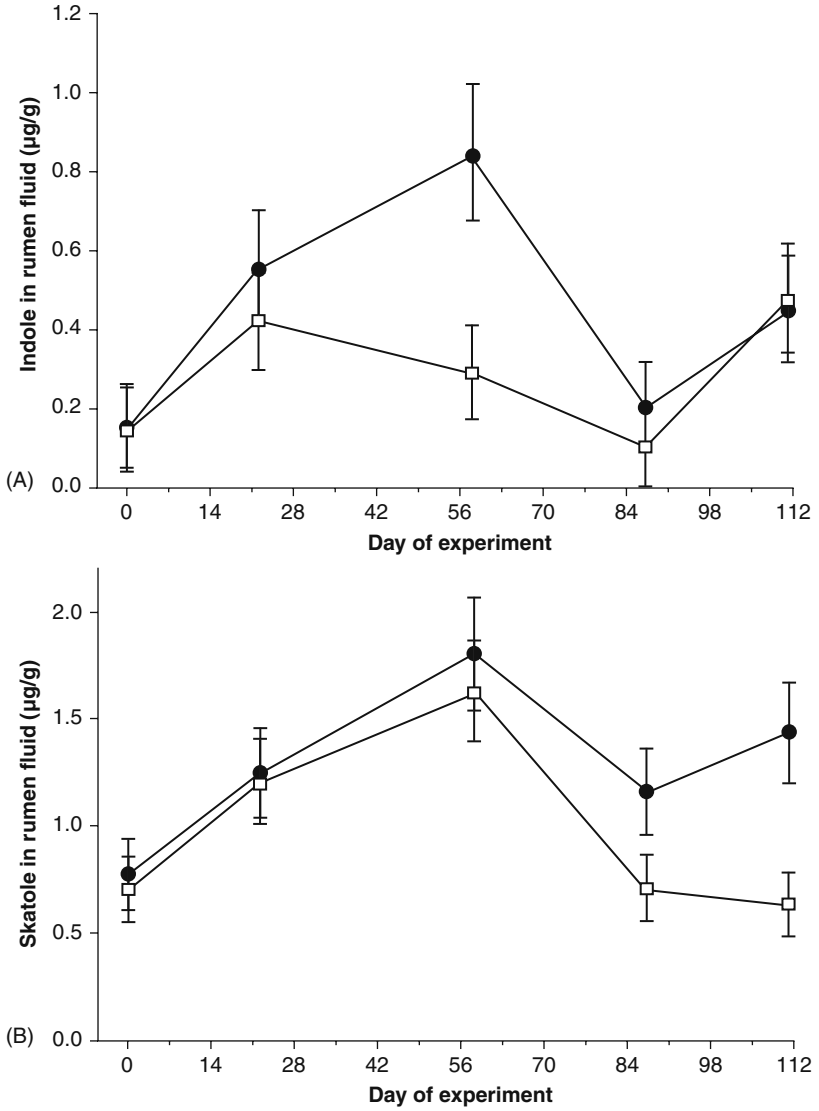

Fig. 2.16 Indole (**A**) and skatole (**B**) concentration in rumen fluid obtained from lambs (•; $n = 12$) grazing perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or (□ $n = 12$) *Lotus corniculatus* L. (cv. Grasslands Goldie). Vertical bars represent the 95% confidence interval. Reprinted with permission from Schreurs et al. (2007)

GC methods have been applied not only for the separation and quantitative determination of the volatile compounds present in meats and meat products but also for the assessment of the binding of aroma substances to various proteins. The aim of the experiments was the promotion of the better understanding of the interaction between the volatile and nonvolatile compounds in meat proteins. The interaction between proteins (actomyosin, G-actin, F-actin) and selected volatile substances

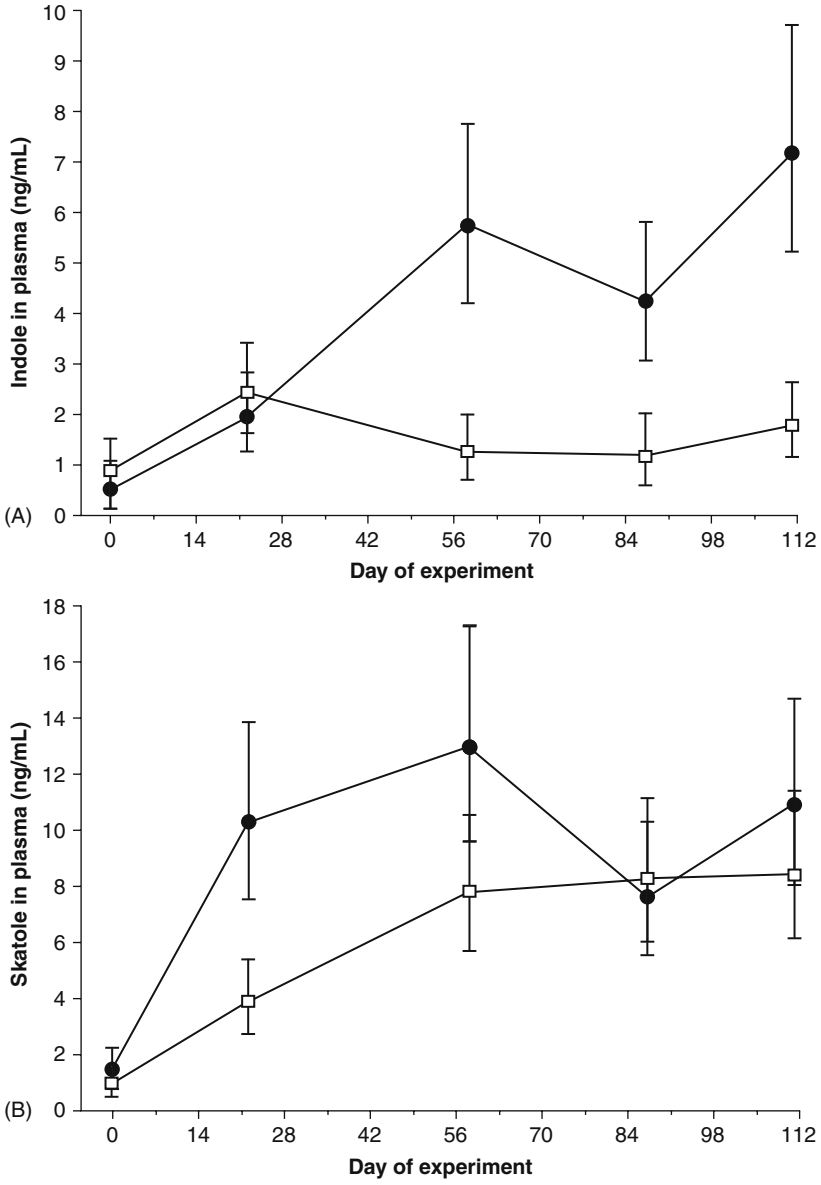

Fig. 2.17 Indole (**A**) and skatole (**B**) concentration in plasma obtained from jugular blood of lambs grazing (•; $n = 12$) perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or (□; $n = 12$) *Lotus corniculatus* L. (cv. Grasslands Goldie). Vertical bars represent the 95% confidence interval. Reprinted with permission from Schreurs et al. (2007)

(3-methyl-butanal, 2-methyl-butanal, 2-pentanone, hexanal, methional, octanal) was investigated by HS-SPME coupled to GC-FID. It was established that actomyosin and F-actin readily binds each volatiles while the binding capacity of G-actin was negligible (Pérez-Juan et al., 2007). A similar study was carried out to measure the

binding of the same volatiles to sarcoplasmic, myofibrillar and isolated actin and actomyosin. GC analyses were performed on a capillary column (60 m × 0.32 mm i.d., film thickness, 1.8 μm). The initial oven temperature was 38°C for 6 min, then ramped to 105°C at 6°C/min, then to 220°C at 15°C/min, final hold 5 min. The influence of protein concentration on the binding of volatile substances is presented at Fig. 2.18. The data indicated that the binding capacity of sarcoplasmic homogenates were higher than that myofibrillar homogenates (Pérez-Juan et al., 2008).

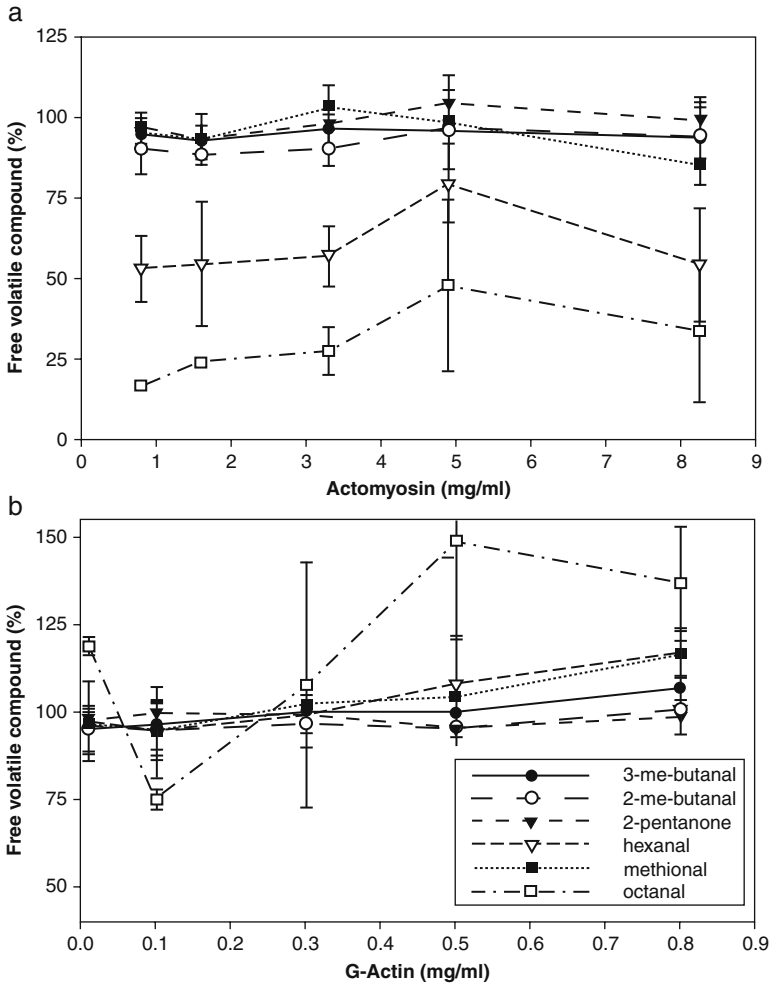

Fig. 2.18 Effect of protein concentration on the binding of volatile compounds: (**a**) actomyosin (0.8–8.2 mg/ml) and (**b**) G-actin (0.01–0.8 mg/ml). Results are expressed as percentage of the free volatile compound found in the headspace without protein in solution. Reprinted with permission from Pérez-Juan et al. (2008)

2.6 Milk and Dairy Products

The composition of volatile aroma compounds in milk and in cheeses has also been vigorously investigated. These measurements were motivated by the fact that the composition of volatile ingredients in milk can result in the modification of the sensory characteristic of the cheese influencing consumer acceptance. However, not only the milk but also the bacterial strains used for cheese ripening exert a considerable impact on the quality of the end product (Andrighetto et al., 2002). The biochemical pathways of the production of flavour compounds have been previously reviewed (McSweeny and Sousa, 2000). It was many times established that the aroma composition of cheeses made form raw and pasteurised milk differs considerably (Awad, 2006; Fernandez-Garcia et al., 2002a). The effect of ewes, raw milk and animal rennet on the sensory characteristics of cheeses has also been established (Barron et al., 2005). The influence of seasonal variation on the quantity and quality of aroma compounds was also illustrated (Carbonell et al., 2002; Fernandez-Garcia et al., 2002b). The majority of studies apply GC for the measurements. Thus, the use of purge and trap and SPME (Mallia et al., 2005) as well as SPME-GC has been reported (Ziino et al., 2005). GC-olfactometry has also been employed for the analysis of odorants in various cheese types (Curioni and Bosset, 2002).

Similarly to other foods and food products, the method of preference for the analysis of volatiles is HS-SPME followed by various capillary GC techniques such as GC-FID and GC-MS. Thus, HS-SPME method was employed for the investigation of the effect of packing material and storage time on the aroma substances in whole pasteurised milk. It was found that light-induced oxidation and/or autooxidation influence the quality of the milk. The application of dimethyl disulphide, pentanal, hexanal and heptanal as markers of the fresh milk quality was proposed (Karapatanis et al., 2006).

The impact of short-chain free fatty acids (FFA) on the formation of cheese-like off-flavour in pasteurised yoghurt has also been investigated by SPE-GC. The data indicated that FFAs are responsible for the cheese-like off-flavour and they are the result of the activity of a heat-resistant lipase enzyme (Rychlik et al., 2006).

The aroma profile of four different creams were determined by headspace sorptive extraction followed by GCO-MS. It was established that "yoghurt" cream flavour depended on the amount of diacetyl, acetoin, dimethyl trisulphide, 2-nonanone, butanoic acid, dimethyl sulphide, 2-butanone while 2-pentanone, dimethyl trisulphide, 2-nonanone contributed to the animalic cream flavour. The "sterilised" cream flavour was correlated with the concentration of dimethyl trisulphide, 2-nonanone, 2-pentanone, 2-heptanone, 2-furfural and 2-furanmethanol (Pionnier and Hugelshofer, 2006).

The application possibilities of two-dimensional gas chromatography with TOFMS detection in the analysis of flavour substances in foods were previously reviewed. The efficacy of various sample preparation methods, such as solvent-assisted flavour evaporation (SAFE), high-vacuum distillation (HVD) and cold finger distillation (CF), were compared. Dairy spread extract, dairy and non-dairy sour cream samples were analysed. GC × GC system consisted of capillary columns (first

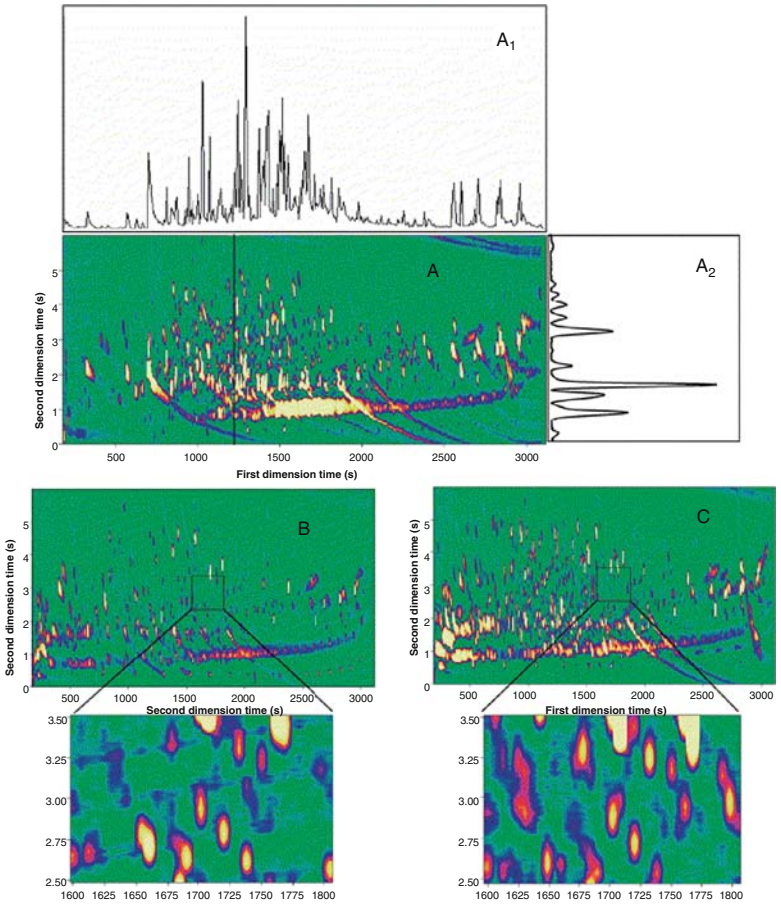

Fig. 2.19 Details of full-scan (*m/z* 40–400) GC×GC–TOF-MS chromatograms of sour cream extracts. (**A**) CF distillation of a non-dairy sour cream extract with (**A_1**) its reconstructed 1D chromatogram and (**A_2**) the intersection across the second dimension of the plane of the marked region; (**B**) SAFE of the same non-dairy sour cream extract and (**C**) SAFE of a dairy sour cream extract. Blow-ups of the (identical) marked areas in (**B**) and (**C**) are also shown; they were generated by using a different contrast. Reprinted with permission from Adahchour et al. (2003)

dimension column, 15 m × 0.25 mm, film thickness, 0.25 μm, second-dimension column, 10.8 m × 0.1 mm, film thickness, 0.1 μm). Temperature program was identical for the two columns: started at 50°C (4 min hold), 5°C/min 280, final hold 3 min. The chromatograms of sour cream extracts and daily spread extracts are shown in Figs. 2.19 and 2.20, respectively. The chromatograms in the Figs. 2.19 and 2.20 demonstrate that the application of two-dimensional GC increases considerably the efficacy of separation, enhances sensitivity and facilitates identification of volatiles (Adahchour et al., 2003).

The influence of the temperature on the release of model aroma substances from dairy custard was followed by SPME-GC/FID. Ethyl butyrate, ethyl hexanoate and

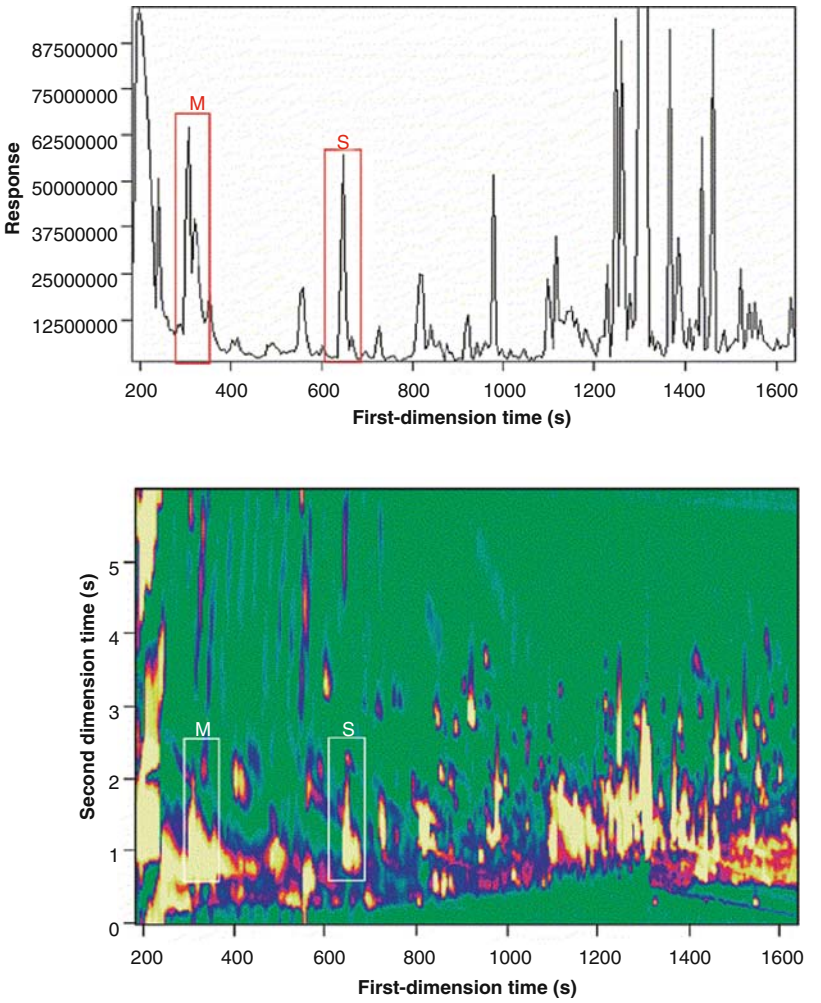

Fig. 2.20 Detail of the GC×GC–TOF-MS TIC chromatogram of a dairy spread extract: (*top*) reconstructed 1D-GC–TOF-MS and (*bottom*) GC×GC colour plot. Regions marked M and S are the elution regions of methional and sotolon, respectively. Reprinted with permission from Adahchour et al. (2003)

cis-3-hexenol were separated in a packed column (3 m × 2.2 mm i.d.). The results demonstrated that the aroma substances are adsorbed more strongly to the custard and then to the water and their adsorption markedly depends on the temperature (Seuvre et al., 2008). The concentration of diacetyl (2,3-butanedione) typical for butter aroma has been many times determined in milk, milk products and synthetic matrices (Haahr et al., 2000). A rapid GC method for the analysis of diacetyl in milk, fermented milk and butter has been recently published. Samples were mixed with acetone, centrifuged and the filtered supernatant was injected in GC without

other prepurification procedure. Separations were performed on a capillary column (30 m × 0.32 mm i.d., film thickness, 0.5 μm), injector and detector temperatures being 250° and 260°C, respectively. Temperature program started at 50°C and raised to 240°C at 7°C/min. Helium was employed as carrier gas. Analytes were detected by FID. A typical chromatogram is shown in Fig. 2.21. It was established that the diacetyl content of the samples shows high variation and the low coefficient of variation makes the method suitable for the quantitative determination of diacetyl in milk and milk products (Macciola et al., 2008).

The overwhelming majority of studies dealing with the analysis of aroma compounds in dairy products is concentrated on various cheeses. These phenomena can be explained by the fact that the taste and flavour of cheese play a decisive role in its commercial value. The role of metabolic activity of bacteria (glycolysis, lipolysis and proteolysis) and the catabolism of amino acids in the formation of characteristic cheese flavour have been previously reviewed (Marilley and Casey, 2004).

Dynamic headspace extraction combined with GC-MS was applied for the characterisation of the "Fontina Valle d,Aosta", a protected designation of origin (PDO) cheese. Samples were extracted at 40°C for 30 min using a nitrogen flow of 60 ml/min. Volatiles were preconcentrated, then thermally desorbed and injected into the GC column (30 m × 0.25 mm, film thickness, 0.25 μm). Temperature program started at 35°C (8 min hold), 6°C/min to 60°C, 4°C/min to 160°C, 20°C/min to 200°C, final hold 1 min. Electron impact (EI) mass spectra were recorded at 70 eV ionisation energy. The volatile compounds identified in the cheese samples are listed in Table 2.32. It was established that the main components were alcohols (2-butanol), sulphur (dimethyldisulphide) and carboxylic compounds. The method was proposed for the authenticity test of this variety of cheese (Berard et al., 2007).

Another study applied headspace sorptive extraction (HSSE) coupled to thermal desorption unit and GC-MS for the separation and quantitative determination of volatile compounds in a mountain cheese. HSSE was performed by suspending a stir bar in a 20 ml headspace vial containing 2 g of grated cheese. Adsorption was carried out at 50°C for 60 min. After finishing adsorption, the stir bar was thermally desorbed, the analytes were cryofocused and injected in the capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started from 35°C to 120°C at 2°C/min, then to 280C at 5°C/min, final hold 5 min. Helium was employed as carrier gas at a flow rate of 1 ml/min. The relative abundance of aldehydes, ketones, free fatty acids, esters, alcohols, hydrocarbons and terpenes are listed in Table 2.33. It was concluded from the results that the method is suitable for the measurement of volatile analytes in this type of cheese and can be applied for the authenticity test (Panseri et al., 2008).

The cooperation between various lactococcal strains for the formation of cheese flavour has also been investigated. The aroma compounds in the samples were separated and quantitated by SPME coupled to GC-MS. The data illustrated that the combination of different *Lactococcus lactis* strains may result in higher amount of volatile flavour compounds (Amórita et al., 2006). Another study investigated the capacity of *Propionibacterium freudenreichii* to form volatile aroma compounds in Emmenta cheese. The volatiles were separated by GC-MS. The data demonstrated

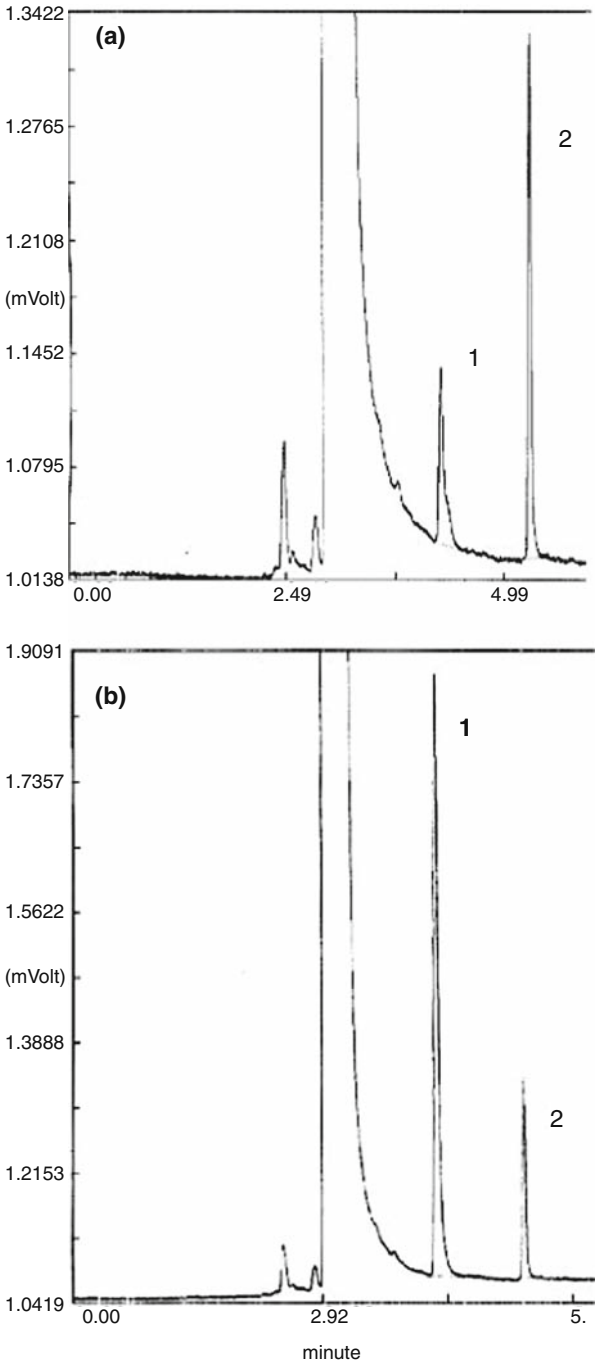

Fig. 2.21 Example of GLC-FID chromatograms of diacetyl in a UHT cow's milk: (**a**) without diacetyl added; (**b**) with diacetyl added (200 μg g^{-1}). The peaks are: no. 1. diacetyl; no. 2. 2,3-pentanedione (IS). Reprinted with permission from Macciola et al. (2007)

Table 2.32 Volatile compounds identified in the Fontina PDO samples ($n = 24$)

	ID[a]	KI_{calc}	KI_{tab}[b]	Occurrence[c]	Chromatographic response (peak area) mean ± Std. Dev[d] × 10^3
Linear aldehydes					
Butanal	MS; KI	880	878	16	4300 ± 4600
Pentanal	MS; KI	985	977	16	3600 ± 3800
Hexanal	MS; KI	1082	1080	24	34,000 ± 54,000
Heptanal	MS; KI	1191	1186	14	1300 ± 1500
Octanal	MS; KI	1290	1286	10	1600 ± 1800
Nonanal	MS; KI	1398	1396	9	1100 ± 1200
Branched-chain aldehydes					
2-Methylpropanal	MS; KI	812	814	23	690 ± 540
2-Methylbutanal	MS; KI	916	914	23	4200 ± 3200
3-Methylbutanal	MS; KI	920	917	24	18,000 ± 16,000
Ketones					
Acetone	MS; KI	819	814	24	7300 ± 6600
2-Butanone	MS; KI	907	901	24	330,000 ± 280,000
3-Buten-2-one	MS; KI	946	953	14	1500 ± 1700
2-Pentanone	MS; KI	979	980	10	20,000 ± 23,000
4-Methyl-2-pentanone	MS; KI	999	1008	12	1900 ± 1400
(?)-Penten-(?)-one	MS	1035		4	2300 ± 1100
2-Heptanone	MS; KI	1188	1185	12	7300 ± 1000
2-Nonanone	MS; KI	1392	1394	10	800 ± 1000
Diketones					
2,3-Butanedione	MS; KI	988	986	23	7600 ± 7500
2,3-Pentanedione	MS; KI	1069	1071	14	8000 ± 10,000

Table 2.32 (continued)

	ID[a]	KI_{calc}	KI_{tab}[b]	Occurrence[c]	Chromatographic response (peak area) mean ± Std. Dev[d] × 10^3
Primary alcohols					
Ethanol	MS; KI	937	932	24	25,000 ± 28,000
1-Propanol	MS; KI	1051	1052	24	30,000 ± 31,000
1-Butanol	MS; KI	1153	1152	19	14,000 ± 19,000
1-Pentanol	MS; KI	1261	1256	21	2000 ± 3000
1-Hexanol	MS; KI	1359	1354	13	1100 ± 1200
Secondary and tertiary alcohols					
2-Propanol	MS; KI	970	975	10	5700 ± 5600
2-Butanol	MS; KI	1039	1035	24	600,000 ± 350,000
3-Pentanol	MS; KI	1113	1112	5	1100 ± 1000
1-Penten-3-ol	MS; KI	1177	1176	10	1500 ± 1400
3-Hexanol	MS; KI	1194	1207	5	1800 ± 1800
2-Heptanol	MS; KI	1326	1334	17	900 ± 1200
Branched-chain alcohols					
2-Methyl-1-propanol	MS; KI	1107	1097	23	7000 ± 8000
3-Methyl-1-butanol	MS; KI	1222	1215	24	50,000 ± 80,000
2-Ethyl hexanol	MS; KI	1492	1492	4	540 ± 530
Ethyl esters					
Ethyl acetate	MS; KI	891	893	24	61,000 ± 51,000
Ethyl propanoate	MS; KI	959	957	24	14,000 ± 17,000
Ethyl isobutanoate	MS; KI	969	960	21	1700 ± 1700
Ethyl butanoate	MS; KI	1046	1040	22	17,000 ± 20,000
Ethyl isocaproate	MS	1197		6	3000 ± 3800
Ethyl hexanoate	MS; KI	1242	1238	23	2400 ± 3200
Ethyl heptanoate	MS; KI	1327	1331	5	1300 ± 1300
Ethyl octanoate	MS; KI	1439	1438	10	550 ± 470

Table 2.32 (continued)

	ID[a]	KI_{calc}	KI_{tab}[b]	Occurrence[c]	Chromatographic response (peak area) mean ± Std. Dev[d] × 10^3
Other esters					
Propyl acetate	MS; KI	977	976	11	11,000 ± 14,000
Butyl acetate	MS; KI	1085	1077	10	2200 ± 1500
Propyl butanoate	MS; KI	1133	1123	11	3200 ± 1900
Aromatic hydrocarbons					
Benzene	MS; KI	938	936	24	3100 ± 1500
Toluene	MS; KI	1043	1040	24	76,000 ± 59,000
Ethylbenzene	MS; KI	1121	1125	11	3100 ± 3700
p-Xylene	MS; KI	1129	1127	10	310 ± 250
m-Xylene	MS; KI	1136	1132	10	270 ± 260
o-Xylene	MS; KI	1175	1182	9	670 ± 720
Terpenes					
α-Pinene	MS; KI	1017	1010	24	6400 ± 6700
Terpene (not identfied)	MS	1027		10	1000 ± 570
Camphene	MS; KI	1060	1053	14	3500 ± 4700
β-Pinene	MS; KI	1102	1095	20	4500 ± 3500
Limonene	MS; KI	1193	1194	10	1000 ± 700
p-Cymene	MS; KI	1270	1266	4	1300 ± 890
α-Terpinolene	MS; KI	1280	1276	10	1100 ± 1000
Aliphatic hydrocarbons					
Hydrocarbon (not identfied)	MS	<800	<800	19	1900 ± 1600
Hydrocarbon (not identfied)	MS	<800	<800	10	2000 ± 2200
Octane	MS; KI	800	800	22	5500 ± 5500
2-Octene	MS; KI	843	846	23	5500 ± 4800

Table 2.32 (continued)

	ID[a]	KI_{calc}	KI_{tab}[b]	Occurrence[c]	Chromatographic response (peak area) mean ± Std. Dev[d] × 10^3
Sulfur compounds					
S-Methyl-thioacetate	MS	1056		20	3900 ± 4700
Dimethyl disulphide	MS; KI	1080	1075	24	130,000 ± 140,000
S-Methyl thiopropionate	MS	1131		8	3600 ± 4300
Dimethyl trisulphide	MS; KI	1381	1383	9	520 ± 670
Organic acids					
Acetic acid	MS	1480		5	240 ± 130
Propanoic acid	MS	1554		4	190 ± 150
Butanoic acid	MS	1630		4	100 ± 80
Furans					
2-Methyl furan	MS; KI	872	876	16	1900 ± 1700
2-Ethyl furan	MS; KI	950	945	21	700 ± 570
2-Pentyl furan	MS; KI	1236	1240	18	500 ± 290
Halogen compounds					
Halogen compound (not identfied)	MS	< 800	< 800	6	500 ± 100
Dichloromethane	MS; KI	933	927	14	600 ± 500
Chloroform	MS; KI	1020	1018	16	2400 ± 2000

[a]ID: MS = identification by comparison with NIST mass spectrum, KI = identification by comparison with Kovats indices.
[b]KI: identification by comparison with KI home-made database.
[c]Number of samples (out of 24) in which the component was detected.
[d]Referred to all the measurements performed on the 24 samples.
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Table 2.33 Relative abundance[1] (mean±SEM) of aldehydes, ketones, free fatty acids, and esters detected in the headspace of Bitto cheese samples made in different farms

Compound	Farm1 ($n=4$)	Farm2 ($n=3$)	Farm3 ($n=3$)	Farm4 ($n=2$)	Farm5 ($n=2$)	Odour description[2]	P
Relative abundance[1] (mean±SEM) of ketones detected in the headspace of Bitto cheese samples made in different farms							
3-Methyl-1-butanal	0.19±0.11	0.49±0.03	n.d.	n.d.	1.92±0.14	Mild, oil, butter	NS
pentanal	n.d.	n.d.	n.d.	n.d.	6.64±0.28	–	NS
2-Pentenal	0.31±0.05	0.27±0.05	0.35±0.13	0.51±0.22	1.18±0.01	Green, vegetable	NS
n-Hexanal	4.14±0.42	1.86±0.04	1.62±0.68	12.35±0.69	19.40±9.77	Herbaceous, woody	NS
2-Hexenal	0.37±0.17	n.d.	1.52±0.74	0.52±0.09	0.54±0.12	Fatty, grassy	NS
n-Heptanal	3.06^{a}±0.65	2.11^{a}±0.15	2.15^{a}±0.21	9.48^{b}±2.36	13.18^{b}±0.05	Sour milk, dairy, bitter almond	<0.05
Benzaldehyde	1.06±0.25	1.13±0.06	0.55±0.12	0.75±0.01	n.d.	Sweet	NS
1,4 Hexadienal	$1.48^{a,b}$±0.01	0.64^{a}±0.05	2.61^{b}±0.22	$1.43^{a,b}$±0.12	0.94^{a}±0.23	Fresh, green, Floral, cinnamon-like	<0.05
2,4 Heptadienal	1.43±0.23	0.65±±0.06	2.16±0.20	1.09±0.54	2.08±0.20	Nutty	NS
Benzenacetaldehyde	$0.93^{a,b}$±0.03	1.26^{b}±0.04	0.46^{a}±0.00	1.18^{b}±0.03	$0.83^{a,b}$±0.17	Almond-like, Nutty	<0.05
n-Octanal	0.28^{a}±0.04	0.19^{a}±0.02	0.35^{a}±0.04	0.90^{b}±0.04	1.52^{c}±0.01	Green, herbaceous	<0.05
2-Nonenal	1.88^{a}±0.87	0.52^{a}±0.02	0.93^{a}±0.15	8.97^{b}±0.04	8.22^{b}±0.45	Penetrant, fatty, waxy	<0.05
n-Nonanal	1.37±0.13	1.17±0.05	0.74±0.27	2.10±0.70	3.01±0.80	Floral, citrus, green	NS
2-Decanal	0.70±0.34	n.d.	0.17±0.06	1.20±0.02	2.16±0.11	Orange, fatty, fried	NS
2,4-Decadienal	1.62^{a}±0.47	0.54^{a}±0.03	1.63^{a}±0.23	1.77^{a}±0.12	5.76^{b}±0.19	Powerful, fatty	<0.05
n-Decanal	0.47±0.12	n.d.	n.d.	0.13±0.08	0.09±0.05	Penetrant, sweet, waxy, floral, citrus	NS
n-Hexadecanal	0.50±0.26	0.45±0.07	0.45±0.05	n.d.	0.06±0.04	Waxy, floral	NS
Relative abundance[1] (mean±SEM) of ketones detected in the headspace of Bitto cheese samples made in different farms							
2-Pentanone	2.29±0.37	1.67±0.03	2.01±0.27	0.17±0.10	n.d.	Sweet, floral, ethereal	NS
3-Hydroxy-2-butanone	n.d.	n.d.	n.d.	n.d.	3.10±0.39	Buttery	NS
2-Heptanone	12.33±2.61	18.26±4.92	13.27±3.44	6.01±2.15	6.44±1.47	Blue cheese, spicy	NS
8-Nonen-2-one	0.58±0.18	0.68±0.27	0.74±0.15	0.24±0.08	0.59±0.13	–	NS
2-Nonanone	9.88±2.67	15.99±3.46	10.54±3.36	6.20±2.34	6.31±1.71	Fruity, floral	NS
2-Undecanone	5.34±0.28	6.41±0.27	3.98±1.18	4.17±0.42	5.96±0.29	Citrus, rose, iris	NS
2-Tridecanone	3.03±0.36	4.90±0.73	4.85±0.21	2.26±0.16	3.74±0.18	Warm, herbaceous	NS
Diphenylmetanone	0.28^{a}±0.04	0.74^{b}±0.02	$0.38^{a,b}$±0.09	0.12^{a}±0.07	0.15^{a}±0.09	–	<0.05

Table 2.33 (continued)

Compound	Farm1 ($n=4$)	Farm2 ($n=3$)	Farm3 ($n=3$)	Farm4 ($n=2$)	Farm5 ($n=2$)	Odour description[2]	P
2-Pentadecanone	1.16±0.28	1.76±0.30	2.01±0.12	0.79±0.02	2.00±0.14	Delicate musk	NS
5,9-Undecadien-2-one	0.24±0.01	n.d.	0.33±0.06	0.09±0.05	n.d.	–	NS
Relative abundance[1] (mean±SEM) of free fatty acids detected in the headspace of Bitto cheese samples made in different farms							
Butanoic acid	6.16±2.49	7.04±0.05	5.32±1.55	5.32±0.33	1.24±0.19	Sharp, cheesy	NS
Pentanoic acid	1.89±0.20	0.23±0.06	1.84±0.53	0.07±0.04	n.d.	Putrid, sweety, rancid	NS
Hexanoic acid	4.37±0.54	3.24±0.32	5.20±1.31	2.18±0.03	3.87±0.80	Sickening, sour	NS
Octanoic acid	1.22±0.29	2.26±0.06	2.49±1.05	0.82±0.23	1.87±0.53	Unpleasant, oily	NS
Decanoic acid	3.21±0.26	2.52±1.46	2.16±1.16	1.42±0.19	3.66±0.24	Fatty	NS
Dodecanoic acid	0.92±0.34	0.25±0.14	0.82±0.03	0.39±0.12	n.d.	Fatty	NS
Tetradecanoic acid	0.05±0.03	n.d.	0.16±0.03	n.d.	0.05±0.03	Faint, waxy, oily	NS
Tetradecenoic acid	1.43^{a}±0.83	5.36^{b}±0.07	1.30^{a}±0.08	2.64^{a}±0.38	0.17^{a}±0.04	–	<0.05
Hexadecanoic acid	3.83±0.74	0.67±0.19	2.96±0.04	4.34±0.21	n.d.	Virtually, odourless	NS
Octadecanoic acid	0.66±0.38	1.16±0.18	0.92±0.11	21.76±0.08	n.d.	Odourless	NS
3-Methylbutanoic acid	3.05±0.31	n.d.	1.30±0.02	1.24±0.02	0.32±0.01	–	NS
2-Methylbutanoic acid	1.59±0.13	0.91±0.13	0.35±0.20	n.d.	0.09±0.05	–	NS
Relative abundance[1] (mean±SEM) of esters detected in the headspace of Bitto cheese samples made in different farms							
Ethylacetate	7.00±4.04	5.45±0.06	24.69±0.43	n.d.	n.d.	Fruity, fragrant, banana–pineapple	NS
Methylbutyrate	1.99±0.15	0.69±0.40	1.36±0.01	0.27±0.04	n.d.	Fragrant, ethereal, sweet	NS
Ethylbutanoate	10.65±4.33	4.35±0.55	4.52±2.05	12.40±5.59	n.d.	Sharp, cheesy	NS
Propylbutanoate	0.13±0.08	0.44±0.09	0.32±0.00	n.d.	n.d.	Fruity, sour	NS
Methylpentanoate	0.30±0.08	0.59±0.29	0.35±0.05	0.10±0.06	0.24±0.06	Cheese, parmesan	NS
Methylhexanoate	2.49^{a}±0.29	2.05^{a}±1.18	2.13^{a}±0.20	17.08^{b}±1.07	1.00^{a}±0.13	Pineapple, ethereal	<0.05
Ethylhexanoate	7.72±1.23	5.33±0.92	4.96±0.59	2.86±0.08	1.70±0.11	Sickening, sour	NS
Propylhexanoate	0.17±0.10	0.16±0.09	0.10±0.06	0.18±0.04	0.00	Wine-like, cheese	NS
Methyloctanoate	0.43±0.01	0.37±0.21	0.17±0.10	0.25±0.00	0.37±0.09	Green, fruity	NS
Ethyloctanoate	2.89±0.59	1.36±0.05	1.92±0.37	1.12±0.25	0.41±0.06	Unpleasant, oily	NS

Table 2.33 (continued)

Compound	Farm1 ($n=4$)	Farm2 ($n=3$)	Farm3 ($n=3$)	Farm4 ($n=2$)	Farm5 ($n=2$)	Odour description[2]	P
Ethyldecanoate	1.65±0.53	0.69±0.19	1.45±0.02	0.10±0.04	0.21±0.05	Fatty	NS
Ethyldodecanoate	1.52±0.57	0.11±0.06	1.14±0.28	n.d.	0.06±0.03	Fatty	NS
Ethyltetradecanoate	0.33±0.08	0.11±0.07	0.27±0.09	0.07±0.04	0.03±0.02	Mild waxy, soapy	NS
Methyl hexadecanoate	n.d.	1.73±0.63	1.83±0.42	0.07±0.04	n.d.	Virtually, odourless	NS
Methyl octadecanoate	n.d.	0.28±0.16	0.75±0.34	0.04±0.02	n.d.	Virtually, odourless	
Relative abundance[1] (mean±SEM) of alcohols detected in the headspace of Bitto cheese samples made in different farms							
Ethanol	1.57±0.91	n.d.	n.d.	n.d.	n.d.	–	NS
2-Butanol	5.04±2.91	5.02±0.03	n.d.	n.d.	n.d.	Medicinal	NS
1-Butanol	1.60±0.52	3.23±0.88	1.80±0.56	0.99±0.07	1.61±0.66	Medicinal	NS
2-Pentanol	1.23±0.71	n.d.	n.d.	n.d.	n.d.	Mild green, fusel oil	NS
3-Methyl-1-butanol	2.34±0.40	2.35±0.01	1.72±0.03	n.d.	n.d.	Herbaceous, hearthy, oily	NS
2-Methyl-1-butanol	0.88±0.21	0.95±0.16	0.27±0.16	n.d.	n.d.	–	NS
2-Furanmethanol	0.79±0.02	1.26±0.05	0.00	0.00	1.21±0.01	–	NS
1-Hexanol	$0.31^{a,b}$±0.01	0.04^{a}±0.02	$0.41^{a,b}$±0.05	0.77^{b}±0.13	$0.51^{a,b}$±0.04	herbaceous, fragrant, green, woody	<0.05
2-Heptanol	n.d.	n.d.	n.d.	0.07±0.04	n.d.	Earthy, Oily	NS
Phenylethylalcohol	1.59^{a}±0.27	0.45^{b}±0.08	0.33^{b}±0.01	0.12^{b}±0.07	0.09^{b}±0.05	–	<0.05
Benzenethanol	0.34±0.02	2.58±0.14	n.d.	0.13±0.08	n.d.	–	NS
Relative abundance[1] (mean±SEM) of hydrocarbons detected in the headspace of Bitto cheese samples made in different farms							
Toluene	2.30±0.41	0.93±0.54	n.d.	0.71±0.10	n.d.	–	NS
Methylbenzene	1.81±0.45	2.94±0.37	1.02±0.58	1.37±0.30	1.15±0.11	Fruity, fragrant	NS
Ethylbenzene	0.81±0.23	1.29±0.09	0.82±0.21	0.35±0.05	0.40±0.06	Heavy, floral	NS
p-Xylene	1.68±0.33	1.14±0.57	1.38±0.14	0.98±0.24	1.36±0.42	–	NS
Benzene1,4 dimethyl	0.24±0.09	0.39±0.22	0.46±0.26	n.d.	0.02±0.01	Sweet, almond, cherry, spicy	NS
Tetradecane	0.58±0.24	0.82±0.32	0.77±0.07	9.92±5.62	0.27±0.08	–	NS
2-Hexadecene	1.16±0.46	1.75±0.62	2.03±0.44	0.04±0.02	0.65±0.06	–	NS
Naphthalene	0.99±0.18	0.88±0.04	3.46±1.23	0.48±0.00	0.45±0.13	–	NS

Table 2.33 (continued)

Compound	Farm1 ($n=4$)	Farm2 ($n=3$)	Farm3 ($n=3$)	Farm4 ($n=2$)	Farm5 ($n=2$)	Odour description[2]	P
Relative abundance[1] (mean±SEM) of terpenes detected in the headspace of Bitto cheese samples made in different farms							
α-Pinene	1.02±0.24	1.31±0.76	1.55±0.25	0.14±0.08	0.47±0.08	Sharp, pine	NS
Camphene	0.43±0.25	0.17±0.10	n.d.	0.29±0.10	0.84±0.05	Camphoraceous	NS
Sabinene	0.47±0.27	0.90±0.52	0.73±0.42	0.61±0.03	1.26±0.08	–	NS
β-Pinene	0.70±0.40	0.62±0.36	1.94±0.05	1.08±0.03	n.d.	Woody, pine	NS
β-Myrcene	0.98±0.41	1.09±0.63	1.24±0.06	0.30±0.02	1.55±0.19	Sweet, balsamic, plastic	NS
Cumene	0.17±0.10	0.46±0.26	0.51±0.04	0.25±0.02	n.d.	Pungent, acid	NS
δ-3-Carene	0.56±0.09	0.21±0.12	0.42±0.06	0.39±0.09	0.29±0.09	–	NS
Limonene	1.26±0.32	1.56±0.26	1.13±0.21	0.83±0.13	0.77±0.17	Warm, spearmint	NS
γ-Terpinene	0.34±0.04	0.19±0.11	0.20±0.11	0.05±0.03	0.37±0.22	Herbaceous, citrus	NS
Verbenol	0.33±0.19	0.23±0.13	n.d.	n.d.	1.21±0.13	Spicy, minty, camphoraceous	NS
Trans-β-caryophyllene	0.20^{a}±0.01	0.06^{a}±0.03	0.46^{b}±0.02	0.15^{a}±0.01	0.11^{a}±0.06	Terpene odour, woody, spicy	

Means within rows with different superscripts are significantly ($P<0.05$). NS, not significant.
[1] Relative abundance expressed as percentage on total volatile compounds detected.
[2] Odour description from *Flavors & Fragrances*, Aldrich International Edition 2003–2004.
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that propionibacteria exert a considerable influence on the formation of aroma substances, increasing the amount of short-chain carboxylic acids (acetic, propionic, butanoic, hexanoic, isovaleric acids), esters of acetic and propionic acids, ketones and alcohols (Thierry et al., 2004). The rheological properties and aroma profile of the Egyptian Ras cheese were determined too. Volatiles were concentrated by thermal desorption cold-trap (TDCT) and separated by GC-MS. The method identified 68 volatile substances, 13 alcohols, 11 aldehydes, 17 ketones, 25 esters and 4 other compounds. The concentration of aroma compounds depended considerably on the ripening time of the cheeses. A good relationship was found between the chromatographic data and organoleptic descriptors (Ayad et al., 2004). The influence of a hygienised rennet paste and a defined strain of starter on the volatile substances in Majorero goat cheese was investigated by using static headspace extraction and GC-MS. The measurements indicated that both the starter culture and the hygienised rennet paste exert a marked effect on the volatile fraction (Castillo et al., 2007). Another study analysed the water-soluble extract of goat cheese using TLC-FID and GC-MS technologies. The measurements found 43 volatile compounds, the short-chain FFAs being the most abundant (Engel et al., 2002).

A complex analytical program was applied for the characterisation of Kufu cheese, a mould-ripened variety. Urea-polyacrylamide gel electrophoresis (urea-PAGE) was employed for the study of the proteolysis of the pH 4.6-insoluble fraction. Peptide profiles of the samples were measured by RP-HPLC and the volatiles were separated and identified by SPME-GC-MS. GC measurements were performed on a capillary column (50 m × 0.2 mm i.d., film thickness, 0.33 μm). Temperature program started at 40°C (2 min hold), ramped to 170°C at 5°C/min 1 min hold), then to 240°C at 10°C/min. MS detection range was 33–450 *m/z*. Urea PAGE electrophoregrams are shown in Fig. 2.22. Urea-PAGE measurements indicated that degradation rate of α_{s1} casein was considerably higher than that of β casein. Peptide profiles of some samples are depicted in Fig. 2.23. The chromatograms illustrated that the peptide profiles of the samples show marked differences. GC-MS separated and identified 138 volatile substances. Ketones and alcohols were present in

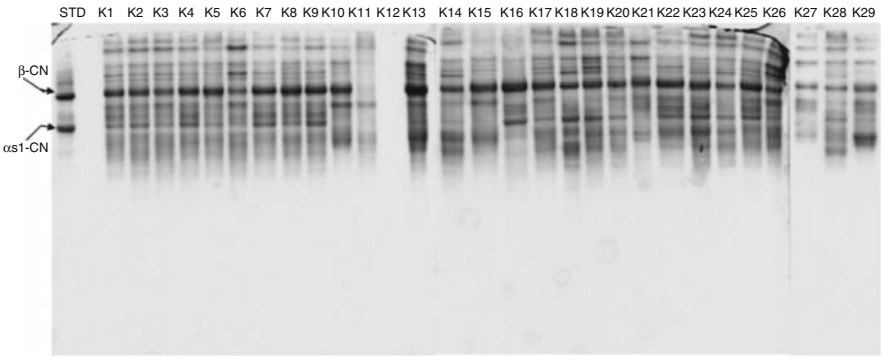

Fig. 2.22 Urea-polyacrylamide gel electrophoretograms of sodium caseinate (STD) and the pH 4.6-soluble fractions of Kuflu cheese samples (K1–K29). Reprinted with permission from Hayaloglu et al. (2008)

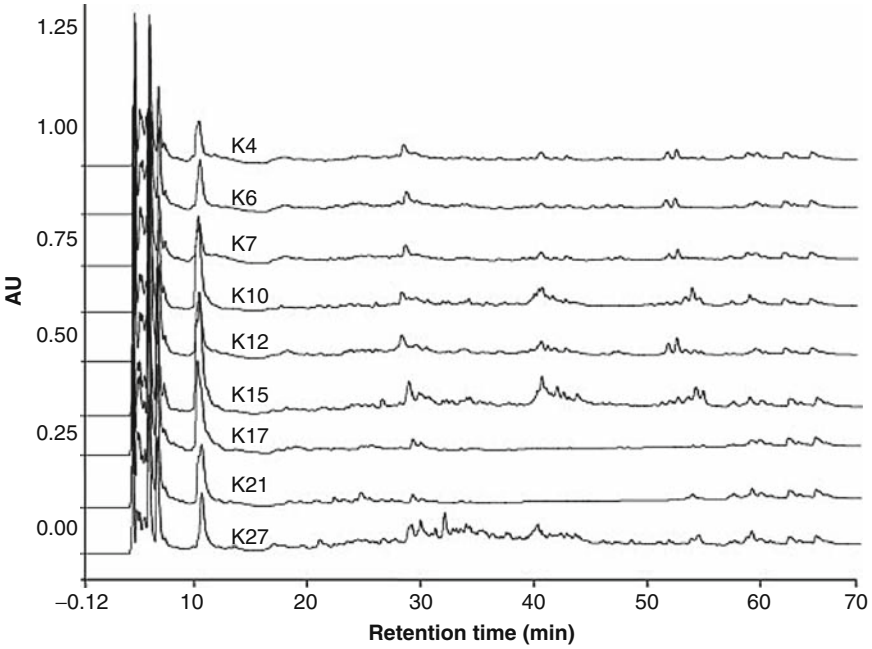

Fig. 2.23 RP-HPLC peptide profiles of the pH 4.6-soluble fractions from randomly chosen samples of Kuflu cheese. Reprinted with permission from Hayaloglu et al. (2008)

the highest amount followed by terpenes and sulphur compounds. The quantity of aldehydes and lactones was fairly low (Hayaloglu et al., 2008).

The culture-independent fingerprinting technique PCR and denaturing gradient gel electrophoresis (DGGE) were employed for the investigation of microbial dynamics during the manufacture of Pecorino Siciliano cheese. Some DGGE electrophoregrams are shown in Figs. 2.24 and 2.25. The electrophoregram illustrates the diversity of the strains participating in the ripening of Pecorino Siciliano. The presence of *Lactococcus lactis, Streptococcus thermophius, Enterococcus faecalis, Leucunostoc mesenteroides* was established. The dominance of *Strepcococcus bovis* and *Lactococcus lactis* species in the cheeses prepared by the traditional technology was also demonstrated (Randazzo et al., 2006).

The composition and sensory characteristics of Halloumi cheese kept in brine was investigated in detail. Lactose and organic acid were measured by HPTLC on an ion-exchange column (300×7.8 mm). Separations were carried out isocratically at 35°C using 5 mM H_2SO_4 as mobile phase. Analytes were detected by a refractive index detector. Volatiles were measured by GC on a capillary column ($60\,m \times 0.25$ mm i.d., film thickness, $0.25\,\mu m$). Temperature program started at 35°C (3 min hold), ramped to 80°C at 4°C/min (12 min hold), then to 200°C at 7°C/min (6 min hold). MS detection range was 33–450 *m/z*. Electron energy was 70 eV. FFAs were determined by GC-FID, column dimensions were: $30\,m \times 0.25$ mm i.d., film thickness, $0.25\,\mu m$). Column temperature started at

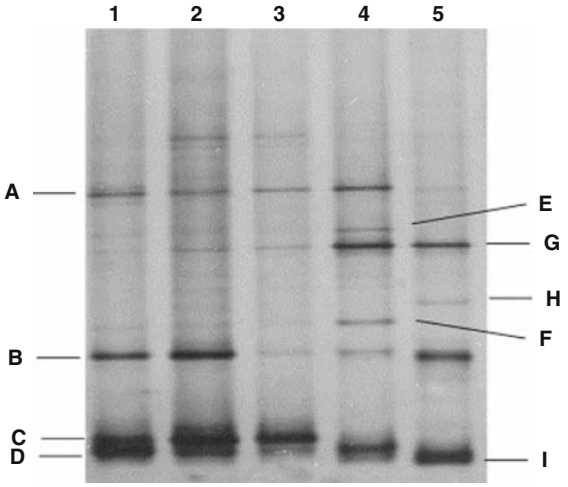

Fig. 2.24 DGGE of PCR products of the V6 to V8 regions of the 16S rDNA of samples taken during artisanal Pecorino Siciliano cheese manufacturing. Lanes: 1, raw milk; 2, milk plus rennet; 3, curd; 4, curd after fermentation; 5, 15-day ripened cheese. Reprinted with permission from Randazzo et al. (2006)

60°C (2 min hold), ramped to 70°C at 1°C/min (12 min hold), then to 220°C at 10°C/min (18 min hold). The concentrations of volatiles found in cheeses are listed in Table 2.34. It was found that ethanol and acetic acid were the dominant aroma compounds. Palmitic and oleic acids were also present in considerable amount (Kaminarides et al., 2007).

RP-HPLC was applied for the investigation of the effect of viable cells and cell-free extracts of *Lactococcus lactis* and *Debaryomyces vanrijiae* on the formation of

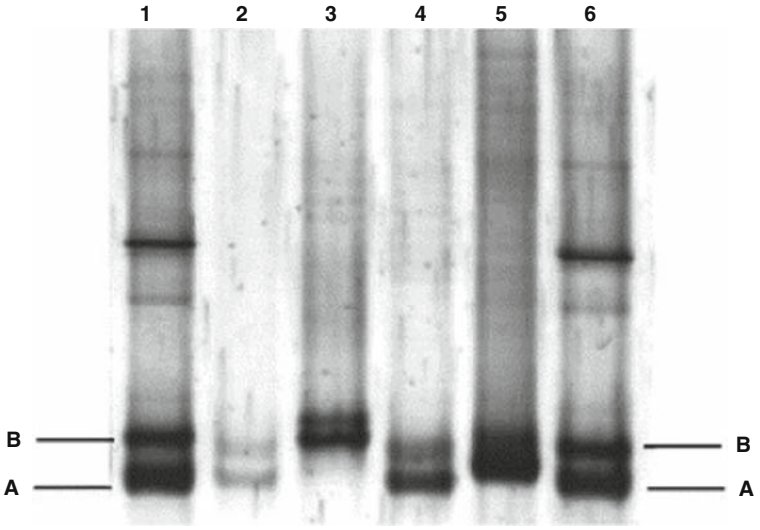

Fig. 2.25 DGGE separation patterns of PCR-amplified 16S rDNA segments derived from strains isolated from artisanal Pecorino Siciliano cheese. Lanes: 1, 15-day ripened cheese; 2, KF63 strain; 3, MF83 strain; 4, 17F73 strain; 5, SF73 strain; 6, 15-day ripened cheese. Reprinted with permission from Randazzo et al. (2006)

Table 2.34 Volatile aroma compounds of Halloumi cheese at 1, 15, 30 and 45 days (peak area $X10^3$ TIC in arbitrary units)

	Age of cheese (days)			
Volatile aroma compounds	1	15	30	60
Alcohols				
Ethanol	6686	4105	10107	19445
3-Methyl-1-butanol	1385	2596	580	1132
2-Methyl-1-propanol	257	–	–	–
Aldehydes				
Acetaldehyde	15	–	2037	16284
3-Methyl-butanal	455	31667	487	303
3-hydroxy-butanal	2054	1137	1745	–
Pentanal	10998	–	–	–
Heptanal	–	1193	–	–
Ketones				
Acetone	3674	2586	810	2267
3-Methyl-2-butanone	195	–	–	333
Diacetyl	473	638	483	827
3-hydroxy-2-butanone (acetoin)	–	435	417	261
Volatile acids				
Acetic acid	433	590	671	1105
2-Methyl-propanoic acid	–	–	154	150
Butanoic acid	228	–	177	1726
3-Methyl-butanoic acid	405	159	–	163
Hexanoic acid	202	–	357	1939
Esters				
Butanoic acid, methyl ester	–	–	–	3129
Hydrocarbons				
1-Chloro-3-methyl-butane	–	73	–	–
1-Methoxy-4[1-propenyl]-benzene	2038	222	835	3906
Sulphur compounds				
1-Propanethiol	–	1284	–	–
2-Methyl-2-propanethiol	612	–	–	–
2-Methyl-2-butanethiol	–	–	–	172
1-Pentanethiol	2445	3277	2485	3141
Other compounds				
Acetonitrile	1816	883	281	997

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FFAs in ovine, bovine and caprine milk fats. RP column was 250×4 mm, particle size, 5 μm. Measurements were carried out at 33°C using gradient elution (water, methanol, ACN). The results are depicted in Figs. 2.26, 2.27, and 2.28. The data indicated that the formation of FFAs depends on both the character of the natural microflora and the type of substrate (Regado et al., 2007).

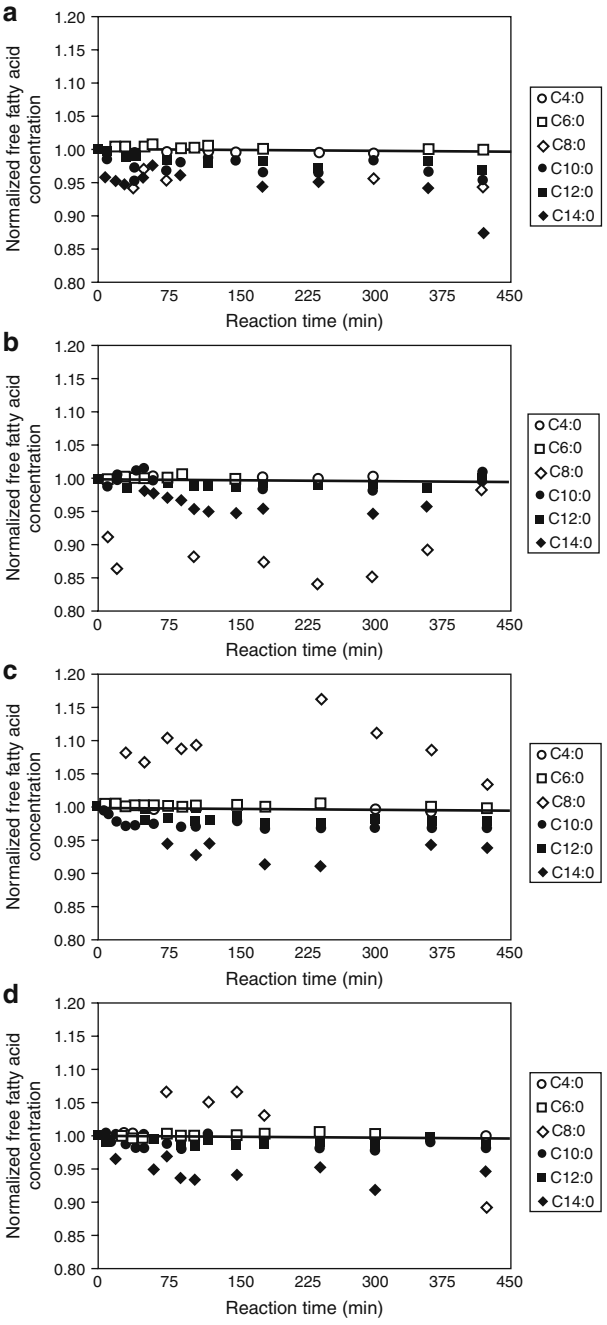

Fig. 2.26 Evolution of normalised free fatty acid concentrations in lipolysed bovine milk fat with reaction time, effected by *Lactococcus lactis* as (**a**) viable cells and (**b**) cell-free extract, and by *Debaryomyces vanrijiae* as (**c**) viable cells and (**d**) cell-free extract. Reprinted with permission from Regado et al. (2007)

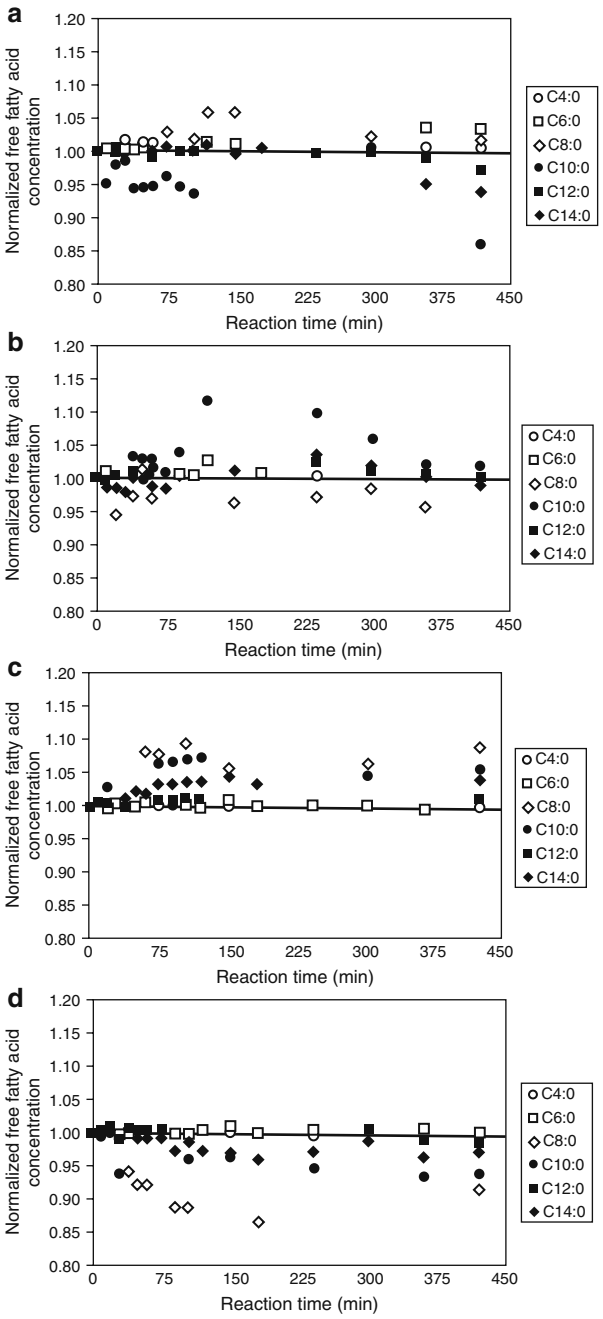

Fig. 2.27 Evolution of normalised free fatty acid concentrations in lipolysed ovine milk fat with reaction time, effected by *Lactococcus lactis* as (**a**) viable cells and (**b**) cell free extract, and by *Debaryomyces vanrijiae* as (**c**) viable cells and (**d**) cell-free extract. Reprinted with permission from Regado et al. (2007)

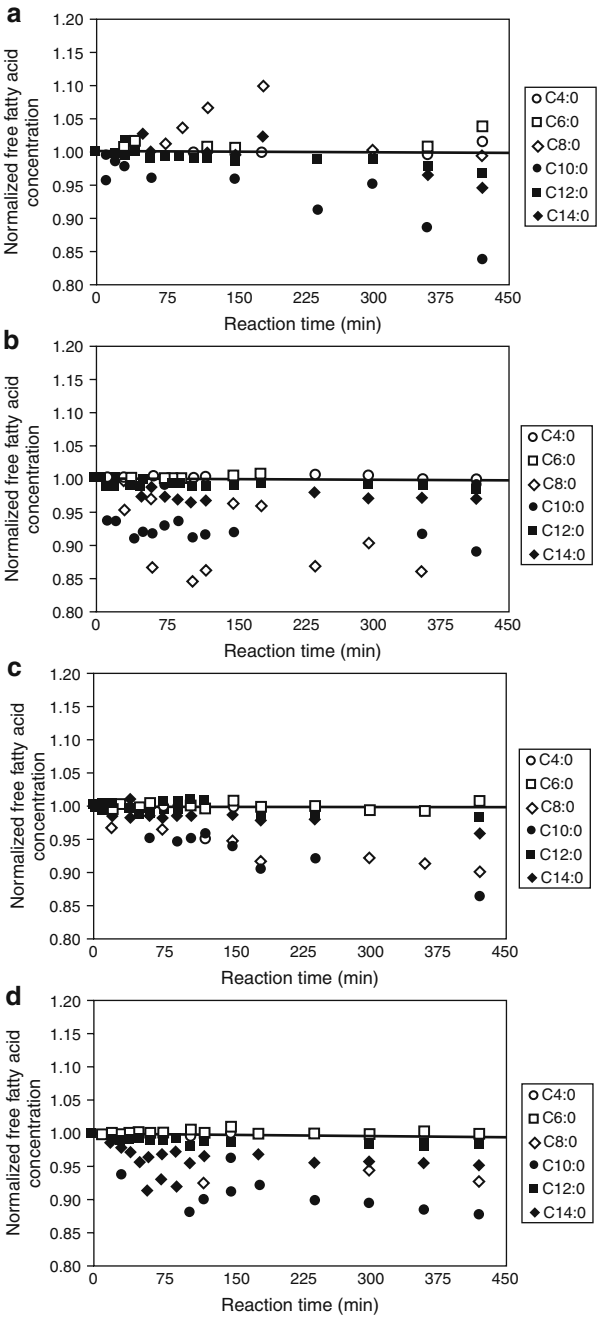

Fig. 2.28 Evolution of normalised free fatty acid concentrations in lipolysed caprine milk fat with reaction time, effected by *Lactococcus lactis* as (**a**) viable cells and (**b**) cell-free extract, and by *Debaryomyces vanrijiae* as (**c**) viable cells and (**d**) cell-free extract. Reprinted with permission from Regado et al. (2007)

2.7 Non-alcoholic Beverages

The fruit juice industry is one of the world's major businesses. As aroma substances influence the sensorial quality of fruit juices and other non-alcoholic liquid products, the separation and quantitation of volatiles in these classes of food products have been extensively investigated. Also in the case of fruit juices the method of preference for the analysis of aroma compounds is the HS-SPME followed by GC-MS of by GC-FID. The conditions of headspace microextraction (HP-SPME) were studied in detail for the optimisation of the measurement of the main volatile compounds in orange (*Citrus sinensis*) beverage emulsions. The efficacy of SPME fibres (PDMA, 100 μm; CAR/PDMAS, 75 μm; PDMS/DVB, 65 μm and DVB/CAR/PDMS 50/30 μm), the adsorption temperature (25–45°C), adsorption time (5–25 min), sample concentration (1–100%), sample amount (5–12.5 g), pH (2.5–9.5), salt type (K_2CO_3, Na_2CO_3, NaCl, Na_2SO_4), salt concentration (0–30%) and mode of stirring were evaluated. Aroma compounds were separated by GC-FID and GC-electron ionisation time-of-flight mass spectrometry (TOFMS) using capillary column (30 mm length, i.d. 0.25 mm, film thickness, 0.25 μm). Temperature gradient began at 45°C for 5 min, increased to 51°C at 1°C/min, 5 min hold, then to 160°C at 5°C/min, then to 250°C at 12°C/min, final hold 15 min. Helium was employed as carrier gas, injector and detector temperatures were 250°C and 270°C, respectively. It was established that the optimal analytical conditions were: CAR/PDMS fibre, 45°C adsorption temperature for 15 min, 5 g of beverage emulsion diluted to 1:100, 15% w/w of NaCl with stirring, pH 4. The samples contained 14 aroma compounds (ethyl acetate, α-pinene, ethyl butyrate, β-pinene, 3-carene, myrcene, limonene, γ-terpinene, octanal, decanal, linalool, 1-octanol, neral and geranial). The ratio of limonene was 94.9%. Because of the low LOD and LOQ values, the good linearity and reliability, the method was proposed for the analysis of orange beverage emulsions (Mirhosseini et al., 2007).

The aroma substances in orange cultivar Kozan (Turkey) were also measured by extraction on a polymeric resin, eluted by pentane/dichloromethane and separated by GC-FID and GC-MS. Thirty four substances were detected and identified (7 esters, 2 aldehydes, 5 alcohols, 5 terpenes, 12 terpenols, 3 ketones). It was established that the main components of the aroma profile of orange juice are linalool, limonene, β-phellandrene, terpinene-4-ol and ethyl 3-hydroxy hexanoate (Selli et al., 2004).

The composition of odour-active compounds of hand-squeezed juices from different orange varieties was investigated by HS-SPME followed by GC-O, GC-FID and GC-MS. HS-SPME was performed with a DVB/CAR/PDMS fibre at 40°C for 30 min in stirred samples. GC measurements were carried out in a capillary column (30 m × 0.32 mm i.d., film thickness, 0.5 μm). Temperature gradient began at 70°C for 7 min, then ramped to 220°C at 4°C/min, final hold 20 min. Helium was employed as carrier gas. The odour-active compounds are compiled in Table 2.35. The measurements demonstrated that the amount of limonene is the highest in each varieties, changing from 90% to 97%. Besides limonene, the concentration of other four compounds (β-myrcene, methyl butanoate, α-pinene, ethyl hexanoate) contributed markedly to the aroma profile of the orange varieties (Arena et al., 2006).

Table 2.35 Area distribution (% – GC/FID data) of odour-active compounds present in the juices of different varieties of oranges

No.	LRI	Compounds[a]	Moro	Tarocco	Washington navel	Valencia late
1	993	Methyl butanoate	1.528	3.783	0.578	0.850
2	995	n.i.[b]	–	0.136	n.d.[c]	n.d.[c]
3	1030	α-Pinene	0.669	0.282	0.242	0.442
4	1049	Ethyl butanoate	0.317	1.227	0.192	0.106
5	1063	Ethyl 2-methyl butanoate	0.018	0.047	0.009	0.002
6	1099	Hexanal	–	0.510	0.221	0.017
7	1118	β-Pinene	0.018	0.043	0.010	0.019
8	1153	*Z*-3-hexenal	n.d.[c]	0.044	–	n.d.[c]
9	1175	β-Myrcene	4.828	2.395	0.801	3.651
10	1235	Limonene	91.91	90.12	97.36	94.35
11	1246	Ethyl hexanoate	0.477	0.674	0.389	0.377
12	1319	α-Terpinolene/octanal	0.005	0.293	0.016	0.018
13	1366	Hexanol	0.023	0.206	0.040	0.009
14	1411	Nonanal	0.007	0.018	0.024	0.004
15	1448	Ethyl octanoate	0.032	0.049	0.012	0.037
16	1473	n.i.[b]	0.050	0.012	0.018	0.072
17	1501	Decanal	n.d.[c]	0.016	0.015	n.d.[c]
18	1560	Linalool	0.032	n.d.[c]	0.003	0.006
19	1640	No peak	–	–	–	–
20	1704	n.i.[b]	0.056	0.133	0.070	0.031
21	1867	n.i.[b]	0.026	0.013	–	0.011
22	1887	n.i.[b]	–	–	–	–

[a] Identified by comparing LRI, mass spectra and odour note.
[b] Not identified.
[c] Not detectable.
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The impact of various technological steps and different additives on the composition of aroma substances have also been studied in detail. Thus, the effect of Arabic gum, xanthan gum and orange oil on flavour release from diluted orange beveraged emulsion was investigated by HS-SPME (CAR/PDMS fibre, extraction at 45°C for 15 min) coupled to GC-TOFMS and GC-FID. Analytes were separated on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature gradient began at 45°C for 5 min, then increased to 51°C at 1°C/min (5 min hold), to 160°C at 5°C at 5°C/min, to 250°C at 12°C, final hold 15 min. Helium was employed as carrier gas and the detector temperature was 270°C. Calculations indicated that the addition of 15.87% (w/w) Arabic gum, 0.5% (w/w) xanthan gum and 10% (w/w) orange oil minimalise the overall flavour release (Mirhosseini et al., 2008).

Another experimental setup was applied for the analysis of flavour and off-flavour substances in orange juice. Analytes were concentrated by pervaporation and separated by GC-MS using a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature gradient began at 30°C for 5 min, then increased to 130°C at 6°C/min, then to 155°C at 30°C/min, to 200°C at 50°C, final hold 5 min. Helium was employed as carrier gas and the detector temperature was gradually increased during the separation process. The concentration of the six aroma compounds in fresh hand-squeezed and frozen concentrated orange juice are listed in Table 2.36. It was found that the pervaporation method is suitable for the rapid and cheap separation of the aroma compounds and can be successfully coupled to GC-MS (Gómez-Ariza et al., 2004a).

A different GC method was employed for the measurement of aroma compounds (limonene, α-terpineol, carvone, γ-decalactone, ethyl 2-methylbutanoate, methyl 2-methylbutanoate) in orange and apple juices, and in concentrates. Measurements were performed on a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Oven temperature was 50°C for 3 min, then increased at 4°C. It was stated that the GC-MS results offer an objective possibility for the evaluation of the sensory characteristics of samples (Elss et al., 2007).

Table 2.36 Amounts (mg/l) of volatile constituents in fresh and processed orange juices

	Fresh hand-squeezed orange juice		Frozen concentrated orange juice	
Analyte	Mean[a]	Range	Mean[a]	Range
Ethyl butanoate	0.26	0.2–0.9	0.16	0.01–0.6
α-Pinene	0.55	0.4–1.3	1.55	0.6–1.9
Limonene	61.9	29–80	151	99–256
Linalool	0.69	0–1.9	0.80	0.3–1.6
α-Terpineol	0.19	0.05–1.9	0.39	0–1.7
Citral-a geranial–b neral	0.20	0.1–0.5	0.50	0.06–0.6

[a] Fifteen samples of each type of orange juice; internal standard: *n*-dodecanol.
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Table 2.37 Volatile compounds in Jonagold apple juice

Volatile name[a]	KI[b]	Odour property	Odour threshold[c] (mg/l)	Concentration (mg/l)[d]	Control juice[e]	Ascorbic acid juice[f]	Aroma value[g]
Ester							
Propyl acetate	973	Strong-sweet	2(1)	0.03 ± 0.01	0.04 ± 0.02	0.0	0.0
2-Methylpropyl acetate*	1024	Sweet, fresh	0.065(1)				
Ethyl butanoate	1041	Sweet, fruity	0.001(1)	0.17 ± 0.06	0.19 ± 0.05	170	190
Butyl acetate	1071	Sweet, fruity	0.066(1)	1.37 ± 0.15	1.42 ± 0.22	21	22
2-Methylbutyl acetate	1121	Fresh	0.011(1)	0.18 ± 0.05	0.17 ± 0.04	16	15
Pentyl acetate	1173	Fruity, fresh	0.043(1)	0.02 ± 0.01	0.03 ± 0.01	0.5	0.7
Butyl butanoate	1210	Fresh	0.1(1)	0.37 ± 0.31	0.43 ± 0.36	3.7	4.3
2-Methylbutyl butanoate	1253	Fresh		0.03 ± 0.03	0.04 ± 0.03		
Hexyl acetate	1273	Sweet, fruity	0.002(1)	4.34 ± 0.83	4.04 ± 0.69	2170	2020
Hexyl 2-methylbutanoate	1415	Pungent	0.022(2)	0.30 ± 0.05	0.30 ± 0.07	14	14
Hexyl hexanoate*	1620			0.03 ± 0.00	0.04 ± 0.01		
Aldehyde							
Hexanal	1080	Green, grassy	0.005(2)	0.07 ± 0.04	0.27 ± 0.06	14	54
trans-2-Hexenal	1215	Green, grassy	0.017(2)	0.16 ± 0.04	0.80 ± 0.08	9.4	47
Alcohol							
1-Butanol	1148	Light-fruity	0.5(1)	4.17 ± 2.29	5.87 ± 2.87	8.3	12
1-Pentanol	1238		4(1)	0.03 ± 0.01	0.03 ± 0.01	0.0	0.0
1-Hexanol	1356	Light-apple	0.5(1)	1.85 ± 1.39	2.10 ± 1.32	3.7	4.2
6-Methyl-5-hepten-2-ol	1455			0.07 ± 0.02	0.04 ± 0.01		
1-Octanol	1560		0.13(3)	0.04 ± 0.01	0.04 ± 0.01	0.3	0.3
3-Methylthio-l-propanol	1723			0.20 ± 0.09	0.16 ± 0.04		

Table 2.37 (continued)

Volatile name[a]	KI[b]	Odour property	Odour threshold[c] (mg/l)	Concentration (mg/l)[d]	Control juice[e]	Ascorbic acid juice[f]	Aroma value[g]
Hydrocarbon							
Undecane	1096	Light-sweet		0.07 ± 0.02	0.10 ± 0.02		
α-Farnesene	1766			0.04 ± 0.01	0.01 ± 0.01		
Acid							
Acetic acid	1442			2.03 ± 0.41	2.07 ± 0.49		
Phenol							
Methyl chavicol*	1663			0.07 ± 0.01	0.07 ± 0.00		

[a] Volatile compounds with * are tentatively identified compounds.
[b] Kovat Index.
[c] Odour thresholds-in-water (mg/l).
[d] Concentrations of volatile compounds (mg/l) were calculated relative to internal standard, cyclohexanol. Data show the means with standard errors of three replications.
[e] Concentration of the ascorbic acid in the control apple juice was 0% w/v.
[f] Concentration of the ascorbic acid in the apple juice was 0.2% w/v.
[g] Aroma values were calculated from the ratios of the volatile concentrations to the odour thresholds.
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The effect of ascorbic acid on the sensory characteristics of cloudy apple juice was investigated by liquid–liquid direct extraction using the mixture of diethyl ether-pentane (1:1, v/v) and by the separation of analytes by GC-FID and GC-MS. Measurements were performed on a capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was 40°C for 3 min, then increased at 3°C/min to 230°C. The volatile compounds found in the control and treated juices are compiled in Table 2.37. Sensory evaluation revealed that the odour values of the aroma substances is higher in the apple juice samples containing ascorbic acid (Komthong et al., 2007).

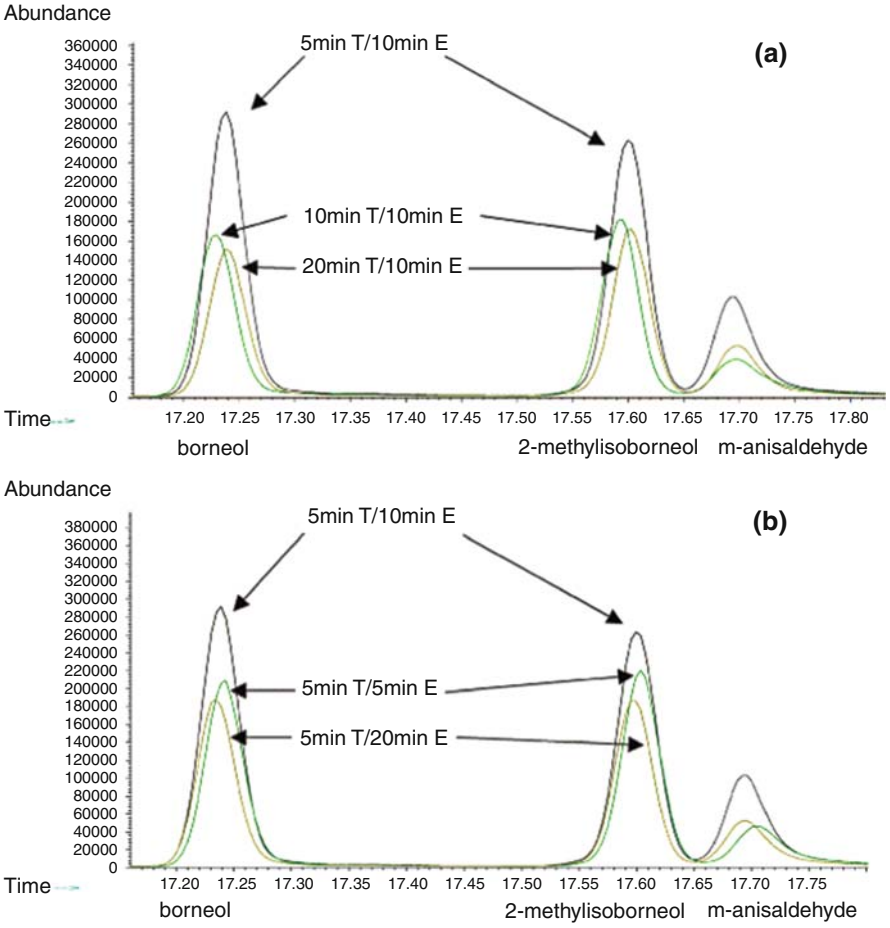

Fig. 2.29 GC–MS chromatograms of borneol, 2-methylisoborneol and *m*-anisaldehyde (0.1 mg L^{-1}) at a sample temperature of 60°C: (**a**) different thermostating times (*T*) (5, 10, 20 min); (**b**) different extraction times (*E*) (5, 10, 20 min); used fibre: DVB/CAR/PDMS. Reprinted with permission from Zierler et al. (2004)

Table 2.38 Characteristic mass/charge ratios, retention times (t_R) and retention indices (I) on HP5 for the GC–MS analysis

Compound	Formed by	Odour thresholds in water (μg/l)	*m/z*	T_R(min)	I(HP5)	Group
2,3-Dimethylpyrazine	*Actinomycetes*	400–2500 (33)	67, 108, 109	14.13	816.1	*I*
1-Octen-3-ol	*Actinomycetes*	0.005–100 (33)	57, 72, 85	15.50	878.9	*II*
3-Octanone	*Actinomycetes*	28–50 (33)	57, 72, 99	15.71	888.5	*I*
2-Isopropyl-3-methoxypyrazine	*Actinomycetes*	0.002–10 (33)		18.27	1015.0	*I*
3-Isopropyl-2-metoxypyrazine	*Actinomycetes*	0.002–10 (34)		18.27	1015.0	*II*
Fenchyl alcohol	*Actinomycetes*	–		18.94	1050.8	*II*
Borneol	*Actinomycetes*	140 (33)	95, 110, 139	20.13	1115.8	*I*
2-Isobutyl-3-methoxypyrazine	*Actinomycetes*	0.002–10 (33)	94, 124, 151	20.16	1117.5	*II*
α-Terpineol	*Actinomycetes*	4.6–350 (33)	93, 121, 136	20.54	1139.8	*I*
2-Methylisoborneol	*Actinomycetes*	0.002–0.1 (33)	95, 108, 135	20.57	1141.5	*II*
m-Anisaldehyde	*Actinomycetes*	50–200 (33)	107, 135, 136	20.70	1149.1	*I*
o-Anisaldehyde	*Actinomycetes*	50–200 (33)	77, 119, 136	21.61	1202.5	*II*
p-Anisaldehyde	*Actinomycetes*	50–200 (33)	77, 92, 135	21.87	1218.8	*II*
Geosmin	*Actinomycetes*	0,01–0.36 (33)	97, 112, 125	25.11	1431.2	*I*
Guaiacol	*A. acidoterrestris*	2–100 (33)	81, 109, 124	15.70	888.1	*III*
2,6-Dibromophenol	*A. acidoterrestris*	0.0005 (12)	63, 143, 252	20.77	1153.2	*III*

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The off-flavour substances produced by microorganisms (*Alicyclobacillus acidoterrestries* and *Actinomycetes*) in apple juice have also been investigated applying HS-SPME-GC-MS. Separations were performed on a capillary column (30 m × 0.25 mm i.d., film thickness, 1 μm). Oven temperature was 10°C for 1 min, then ramped at 8 or 10°C/min to 250°C. Typical chromatograms showing the separation of some volatile substances are depicted in Fig. 2.29. Volatile compounds produced by the microorganisms are compiled in Table 2.38. It was stated that the method is suitable for the sensitive detection of off-flavours in apple juice produced by microorganisms (Zierler et al., 2004).

Besides the very popular orange and apple juices, the aroma profile of some other juices has also been investigated. Thus, a HS-SPME GC-FID and HS-SPME GC-MS methods were employed for the measurement of the aroma profile of apricot, peach and pear juices. Analytes were extracted by HS-SPME at 40°C for 30 min with stirring. Measurements were performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Column temperature started at 60°C (5 min hold), ramped to 240°C at 3°C/min (10 min hold). The injector and detector temperatures were 250°C and 280°C, respectively. Analytes were identified by GC-MS in separate experiments. The chromatograms of apricot, peach and pear juices are depicted in Fig. 2.30. The chromatograms illustrate that a considerable number of volatiles can be detected in juices (37 compounds in apricot juice, 60 in peach and 49 in pear juice). It was stated that the method applied can be used for the differentiation between juices and can further detect the addition of flavourings (Riu-Aumatell, et al., 2004).

The volatile composition of fresh, clarified and fermented coconut sap was investigated by simultaneous distillation, solvent extraction and GC-MS. The main volatile components of the various products were: ethyl acetate, phenyl ethyl alcohol, ethyl lactate, 3-hydroxy-2-pentanone, farnesol, 2-methyl tetrahydrofuran, tetradecanone (fresh coconut sap); ethyl lactate, phenyl ethyl alcohol, 1-hexanol, 2-methyl tetrahydrofuran, 3-hydroxy-2-pentanone, 2-hydroxy-3-pentanone (clarified coconut sap) and ethyl lactate, phentl ethyl alcohol, farnesol (fermented coconut sap) (Borse et al., 2007).

The aroma substances have also been analysed in vinegars. The effect of technological steps used for the acceleration of the aging of wine vinegars was studied by SPME followed by GC. The composition of the volatile substances in the samples demonstrated that the addition of toasted oak chips increases the concentration of vanillin 20-fold (Morales et al., 2004). Another study compared stir bar sorptive adsorption (SBSE) and SPME for the preconcentration of volatile substances in vinegar. GC analyses were performed on a fused silica capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). Column temperature started at 35°C (10 min hold), ramped to 100°C at 5°C/min, to 210°C at 3°C (40 min hold). Carrier gas was helium, the detector temperature was set to 250°C. A total ion chromatogram is depicted in Fig. 2.31, showing the complexity of the aroma profile of vinegar. It was found that the detection and quantitation limits were lower for SBSE while the repeatabilty and reproducibility were better (Guerrero et al., 2007).

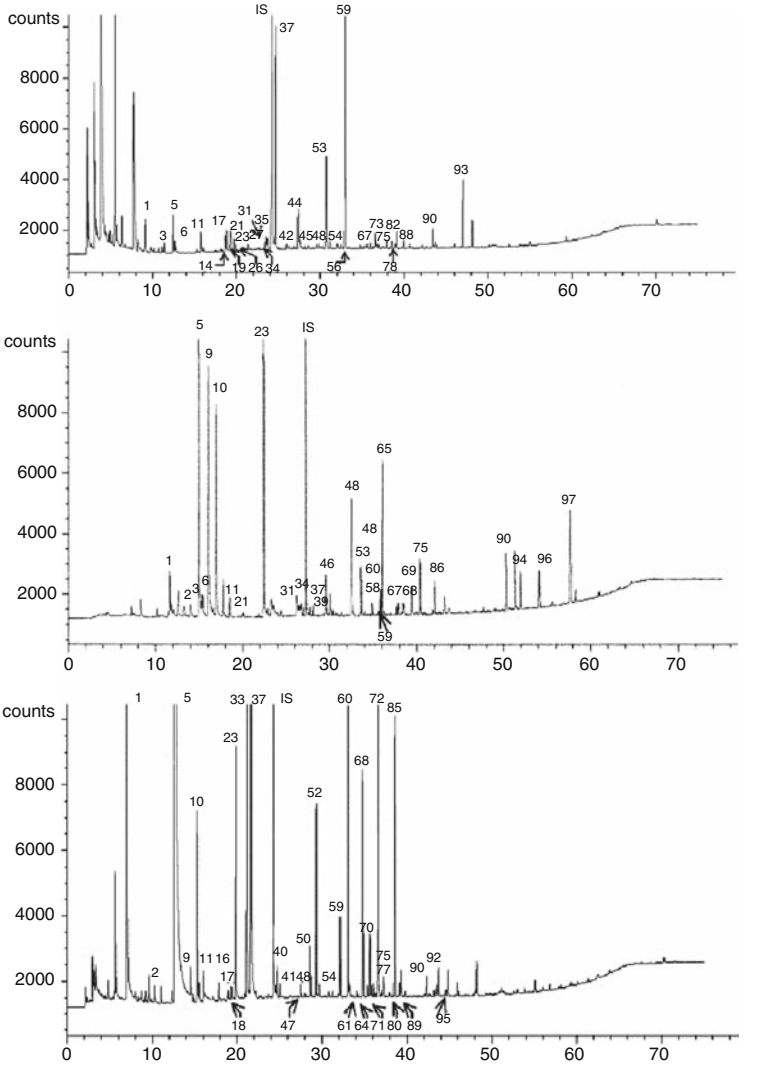

Fig. 2.30 Chromatogram of apricot sample obtained by CW column and FID detector. Chromatogram of peach sample. Peak identification: limonene (1), β-ocimene (3), isoamylbutyrate (4), hexylacetate (5), α-terpnolene (6), isoamylvalerate (7), 1-hexanol (11), butylhexanoate (14), hexyl butyrate (17), hexyl isovalerate (19), 3,8,8-Trimethyltetrahydronaphthalene (21), 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene (22), ethyl octanoate (23), acetic acid (26), vitispirane (31), linalool (37), hexyl hexanoate (44), 1,2,3,4-tetrahydro-1,6,8-trimethyl naphthalene (46), ethyl decanoate (48), α-terpineol (53), estragole (58), geranyl acetate (60), benzyl acetate (65), geraniol (67), β-damascenone (68), 1,2-dihydro-1,4,6-trimethylnaphthalene (69), anthole (70), ethyl dodecanoate (75), α-ionone (82), cinnamaldehyde (88), ethyl teteradecanoate (90), amyl benzoate (92), γ-decalactone (93), methyl tetradecadienoate (95), γ-undecalactone (96), γ-dodecalactone (97). Reprinted with permission from Riu-Aumatell et al. (2004)

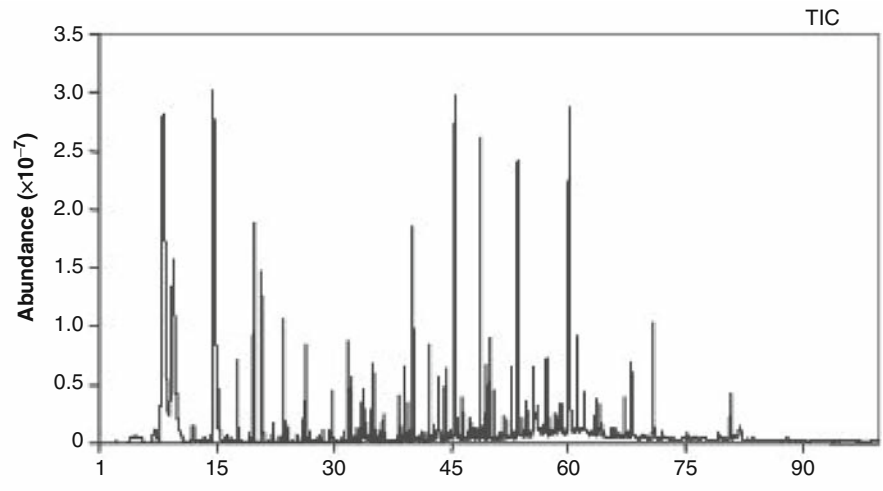

Fig. 2.31 Total ion chromatogram obtained for a vinegar sample by means of SBSE. Retention times (min): ethyl isobutyrate (13.62); propyl acetate (13.99); isobutyl acetate (15.76); ethyl butyrate (16.84); *n*-butyl acetate (18.38); ethyl isopentanoate (18.46); hexanal (18.70); isobutanol (19.71); isopentyl acetate (20.57); ethyl pentanoate (20.77); 1-butanol (21.84); *trans*-2-hexenal (24.01); isoamyl alcohol (23.84); 2-methyl-1-butanol (24.12); ethyl hexanoate (24.65); hexyl acetate (25.80); 3-hydroxy-2-butanone (26.62); *cis*-3-hexenyl acetate (27.59); ethyl lactate (28.51); hexan-1-ol (28.87); *cis*-3-hexen-1-ol (30.04); *trans*-2-hexen-1-ol (30.82); ethyl octanoate (31.87); 2-furaldehyde (32.87); benzaldehyde (35.15); isobutyric acid (36.84); 5-methyl-2-furaldehyde (36.95); 2-acetyl-5-methylfuran (38.54); butyric acid (38.89); isovaleric acid (40.28); diethyl succinate (40.58); ∗-terpineol (41.51); benzyl acetate (42.64); ethyl-2-phenyl acetate (44.59); phenylethyl acetate (45.95); hexanoic acid (46.57); benzyl alcohol (47.03); 2-phenylethanol (49.21), 2-ethyl hexanoic acid (50.17); 4-ethylguaiacol (52.87); octanoic acid (53.75); eugenol (57.21); 4-ethylphenol (57.36); 5-acetoxymethyl-2-furaldehyde (58.00); decanoic acid (60.39); diethyl ftalate (63.87); 5-hydroxymethyl-2-furaldehyde (68.90). Reprinted with permission from Guerrero et al. (2007)

2.8 Alcoholic Beverages

2.8.1 Wines

Because of their considerable commercial value, the aroma profile and the origin and identification of off-flavours in wines have been vigorously investigated (Cuilleré et al., 2004: Ferreira et al., 2002, Hoenicke et al., 2000). The presence of free and conjugated indole-3-acetic acid (Hoenicke et al., 2001), 2-aminoacetophenone (2-AAP) (Hoenicke et al., 2002a, b) was established causing off-flavour of wine. Direct-immersion SPME followed by GC-MS was employed for the measurement of 2-AAP in wines. Analyte was separated on a capillary column (30 m × 0.32 mm i.d., film thickness, 0.25 μm). Oven temperature started at 40°C and increased to 250°C at 10°C/min. It was stated that the method is rapid, accurate, highly sensitive (1 ppt) and can be applied for the measurement of 2-AAP in wines (Fan et al., 2007). 2-AAP in wine was also determined by using stable isotope dilution assay and

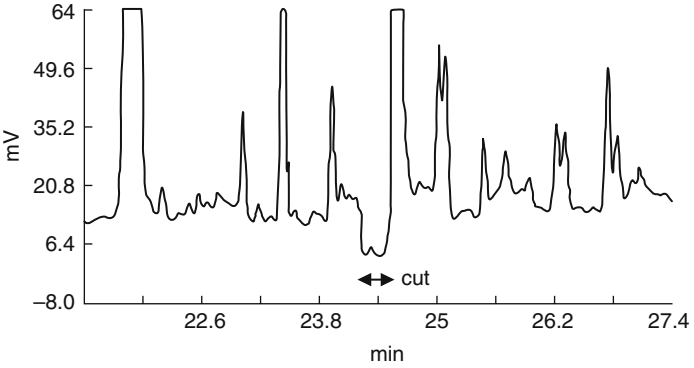

Fig. 2.32 FID chromatogram (section) of a wine extract after first dimension separation and "cut-window" for AAP. Reprinted with permission from Schmarr et al. (2007)

multidimensional GC-MS. Analyte was preconcentrated by SPE and was analysed by two-dimensional GC. A FID chromatogram (section) of a wine extract after first-dimension separation and "cut-window" for AAP is shown in Fig. 2.32. It was stated that the technique is robust, precise and it can be applied for the quantitative determination of AAP in wines (Schmarr et al., 2007). One of the most important off-flavour of wines, the so-called cork taint, is caused by the compound 2,4,6-trichloroanisole (2,4,6-TCA) (Chatonnet et al., 2003; Sefton and Simpson, 2005). It can be produced by fungal metabolism of chlorophenols (Alvarez-Rodriguez et al., 2002) and it can permeate from cork to wine (Capone et al., 2002). The separation and quantitative determination of 2,4,6-TCA was mainly achieved by GC-ECD and GC-MS technologies (Alzaga et al., 2003; Jonsson et al., 2006; Riu et al., 2002, 2005, Juanola et al., 2002). A rapid method was developed for the measurement of 2,4,6-TCA and 2,3,6-trichlorotoluene in synthetic and commercial wines and in cork soaks. Analytes were preconcentrated by HS-SPME and analysed by GC-ECD. Separation was performed in a capillary column (50 m × 32 mm × film thickness, 0.20 μ). Helium was applied as carrier gas at a flow rate of 2 ml/min. Starting column temperature was 70°C and it was increased to 250°C. Same typical chromatograms showing the baseline separation of analytes are shown in Fig. 2.33. The repeatability of the method was 5.72%, the LOD values varied between 0.177 and 0.368 ng/l (Vlachos et al., 2007).

Not only various GC technologies but also HPLC methods were employed for the measurement of flavour compounds in wine. Thus, the taste-modulating flavour compound *N*-glucosyl ethanolamine was measured in wines using LC-MS methods (ion trap mass spectrometer with negative electrospray ionisation and triple-quadrupole mass spectrometer). Separation was carried out on a carbohydrate column (250 × 4.6 mm i.d., particle size, 5 μm). The analyte was separated by gradient elution from 100% water to 95% acetonitrile (ACN) in 30 min. A typical chromatogram illustrating the separation capacity of the system is shown in Fig. 2.34. The flow rate was 0.8 ml/min. It was established that the concentration of the analytes in the samples varied between 1.1 and 4.0 μg/l, the LODs were 9 μl (Rijke et al., 2007).

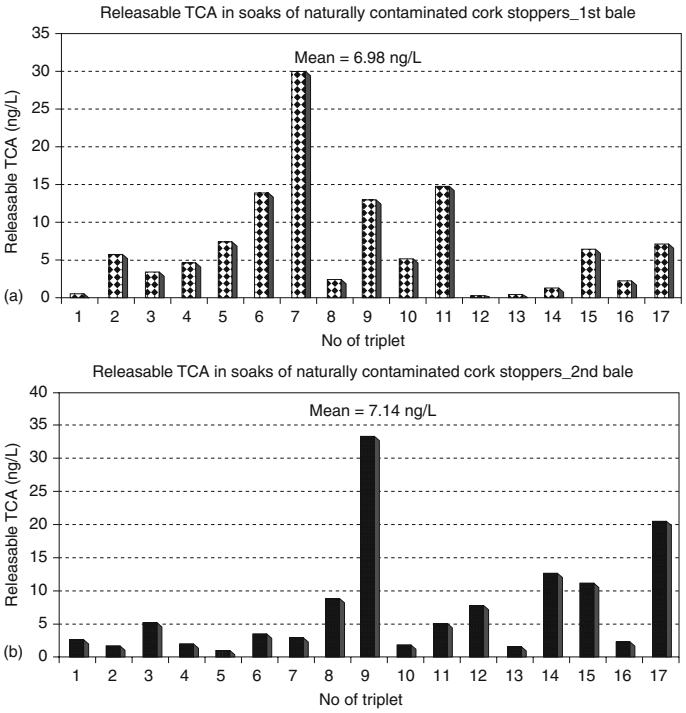

Fig. 2.33 Releasable TCA (2,4,6-trichloroanisole) of 50 rejected cork stoppers by sensorial analysis, analysed in triplets, in order to avoid matrix effects (**a**) 1st bale and (**b**) 2nd bale. Reprinted with permission from Vlachos et al. (2007)

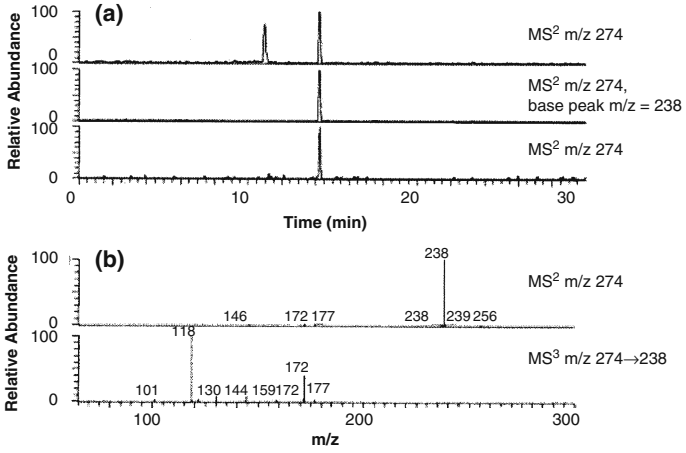

Fig. 2.34 LC–ESI(−)-MS, MS^2 and MS^3 with post-column addition of chloroform of isolated Burgenland wine fraction. Reprinted with permission from Rijke et al. (2007)

A slightly different SPME-GC-MS technique was applied for the measurements of the volatiles causing off-flavours in wine. SPME was carried out at 45°C for 60 min analytes were separated in capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Initial oven temperature was 50°C for 2 min, raised to 190°C at 3.0°C/min, then to 320°C at 50°C/min, final hold 1 min. Some data are summarised in Table 2.39. It was found that the analytical method is linear, specific, accurate and repeatable and its application for the control of the results of sensorial analysis was advocated (Boutou and Chatonnet, 2007).

Polyfunctional mercaptans in wines have been separated and quantitatively determined as pentafluorobenzyl derivatives by GC-negative chemical ionisation (NCI)-MS system. Wines were extracted with benzene and derivatised by pentafluorobenzyl bromide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene. Analytes were separated on a capillary column (20 m × 0.15 mm i.d., film thickness, 0.15 μm). Separation started at 70°C for 3 min, raised to 140°C at 20°C/min, to 180°C at 15°C/min, to 210°C at 30°C and to 300°C at 250°C/min. A typical chromatogram is shown in Fig. 2.35. It was demonstrated that the method is suitable for the simultaneous determination of 2-furfurylthiol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexylacetate and 3-mercaptohexanol. It was assumed that the method can be applied for the measurement of these aroma compounds in different other matrices too (Mateo-Vivaracho et al., 2007) innen.

Multidimensional chromatography was employed for the identification of odorants in aged wines. The best results were achieved by using dynamic headspace extraction followed by fractionation on a normal-phase HPLC column and by the GC-MS analysis of the HPLC fractions. The high odorant activity of ethyl cyclohexanoate, ethyl-2-hydroxy-3-methylbutyrate, ethyl-2-hydroxy-4-methylpentanoate was established (Campo et al., 2006a).

HS-SPME combined with GC-FID has been successfully applied for the investigation of the interaction between the polymeric fraction of wine and volatile substances such as ethyl hexanoate, ethyl octanoate and ethyl decanoate. The method was proposed for the study of the binding of aroma substances to the polymeric fraction of wine (Rocha et al., 2007). GC-O, detection frequency analysis (DFA) and GC-MS were simultaneously applied for the investigation of the aroma substances in Brazilian Cabernet Sauvignon wines. Aroma compounds were extracted by dichloromethane and analysed by GC-FID-GC-O and by GC-MS. GC-FID measurements were performed on a capillary column (21.5 m × 0.32 mm i.d., film thickness, 0.25 μm). Injector and detection temperatures were set to 250°C. Separation started at 40°C for 1 min, ramped to 220°C at 3°C/min (final hold 25 min). Samples were also analysed by GC-flame photometric detector (FPD). The concentrations of the eight main compounds responsible for the characteristic odour of Cabernet Sauvignon wines are compiled in Table 2.40. It was concluded from the data that the location of the vineyard exerts a marked influence on the composition and concentration of aroma active substances (Falcao et al., 2008).

Table 2.39 Application of the method to a red wine and a white wine

		White wine $n = 3$ replications					Red wine $n = 3$ replications				
Molecule	Units	Concentration by validated method[a,b,c]	Concentration by test method	RSD (%)	Added	Retrieved (%)	Concentration by validated method[a,b,c]	Concentration by test method	RSD (%)	Added	Retrieved (%)
1-Octen-3-ol	µg/l		1.5	17.6	2.1	100.0		2.8	5.5	2.1	81.1
Fenchone	µg/l		0.1	0.0	2.2	103.4		nd		2.2	90.1
Fenchol	µg/l		0.1	35.3	2.5	118.7		nd		2.5	83.5
Guaiacol	µg/l	11.3[b]	9.1	5.2	48.5	115.6	32.6[b]	30.9	10.4	48.5	93.2
2MIB	ng/l		nd		40.4	98.3		nd		40.4	90.2
Geosmin	ng/l		nd		41.2	124.9		nd		41.2	104.9
2M35DP	ng/l		Traces	6.3	10.7	86.8		Traces	10.4	10.7	88.7
IPMP	ng/l		nd		19.9	94.4		nd		19.9	106.5
IBMP	ng/l		nd		19.8	114.8		nd		19.8	96.5
TCA	ng/l	Traces[a]	0.7	7.3	2.0	90.2	2.2[a]	1.2	13.3	2.0	86.4
TeCA	ng/l	2.0[a]	2.2	3.6	2.1	102.2	2.3[a]	2.7	9.8	2.1	88.0
TBA	ng/l	nd[b]	nd		2.6	102.1	nd[b]	nd		2.6	104.8
PCA	ng/l	5.3[a]	5.0	3.0	2.0	98.2	6.4[a]	6.9	6.3	2.0	84.2
E4P	µg/l	nd[c]	nd		86.9	101.4	856[c]	742.8	1.0	86.9	95.1
E4G	µg/l	nd[c]	nd		20.7	101.6	149[c]	137.8	2.4	20.7	111.6
V4P	µg/l	32.0[c]	31.5	15.2	21.5	76.3	Traces[c]	20.9	10.2	21.5	84.2
V4G	µg/l	121.0[c]	130.9	2.1	19.9	126.6	Traces[c]	14.8			

Comparison with two methods already used in the same laboratory. nd: not detected; traces: L_d < traces < L_q.

[a] Method 1 COFRAC accredited for assay of TCA, TeCA and PCA.

[b] Molecules (guaiacol and TBA) assayed concurrently by method 1.

[c] Method 2 validated for the assay of volatile phenols.

2MIB=2-Methylisoborneol, 2M35Dp=2-Methoxy-3,5-dimethylpyrazine, IPMP=2-Isopropyl-3-methoxypyrazine, IBMP=2-Isobutyl-3-methoxypyrazine, TCA=2,4,6-Trichloroanisole, TeCA=2,3,4,6-Tetrachloroanisole, TBA=2,4,6-Tribromoanisole, PCA=Pentachloroanisole, E4P=Ethyl-4-phenol, E4G=Ethyl-4-guaiacol, V4G=Vinyl-4-guaiacol, V4P=Vinyl-4-phenol.

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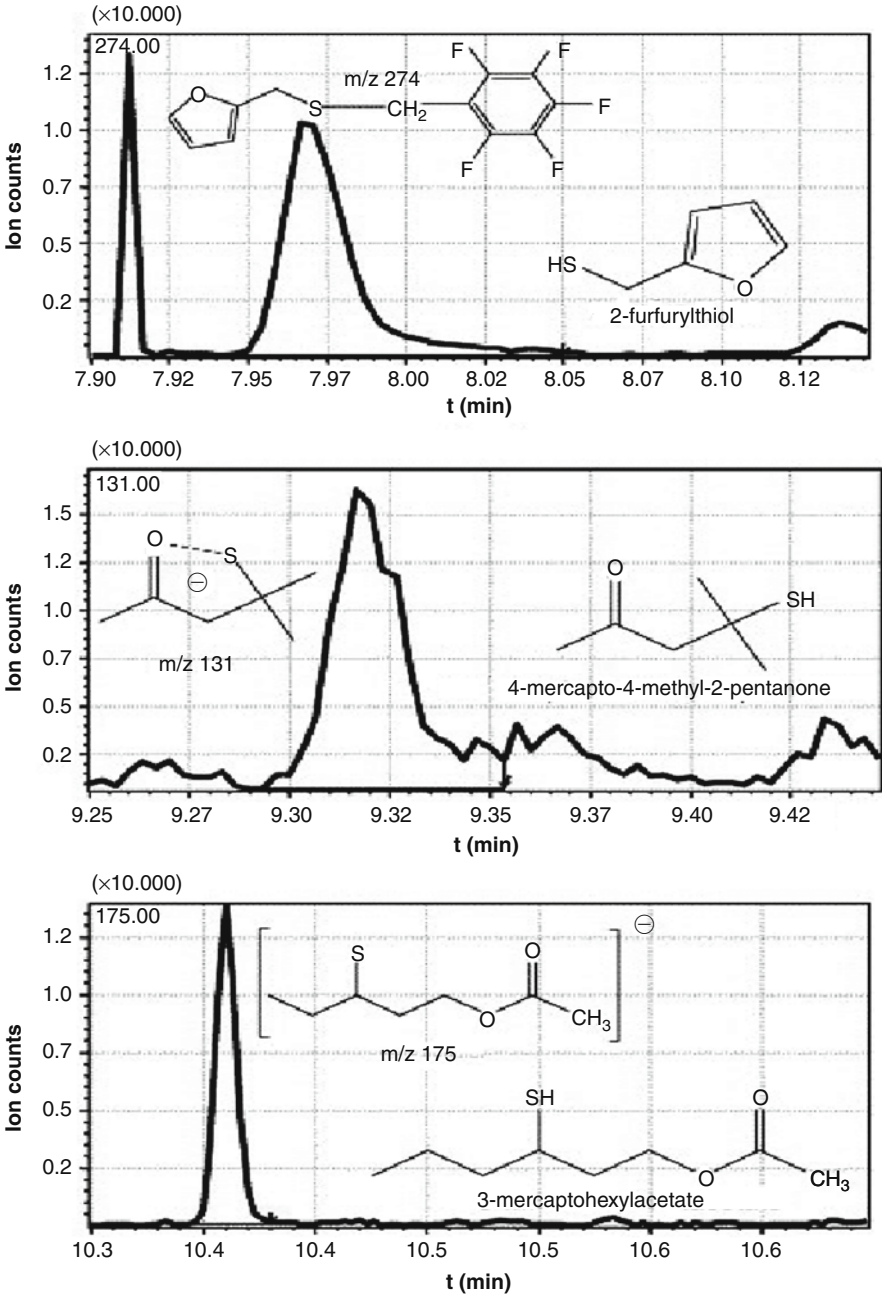

Fig. 2.35 Chromatograms obtained in the analysis, following the proposed procedure, of a wine containing 26 ng/l FFT (2-furanmethanethiol), 4 ng/l MP (4-mercapto-4-methyl-2-pentanone) and 66 ng/l MHA (3-mercaptohexylacetate). Reprinted with permission from Mateo-Vivaracho et al. (2007)

Table 2.40 Quantification of the eight principal compounds responsible for the odorant-active zones in Cabernet Sauvignon wines and odour thresholds

Compounds (μg/l)	BR wine	SJA wine
Acetic acid	n.a.	n.a.
3-Methoxy 2-isobutyl pyrazine***	0.018 ± 0.00	0.040 ± 0.00
Butyric acid*	8160 ± 220	11430 ± 600
Isovalerianic acid*	8830 ± 290	9330 ± 990
Methional**	153.00 ± 0.00	n.d.
β-Damascenone***	13.33 ± 0.47	17.20 ± 1.91
2-Phenylethanol*	90,160 ± 63810	42,730 ± 8140
β-Ionone***	0.08 ± 0.01	0.14 ± 0.00
Furaneol***	252.21 ± 3.90	111.47 ± 2.10

n.a. = not analyzed. n.d. = not detected. Compounds analysed by *GC–FID; **GC–FPD; ***GC–MS.
BR wine = wine with red fruits and jam aromas.
SJA wine = wine with vegetative characteristics.
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An SPE GC-MS method was developed and optimised for the isolation and quantitative determination of aroma thiols. Separation was carried out on a capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). Initial oven temperature was 40°C for 6 min, increased to 200°C at 2°C/min. Mass detection ranged from *m/z* 40 to *m/z* 250. The GC-Ms chromatogram of a spiked wine sample is depicted in Figs. 2.36 and 2.37. The chromatograms in Figs. 2.36 and 3.36 illustrate the good separation capacity of the SPE GC-MS system. Because of the good validation parameters, the method was proposed for the separation and quantitative determination of aroma thiols in wine (Ferreira et al., 2007). A HS-SPME method followed by GC-nitrogen-phosphorous detection (NPD) and by stable isotope dilution GC-MS was employed for the analysis of 3-isobutyl-2-methoxypyrazine in wine. Measurements for the optimisation of the HS-SPME procedure were performed in a GC-NPD system using a capillary column (30 m × 0.32 mm i.d., film thickness, 0.25 μm). Starting column temperature was 50°C for 3 min, increased to 90°C at 40°C/min, then to 140°C at 4°C, to 230°C at 20°C/min (final hold 10 min). The temperature of the NPD was set to 300°C. HS-SPME-GC-MS/MS system was employed for analysis using a different capillary column (30 m × 0.25 mm i.d., film thickness, 0.5 μm) and the same temperature gradient. It was stated that the method can be employed for the study of the efficacy of some viticultural and enological techniques (Prouteau et al., 2004). SPME and GC-MS were employed for the determination of the aroma profile of traditional wine fermentation and the use of immobilised yeast cells on brewer's spent grains. Immobilised cells produced more ethyl and acetate esters and volatile fatty acids. It was further established that the amount of aroma substances decreased with decreasing fermentation temperature. The flavour of wines produced with immobilised yeast was better than that fermented by free yeast cells (Mallouchos et al., 2007).

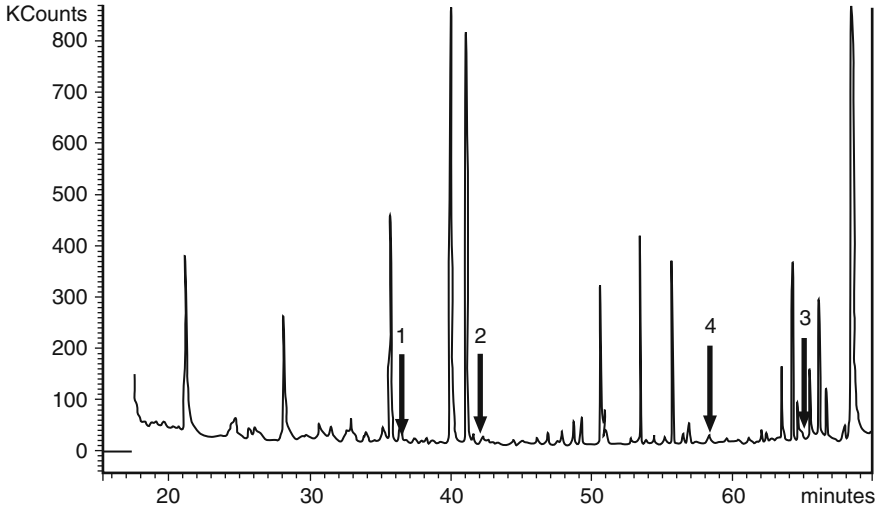

Fig. 2.36 General GC–MS chromatogram obtained in the analysis of a wine sample spiked with different levels of the analytes. The numbered arrows indicate the elution areas of each one of the analytes; 1, 4-methyl-4-mercaptopentanone; 2, furfurylthiol; 3, 3-mercaptohexanol; 4, 3-mercaptohexyl acetate. Reprinted with permission from Ferreira et al. (2007)

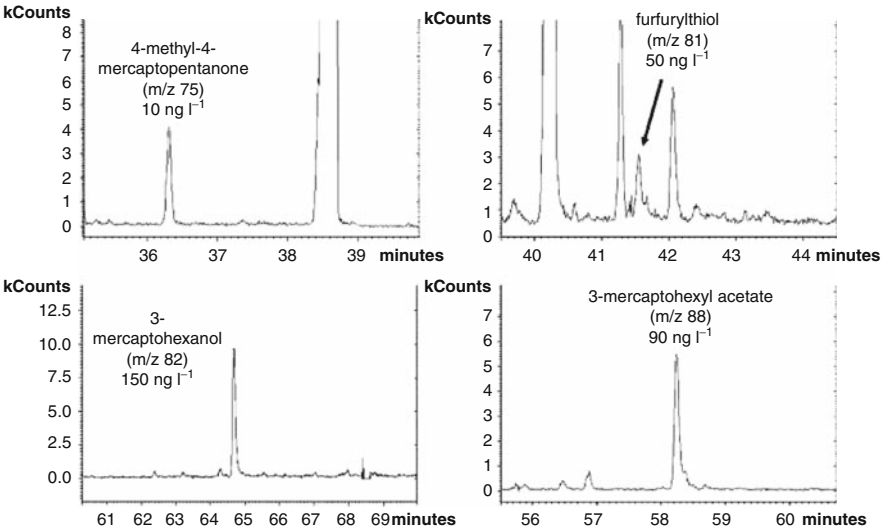

Fig. 2.37 Detailed ion chromatogram showing the ion peaks of the four analytes studied. Reprinted with permission from Ferreira et al. (2007)

The interaction between aroma substances and whole mannoprotein isolated from *Saccharomyces cerevisiae* was investigated by using dynamic and static SPME, GC and HPLC. Isoamyl acetate, hexanol, ethyl hexanoate and β-ionone were applied as model compounds. The measurements demonstrated that the binding of aroma compounds to whole mannoprotein depends on both the character of the aroma substances and the origin of mannoprotein (Chalier et al., 2007).

The aroma production of *Saccharomyces cerevisiae, S. bayanus, S. cerevisiae* × *S. bayanus, Brettanomyces bruxellensis, Hanseispora uvarum, Kloekcera apiculata, Torulaspora delbreckii* and *Debaryomyces carsonii* was investigated during the fermentation of synthetic and natural must. Volatiles were separated and identified by GC-MS and GC-FID. GC-MS was performed by using a capillary column (60 m × 0.25 mm i.d., film thickness, 0.5 μm). Starting column temperature was 40°C for 3 min, increased to 90°C at 10°C/min, then to 230°C at 2°C/min (final hold 37 min). Injector temperature was enhanced during the analytical process. The data illustrated that the different genera of yeasts produce different aroma substances from odourless precursors such as nor-isoprenoids, terpenols, benzenoids, volatile phenols, vanillines and lactones (Hernández-Orte et al., 2008).

2.8.1.1 White Wines

The aroma composition in white wines of different origin has also been extensively investigated. Thus, the concentration of 3-mercaptohexan-1-ol (3-MH) and 3-mercaptohexyl acetate (3-MHA) was determined in wines prepared from grapes Sauvignon Blanc, Traminer, Verdicchio and Mueller Thurgau. Analytes were extracted by HS-SPME and SPE, and the efficacy of the two extraction procedures was compared. GC-MS was carried out using two capillary columns (30 m × 0.32 mm i.d., film thickness, 0.25 μm and 10 m × 0.32 mm i.d., film thickness, 0.25 μm). It was established that the detection limit was lower for HS-SPME and the results determined by both methods were commensurable (Fedrizzi et al., 2007).

GC-FID, GC-MS and GC-O were simultaneously applied for the study of the influence of grape overripeness, drying and *Botrytis cinerea* infection on the aroma profile of the sweet Fiano wine (Italy). SPE preconcentration of volatiles was carried out on cartridges filled with C-18 support. Analytes were separated by GC-FID and identified by GC-MS using the same capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm) and the same chromatographic conditions. Initial oven temperature was 40°C for 3 min, increased to 220°C at 2°C/min final hold 15 min. The volatiles identified by these methods are compiled in Table 2.41. It was found that the most important odour impact aroma substances were nerol, geraniol, linalool, vitispirane (camphor), γ-nonalactone, δ-decalactone, γ-decalactone, and 1-octen-3-ol. It was further established that overripeness and the drying enhance the amount of aroma substances in the Fiano wine (Genovese et al., 2007).

Table 2.41 Quantitative data of volatile compounds identified in the sweet Fiano wine (A) and base Fiano wine (B)

Compound	Concentration (μg/l)[a]		Compound	Concentration (μg/l)[a]	
	A	B		A	B
Esters			*Terpenes*		
Ethyl 2-methylpropanoate	156±14	776±181	*p*-Cimene	8.1±0.5	nf
2-Methylpropyl acetate	1759±185	1959±1110	*cis*-Linalool oxide	70.4±3.6	49.1±1.9
Ethyl butanoate	375±17	925±14	*trans*-Linalool oxide	31.3±1.4	14.6±0.6
Ethyl 2-methylbutanoate	61.1±4.1	184±1.8	Linalool	120±5.0	11.8±0.6
Ethyl 3-methylbutanoate	107±5	340±10	Terpinen-4-ol	190±7	8.0±1.6
3-Methylbutyl acetate	768±28	938±20	∗-Terpineol	196±8	129±6
Ethyl hexanoate	1147±30	3781±116	Nerol	nd	nd
Hexyl acetate	<3.7	24.7±1.4	Geraniol	22.4±0.4	<3.7
Ethyl heptanoate	17.6±0.6	<3.7			
Ethyl lactate	287±23	1283±75	*Lactones*		
Ethyl 2-hydroxy-3-methylbutanoate	8.8±0.8	24.3±1.1	γ-Butyrolactone	219±22	209±13
Ethyl octanoate	1246±38	6152±284	Valerolactone	76.0±3.1	nf
Ethyl 2-hydroxycaprinoate	94.9±9.8	276±6	*cis*-Wisky lactone	165±7	nf
Diethyl propandioate	36.8±3.4	36.5±2.4	*trans*-Wisky lactone	274±9	20.3±3.1
4-Oxoethyl-pentanoate	45.4±4.3	nf	γ-Nonalactone	43.2±6.1	6.3±0.3
Ethyl 2-furoate	40.9±2.6	104±4	γ-Decalactone	15.5±0.9	3.7±0.1
Ethyl decanoate	95.6±3.1	1529±214	δ-Decalactone	22±1	4.0±0.1
Isoamyl octanoate	<3.7	52.2±5.2			
Diethyl succinate	13,011±589	22673±1179	*Aldehydes and ketones*		
Ethylphenyl acetate	181±6.7	166±8	Diacetyl	nd	nd
2-Phenylethyl acetate	144±7	308±12	Acetoin	<3.7	<3.7
Ethyl 3-hydroxyhexanoate	15.5±2.0	nf	Furfural	1260±65	<3.7
Diethyl malate	1134±79	2908±114	Benzaldehyde	204±41	9.9±0.6
Ethyl cinnamate	27.3±1.2	10.4±1.2	5-Methylfurfural	30.6±3.7	24.4±0.9
Ethyl vanillate	138±18.1	nf	Acetophenone	nd	nf
			Furaneol	nd	nd

Table 2.41 (continued)

Compound	Concentration (μg/l)[a]		Compound	Concentration (μg/l)[a]	
	A	B		A	B
Alcohols			Homofuraneol	nd	nd
2-Methyl-1-propanol	118±15	202±29	3,4-Dihydro-8-hydroxy-3-methyl-1-*H*-2-Benzopyran-1-one	31.2±6.9	nf
1-Butanol	<3.7	7.8±2.0			
3+2-Methyl-1-butanol	16,800±1197	33663±2401			
4-Methyl-1-pentanol	11.3±1.5	<3.7	*C13-norisoprenoid*		
1-Hexanol	805±41	12493030	Vitispirane	nd	nf
3-Hexen-1-ol	35.8±3.4	69.4±2.1	TDN	7.8±1.3	9.1±0.8
1-Octen-3-ol	213±8	<3.7	β-Damascenone	10.4±0.8	<3.7
1-Heptanol	27.9±1.8	nf			
2-Ethylhexanol	18.6±2.0	nf	*Phenols*		
2-Phenylethanol	7335±456	19328±502	Guaiacol	<3.7	<3.7
			Eugenol	41.1±2.5	11.2±1.0
Acids			4-Vinylguaiacol	265±12	80.9±3.7
Acetic acid	122±37	141±27	Syringol	75±3.1	20.0±1.5
2-Methylpropanoic acid	285±7	225±21	Isoeugenol	nd	nd
Butanoic acid	93.0±6.2	154±15			
3-Methylbutanoic acid	342±28	513±25	*Other*		
Hexanoic acid	1024±133	4381±103	3-Methylthio-1-propanol	10.5±2	17.6±4
Heptanoic acid	14.8±2.7	nf	*N*-3-methylbutyl acetamide	59.6±4.4	nf
2-Hexanoic acid	31.2±3.0	125±8			
Octanoic acid	3010±411	19451±478			
Nonanoic acid	<3.7	nf			
Decanoic acid	484±87	5655±225			
Benzoic acid	87.6±10.1	<3.7			
Phenyl acetic acid	nd	nd			

nf, not found; nd, not determined since the relative peak contained more than one component as confirmed by mass spectrometry.

[a] Means of triplicate analysis.

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An elegant and simple method was developed and successfully employed for the determination of odour-active aldehydes in wine. Analytes octanal, nonanal, decanal, (*E*)-2-nonenal and (*E,Z*)2,6-nonadienal were retained on a SPE cartridge and derivatised in situ by *O*-(2,3,4,5,6-pentafluorobenzyl)oxime. Aroma substances were separated in a capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). Initial oven temperature was 40°C for 5 min, ramped to 140°C at 10°C/min, then to 190°C at 2°C, to 210°C at 20°C/min.

Mass range was 45–350 *m/z*. The separation capacity of the method is illustrated in Fig. 2.38. The detection limit of the method varied between 12 and 20 ng/l (Ferreira et al., 2004).

The various aspects of the vinification process and their impact on the amount and composition of aroma substances have been vigorously investigated. GC-FID and GC-MS were applied for the determination of the effect of skin contact on the aroma substances in cv. Muscat of Bornova wines. It was established that 6 h skin contact increased markedly the amount of aroma compounds. The most odour-active compounds were: β-damascenone, ethyl hexanoate, ethyl butanoate,

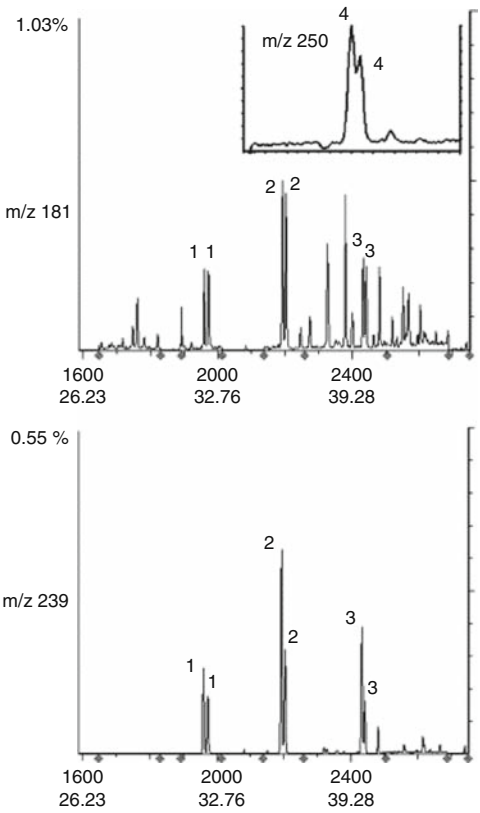

Fig. 2.38 GC–MS chromatograms of a Merlot wine showing the detail of the different peaks. Peaks: 1, octanal-PFBHA; 2, nonanal-PFBHA; 3, decanal-PFBHA; 4, *E*-2-nonenal-PFBHA (PFHBA=*O*-2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride). The concentration of these compounds was (μg/l): octanal, 0.972; nonanal, 3.301; decanal, 1.003; (*E*)-2-nonenal, 0.036. Double peaks occur because two isomers are obtained from each analyte. Reprinted with permission from Ferreira et al. (2004)

isoamyl acetate, 2-phenyl ethyl acetate, linalool, geraniol and 2-phenyl ethanol (Selli et al., 2006). The aroma components of Zalema wines (southern Spain) were investigated with similar separation technologies. The measurements indicated the involvement of the following substances in the aroma formation of the wine: β-damascenone, β-ionone, isoamyl alcohol, β-phenylethanol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexyl acetate, 3-mercapto-1-hexanol, acetaldehyde, phenylacetaldahyde, isoamyl acetate, ethyl hexanoate, ethyl butyrate, ethyl isovalerate, and ethyl octanoate (Gómez-Miguez et al., 2007). GC-MS was employed for the investigation of the effect of added fungal glycosidase enzyme on the aroma composition. The measurements indicated that the addition of enzyme slightly increased the amount of glycosidically bound aroma substances (Palomo et al., 2005).

Stir bar sorptive extraction(SBSE) followed by thermal desorption-GC-MS was employed for the measurement of the aroma profile of Chardonnay, Muscat, Eva, Cayetana and Pardina wines. The optimal extraction method was performed at 60°C for 90 min. Samples were stirred at 700 rpm during the extraction. Analytes were separated on a capillary column (50 m × 0.22 mm i.d., film thickness, 0.25 μm). Initial oven temperature was 50°C for 2 min, increased to 1,230°C at 12°C/min, final hold 20 min. Mass range varied between 35 and 500 *m/z*. Calculations proved that the aroma composition can be employed for the differentiation between wine varieties limonene, linalool, nerolidol and 1-hexanol being the most important differentiating compounds (Zalacain et al., 2007).

The flavonoids and aroma compounds were analysed in cv. Albarin bianco (North Spain) by HPLC and GC, respectively. The free and bound aroma substances were separated on a C-18 SPE cartridge, and analysed separately. Measurements were performed on a capillary column (50 m × 0.25 mm i.d., film thickness, 0.20 μm). Starting oven temperature was 60°C for 5 min, ramped to 220°C at 3°C/min, final hold 15 min. Analytes were detected by FID detector temperature being 260°C. The concentrations of free and bound aroma compounds in Albarin Blanco wines are compiled in Table 2.42. The most abundant volatiles were linalool, β-ionone, isoamyl alcohols, ethyl acetate, isoamyl acetate, ethyl hexanoate and ethyl octanoate. It was concluded from the data that the results may contribute to the better understanding of the similarities and dissimilarities between Albarin blanco wines (Masa and Villanova, 2008).

Multidimensional GC has also been used for the investigation of the aroma profile of white wines. The first system consisted of a semi capillary column (30 m × 0.53 mm i.d., film thickness, 5 μm), FID and a sniffing port. The second part contained a programmable temperature vaporisation (PTV) injector, a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm) and simultaneous MS and sniffing port detection. The starting temperature of the first column was 40°C for 5 min, then raised to 230°C at 3°C/min. The oven temperature of the second column was 60°C for 6 min, then raised to 230°C at 4°C/min. MS mass range was 35–250 *m/z*. The spectra of the novel compounds (ethyl 3-methylpentanoate, ethyl 4-methylpentanoate, ethyl 2-methylpentanoate) are depicted in Fig. 2.39. It was assessed that these novel aroma compounds can play a considerable role in the aroma formation of some wine varieties (Campo et al., 2006).

Table 2.42 Bound and free compound levels (μg/l) in Albarín Blanco wine over three vintages, mean and standard deviation

	Vintages				
Compound	I	II	III	Mean	SD
Bound compounds					
Linalool	ND	46	59	35	31.0
Terminen-4-ol	121	146	ND	89	78.1
∗-Terpineol	50	34	31	38.3	10.2
Nerol	42	20	5	22.3	18.6
Geraniol	17	25	18	20	4.36
Benzyl alcohol	51	51	ND	34	29.4
2-Phenylethanol	1222	640	ND	621	611
β-Ionone	21	16	10	15.7	5.51
Eugenol	45	34	ND	26.3	23.5
Free compounds					
Terpenes					
Linalool	81	236	122	146	80.3
Terpinen-4-ol	87	273	ND	120	139
∗-Terpineol	305	208	19	177	145
Citronellol	ND	12	5	5.67	6.03
Nerol	ND	17	5	7.33	8.74
Geraniol	62	78	7	49	37.2
C_{13}-norisoprenoids					
Theaspirane-b	254	72	ND	163	129
β-Ionone	24	18	12	18	6.00
Alcohols					
Methanol	7300	22920	22080	17433	8786
1-Propanol	17890	24390	69180	37153	27926
1-Butanol	450	ND	1200	550	606
2-Methyl-1-propanol	68390	81950	63380	71240	9607
Isoamyl alcohol	267350	328590	199950	265297	64345
Benzyl alcohol	106	70	ND	58.7	53.9
2-Phenylethanol	95420	58140	28740	60767	33418
Acetates					
Ethyl acetate	60250	61110	54410	58590	3645
Isoamyl acetate	170	140	270	193	68.1
Hexyl acetate	270	250	ND	173	150
Ethyl esters					
Ethyl lactate	12440	11900	17250	13863	2945
Ethyl butyrate	4820	170	2100	2363	2336
Ethyl hexanoate	280	290	1130	567	488
Ethyl octanoate	330	300	1840	823	881
Ethyl decanoate	90	90	2210	797	1224
Diethyl succinate	7570	1790	ND	3120	3956
Ethyl myristate	190	50	ND	80	98.5

ND – not detected.
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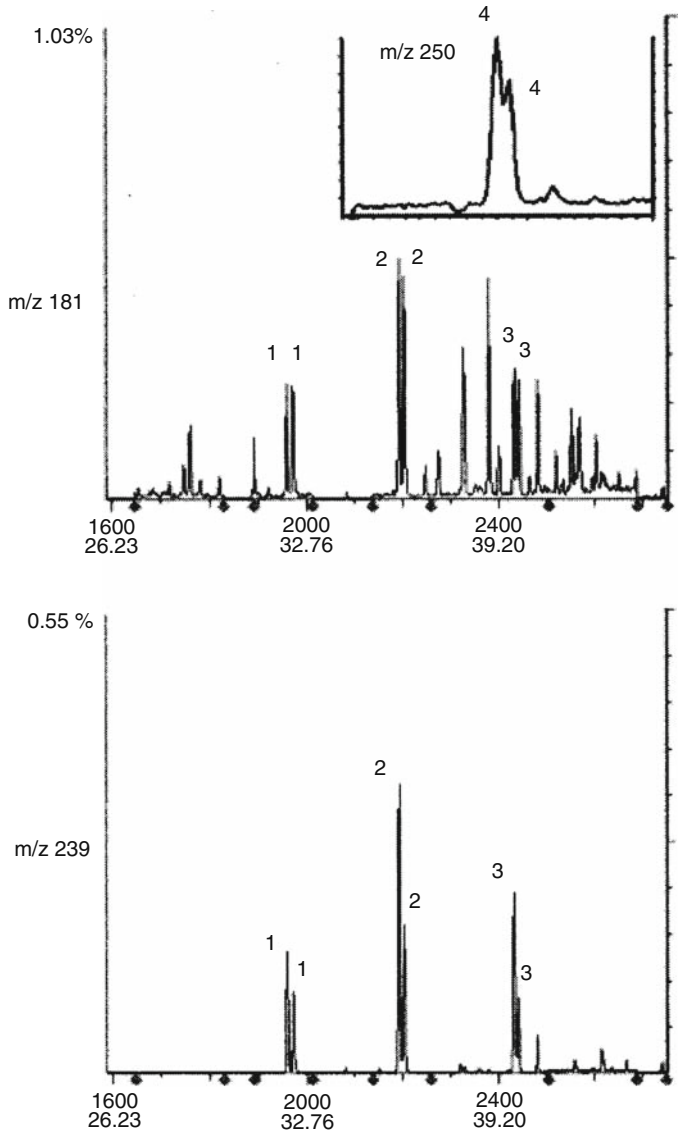

Fig. 2.39 Mass spectrum of ethyl 2-hydroxy-3-methylbutyrate isolated from a Sherry wine extract. Reprinted with permission from Campo et al. (2006)

GC-O, GC-FID and GC-MS were applied for the elucidation of the differences between the aroma profiles of dry white wines and botrytised wines. Aroma compounds were preconcentrated by traditional liquid–liquid extraction and the aroma substances were separated in a capillary column (50 m × 0.25 mm i.d., film thickness, 1.0 μm), and detected simultaneous by FID and sniffing port. The starting

temperature was 45°C for 1 min, then raised to 230°C at 3°C/min. MS detection was performed at the mass range 40–300*m/z*.

The results demonstrated that the botrytisation influence considerably both the amount and the composition of aroma compounds (Sarrazin et al., 2007).

Because of their worldwide consumption, the aroma composition of Madeira wines has been extensively investigated. Thus, the musts of Boal, Malvasia, Sercial and Verdelho grape varieties were analysed using HS-SPME and GC-MS. The similarities and differences between the aroma profiles of musts were assessed by PCA and linear discriminant analysis. Volatiles were separated on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25). The starting temperature was 40°C for 1 min, then raised to 120°C at 1°C/min, to 180°C at 1.7°C/min, ant to 220°C at 25°C/min. MS detection was performed at the mass range 30–300*m/z*. A typical chromatogram depicting the separation of some terpenoids is shown in Fig. 2.40. Calculation demonstrated that the grape varieties can be separated according to their aroma profile using multivariate mathematical statistical methods (Camara et al., 2004).

The aroma substances were determined by GC-O, GC-FID and GC-MS in four Madeira wines Malvasia, Boal, Verdelho and Sercial). Both liquid–liquid extraction and SPE were employed for the preconcentration of analytes. The chromatograms demonstrated that the aroma profiles of Madeira wines are complicated, containing a considerable number of identified and non-identified aroma substances sotolon, phenylacetaldehyde being the most important odour-active components (Campo et al., 2006).

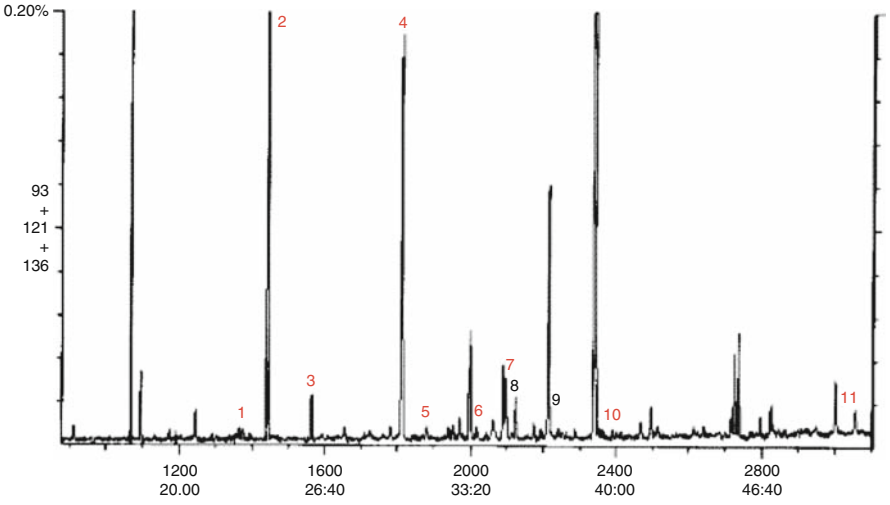

Fig. 2.40 Chromatogram (SIM, *m/z* = 93+121+136) of some terpenoids obtained from HS–SPME/GC–MS analysis of a 1999 Verdelho must sample (1, (*E,E*)-farnesal; 2, linalool; 3, 4-terpineol; 4, α-terpineol; 5, neral; 6, citronellol; 7, nerol; 8, β-damascenone; 9, geraniol; 10, β-ionone; 11, farnesol). Reprinted with permission from Camara et al. (2004)

SPME and SBSE followed by GC-MS were also employed for the study of the aroma composition of Madeira wines. The main components of the aroma composition were ethyl octanoate, ethyl decanoate, ethyl decenoate, diethyl succinate, ethyl dodecanoate, ethyl nonanoate, ethyl hexanoate, isoamyl octanoate, vitispirane, 1,1,6-trimethyl 1,2-dihydro naphthalene and phenyl ethanol (Alves et al., 2005).

2.8.1.2 White and Red Wines

Similar to white wines, the aroma substances in red wines have also been extensively investigated and the volatiles of white and red wines were frequently compared. The volatile sulphur compounds in wines were determined by employing automated HS-SPME and GC-pulsed flame photometric detection (PFDT). CAR-PDMS fibres were applied for the extraction of sulphur compounds, the extraction temperature being 35°C and the extraction time 20 min. Analytes were separated on a capillary column (30 m × 0.32 mm i.d., film thickness, 1 μ). The starting oven temperature was 35°C for 3 min, then raised to 100°C at 10°C/min, to 220°C at 20°C/min. Injector and detector temperatures were 300°C. A typical chromatogram showing the separation of sulphur compounds is depicted in Fig. 2.41. The average, minimal and maximal concentrations of hydrogen sulphide (H_2S) methanethiol (MeSH), ethanethiol (EtSH), dimethylsulphide (DMS), diethylsulphide (DES) and dimethylsulphide (DMDS) are compiled in Table 2.43. The data illustrate that the amount of volatile sulphur compounds is markedly different in white and red wines (López et al., 2007).

The results obtained by GC-MS and e-nose responses were compared by the sensorial descriptors identified by a sensory panel. Correlations were calculated by PLS. GC separations were carried out in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25). The initial oven temperature was 55°C for 2 min, then

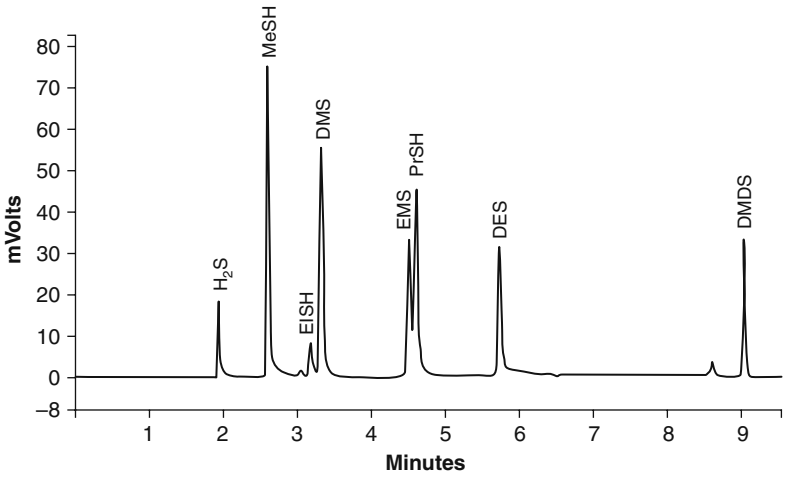

Fig. 2.41 Chromatogram of a red wine analysed with the proposed method. Reprinted with permission from López et al. (2007)

Table 2.43 Average, maximum and minimum concentrations found in red and whites wines

	White wines ($n = 21$)			Red wines ($n = 13$)		
	Average	Minimum	Maximum	Average	Minimum	Maximum
H_2S	7.6	N.D.	30	2.8	N.D.	13
MeSH	7.5	5.3	12	9.4	8.2	11
EtSH	2.6	1.0	7.1	3.7	2.5	6.3
DMS	33	9.3	65	44	18	106
DES	1.7	1.5	1.9	2.4	2.2	2.6
DMDS	2.2	1.4	2.9	3.7	2.1	5.2

All data expressed in μg/l. N.D.: not detected.
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ramped to 160°C at 1.5°C/min. MS conditions were: ion source and interface temperatures, 200°C; electron impact energy, 70 eV; mass range *m/z*, 25–500. A typical chromatogram depicting the separation of aroma substances is shown in Fig. 2.42. Calculations demonstrated that the e-nose responses correlated better with the sensorial descriptors of the GC-MS data (Lozano et al., 2007).

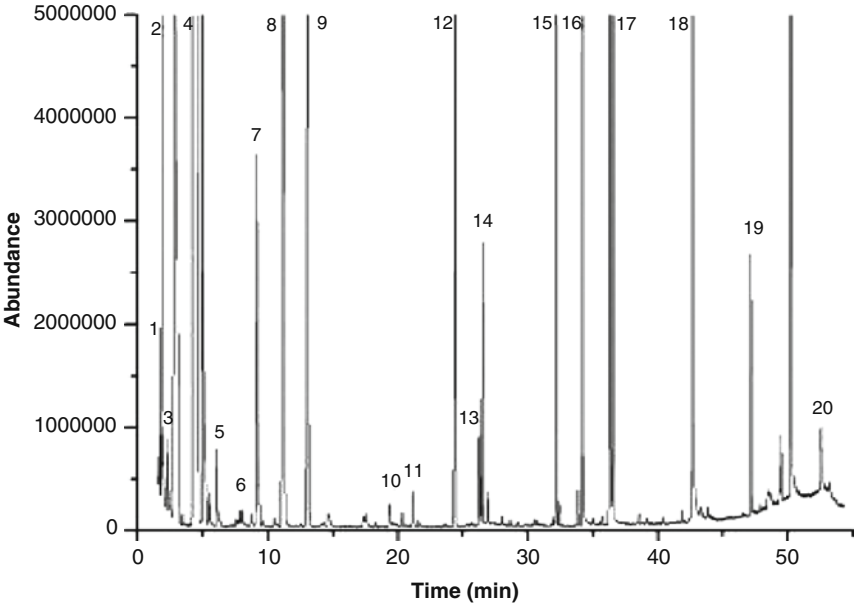

Fig. 2.42 Chromatogram of minor compounds in Malvar wine: (1) isobutyl acetate; (2) ethyl butyrate; (3) isoamyl acetate; (4) ethyl hexanoate; (5) hexyl acetate; (6) ethyl lactate; (7) 1-hexanol; (8) 3-octanol (internal standard); (9) ethyl octanoate; (10) isobutyric acid; (11) butyric acid; (12) ethyl decanoate; (13) isovaleric acid; (14) diethyl succinate; (15) phenyl ethyl acetate; (16) hexanoic acid; (17) 2-phenylethanol; (18) octanoic acid; (19) 4-vinyl-guaiacol; (20) decanoic acid. Reprinted with permission from Lozano et al. (2007)

SPE coupled to multidimensional GC-FID and GC-MS were applied for the determination of four novel odour-active ethyl esters (ethyl 2-, 3-, 4-methylpentanoate, ethyl cyclohexanoate) in white and red wines, in brandy and whisky. The first GC system consisted of a capillary column (30 m × 0.32 mm i.d., film thickness, 0.50 μm). The oven temperature started at 40°C for 7 min, then ramped to 100°C at 4°C/min, then to 140°C at 6°C/min, to 200°C at 20°C/min. The second GC system also employed a capillary column (30 m × 0.32 mm i.d., film thickness, 1 μm). The oven temperature started at 40°C for 16 min, then increased to 130°C at 5°C/min, then to 250°C at 20°C/min. The temperatures of transfer line and ion trap were 170°C and 150°C, respectively. Mass range was 45–200 *m/z*. It was stated that he method separates well the novel aroma substances as illustrated in Fig. 2.43. The concentrations of these odour-active compounds are compiled in Table 2.44. It was concluded from the results that these compounds are the main contributors to the aroma of sweet wines, whiskies and brandies (Campo et al., 2007).

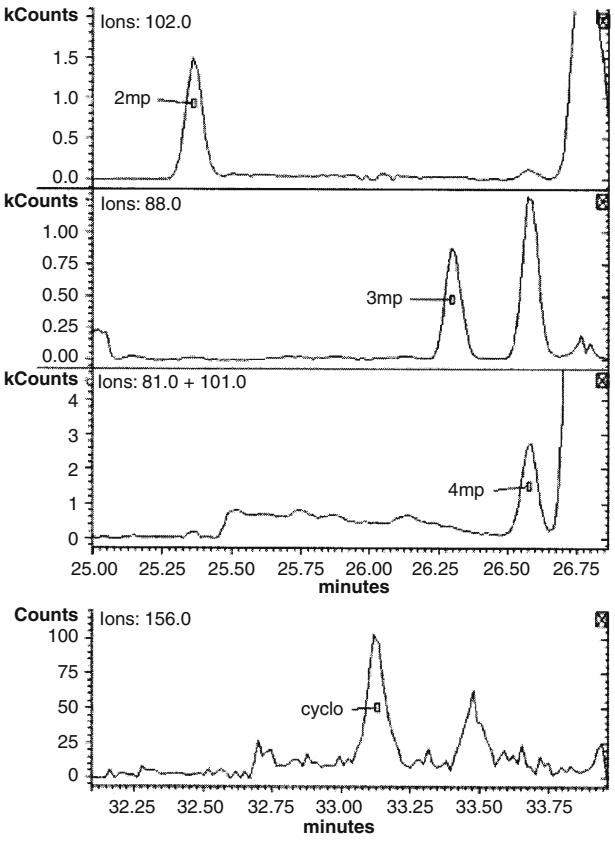

Fig. 2.43 Example of the ionic chromatograms obtained for ethyl 2-, 3- and 4-methylpentanoate and ethyl cyclohexanoate in Cava wine. Reprinted with permission from Campo et al. (2007)

Table 2.44 Levels of ethyl 2-, 3- and 4-methylpentanoate and ethyl cyclohexanoate (expressed as ng/l) found in the studied samples and odour activity values (OAV[a])

			Et. 2mp		Et. 3mp		Et. 4mp		Et. ciclo	
Sample type	Year	Brand	Conc.	*OAV*	Conc.	*OAV*	Conc.	*OAV*	Conc.	*OAV*
White young	2004	Pazo	<q.l.	–	<q.l.	–	14	1.4	<q.l.	–
	2004	Viña Albada	<q.l.	–	<q.l.	–	56	5.6	<q.l.	–
	2004	Marqués de Riscal	<q.l.	–	<q.l.	–	120	12	<q.l.	–
Red young	2005	Montesierra	<q.l.	–	<q.l.	–	175	17	<q.l.	–
	2005	Borsao	<q.l.	–	<q.l.	–	175	17	<q.l.	–
	2004	Viñas del Vero	<q.l.	–	<q.l.	–	262	26	<q.l.	–
Red barrel aged	1999	Lan	22	7.5	36	4.5	258	26	4.9	4.9
	1998	Viña Pomal	18	5.9	32	4.0	211	21	5.5	5.5
	1995	Faustino	22	7.2	35	4.3	253	25	4.9	4.9
Porto	–	Ruby	22	7.4	35	4.4	335	34	3.5	3.5
	–	Tawny	36	12	43	5.4	422	42	14	14
	–	White	53	18	39	4.8	442	44	50	50
Noble rot	2002	Saut. Laribotte	20	6.8	23	2.9	51	5.1	<q.l.	–
	2002	Saut. Baron	8.5	2.8	19	2.4	116	12	<q.l.	–
	2003	Saut. Aureus	14	4.5	23	2.8	63	6.3	37	37
	2002	Tokaji Oremus	33	11	38	4.8	195	20	4.2	4.2
Cava	2001	Gramona	369	123	89	11	228	23	13	13
Fino	3[b]	Cobos	18	5.9	112	14	748	75	8.5	8.5
	5[b]	Quinta	12	3.9	145	18	853	85	<q.l.	–
	5[b]	Tío Pepe	2.8	0.9	514	64	1356	136	13	13
Cream	5[b]	Cream Canasta	17	5.6	48	6.0	376	38	4.7	4.7
	8[b]	Cream Ibérica	26	8.5	180	23	1439	144	36	36
Pale Cream	2002	Cartojal	9.6	3.2	18	2.3	142	14	<q.l.	–

Table 2.44 (continued)

Sample type	Year	Brand	Et. 2mp		Et. 3mp		Et. 4mp		Et. ciclo	
			Conc.	*OAV*	Conc.	*OAV*	Conc.	*OAV*	Conc.	*OAV*
Pedro Ximénez	8[b]	Duquesa	9.9	3.3	26	3.2	197	20	18	18
	10[b]	Leyenda	<q.l.	–	<q.l.	–	110	11	<q.l.	–
	1971	Don PX	17	5.8	126	16	594	59	30	30
	1975	Don PX	1066	355	518	65	972	97	63	63
Brandy	8[b]	Marqués Misa	38	13	36	4.5	566	57	48	48
	8[b]	Duque de Alba	82	27	85	11	938	94	85	85
Whisky	12[b]	Knockando	246	82	457	57	1336	134	21	21
	12[b]	Cardhu	862	287	1035	129	2724	272	22	22

[a] Odour thresholds.
[b] Sample with no attributable vintage date on the bottle. Instead, the aging period (years) is indicated <q.l.: below the quantification limit.
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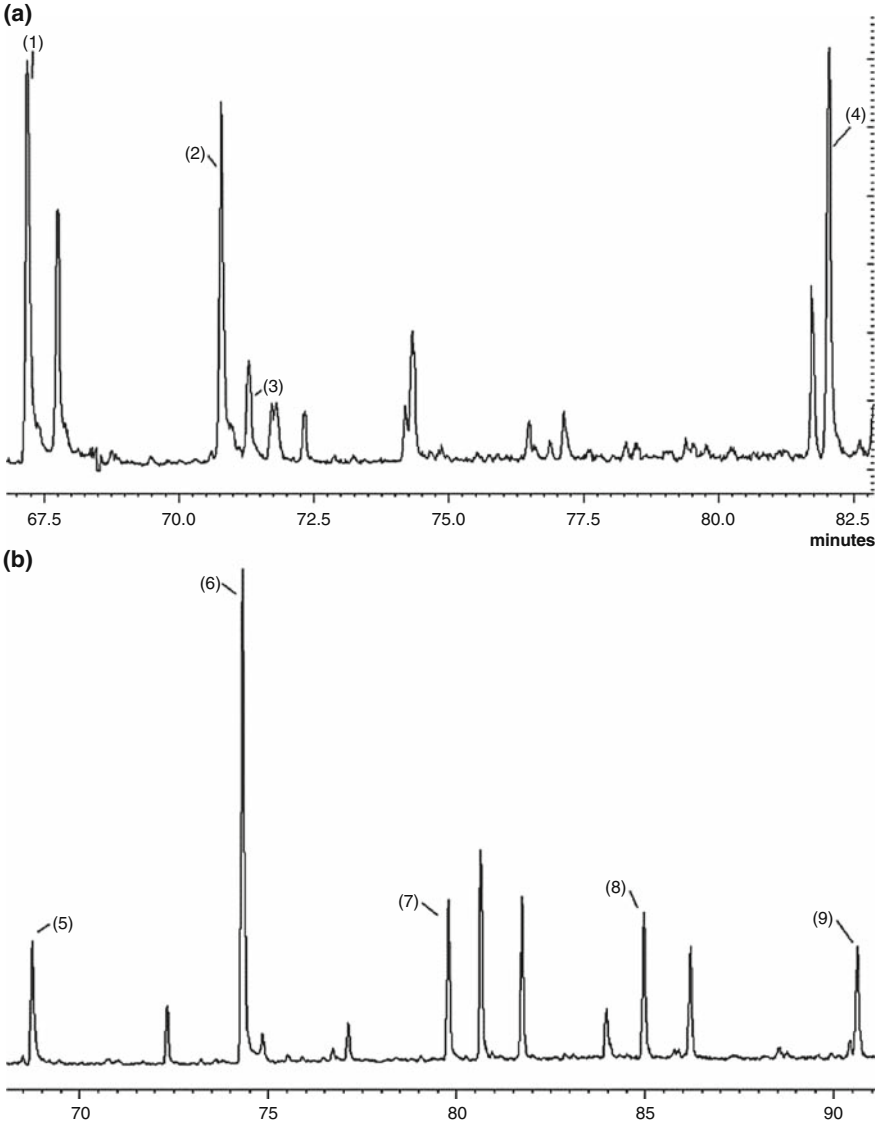

Fig. 2.44 (**a**) Ion chromatogram (*m/z* 99) from a dichlomethane extract obtained from a red wine spiked with 10 μg/ml of analytes. Peak identification: 1, *trans*-whiskylactone; 2, *cis*-whiskylactone; 3, δ-octalactone; 4, δ-decalactone. (**b**) Ion chromatogram (*m/z* 85) from a dichlomethane extract obtained from a red wine spiked with 10 μg/ml of analytes. Peak identification: 5, γ-octalactone; 6, γ-nonalactone; 7, γ-decalactone; 8, γ-undecalactone; 9, γ-dodecalactone. Reprinted with permission from Ferreira et al., 2004

2.8.1.3 Red Wines

The aroma composition of red wines was separately investigated too using similar experimental setup as used for the analysis of white wines. Because of its paramount importance, the various preconcentration techniques have been extensively investigated, optimising existing support or synthesising novel ones. The synthesis and application of the hydroxy-terminated silicone oil-butyl methacrylate-divinylbenzene were reported. It was stated that the new support is suitable for the simultaneous extraction of polar alcohols, fatty acids and nonpolar esters from red wine (Liu et al., 2005). Another study developed a simple strategy for the optimisation of SPE technology and the new method was applied for the determination of aliphatic lactones in wine. SPE parameters were calculated from the solid–liquid extraction coefficients and from some measured bed parameters. The extracts were analysed by GC-FID and GC-MS. GC-FID was performed using a capillary column. (50 m × 0.32 mm i.d., film thickness, 0.5 μm). The oven temperature started at 40°C for 5 min, then ramped to 190°C at 5°C/min. GC-MS applied a slightly different capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). The oven temperature started at 40°C for 5 min, then increased to 200°C at 2°C/min. Detection range of MS was 35–200 *m/z*. Ion chromatograms of dichloromethane extracts are depicted in Fig. 2.44. The chromatograms illustrate the baseline separation of the aroma substances. The quantitative results are compiled in Table 2.45. It was established that the concentrations of this class of volatiles are higher in red wines than in white wines (Ferreira et al., 2004).

Volatile phenols have also been frequently investigated in red wines. Dispersive liquid–liquid microextraction (DLLME) using a mixture of water–chloroform–acetone was employed for the preconcentration of 4-ethylphenol and 4-vinylguajacol from wine samples. GC-MS analysis of the phenol derivatives was

Table 2.45 Quantitative analysis of wines (all data given in μg/l)

	Threshold (μg/l)	Aged red ($n=5$)			White wine ($n=4$)			Young red ($n=4$)		
		Average	Min	Max	Average	Min	Max	Average	Min	Max
trans-Whiskylactone	69	74.5	22.6	158	0.9	0.0	3.7	1.5	1.0	2.4
cis-Whiskylactone	28	205	64.9	386	1.6	0.0	6.5	0.0	0.0	0.0
γ-Octalactone	7	2.3	1.4	4.6	0.0	0.0	0.0	1.4	1.3	1.6
γ-Nonalactone	25	13.4	3.7	27.0	5.9	2.2	9.6	10.2	6.1	16.4
γ-Decalactone	0.7	0.5	0.0	1.5	0.1	0.0	0.4	0.2	0.1	0.3
δ-Decalactone	100	0.7	0.0	3.3	13.2	0.0	52.7	0.0	0.0	0.0
γ-Undecalactone	60	1.2	0.0	5.7	0.0	0.0	0.0	0.0	0.0	0.0
γ-Dodecalactone	7	4.6	0.7	17.7	0.0	0.0	0.0	1.6	0.4	2.5

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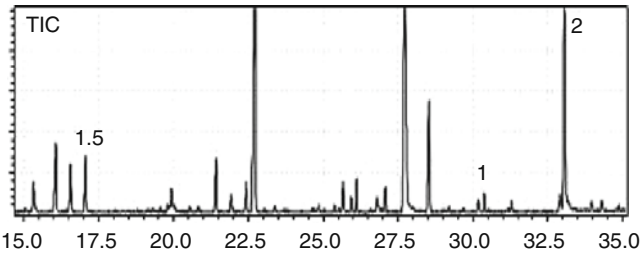

Fig. 2.45 Total ion current obtained from a wine contaminated with *Brettanomyces*. Peak 1, 4-ethylguaiacol; peak 2, 4-ethylphenol; i.s., internal standard. Reprinted with permission from Farina et al. (2007)

carried out on a capillary column (25 m × 0.25 mm i.d., film thickness, 0.25 μm). The initial oven temperature was set to 50°C, then increased to 215°C at 5°C/min, to 220°C at 20°C/min, final hold 15 min. Detection range of MS was 40–400 m/z. The total ion current of a wine extracts is depicted in Fig. 2.45 and illustrates the good separation of the aroma substances. The concentrations of volatiles found in wines are listed in Table 2.46. LOD and LOQ values were 28 and 95 μ/l for 4-ethylguajacol and 44 and 147 μl for 4-ethylphenol, respectively. It was stated that the method is rapid, simple and economic and can be applied for the solving of commercial problems (Farina et al., 2007).

The concentration of 4-ethylphenol and 4-ethyl-2-methoxyphenol was measured in wines by a stable isotope dilution assay. GC-MS analysis of the volatile phenols was performed on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The starting oven temperature was 40°C for 1 min,, then raised to 260°C at 8°C/min, final hold 1 min. The results are compiled in Table 2.47. The LOQ values were 500 ng/l for 4-ethylphenol and 100 ng/l for 4-ethyl-2-methoxyphenol. It was concluded from the experiments that individual stable isotope derivatives are necessary for the reliable quantitative determination of these volatiles (Rayne and Eggers, 2007). HS-SPME-GC-MS and RP-HPTC/MS were employed for the investigation of the polyphenols

Table 2.46 Quantification of volatile phenols in samples of Tannat wines

Sample	4-Ethylguaiacol (μg/l)	4-Ethylphenol (μg/l)	Bretty flavour
1	120	1120	Yes
2	n.q.	170	Yes
3	n.d.	n.q.	No

n.d.: below LOD.
n.q.: detected but below LOQ.
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Table 2.47 Determination of 4-ethylphenol and 4-ethyl-2-methoxyguaiacol concentrations in 54 commercial 2005 vintage barrelled Okanagan Valley red wines

Variety	4-Ethylphenol (μg/l) (%)	4-Ethyl-2-methoxyphenol (μg/l) (%)
Blend of Cabernet Sauvignon/Merlot/Cabernet Franc	16.3 ± 0.7 (49.1)	43.7 ± 0.9 (94.4)
Cabernet Franc	<0.5 (88.5)	24.0 ± 0.8 (63.2)
Cabernet Sauvignon	<0.5 (56.4)	343.5 ± 14.3 (43.0)
Cabernet Sauvignon	135.1 ± (82.4)	120.3 ± 7.1 (36.8)
Cabernet Sauvignon	<0.5 (55.0)	56.5 ± 3.6 (17.4)
Cabernet Sauvignon	147.5 ± 12.3 (42.0)	42.2 ± 3.4 (35.9)
Cabernet Sauvignon	144.0 ± 2.8 (73.7)	29.1 ± 1.0 (12.1)
Cabernet Sauvignon	7.3 ± 0.4 (93.6)	39.2 ± 3.3 (22.7)
Cabernet Sauvignon	57.1 ± 1.4 (84.5)	34.4 ± 2.6 (57.0)
Merlot	<0.5 (52.8)	130.3 ± 4.3 (19.4)
Merlot	<0.5 (92.3)	78.6 ± 7.3 (19.7)
Merlot	29.6 ± 0.9 (87.8)	128.1 ± 1.7 (23.2)
Merlot	39.5 ± 1.8 (80.0)	94.8 ± 5.2 (31.5)
Merlot	106.7 ± 0.4 (67.7)	57.1 ± 3.3 (47.3)
Merlot	119.8 ± 6.4 (59.6)	35.0 ± 3.2 (70.9)
Merlot	18.2 ± 0.4 (59.6)	44.1 ± 2.6 (22.3)
Merlot	<0.5 (77.1)	45.7 ± 1.7 (35.3)
Merlot	16.2 ± 0.9 (69.0)	102.8 ± 8.3 (33.7)
Merlot	<0.5 (63.2)	24.4 ± 0.4 (26.4)
Merlot	84.9 ± 6.6 (45.0)	61.7 ± 4.0 (25.6)
Merlot	199.7 ± 5.8 (78.1)	35.1 ± 3.1 (108.1)
Merlot	55.2 ± 3.6 (78.1)	188.0 ± 10.0 (40.1)
Merlot	74.9 ± 0.3 (57.6)	47.4 ± 1.4 (21.7)
Merlot	<0.5 (55.0)	4.3 ± 0.1 (16.0)
Merlot	22.1 ± 1.7 (56.1)	21.7 ± 1.6 (25.0)
Merlot	0.6 ± 0.1 (43.6)	100.1 ± 6.5 (64.0)
Merlot	21.4 ± 0.1 (55.9)	36.4 ± 2.4 (14.7)
Merlot	5.8 ± 0.3 (82.8)	57.0 ± 2.6 (13.8)
Pinot Noir	<0.5 (78.4)	21.0 ± 0.2 (36.3)
Pinot Noir	54.6 ± 0.1 (44.7)	125.7 ± 4.2 (14.2)
Pinot Noir	3.9 ± 0.4 (67.5)	94.7 ± 3.3 (9.8)
Pinot Noir	80.9 ± 3.8 (50.6)	148.1 ± 11.4 (19.3)
Pinot Noir	1.0 ± 0.2 (43.5)	32.4 ± 1.1 (12.8)
Pinot Noir	23.6 ± 0.7 (84.0)	81.4 ± 3.1 (13.1)
Pinot Noir	51.2 ± 2.4 (88.5)	45.1 ± 2.7 (19.6)
Pinot Noir	31.7 ± 2.9 (98.1)	68.7 ± 5.0 (35.3)

Table 2.47 (continued)

Variety	4-Ethylphenol (μg/l) (%)	4-Ethyl-2-methoxyphenol (μg/l) (%)
Pinot Noir	91.0 ± 2.3 (92.9)	111.0 ± 7.5 (13.8)
Pinot Noir	39.5 ± 0.6 (85.1)	67.9 ± 7.0 (11.9)
Pinot Noir	586.2 ± 10.1 (43.1)	410.5 ± 19.8 (19.0)
Pinot Noir	33.9 ± 2.0 (93.7)	53.5 ± 3.5 (10.0)
Pinot Noir	19.7 ± 1.4 (64.1)	96.9 ± 3.5 (12.8)
Pinot Noir	72.9 ± 4.2 (87.7)	35.6 ± 2.0 (19.6)
Pinot Noir	<0.5 (55.9)	131.7 ± 12.6 (8.2)
Pinot Noir	117.4 ± 0.5 (68.5)	400.3 ± 35.8 (10.3)
Pinot Noir	2.9 ± 0.3 (64.7)	184.3 ± 16.0 (6.8)
Syrah	200.6 ± 13.7 (91.2)	24.8 ± 1.2 (67.1)
Syrah	92.7 ± 5.5 (93.4)	55.9 ± 1.8 (69.6)
Syrah	39.3 ± 1.2 (90.7)	44.4 ± 1.8 (12.1)
Syrah	125.4 ± 0.2 (55.8)	93.3 ± 7.4 (75.3)
Syrah	44.7 ± 1.3 (80.7)	46.2 ± 1.2 (22.3)
Syrah	18.8 ± 1.7 (54.4)	43.6 ± 2.7 (23.7)
Syrah	28.0 ± 0.6 (65.9)	19.2 ± 0.7 (19.1)
Syrah	56.4 ± 2.7 (61.2)	96.0 ± 5.7 (15.2)
Syrah	23.1 ± 0.0 (76.5)	119.2 ± 12.0 (21.2)

Concentrations shown are the average ± range of duplicate analyses with the percent recovery of the isotopically labelled internal standard given in parentheses.
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and aroma substances in red wines. GC analysis of aroma compounds was performed on a capillary column (25 m × 0.32 mm i.d., film thickness, 0.52 μm). Column temperature started at 40°C for 4 min,, then raised to 200°C at 5°C/min. Injector and detector temperatures were 250 and 300°C, respectively. Mass range was 20–450 *m/z*. Phenolic compounds were separated on an ODS column (100 × 4.6 mm, particle size, 5 μm) at 35°C. Isocratic mobile phase consisted of 0.4% (v/v) *ortho*-phosphoric acid (pH 2.3) and 80% (v/v) acetonitrile and 20% (v/v) 0.4% *ortho*-phosphoric acid. Hydroxystylbenes and quercetin were separated by a mobile-phase gradient. Analytes were detected at 285 and 306 nm wavelengths. The HPLC and GC separations of analytes are depicted in Figs. 2.46 and 2.47, respectively. Aroma substances measured by GC are compiled in Table 2.48. It was established that because of the good recovery, linearity, precision and sensitivity, both RP-HPLC and GC-MS methods can be used for the analysis of phenolic compounds and aroma substances in red wines (Baptista et al., 2001).

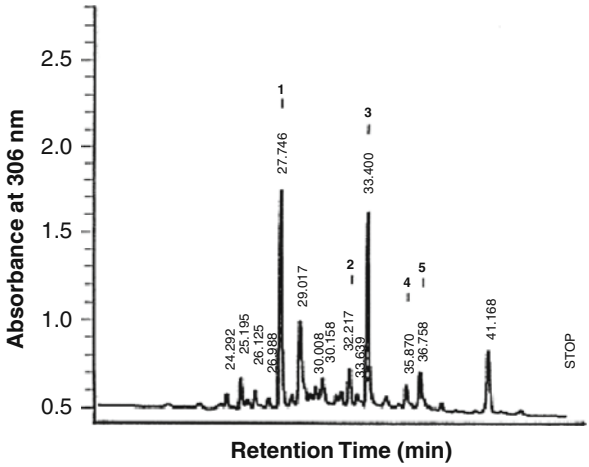

Fig. 2.46 Chromatogram of a reverse-phase HPLC of Azorean (Basalto) red wine monitored at 306 nm. Peaks: 1, *trans*-pieced; 2, *cis*-piceid; 3, *trans*-resveratrol; 4, *cis*-resveratrol; 5, quercetin. Reprinted with permission from Baptista et al. (2001)

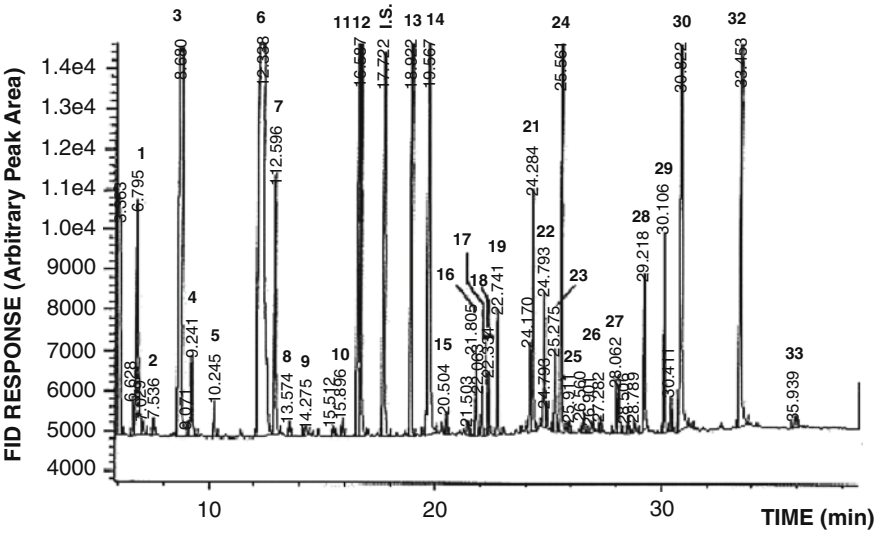

Fig. 2.47 7. HS-SPME/GC of "Basalto" Azorean red wine. GC conditions were as follows: 25 m × 0.32 mm FS-WCOT column coated with 0.52 μm film of HP-FFAP, oven 40°C (held for 5 min) and then programmed to 200°C at 5°C min^{-1}. The helium carrier gas flow rate was 28 cm s^{-1}. The injector and detector (FID) temperature were 250°C and 300°C, respectively. Identified compounds are listed in Table 2.48. Reprinted with permission from Baptista et al. (2001)

Table 2.48 Volatile compound peak area: internal standard peak area ratio of the most significant red wine aroma compounds. Red wines: (A) Basalto (Azorean), (B) Terras do Conde (Azorean), (C) S. Miguel (Azorean), (D) Douro, (E) Dão, (F) Ouro velho, (G) Bairrada, (H) Uva do Monte, (I) Frei Bernardo, (J) Talha, (K) J. P. and (L) Lagoa

IP[a]	RT[b]	Compound name	A	B	C	D	E	F	G	H	I	J	K	L
1	6.795	Ethyl 3-methylbutanoate	0.42	0.02	0..04	0.03	0.06	0.04	0.04	0.06	0.04	0.03	0.10	0.03
2	7.536	2-Methyl-1-propanol	0.03	0.04	0.02	0.05	0.05	0.06	0.05	0.01	0.03	0.01	0.02	0.04
3	8.680	Isobutanol+3-methylbutyl Acetate	4.01	1.61	3.75	1.18	2.66	2.63	3.59	2.97	2.15	2.17	1.68	1.79
4	9.241	Isoamyl acetate	0.13	0.20	0.35	0.18	0.26	0.32	0.24	0.20	0.19	0.37	0.18	0.15
5	10.245	1-Butanol	0.04	Nd	0.01	Tr	0.01	Tr	0.01	Tr	0.01	0.01	0.01	0.01
6	12.336	Ethylhexanoate	32.45	15.00	30.23	22.28	29.82	31.41	35.66	26.54	26.27	34.17	13.94	20.46
7	12.896	3-Methyl-1-butanol+2methyl-1-butanol	0.31	0.18	0.22	0.40	0.31	0.41	0.37	0.22	0.41	0.29	0.18	0.23
8	13.574	1-Hydroxy-2-propanone	0.02	Tr	0.01	Nd	Nd	Nd	Nd	Nd	Tr	Nd	Tr	Tr
9	14.275	4-Methyl-1-pentanol	0.02	Tr	Nd	Tr	0.03	Tr	0.08	Tr	0.02	0.01	0.09	Tr
10	15.896	3-Methyl-1-pentanol	0.02	0.02	Nd	Tr	Tr	Tr	0.02	0.01	Tr	Tr	0.01	Tr
11	16.575	2-Hydroxyethyl propanoate	1.97	0.81	1.22	0.76	1.45	0.93	1.25	0.81	1.05	0.47	2.58	0.41
12	16.687	1-Hexanol	0.25	0.59	0.51	0.42	0.93	0.99	1.29	0.78	0.88	1.12	0.53	0.65
IS	17.722	Internal standard	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13	18.922	Methyl-2-propeonate	0.99	0.32	0.35	1.12	0.62	0.95	0.84	0.47	0.67	0.55	0.35	0.48
14	19.667	Ethyl octanoate	1.11	0.39	1.79	0.04	2.09	0.65	1.59	1.15	1.01	0.57	5.91	1.32
15	20.504	Acetic acid	0.03	0.01	Nd	Nd	Nd	Tr	0.07	0.03	Tr	Nd	Nd	Tr

Table 2.48 (continued)

IP[a]	RT[b]	Compound name	A	B	C	D	E	F	G	H	I	J	K	L
16	21.805	Nerol oxide	0.09	4.52	0.15	0.06	0.23	0.44	0.16	0.08	0.28	0.05	0.10	0.14
17	22.053	Formic acid	0.05	0.04	0.06	0.04	0.11	0.06	0.12	0.05	Nd	Tr	1.41	0.10
18	22.338	2,3-Butanodiol	0.06	0.03	0.06	0.04	0.005	0.03	0.42	0.05	0.06	Tr	0.15	0.03
19	22.741	Linalcol	0.18	0.14	0.12	0.13	0.10	0.09	0.31	0.13	0.10	0.06	0.08	0.06
20	22.090	Linanyl acetate	0.05	Tr	Tr	Tr	0.05		0.10	0.05	0.03	0.02	0.29	0.03
21	24.288	1-Octanol+2-hydroxypropionc acid	0.27	0.06	0.05	0.23	0.19	0.22	0.32	0.09	0.15	0.17	0.07	0.12
22	24.793	2-Hydroxy-3-hexanone+2,3-butanediol	0.15	0.06	0.09	0.08	0.26	0.10	0.09	0.07	0.04	0.04	0.23	0.04
23	25.275	Hotrienol	0.10	0.09	Tr	Tr	Tr	Tr	0.14	0.09	Tr	Tr	Tr	Tr
24	25.551	Ethyl decanoate	1.32	1.04	1.12	1.56	1.26	1.26	1.57	0.46	1.36	0.71	0.82	0.94
25	26.911	Butanoic acid	0.01	Tr	0.19	0.01	Nd	Tr	0.05	0.01	0.01	0.01	Tr	Tr
26	26.560	Diethyl succinate	0.02	0.02	0.04	0.03	Tr	0.02	0.03	0.03	0.06	Nd	Tr	0.01
27	28.092	Hexanoic acid	0.05	0.29	0.03	0.03	Tr	0.57	0.12	0.05	Tr	Tr	0.04	0.03
28	29.218	Geraniol	0.18	0.37	0.18	0.16	0.21	0.16	0.59	0.21	0.19	0.20	0.32	1.02
29	30.106	Benzyl alcohol	1.20	Tr	0.13	0.13	Tr	Tr	0.41	0.19	0.10	0.08	Tr	Tr
30	30.822	Phenylethyl alcohol	4.27	3.38	5.50	5.89	4.51	4.38	5.72	4.30	2.97	4.98	2.74	2.88
31	33.033	3-Hexenoic acid	Nd	0.09	0.08	Nd	0.14	Tr	0.32	Tr	Tr	Nd	0.03	Tr
32	33.436	Octanoc acid	0.53	0.37	0.30	0.38	0.33	0.28	2.66	0.53	0.34	0.12	0.16	0.19
33	35.939	Ethyl 2-hydroxypropanoate	0.03	0.02	0.02	0.03	0.03	0.03	0.02	0.03	Tr	0.02	0.01	0.01

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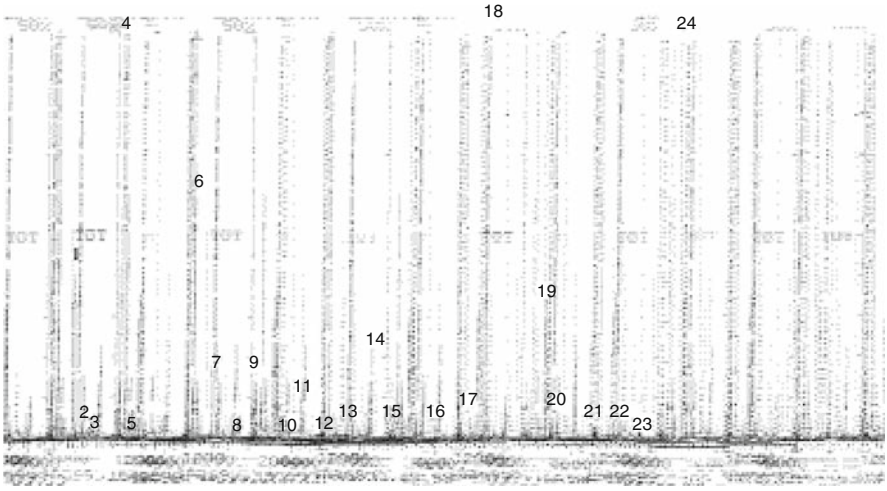

Fig. 2.48 TIC chromatogram of a TNM-MD red wine dichloromethane extract. Peak identification: (1) 2-methylpropan-1-ol; (2) isoamyl acetate; (3) butan-1-ol; (4) 3-methylbutan-1-ol; (5) ethyl hexanoate; (6) ethyl lactate; (7) octan-3-ol (internal standard); (8) ethyl octanoate; (9) acetic acid; (10) *cis*-dioxane; (11) (D,L)-butan-2,3-diol; (12) 2-methylpropanoic acid; (13) (*R*,*S*)-butan-2,3-diol; (14) ethyl decanoate; (15) diethyl succinate; (16) methionol; (17) methyl-2-ethylhexanoate; (18) β-phenylethanol; (19) ethyl 3-hydroxybutyrate; (20) octanoic acid; (21) γ-octalactone; (22) γ-nonalactone; (23) ∗-hydroxyphenylpropanoic acid; (24) ethyl succinate. Reprinted with permission from Perestrelo et al. (2005)

Liquid extraction with dichloromethane followed by GC-MS was applied for the separation and quantitative determination of aroma substances in dry, medium dry, sweet and medium sweet Tinta Negra Mole (TNM) red wines. Extracts were separated in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Column temperature started at 40°C for 1 min, then raised to 220°C at 2°C/min, final hold 10 min. Mass range was 30−300 *m/z*. A typical chromatogram showing the separation of aroma substances is depicted in Fig. 2.48. The concentrations of the aroma compounds are compiled in Table 2.48. It was found that because of the high recovery, good linearity, and low LOQ values, the method can be used for the investigation of the aroma profile of TNM wines (Perestrelo et al., 2006).

The phenolic compounds, major and minor volatile aroma substances were separated and quantitatively determined in wines prepared with various mixtures of Cabernet Sauvignon and Monastrell wines. Anthocyanins such as the 3-glucoside forms of delphidin, cyanidin, petunidin, peonidin and malvidin were analysed by RP-HPLC using a C-18 column (150 × 3.9 mm i.d., particle size, 4 μm). Mobile phase components were 10% aqueous formic acid (A) and acetonitrile (B). Gradient profile started 98% A (1 min), 94% A (4 min), to 86% A (20 min). Analytes were detected at 520 nm. Major volatile components (ethyl acetate, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol and ethyl lactate) were

determined on a capillary column (5 m × 0.85 mm i.d.). The initial column temperature was 33°C (5 min hold), raised to 70°C at 1.5°C/min (2 min hold), to 90°C at 4°C/min (final hold 10 min). Minor volatile compounds (ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, 3-methyl-1-pentanol, 1-hexanol, cis-3-hexen-1-ol, ethyl octanoate, benzaldehyde, ethyl nonanoate, 1-octanol, isobutyric acid, ethyl decanoate, 3-methylbutanoic acid, diethyl succinate, 2-phenylethyl acetate, hexanoic acid, 2-phenylethanol, octanoic acid and decanoic acid) were analysed by GC-MS using a fused-silica capillary (50 m × 0.22.mm i.d., film thickness, 0.25 μm). The initial column temperature was 50°C, raised to 180°C at 2.5°C/min (2 min hold), to 200°C at 1°C/min. MS conditions were: electron impact mode (70 eV), mass range 35–500 *m/z*, detector temperature 150°C. It was stated that the data make possible the differentiation of the wines according to the ratio of Monastrell wines (Lorenzo et al., 2008).

Pervaporation technique (PV) followed by GC-MS was employed for the analysis of 2,6-dichloroanisole, 2,4,6-trichloroanisole, 2,4,6-tribromoanisole in wines. The investigation was motivated by the fact that these compounds are responsible for the off-odour in bottles. GC-MS measurements were performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The initial column temperature was 45°C for 2 min, raised to 265°C at 12°C/min (1 min final hold). A typical chromatogram illustrating the separation capacity of the system is depicted in Fig. 2.49. Because of its high sensitivity, the method using a solid-phase cryogenic trap between PV and GC-MS has been proposed for the quality control of wines (Gómez-Ariza et al., 2004b).

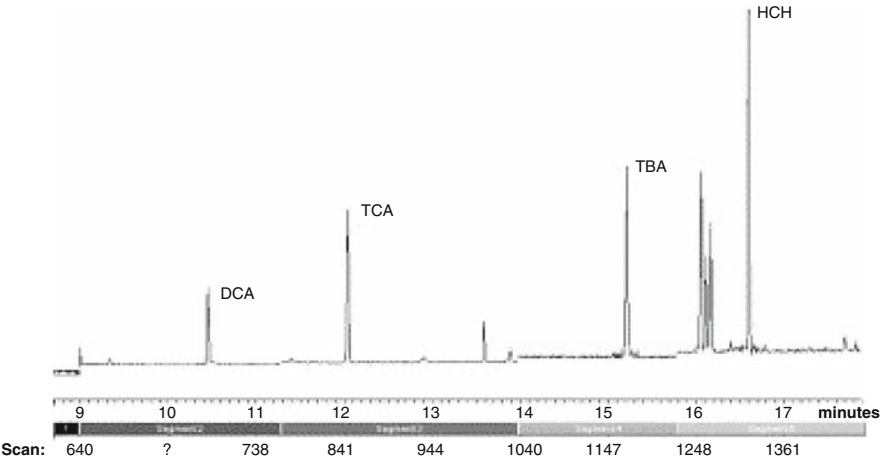

Fig. 2.49 Chromatogram obtained from red wine spiked with 405.5, 404.9, 405.9 and 563.3 pg of DCA (2,6-dichloroanisole), TCA (2,4,6-trichloroanisole), TBA (2,4,6-tribomoanisole) and lindane, respectively. The retention times were 10.5, 12.1, 15.2 and 16.6 min, respectively (Approach B). Reprinted with permission from Gómez-Ariza et al. (2004)

2.8.1.4 Wine Specialities

Besides traditional wines fermented from grapes, the aroma composition of palm wine (*Elaeis guineensis*) has also been investigated using GC-O, LLE-GC-MS and HS-SPME-GC-MS techniques. GC separation was performed in a two-dimensional GC system using two capillary columns of identical dimensions (30 m × 0.32 mm i.d., film thickness, 0.25 μm). A characteristic chromatogram is shown in Fig. 2.50. The chromatogram illustrates the diversity of the aroma compounds of palm wine containing 41 odour-active substances. The concentrations and the odour-activity values (OAVs) of the main aroma components are compiled in Table 2.49 (Lasekan et al., 2007).

A rapid HS-SPME-GC-TOFMS technology was developed and optimalised for the investigation of the aroma profile of ice wines. The HS-SPME method was fully automated. Measurements were performed with a DVB/CAR/PDMS fibre using samples consisting of 3 ml wine with 1 g salt added. Both sample incubation and extraction were carried out at 45°C for 5 min. Volatile compounds were thermally desorbed in the injector during 2 min and separated in a capillary column (10 m × 0.18 mm i.d., film thickness, 0.2 μm). Oven temperature started at 40°C (30 s hold), then raised to 275°C at 50°C/min (final hold 30 s). The conditions of TOF-MS were ionisation type, El; ionisation energy, 70 eV; ion source temperature, 200°C; detector voltage, 1700 V; mass range 35–450 *m/z*; data acquisition rate, 50 spectra/s. The total analysis time was about 20 min. The following aroma substances were included in the optimisation experiments:

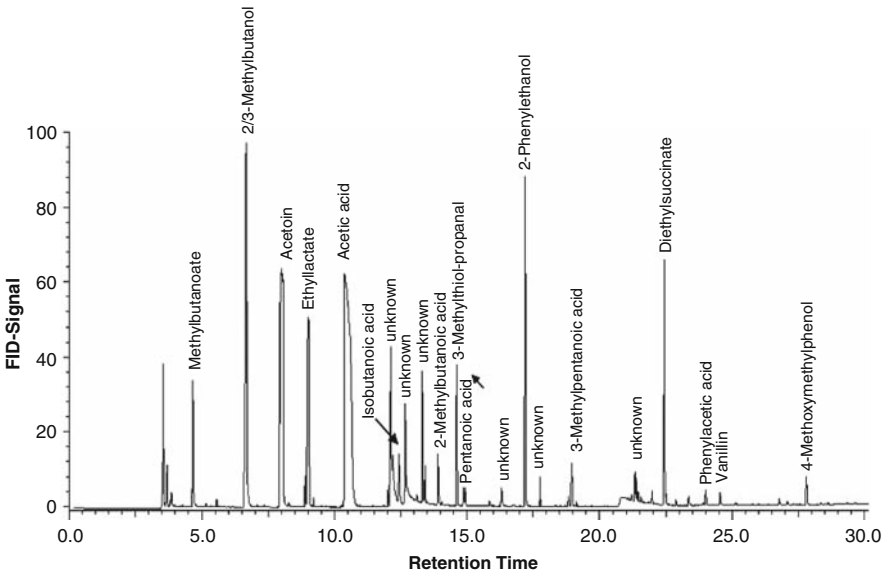

Fig. 2.50 Characteristic gas chromatogram of solvent extracted palm wine (*Elaeis guineensis*). Reprinted with permission from Lasekan et al. (2007)

Table 2.49 Concentrations and odour-activity values (OVAs) of potent odorants of palm wine

Number	Odorants	Concentration (μg/l)[a]	Odour threshold in water (μg/l)[b]	OAVs[c]	Number	Odorants
			o	r	o	r
1	3-Methylbutanol	18,300	1000	250	18	73
2	Ethyl hexanoate	52.2	0.5	0.5	104	104
3	Acetoin	663,500	800	nd	830	nd
4	2-Acetyl-1-pyrroline	11.4	0.1	nd	114	nd
5	2-Acetylpyridine	0.32	19	nd	<1	nd
6	2-Ethyl-3,5-dimethylpyrazine	0.47	0.16	0.08	3	6
7	3-Isobutyl-2-methoxypyrazine	12.0	0.005	nd	2400	nd
8	3-Methylbutyl acetate	61.7	0.88	3	70	21
9	Linalool	11.2	6	1.5	2	8
10	Methylpropanoic acid	1680	8100	1000	<1	2
11	2-Methoxyphenol	0.28	3	0.75	<1	<1
12	2-Phenylethanol	5880	1000	45	6	131
13	Phenylacetic acid	417	10,000	nd	<1	nd

nd: Not determined.
[a] Data are mean values of at least duplicates.
[b] Odour thresholds.
[c] The OAVs (o: orthonasal; r: retronasal) were calculated by dividing the concentration of the odorants by their ortho- and retronasal thresholds in water.
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2-pentanol, ethyl-2-methylbutanoate, γ-butyrolactone,1-hepten-3-ol, linalool, diethyl succinate, α-terpineol, ethyl benzeneacetate, isobutyl octanoate, pentadecyl 2-furanecarboxylic acid, ethyl 9-decenoate, β-damascenone, pyrrolidine, tridecanoic acid, 3-hydroxy-, ethyl ester, ethyl myristate. Because of the rapidity and reliability, the method was proposed for the characterisation and classification of ice wine according to their aroma profiles (Setkova et al., 2007a). The combined method discussed in Setkova et al. (2007a) was successfully applied for the determination of the composition of aroma substances in Canadian and Czech ice wines. The similarities and dissimilarities among the wine samples (origin, grape variety, oak or stainless steel fermentation and ageing conditions) were elucidated by PCA. It was found that ethyl butanoate, furfural, ethyl-2-methylbutanoate, ethyl 3-methylbutanoate, 1-hexanol, isoamyl acetate, 2-octanol, furfuryl alcohol, dihydro-2(3*H*)-furanone, 1-hepten-3-ol, ethyl hexanoate, *cis*-5-trimethyl-2-furanmethanol, *trans*-5-trimethyl-2-furanmethanol, ethyl 2,4-hexadienoate, linalool, 3,7-dimethyl-1,5,7-octatrien-3-ol, etc. play a considerable role in the aroma formation of ice wines. It was further established that the application of PCA may facilitate the classification of ice wines (Setkova et al., 2007b). The same method described in Setkova et al. (2007a, b) was applied for the characterisation of Canadian and

Czech ice wines employing self-organising maps instead of PCA. The results and conclusions were similar as the two previous papers (Giraudel et al., 2007).

The influence of the periodic and controlled microaeration of sherry wines with *Saccharomyces cerevisiae* var. *capensis* on the aroma profile was investigated by GC-MS. Measurements identified 35 aroma substances. It was established that 2,3-butanedione, 2,3-pentanodione, 4-ethylguaiacol exert a marked effect on the aroma formation (Munoz et al., 2007).

2.8.2 Other Alcoholic Beverages

Besides wines, the aroma composition of a wide variety of other alcoholic beverages was determined. Similar to wines, the method of preference for the preconcentration of volatiles is HS-SPME and separations are generally performed by GC. Mainly FID and MS are employed for the quantitation and identification of the components of the aroma profile.

The odour-active compounds in beer have also been vigorously investigated. Thus, the concentration of hydroxycinnamic acids (HCA) and volatile phenols was measured by RP-HPLC using isocratic elution and amperometric electrochemical detection. Analyses were carried out on a C18 column (250×4 mm). The mobile-phase composition was H_2O-CH_3OH-H_3PO_4 (745:245:10, v/v). The concentrations of aroma substances are compiled in Table 2.50. It was established that the simple and rapid method can be employed for the routine monitoring of the concentration of HCA and phenol compounds in wort and beer (Vanbeneden et al., 2006).

The formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids (HCA) has been studied in detail. HCA and volatile phenols were determined by isocratic HPLC employing electrochemical detection as described in Vanbeneden et al. (2006). The concentrations of *p*-coumaric acid (pCA), ferulic acid (FA), sinapic acid (SA), 4-vinylphenol (4VP) and 4-vinylguaiacol (4VG) measured in various beers are compiled in Table 2.51. The data indicate that the amount of analytes

Table 2.50 Amount (ppm) of hydroxycinnamic acids and volatile phenols present in unspiked wort and beer (calculated with the corresponding calibration curves)

	Wort	Pilsner beer	Wheat beer	Dark specialty beer
p-Coumaric acid	1.4794 ± 0.0064	1.4195 ± 0.0208	ND	0.2394 ± 0.0033
Ferulic acid	2.6267 ± 0.0085	2.2253 ± 0.0114	ND	0.3896 ± 0.0043
Sinapic acid	0.1281 ± 0.0031	0.2446 ± 0.0031	0.5035 ± 0.0046	0.3135 ± 0.0041
4-Vinylphenol	ND	0.0453 ± 0.0006	0.3250 ± 0.0031	0.3087 ± 0.0036
4-Ethylphenol	ND	ND	ND	ND
4-Vinylguaiacol	ND	0.1390 ± 0.0020	1.1119 ± 0.0167	0.5870 ± 0.0048
4-Ethylguaiacol	ND	ND	ND	ND

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Table 2.51 Total free (compensated for 4VP and 4VG) and bound alkali-extractable hydroxycinnamic acid content in commercial beers

	$x \pm$ SD[a] (min–max)[b]					
	Total free HCA			Bound HCA		
	pCA + 4VP (ppm)	FA + 4VG (ppm)	SA (ppm)	pCA (ppm)	FA (ppm)	SA (ppm)
Pilsner beer	1.40 ± 0.517	2.18 ± 0.490	0.319 ± 0.082	0.585 ± 0.480	10.1 ± 2.26	1.96 ± 0.333
($n = 5$)[c]	(0.847–2.03)	(1.43–2.61)	(0.208–0.426)	(0.155–1.27)	(7.57–13.8)	(1.58–2.44)
Ale beer	1.33 ± 0.329	1.79 ± 0.481	0.275 ± 0.077	1.04 ± 0.160	11.7 ± 1.28	2.11 ± 0.111
($n = 5$)	(1.05–1.69)	(1.37–2.60)	(0.141–0.331)	(0.926–1.15)	(9.77–13.11)	(1.94–2.19)
Belgian white	1.27 ± 0.378	2.42 ± 0.476	0.393 ± 0.085	1.83 ± 1.10	10.2 ± 1.66	3.68 ± 0.950
($n = 14$)	(0.903–2.06)	(1.54–3.01)	(0.283–0.538)	(0.687–3.43)	(7.25–13.0)	(1.90–5.67)
German Weizen	1.359 ± 0.409	2.24 ± 0.666	0.371 ± 0.150	2.37 ± 0.977	9.80 ± 0.852	3.82 ± 0.781
($n = 9$)	(0.681–1.974)	(1.40–3.30)	(0.133–0.602)	(1.26–4.11)	(8.29–11.1)	(3.04–5.38)
Blond specialty	1.99 ± 1.06	2.77 ± 1.22	0.332 ± 0.149	1.12 ± 0.231	13.6 ± 2.21	2.94 ± 1.01
($n = 16$)	(1.11–3.48)	(1.24–4.85)	(0.155–0.554)	(0.907–1.48)	(9.48–16.3)	(1.59–4.61)
Dark specialty	0.791 ± 0.442	1.82 ± 0.945	0.293 ± 0.153	1.70 ± 0.469	12.0 ± 4.44	2.61 ± 1.43
($n = 8$)	(0.421–1.43)	(0.610–3.27)	(0.092–0.470)	(1.00–2.01)	(9.07–20.8)	(1.62–5.43)
TOTAL	*1.39 ± 0.638*	*2.33 ± 0.858*	*0.344 ± 0.125*	*1.62 ± 0.956*	*11.4 ± 2.62*	*3.08 ± 1.10*
($n = 58$)	*(0.421–3.48)*	*(0.610–4.85)*	*(0.092–0.602)*	*(0.155–4.11)*	*(7.25–20.8)*	*(1.58–5.67)*

[a] $x \pm$ SD: mean ± standard deviation.
[b] (min–max): concentration range.
[c] n: number of samples analysed.
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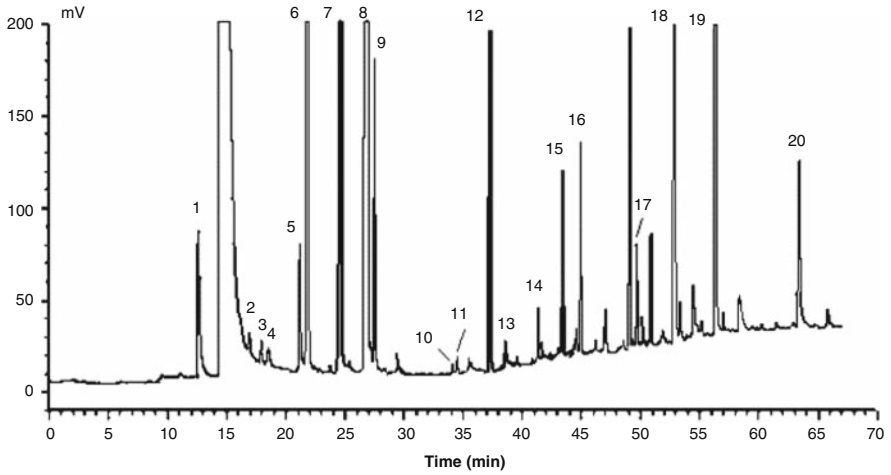

Fig. 2.51 HS-SPME-GC analysis of a real beer sample using the sol–gel-derived TMSPMA-OH-TSO fibre. Peaks: (l) ethyl acetate; (2) isobutyl acetate; (3) ethyl butyrate; (4) 1-propanol; (5) isobutanol; (6) isoamyl acetate; (7) 4-methyl-2-pentanol; (8) isoamyl alcohol; (9) ethyl hexanoate; (10) ethyl lactate; (11) 1-hexanol; (12) ethyl octanoate; (13) acetic acid; (14) linalool; (15) ethyldecanoate; (16) diethyl succinate; (17) hexanoic acid; (18) β-phenylethanol; (19) octanoic acid; (20) decanoic acid. Reprinted with permission from Liu et al. (2005)

show marked differences between the beer varieties contributing to the aroma and taste of beers. The concentrations of total free and bound HCA are compiled in Table 2.52. It was concluded from the results that a suitable brewing yeast strain is the decisive factor in the determination of the phenolic taste of beer (Vanbeneden et al., 2008).

A new SPME microextraction fibre was synthesised and employed for the preconcentration of aroma substances in beer. 3-(Trimethoxysylil)propyl methacrylate (TMSPMA) was bound to hydroxyl-terminated silicone oil and the new SPME phase (TMSPMA-OH-TSO) was used for the investigation of the aroma compounds in beer. The performance of TMSPMA based support was compared with those of PDMS, PDMS-DVB and PA fibres.

GC-FID separations were performed in a capillary column (35 m × 0.32 mm i.d.). Oven temperature started at 40°C (8 min hold), raised to 230°C at 5°C/min (final hold 20 min). A typical chromatogram of real beer sample is depicted in Fig. 2.51. The chromatogram illustrates the good preconcentration power of the new SPME fibre and the adequate separation capacity of the GC-FID system. The influence of the accompanying matrix on the recovery of aroma substances is shown in Table 2.53. It was found that the novel fibre is suitable for the simultaneous extraction of polar alcohols, fatty acids and nonpolar esters, and its performance is higher than those of commercial fibres. The recovery of the method varied between 92.8% and 105.8% Because of the good linearity, precision, low detection limit and accuracy, the new fibre was proposed for the analysis of the aroma compounds in beer (Liu et al., 2005a).

Table 2.52 Total free (compensated for 4VP and 4VG) and bound alkali-extractable hydroxycinnamic acid content in commercial beers

	$x \pm$ SD[a] (min–max)[b]		$x \pm$ SD[a] (min–max)[b]		$x \pm$ SD[a] (min–max)[b]	
	Total free HCA		Total free HCA		Total free HCA	
	pCA + 4VP (ppm)	FA + 4VG (ppm)	SA (ppm)	pCA (ppm)	FA (ppm)	SA (ppm)
Pilsner beer	1.40 ± 0.517	2.18 ± 0.490	0.319 ± 0.082	0.585 ± 0.480	10.1 ± 2.26	1.96 ± 0.333
($n = 5$)[c]	(0.847–2.03)	(1.43–2.61)	(0.208–0.426)	(0.155–1.27)	(7.57–13.8)	(1.58–2.44)
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($n = 58$)	*(0.421–3.48)*	*(0.610–4.85)*	*(0.092–0.602)*	*(0.155–4.11)*	*(7.25–20.8)*	*(1.58–5.67)*

[a] $x \pm$ SD: mean ± standard deviation.
[b] (min–max): concentration range.
[c] n: number of samples analysed.
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Table 2.53 Comparison of peak areas of volatile compositions in various matrices

Volatile compounds	Peak area percentage[a] (%)				
	Water	Water + 4% ethanol	Concentrated synthetic beer	"Volatile-free" beer	Beer 1[b]
Alcohols					
1-Propanol	100	66	79	70	61
Isobutanol	100	68	64	67	64
Isoamyl alcohol	100	66	66	64	63
1-Hexanol	100	53	52	51	51
Linalool	100	76	64	61	57
β-Phenylethanol	100	59	59	59	56
Sum	100	64	63	62	60
Fatty acids					
Acetic acid	100	87	79	77	78
Hexanoic acid	100	68	60	53	52
Octanoic acid	100	63	48	35	34
Decanoic acid	100	80	68	52	50
Sum	100	69	56	43	41
Esters					
Ethyl acetate	100	62	60	59	50
Isobutyl acetate	100	65	63	63	59
Ethyl butyrate	100	71	67	68	62
Isoamyl acetate	100	73	71	66	60
Ethyl hexanoate	100	85	75	74	74
Ethyl lactate	100	83	58	58	55
Ethyl octanoate	100	92	74	77	80
Ethyl decanoate	100	126	119	133	134
Diethyl succinate	100	48	49	47	44
Sum	100	66	61	61	58

[a] Percentage = peak area obtained in other matrix/peak area obtained in the water matrix.
[b] Percentage = (peak area obtained in the spiked beer sample − peak area obtained in the beer sample)/peak area obtained in the water matrix.
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Another study performed the optimisation of SPME technique coupled to GC-MS. The optimal conditions of the CAR-PDMS, PDMA and PA fibres for the preconcentration of beer aroma substances were determined. GC separations were carried out in a capillary column (60 m × 0.32 mm i.d., film thickness, 1.0 μm). Initial oven temperature was 40°C (5 min hold), increased to 200°C at 3°C/min (final hold 5 min). The method separated and identified 182 volatile compounds in beers. The optimised technique was proposed for the comparison of aroma profiles of beers (Pinho et al., 2006).

The bioactive compounds in the extract of hop strobilus were investigated using various chromatographic technologies such as CZE, HPLC-MS-MS and GC-MS. Samples were extracted by SFE and ultrasonic treatment using methanol–acetone–water mixtures in various ratios. CZE applied a fused silica capillary (total length

and effective length being 75 and 50 cm, respectively). Running buffer was 25 mM sodium tetraborate (pH 9.3). Samples were injected hydrodynamically for 15 s. Detection wavelength was set to 210 nm. Applied voltages were +20 and +30 kV. RP-HPLC separations were performed in a C-18 column (150 × 2.1 mm, particle size, 5 μm) at 35°C. Mobile-phase components were 0.1% aqueous formic acid (A) and acetonitrile (B). Analytes were detected by DAD (200–600 nm) and MS (mass range *m/z* 50–1,000). GC-MS separations were carried out in a capillary column (30 m × 0.32 mm i.d., film thickness, 0.25 μm). Starting oven temperature was 100°C, raised to 320°C at 12°C/min. Some electrophoregrams are depicted in Fig. 2.52. The differences in the electrophoregrams emphasise the decisive role of the mode of extraction in the analysis of hop bioactive components. The chromatograms achieved by RP-HPLC-MS-MS are listed in Fig. 2.53. It was assumed that RP-HPLC separates well the clusters of flavonoids and bitter acids. The analytes found in a hop extract by RP-HPLC are compiled in Table 2.54. A GC-MS chromatogram is shown in Fig. 2.54. The data indicated that the main volatile fractions contained lupulone and lupulone isomers (Helmja et al., 2007).

The aroma composition of whiskies has also been investigated in detail. The efficacy of various dynamic HS-SPME fibre coatings for the preconcentration of aroma substances was studied and the extraction method was optimised. PDMS, PA, CAR/PDMS, CW/DVB, and CAR/PDMS/DVB coatings were included in the experiments. Whisky samples were Black Label (BL), Ballantines (Bail) and Highland Clan HC. It was found that the best result can be achieved by employing CAR/PDMS fibre coating at 40°C, stirring at 750 rpm for 60 min. Aroma compounds were separated and quantitated on a fused silica capillary column (30 m × 0.5 mm i.d., film thickness, 0.25 μm). Oven temperature started at 40°C (1 min hold), raised to 120°C at 1°C/min, to 180°C at 1.7°C/min, to 220°C at 25°C/min. Before each step, a constant temperature for 2, 1 and 10 min was held, respectively. The temperature of the transfer line was 220°C, the ionisation energy was 70 eV, the detection range was 30–300 *m/z*. Typical total ion chromatograms showing the aroma profile of whiskies are depicted in Fig. 2.55. Samples contained a high number of volatile substances such as ethyl esters, higher alcohols, acetates, isoamyl esters, fatty acids, terpenes, carbonyl compounds and phenols, but the differences among the aroma profiles of whiskies were mainly quantitative. It was concluded from the measurements that the method is relatively simple, rapid and sensitive and can be applied for the determination of volatiles in whiskies (Camara et al., 2007).

Another study compared the efficacy of dynamic HS-SPME (CAR/PDMS and CW/DVB fibres) and traditional LLE using dichloromethane as extracting agent. Synthetic whisky samples and 24 commercial whisky samples were included in the experiments (Famous Grouse, FG; Dewar's DW; Red Label (RL); Black Label. BL; Grant's, GRA; Long John, LJ; Ballantines. BAL; Highland Clan, HC). Samples were diluted to 12% (v/v) alcohol before extraction. GC-FID analyses were performed in a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.5 μm). Oven temperature started at 40°C (1 min hold), raised to 120°C at 1°C/min (2 min hold), to 180°C at 1.7°C/min (1 min hold), to 220°C at 25°C/min, final

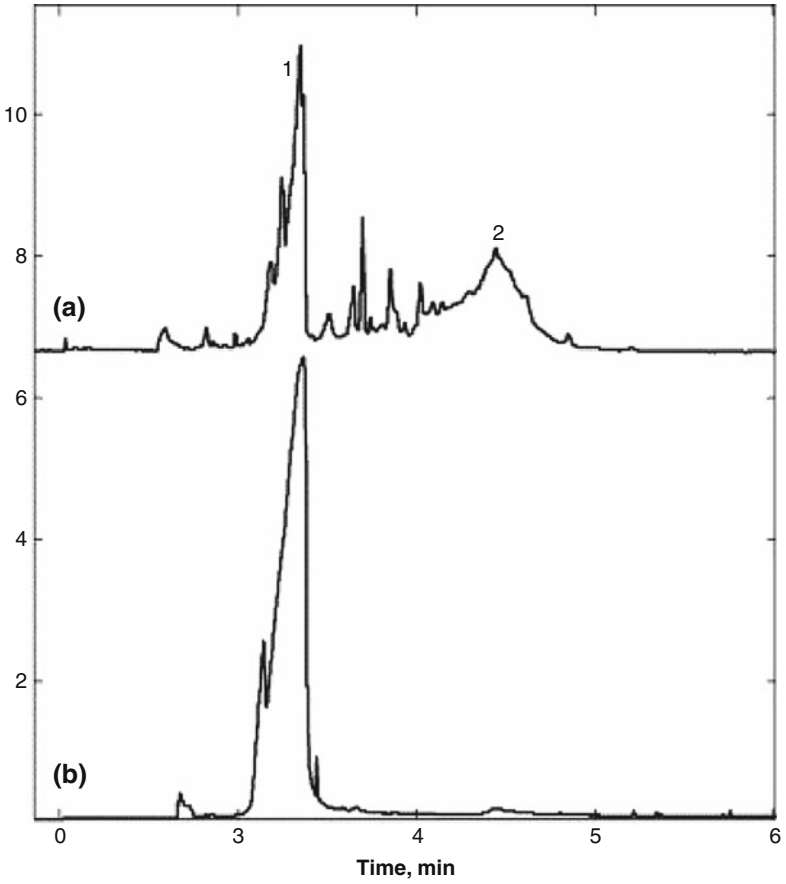

Fig. 2.52 Electropherograms of different hop strobilus extracts. (A) Ultrasonic extraction in 70:30 methanol/water (the extract No. 3); (B) supercritical extraction (the extract No. 6). Separation conditions: separation buffer 25 mM sodium tetraborate (pH 9.3), the effective length of the capillary 50 cm, applied voltage +30 kV, UV detection at 210 nm, injections were performed hydrodynamically during 15 s. Reprinted with permission from Helmja et al. (2007)

hold 10 min. FID and injector temperatures were 300°C and 260°C, respectively. The same chromatographic equipment was employed for GC-MS measurement, the mass range being *m/z* 30–300. Some TIC chromatograms showing the separation of LLE and HS-SPME extracts are depicted in Fig. 2.56. It was established that the extraction efficacy of the methods is markedly different. HS-SPME extracted mainly ethyl esters, higher alcohols and fatty acids, while LLE extracted higher alcohols and ethyl esters. It was further found that ethyl octanoate, isoamyl acetate and isobutyl alcohol are the most potent odour-active compounds (Caldeira et al., 2007).

SPE combined with multidimensional GC was employed for the separation and quantitative determination of four novel odorants (ethyl-2-methylpentanoate, ethyl 3-methylpentanoate, ethyl 4-methylpentanoate and ethyl cyclohexanoate) in wine,

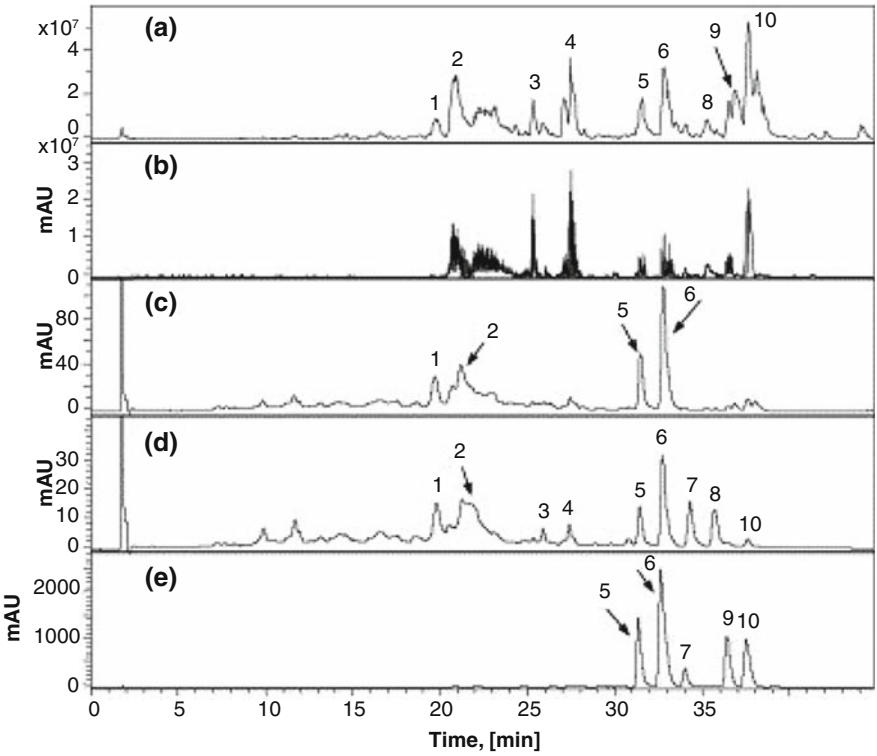

Fig. 2.53 Analysis of hop strobilus extracts by HPLC-MS/MS. Chromatograms (**A**) +MS-BPC of the extract No. 3; (**B**) $+MS^2$ constant neutral loss 56 amu chromatogram of the extract No. 6 characteristic for the bitter acids; (**C**) UV-chromatogram at a wavelength 280 nm of the extract No. 3; (**D**) UV-chromatogram at a wavelength 280 nm of the extract No. 3 after oxidation; (**E**) UV-chromatogram at a wavelength 280 nm of the extract No. 6. Peaks: (1) mainly unidentified compound with $[M + H]^+ = 247$; (2) unidentified bitter acid with $[M + H]^+ = 319$; (3) unidentified bitter acid with $[M + H]^+ = 341$ and 417; (4) xanthohumol/isoxanthohumol; (5) humulol + xanthohumol; (6) humulol + humulone; (7) unidentified bitter acid with $[M + H]^+ = 267$; (8) unidentified bitter acid with $[M + H]^+ = 349$; (9) colupulone; (10) lupulone. Reprinted with permission from Helmja et al. (2007)

distilled beverages, brandy, cognac, gin, whisky etc. Samples of 100 ml were passed through a cartridge containing 200 mg sorbent. Cartridge was washed with water–methanol 1:1 containing 1% sodium bicarbonate, then the analytes were eluted with 1.5 ml of dichloromethane and this solution was injected in the GC-GC-MS system. The first column was 30 m × 0.32 mm i.d., film thickness 0.50 μm connected to a FID detector, while the second column was 30 m × 0.32 mm, film thickness 1 μm using MS detection. The temperature programs were 40°C initial hold 7 min, increased to 100°C at 4°C/min, to 140°C at 6°C/min to 200°C at 20°C/min (columns 1) and 40°C initial hold 16 min, raised to 130°C at 5°C/min, to 250°C at 20°C/min (second column). The concentrations of odorants in the samples are compiled in Table 2.55. The data demonstrate that the highest

Table 2.54 Compounds identified in the hop extract No. 3

Compound	t_R (min)	[M-H- Or [M+H]+	Peak Height (AU)	Main daughter ions (in parentheses the relative intensities – intensity of the first, most abundant ion is taken as 100)	Constant neutral loss 56	Constant neutral loss 69
Unknown bitter acid	20.9	−317	130,373	248; 180 (26); 205 (9); 233 (9); 152 (8); 220 (7)	319 → 263	317 → 248
		+319	14,918	251; 263 (24); 195 (18); 249 (12); 235 (11)		
Xanthohumol/ isoxanthohumol	27.5	−353	32,654	233; 119 (13); 247 (6); 251 (5); 189 (4); 218 (3); 145 (2); 165 (2)		
		+355	34,364	299; 179 (30); 197 (4); 235 (4); 151 (1)	355 → 299	
Posthumulone	29.4	−333	3,140	264; 265 (8); 194 (1.5); 315 (1.4); 219 (1.1); 287 (0.8); 221 (0.8)		333 → 264
		+335	ND	–	ND	
		+335[b]		317; 279; 267; 211; 223; 167		
Humulol	31.5	−221	ND	–		
		+223	10,590	167; 207 (33); 189 (1); 155 (1)	223 → 167	
Cohumulone	31.5	−347	86,207	278; 235 (1.6); 329 (1.1)		347 → 278
		−347[c]		329; 278; 235		
		+349	8,648	223; 293 (85); 281 (32); 331 (23)	349 → 293	
		+349[a]		223; 225 (85); 281 (63); 293 (35); 167 (6); 331 (3)		
Humulol	32.7	−221	ND	–		
		+223	22,607	167; 155 (0.7); 139 (0.3); 175 (0.3); 179 (0.3)	223 → 167	
Humulone/ adhumulone	32.8	−361	132,412	292; 249 (1.5); 343 (1.1); 125 (0.2)		361 → 292
		−361[c]		292; 343; 249		
		+363	17,443	–	363 → 307	
		+363[a]		223; 239 (78); 295 (51); 307 (35); 167 (8); 345 (2)		

Table 2.54 (continued)

Compound	t_R (min)	[M-H- Or [M+H]+	Peak Height (AU)	Main daughter ions (in parentheses the relative intensities – intensity of the first, most abundant ion is taken as 100)	Constant neutral loss 56	Constant neutral loss 69
Prehumulone/ adprehumulone	34.8	−375	5,839	306; 263 (2.0); 357 (1.2); 237 (0.5); 284 (0.4)		375 → 306
		+377	ND	–	ND	
		+377[b]		359; 321; 309; 253; 223; 167		
Postlupulone	35.0	−385	3,212	273; 316 (29); 261 (12); 248 (11); 205 (10); 341 (4)		385 → 316
		+387	791	–	ND	
		+387[b]		387; 331; 319; 263; 207; 275; 219; 163		
Colupulone	36.5	−399	51,288	287; 330 (23); 219 (12); 275 (12); 262 (9); 355 (4); 194 (3); 207		399 → 330
		+401	19,342	275; 345 (75); 277 (58); 219 (18); 333 (17); 381 (10)	401 → 345	
		+401[a]		277; 275 (46); 219 (32); 345 (23); 221 (5); 333 (4); 163 (1)		
Lupulone/ adlupulone	37.6	−413	112,826	301; 344 (22); 289 (12); 233 (11); 276 (9); 369 (5)		413 → 344
		−413[c]		369; 344; 301; 233; 208		
		+415	59,257	275; 359 (79); 291 (48); 219 (25); 347 (13); 395 (5)	415 → 359	
		+415[a]		291; 275 (41); 219 (26); 359 (20); 235 (5); 347 (2); 163 (1)		
Prelupulone/ adprelupulone	39.2	−427	3,088	315; 358 (23); 333 (17); 247 (12); 303 (19); 290 (10); 383 (4)		427 → 358
		+429	771	–	ND	
		+429[b]		429; 373; 361; 305; 249; 275; 219; 163		

AU: arbitrary units; ND: not detectable.
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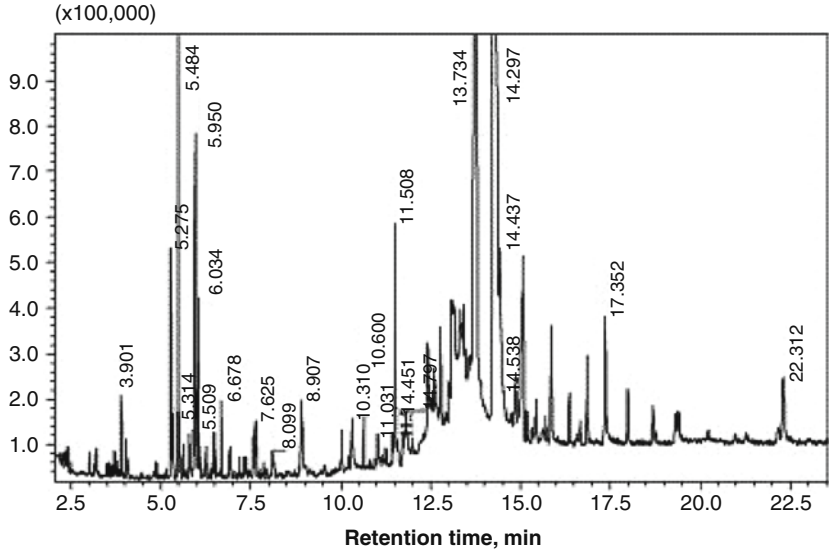

Fig. 2.54 GC–MS chromatogram of the hop strobilus. Reprinted with permission from Helmja et al. (2007)

concentrations occur in sweet wines, whiskeys and brandies. The repeatability of the method was 5–12%, LOD was 1 ng/l. Because of the good separation characteristics of the method, its application in the analysis of these odorants is proposed (Campo et al., 2007).

Two-dimensional GC-FID and two-dimensional GC-TOFMS were employed for the analysis of the Chinese liquor Moutai. The column sets are compiled in Table 2.56. Some chromatograms are shown in Fig. 2.57. It was demonstrated that Moutai liquor contains 528 components including organic acids, alcohols, esters, ketones, aldehydes, acetals, lactones, nitrogen and sulphur-containing compounds, etc. (Zhu et al., 2007).

The aroma composition of a considerable number of other distillates has also been investigated using various extraction techniques and GC separation methods. Thus the composition of the traditional Greek fruit distillate "Mouro" was investigated by using various GC technologies. The measurements indicated that the high concentration of phenylethanol, ethyl octanoate and ethyl decanoate are characteristics for the "mouro" distillate (Soufleros et al., 2004).

The separation and quantitative determination of terpenes in tequila were performed using HS-SPME (PA, PDMS, CW/DVB, PDMS/DVB) at various extraction temperatures, extraction times, stirrings and sodium chloride concentrations. The optimal preconcentration method was followed by GC-MS carried out in a fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.250 μm). The temperature programs were initially held at 40°C for 1 min, increased to 210°C at 5°C/min, to 280°C at 10°C/min. MS detection range was 50–500 *m/z*. The optimal extraction conditions were PDMS/DVB fibre, 100% NaCl, 25°C, 30 min extraction

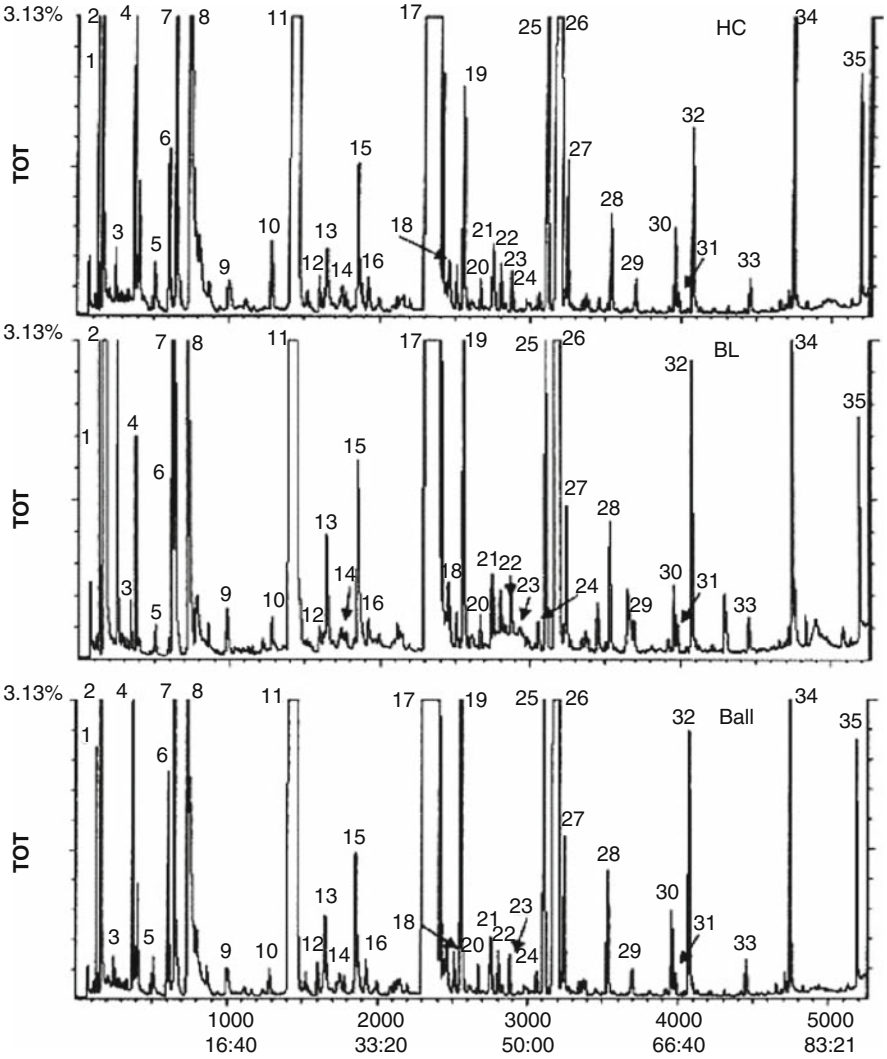

Fig. 2.55 Typical total ion chromatogram of volatile constituents from HC, BL and Balls samples obtained by SPME using a CAR/PDMS coating in the headspace sampling mode. Extraction conditions: extraction temperature: 40°C; extraction time: 60 min; stirring: 750 rpm; sample volume: 30 ml; headspace volume: 30 ml; desorption was performed at 220°C for 6 min. Peak identification: (1) ethyl acetate; (2) ethanol; (3) butan-1-ol; (4) isoamyl acetate; (5) 4-methylpentan-2-ol (IS); (6) 3-methylbutan-1-ol; (7) ethyl hexanoate; (8) estirene; (9) ethyl heptanoate; (10) octan-3-ol (IS); (11) ethyl decanoate; (12) 1,15-pentadecanediol; (13) furfural; (14) VitisI + VitisII; (15) propyl octanoate; (16) butyl caprylate; (17) isoamyl octanoate; (18) cyclodecanemethanol; (19) ethyl 9-decanoate; (20) propyl decanoate; (21) azulene; (22) buthyl decanoate; (23) dodecan-1-ol; (24) β-damascenone; (25) 2-phenylethanol acetate; (26) ethyl decanoate; (27) isoamyl decanoate; (28) phenylethanol; (29) 1,14-tetradecanediol; (30) ethyltetradecanoate; (31) nerolidol; (32) octanoic acid; (33) 1,12 dodecanediol; (34) decanoic acid; (35) dodecanoic acid. Reprinted with permission from Camara et al. (2007)

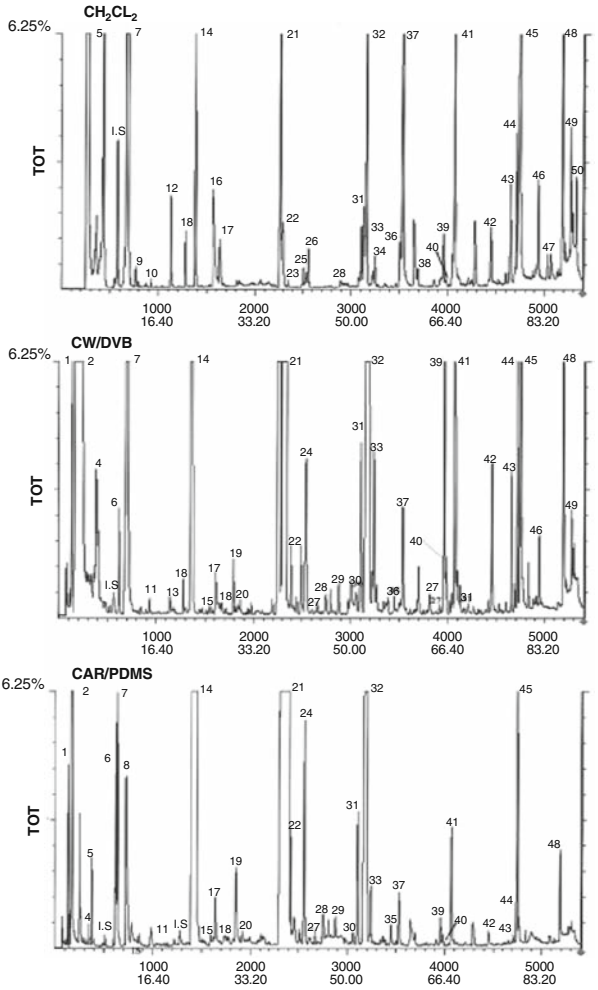

Fig. 2.56 Comparison of TIC chromatograms of the volatile fraction extracted/isolated from BL whiskey sample obtained by LLE method using CH_2Cl_2 as extraction solvent (LLE_{CH2Cl2}) and by dynamic HS-SPME using a 75 μm CAR/PDMS fibre coating ($HS\text{-}SPME_{CAR/PDMS}$). Peak Identification: 1: ethyl acetate; 2: ethanol; 3: 2-methylpropan-1-ol; 4: butan-1-ol; 5: isoamyl acetate; IS: 4-methylpentan-2-ol; 6: ethyl hexanoate; 7: 3-methylbutan-1-ol; 8: styrene; 9: acetal; 10: hexyl acetate; 11: ethyl heptanoate; IS: octan-3-ol; 12: ethyl latate; 13: hexan-1-ol; 14: ethyl octanoate; 15: linalool; 16: acetic acid; 17: furfural; 18: vitispirane (isomer I + II); 19: propyl octanoate; 20: butyl caprylate; 21: ethyl decanoate; 22: isoamyl octanoate; 23: butanoic acid; 24: *trans*-ethyl 2-decenoate; 25: 3-methylbutanoic acid; 26: diethyl succinate; 27: azulene; 28: buthyl decanoate; 29: dodecan-1-ol; 30: β-damascenone; 31: 2-phenylethanol acetate; 32: ethyl dodecanoate; 33: isoamyl decanoate; 34: hexanoic acid; 35: ∗-ionol; 36: benzeneacetaldehyde; 37: β-phenylethanol; 38: *cis*-whiskey lactone; 39: ethyl tetradecanoate; 40: nerolidol; 41: octanoic acid; 42: 1,12 dodecanediol; 43: ethyl hexadecanoate; 44: ethyl 9-hexadecanoate; 45: decanoic acid; 46: cyclododecanemethanol; 47: ethyl succinate; 48: dodecanoic acid; 49: 5-(hydroxymethyl) furfural; 50: vanillin. Reprinted with permission from Caldeira (2007)

Table 2.55 Levels of ethyl 2-, 3- and 4-methylpentanoate and ethyl cyclohexanoate (expressed as ng/l) found in the studied samples and odour activity values (OAV[a])

Sample type	Year	Brand	Et. 2mp	Et. 3mp	Et. 4mp	Et. ciclo	Sample type	Year	Brand	Et. 2mp
			conc.	*OAV*	conc.	*OAV*				conc.
White young	2004	Pazo	<q.l.	–	<q.l.	–	14	1.4	<q.l.	–
	2004	Viña Albada	<q.l.	–	<q.l.	–	56	5.6	<q.l.	–
	2004	Marqués de Riscal	<q.l.	–	<q.l.	–	120	12	<q.l.	–
Red young	2005	Montesierra	<q.l.	–	<q.l.	–	175	17	<q.l.	–
	2005	Borsao	<q.l.	–	<q.l.	–	175	17	<q.l.	–
	2004	Viñas del Vero	<q.l.	–	<q.l.	–	262	26	<q.l.	–
Red barrel aged	1999	Lan	22	7.5	36	4.5	258	26	4.9	4.9
	1998	Viña Pomal	18	5.9	32	4.0	211	21	5.5	5.5
	1995	Faustino	22	7.2	35	4.3	253	25	4.9	4.9
Porto	–	Ruby	22	7.4	35	4.4	335	34	3.5	3.5
	–	Tawny	36	12	43	5.4	422	42	14	14
	–	White	53	18	39	4.8	442	44	50	50
Noble rot	2002	Saut. Laribotte	20	6.8	23	2.9	51	5.1	<q.l.	–
	2002	Saut. Baron	8.5	2.8	19	2.4	116	12	<q.l.	–
	2003	Saut. Aureus	14	4.5	23	2.8	63	6.3	37	37
	2002	Tokaji Oremus	33	11	38	4.8	195	20	4.2	4.2
Cava	2001	Gramona	369	123	89	11	228	23	13	13
Fino	3[b]	Cobos	18	5.9	112	14	748	75	8.5	8.5
	5[b]	Quinta	12	3.9	145	18	853	85	<q.l.	–
	5[b]	Tío Pepe	2.8	0.9	514	64	1356	136	13	13

Table 2.55 (continued)

Sample type	Year	Brand	Et. 2mp	Et. 3mp	Et. 4mp	Et. ciclo	Sample type	Year	Brand	Et. 2mp
Cream	5[b]	Cream Canasta	17	5.6	48	6.0	376	38	4.7	4.7
	8[b]	Cream Ibérica	26	8.5	180	23	1439	144	36	36
Pale Cream	2002	Cartojal	9.6	3.2	18	2.3	142	14	<q.l.	–
Pedro Ximénez	8[b]	Duquesa	9.9	3.3	26	3.2	197	20	18	18
	10[b]	Leyenda	<q.l.	–	<q.l.	–	110	11	<q.l.	–
	1971	Don PX	17	5.8	126	16	594	59	30	30
	1975	Don PX	1066	355	518	65	972	97	63	63
Brandy	8[b]	Marqués Misa	38	13	36	4.5	566	57	48	48
	8[b]	Duque de Alba	82	27	85	11	938	94	85	85
Whisky	12[b]	Knockando	246	82	457	57	1336	134	21	21
	12[b]	Cardhu	862	287	1035	129	2724	272	22	22

[a] Odour thresholds.
[b] Sample with no attributable vintage date on the bottle. Instead, the aging period (years) is indicated <q.l.: below the quantification limit.
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Table 2.56 GC × GC column sets and temperature programs

	First column	Second column
Set 1		
Stationary phase	HP-Innowax	DB-1701
Length (m)	60	1.2
Diameter (mm)	0.25	0.1
Film thickness (μm)	0.25	0.4
Temperature program 50°C, 2° min^{-1} to 230°C, 10 min hold		
Set 2		
Stationary phase	DB-Petro	DB-1701
Length (m)	50	2.6
Diameter (mm)	0.2	0.1
Film thickness (μm)	0.5	0.1
Temperature program 50°C, 3° min^{-1} to 260°C, 30 min hold		

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time and stirring at 1,200 rpm. A characteristic chromatogram depicting the separation of the aroma substances in aged tequila is shown in Fig. 2.58. The baseline separation of terpenes illustrates the good analytical characteristics of the chromatographic system. The terpenes identified in tequila are compiled in Table 2.57. It was stated that the method is simple, rapid, solvent-free and can be successfully applied for the analysis of terpenes in tequila (Pena-Alvarez et al., 2006).

The aroma substances of the Brazilian sugarcane spirit have been extracted by HS-SPME and analysed by two-dimensional GC (GC × GC/TOFMS). HS-SPME preconcentration step was performed on a PA fibre at 60°C for

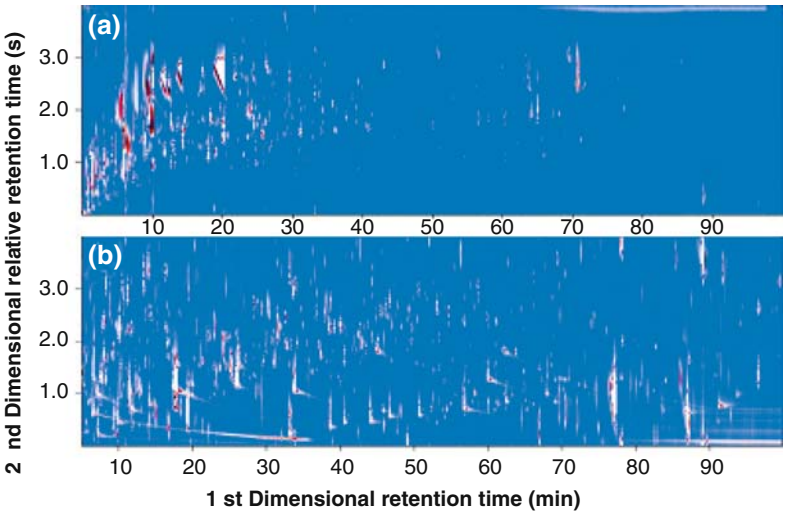

Fig. 2.57 GC × GC/FID contour plots of Moutai liquor sample 1 in different column sets: (a) DB-petro + DB-1701; (b) HP-innowax + DB-1701. Reprinted with permission from Zhu et al. (2007)

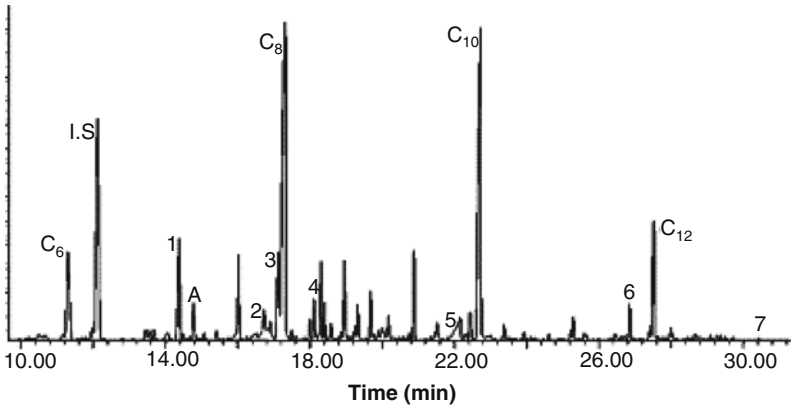

Fig. 2.58 HS-SPME-GC–MS of aged tequila. C_6: ethyl hexanoate; SI: internal standard; 1: linalool; 2: terpinen-4-ol; 3: ∗-terpineol; C_8: ethyl octanoate; 4: β-citronellol; 5: eugenol; C_{10}: ethyl decanoate; 6: *trans*-nerolidol; C_{12}: ethyl dodecanoate; 7: *trans*-farnesol. Reprinted with permission from Pena-Alvarez et al. (2006)

25 min. The dimensions of the primary column for GC × GC/TOFMS system were 30 m × 0.25 mm i.d., film thickness 0.25 μm. The second column was 1.5 m × 0.1 mm i.d., film thickness, 0.1 μm. The temperature program was 35°C, initial hold 5 min, increased to 210°C at 3°C/min, to 240°C at 40°C/min, final hold 10 min. The presence of 70 volatile compounds in the headspace of *cachaca* was demonstrated. Typical GC × GC/TOFMS contour plots are depicted in Fig. 2.59,

Table 2.57 Terpenes identified in tequila

Terpenes	
Linalool oxide (A)[a]	β-Citronellol[b]
Linalool oxide (B)[a]	Cuminol[a]
Linalool[b]	Carvacrol[a]
trans-p-2,8-mentadien-1-ol[a]	Timol[a]
p-Ment-1-en-8-ol[a]	Eugenol[b]
β-Terpineol[a]	β-Farnesene[a]
Ocimenol[a]	*cis*-Nerolidol[b]
*Monoterpene[a]	*Sesquiterpene[a]
Terpinen-4-ol[b]	γ-Eudesmol[a]
p-Cimen-8-ol[a]	δ-Cadinol[a]
∗-Terpineol[b]	Bisabolol oxide(II)[a]
γ-Terpineol[a]	∗-Farnesyl acetate[a]
p-Ment-1-en-9-al[a]	Farnesol (+2H)[a]
β-Ciclocitral[a]	*trans*,*trans*-Farnesol[b]

[a] Identified on the basis of MS data alone.
[b] Identified on the basis of both MS data and retention times of authentic standards.
* Not identified.
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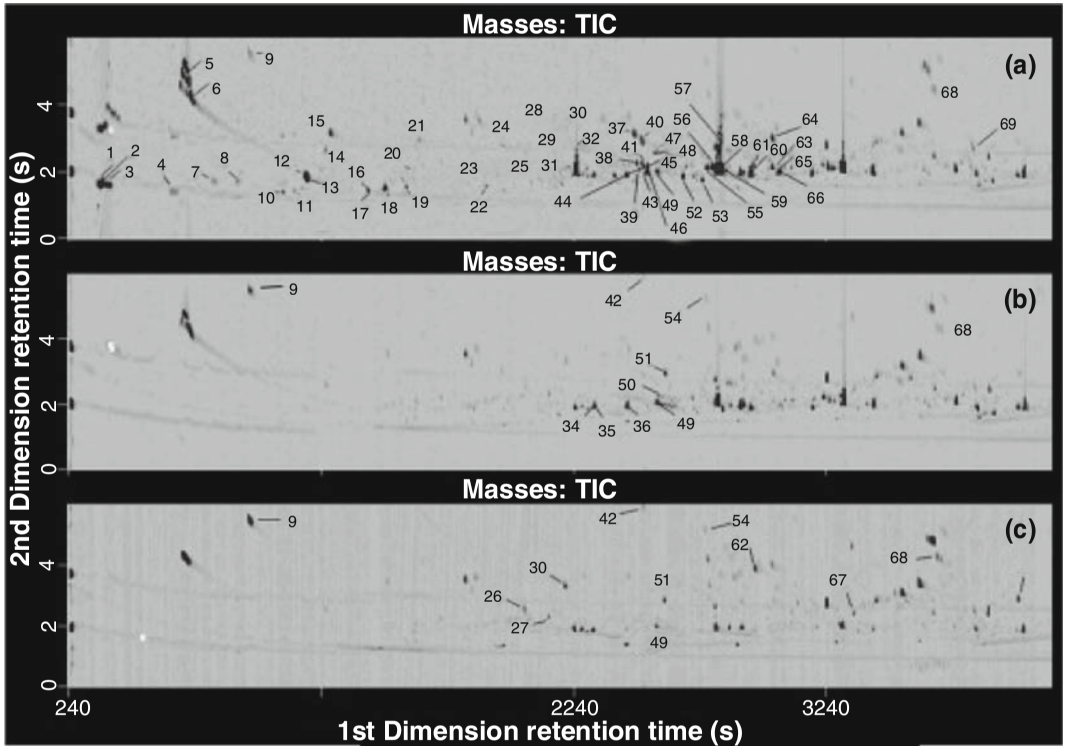

Fig. 2.59 GC × GC/TOFMS contour plots of SPME headspace extracts of the distillation process during *cachaça* distillation. (A) Contour plot obtained for the first fraction (*cabeça* "head") with high content of ethanol (not shown; mass scanning starts after elution of ethanol) and the more volatile and semi-volatile compounds. (B) *coração* "core" fraction depleted of more volatile compounds and richer in the semi-volatile compounds. (C) *cauda* "tail" fraction corresponding to the end of the distillation with enhanced water content and less volatile compounds. Reprinted with permission from Cardeal et al. (2008)

showing the effect of distillation process on the aroma composition of samples. It was concluded from the results that the method is suitable for the monitoring of the industrial production of *cachaca*, quality control and authenticity test (Cardeal et al., 2008).

SFE has also been employed for the extraction of aroma substances from sugar cane spirits (SCS). The optimal conditions for the supercritical CO_2 extraction were 10 Mpa pressure and 313 K. GC-MS was performed in a fused silica capillary column (30 m × 0.25 mm i.d., film thickness 1.245 μm. The temperature program started at 50°C, initial hold 10 min, increased to 225°C at 5°C/min, final hold 15 min.

Mass range was 13–300 *m/z*. The presence of more than 25 volatile compounds in the headspace of sugar cane spirits was demonstrated including alcohols, aromatic alcohols, phenols, fatty acids, esters, ketones, etc. The application of this novel technology in the isolation of aroma compounds from food, pharmaceutical and cosmetic industries has been proposed (Gracia et al., 2007).

The influence of ultrasonic treatments on the aging of rice alcoholic beverage was investigated by the determination of the alcohol content, titratable acidity, sensorial evaluation and GC measurement of the volatile substances. The results demonstrated that 20 kHz treatment influences beneficially the quality of rice alcoholic beverage (Chang, 2005).

2.9 Coffee, Tea and Cocoa

Coffee, tea and various cocoa products are widely consumed all over the world. As the taste and the flavour of the product are the main parameters influencing consumer acceptance, a considerable number of papers dealing with the separation and quantitative determination of the aroma compounds in coffee, tea and cocoa products has been published (Costa Freitas et al., 2001; Sanz et al., 2001: Borse et al., 2002; Rocha et al., 2003; Ryan et al., 2004; Mondello et al., 2004; Zambonin et al., 2005).

The volatile profile of defective (black, immature and sour) and healthy coffee beans was determined by SPME followed by GC/MS. Volatiles were preconcentrated on a triple-phase SPME (divinylbenzene/carboxen/polydimethylsiloxane). The ground coffee was heated for 10 min at 70°C in a vial, then SPME was performed for 40 min at 70°C. Analytes were separated on a capillary column (30 m × 0.25 mm i.d.), helium being the carrier gas. Injector temperature was 250°C, detector and interface temperatures were 300°C and 275°C, respectively. Column temperature was initiated at 40°C (5 min hold), raised to 180°C at 3°C/min, then to 250°C (5 min) at 10°C/min. Typical chromatograms illustrating the good separation capacity of the method are shown in Fig. 2.60. The analytes tentatively identified in the various samples are listed in Table 2.58. Principal component analysis (PCA) of the data suggested that the volatile profile of roasted coffee beans can be employed for the differentiation between healthy and defective coffees (Rawat et al., 2007).

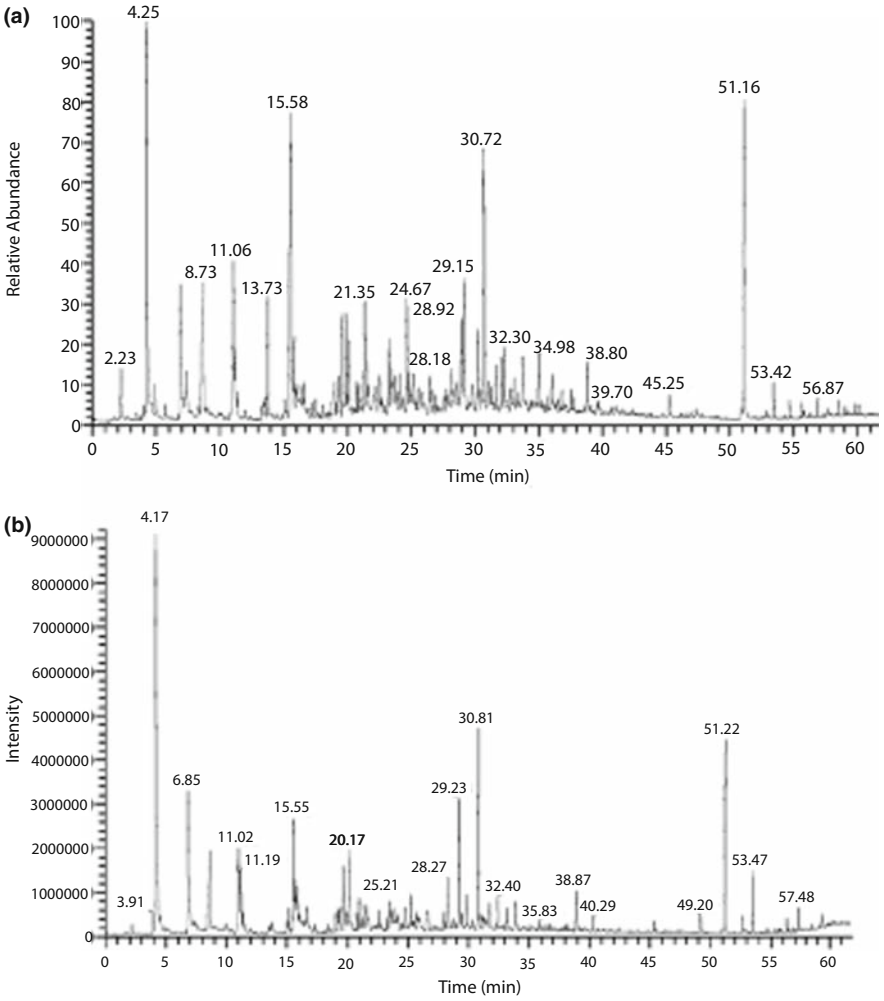

Fig. 2.60 Typical HS–SPME–GC–MS chromatograms of roasted and ground coffee headspace: (**a**) healthy; (**b**) black; (**c**) sour and (**d**) immature. Reprinted with permission from Mancha Agresti et al. (2008)

HS-SPME-GC-MS and HS-SPME-GC-O were simultaneously applied for the study of the influence of various processing steps on the concentration and composition of volatile substances in green coffee beans. The investigations revealed that the microbial removal of mucilage in water exerts a beneficial impact on the sensory characteristics of the coffee (Gonzalez-Rios et al., 2007).

Table 2.58 A comparative of volatile flavour components tentatively identified by different extraction procedures

S. No.	Components	RI	Hydrodistillation Clevenger type	SDE[b] MDA[a]	SDE[b] Enriched
1	Isobutyl alcohol	1103	–	0.37	–
2	*n*-Undecane	1109	–	0.62	–
3	3-Pentanol	1116	–	0.21	–
4	Ethylbenzene	1142	–	40.84	–
5	*p*-Xylene	1149	–	5.30	–
6	*m*-Xylene	1156	–	8.92	–
7	2-Methyl-2-butanol	1162	–	–	0.09
8	1-Penten-3-ol	1167	–	0.21	4.15
9	Isopropylbenzene	1185	–	0.23	–
10	2-Heptanone	1192	–	–	0.23
11	*n*-Heptanal	1195	–	–	0.46
12	*o*-Xylene	1197	–	4.01	–
13	*n*-Tetradecane	1203	–	0.43	–
14	3-Methyl-1-hexene	1213	–	–	1.13
15	Isoamyl alcohol	1215	–	2.80	–
16	(*E*)-2-Hexenal	1230	–	–	16.71
17	1-Pentanol	1259	–	–	2.70
18	2-Methylpyrazine	1276	–	–	0.06
19	Benzene	1290	–	0.14	–
20	3-Hydroxy-2-butanone	1298	1.59	–	–
21	2,2,6-Trimethylcyclohexanone	1323	–	–	0.24
22	(*Z*)-2-Pentenol	1331	–	0.48	2.41
23	6-Methyl-5-hepten-2-one	1347	–	0.27	0.66
24	1-Hexanol	1363	0.12	–	2.25
25	(*Z*)-3-Hexenol	1393	0.90	1.66	6.87
26	Vinylmethylether	1395	0.17	–	–
27	*n*-Nonanal	1398	–	–	0.20
28	2,5,6,6-Tetramethylcyclohexe-2-en-1-one	1405	–	–	0.06
29	€-2-Hexenol	1416	0.05	–	1.31
30	Linalool oxide-I (furanoid)	1449	0.77	2.02	4.51
31	1-Octen-3-ol	1461	–	–	0.36
32	1-Heptanol	1464	–	–	0.26
33	*n*-Heptyl acetate	1469	–	–	0.05
34	2-Propylfuran	1473	0.10	–	0.13
35	Linalool oxide-II (furanoid)	1477	0.90	4.81	10.66
36	2-Ethylhexan-1-ol	1494	–	–	0.04
37	(*E*,*E*)-2,4-Heptadienal	1497	–	–	0.90
38	2-Acetylfuran	1512	0.25	–	0.10
39	Benzaldehyde	1528	–	–	2.96
40	Linalool	1559	5.46	2.14	4.05
41	1-Octanol	1567	0.24	–	0.43
42	3,5-Octadien-2-one	1575	–	–	0.32
43	6-Methyl-3,5-heptadien-2-one	1596	–	0.14	0.31
44	2,6,6-Trimethyl-2-hydroxycyclohexanone	1601	2.75	1.28	1.78
45	1,2-Dimethylpyridone	1611	–	–	0.13

Table 2.58 (continued)

S. No.	Components	RI	Hydrodistillation Clevenger type	SDE[b] MDA[a]	SDE[b] Enriched
46	β-Cyclocitral	1619	–	–	0.11
47	3,7-Dimethyl-1,5,7-octatrien-3-ol	1620	–	–	0.15
48	4-Butanolide	1634	–	0.26	–
49	2-Methoxyphenylethanol	1645	0.11	–	0.05
50	Phenylacetaldehyde	1649	–	–	1.62
51	Acetophenone	1654	0.14	0.88	0.32
52	Methyl tetradeca-10,11-dienoate	1663	0.20	–	–
53	3-Methyl-2,4-nonanedione/4-hydroxybutyl hexanoate	1666	–	–	0.05
54	1-Nonanol	1669	1.62	–	0.16
55	Isovaleric acid	1687	–	1.00	0.63
56	2(*S*)-hydroxy-γ-butyrolactone	1692	0.24	–	–
57	α-Terpineol	1700	0.29	0.43	0.18
58	Unidentified	1736	0.39	–	–
59	(−)-2,6,6-Trimethyl-2-vinyl-4-hydroxy-tetrahydropyran	1746	–	0.88	0.42
60	Valeric acid	1756	–	0.86	0.32
61	(*Z*)-3-(Hydroxymethyl)-7-methylocta-2,6-dien-1-yl acetate	1764	0.27	–	–
62	Epoxylinalol	1772	0.31	2.21	1.39
63	Methyl salicylate	1775	2.63	0.46	1.30
64	2-Butene-2-ol	1802	0.21	–	0.05
65	Nerol	1807	0.35	–	0.53
66	α-Damascone	1812	3.70	–	0.06
67	1-Phenylethanol	1822	0.27	0.12	–
68	€-2-Decenyl acetate	1830	1.40	–	–
69	α-Irone	1850	1.49	–	0.16
70	Geraniol	1857	12.32	0.39	5.74
71	*n*-Hexanoic acid	1864	7.79	5.20	5.58
72	Acetonyl decyl ether	1904	0.12	–	–
73	2-Phenylethanol	1918	–	5.93	1.29
74	β-Ionone	1937	12.15	–	1.26
75	Heptanoic acid	1968	–	–	0.35
76	3-Hexenoic acid	1977	–	1.62	–
77	2-Hexenoic acid	1984	–	1.41	–
78	5,6-Epoxy-β-ionone	1989	–	–	0.48
79	3-Butenen-2-one	1990	0.39	0.12	–
80	2-Methylvaleraldehyde	2024	1.29	–	–
81	Nerolidol	2049	9.29	–	0.47
82	Octanoic acid	2075	–	–	0.38
83	(*Z*)-3-Hexenyl benzoate	2127	0.50	–	–
84	Hexahydrofarnesylacetone	2130	8.34	–	0.63
85	Methyl cyclopropyl ketone	2139	0.35	–	–
86	3,6-Dimethyl-2*H*-pyran-2-one	2152	0.50	–	0.17
87	Nonanoic acid	2181	0.72	–	0.61
88	2,7-Epoxy-megastigma-4,8-diene	2187	0.61	–	–

Table 2.58 (continued)

S. No.	Components	RI	Hydrodistillation Clevenger type	SDE[b] MDA[a]	SDE[b] Enriched
89	Docosane	2202	–	–	0.21
90	Methyl nonanoate	2232	0.87	–	0.23
91	Tridecanoic acid	2316	1.31	–	0.24
92	Tricosane	2328	0.31	–	0.48
93	2(4*H*)-Benzofuranone	2352	–	0.73	0.15
94	(*E*,Z)-Farnesol	2365	–	0.70	0.11
95	(*E*,*E*)-Farnesylacetone	2382	1.04	–	0.21
96	Tetracosane	2402	–	–	0.79
97	Pentacosane	2483	–	–	1.13
98	Dicyclohexyl phthalate	2529	0.20	–	0.23
99	Hexacosane	2602	–	–	1.28
100	Phytol	2619	14.37	–	1.69
101	1,3-Dioxolane	2653	–	–	0.17
102	1-Propene-1-thiol	2635	–	–	0.05
103	Unidentified	*N*	–	–	2.06
	Total % composition		99.40	100	100

N: RI not calculated.
[a] MDA: Mini distillation apparatus.
[b] SDE: Simultaneous distillation extraction.
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GC-MS was also employed for the fingerprint developing of coffee flavour. Measurements were carried out in a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm), helium being the carrier gas. Injector and detector temperatures were 250°C. Column temperature was initiated at 40°C (6 min hold), raised to 230°C at 10°C/min, final hold, 10 min. Typical chromatograms illustrating the good separation capacity of the method are shown in Fig. 2.61. The method isolated and identified 52 volatile compounds. It was suggested that the method can be applied for the quality control of coffee flavour (Huang et al., 2007).

Not only various GC technologies but also HPLC has found application in the analysis of coffee. Thus, the concentration of chlorogenic acids (CGA) and its derivatives have been many times investigated by HPLC. The chemical structure of this class of compounds is shown in Fig. 2.62. Besides their characteristics to influence coffee flavour, they have beneficial health effects showing antioxidant capacity (Moreira et al., 2005; Natella et al., 2002: Pereira et al., 2003). Moreover, they enhance insulin action in conscious rats (Shearer et al., 2003).

Because of the negligible volatility, CGA and CGA derivatives cannot be analysed by GC. The method of preference for their separation and quantification is HPLC-MS (Clifford et al., 2003, 2006a, b).

CGA and related compounds were separated and quantitated in green and roasted beans of *Coffea arabica* and *Coffea canephora*. HPLC measurements were performed in a RP column (150 × 2 mm i.d.; particle size, 5 μm, pore size, 100 Å). The components of the gradient elution were 0.3% aqueous formic acid and methanol.

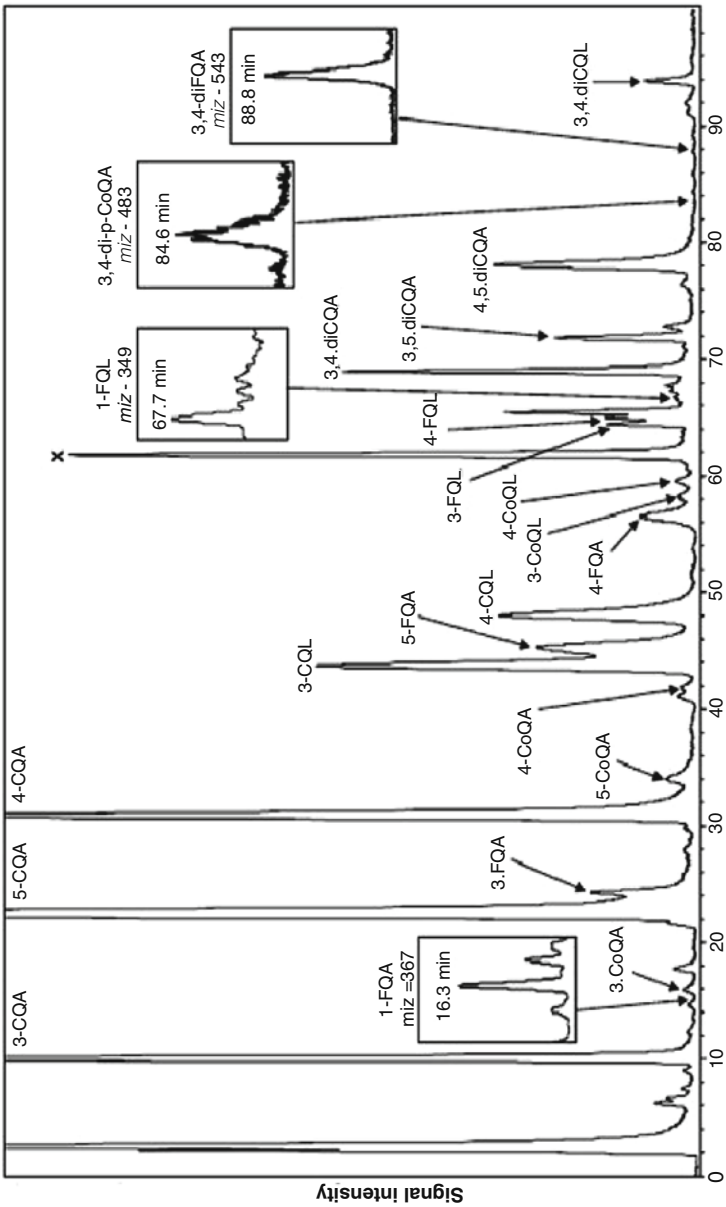

Fig. 2.61 Typical total ion chromatogram (TIC) of chlorogenic acids (CGA) and lactones (CGL) analysis represented by a light roasted *C. canephora* cv. Conillon sample. 3-CQA, 4-CQA and 5-CQA peaks are shown off scale to highlight small peaks. Note that signal intensity of different compounds cannot be directly compared, since ionization efficiency may vary among them. Selected areas of SIM chromatograms of mass-to-charge (*m/z*) 367, 349, 483 and 543 were inserted in the figure to illustrate the elution order of 1-FQA, 1-FQL, 3,4-di-*p*-CoQA and 3,4-diFQA, respectively. The peak marked "X" presented mass-to-charge ratio of 365, consistent with caffeoyl–tryptophan. Reprinted with permission from Perrone et al. (2008)

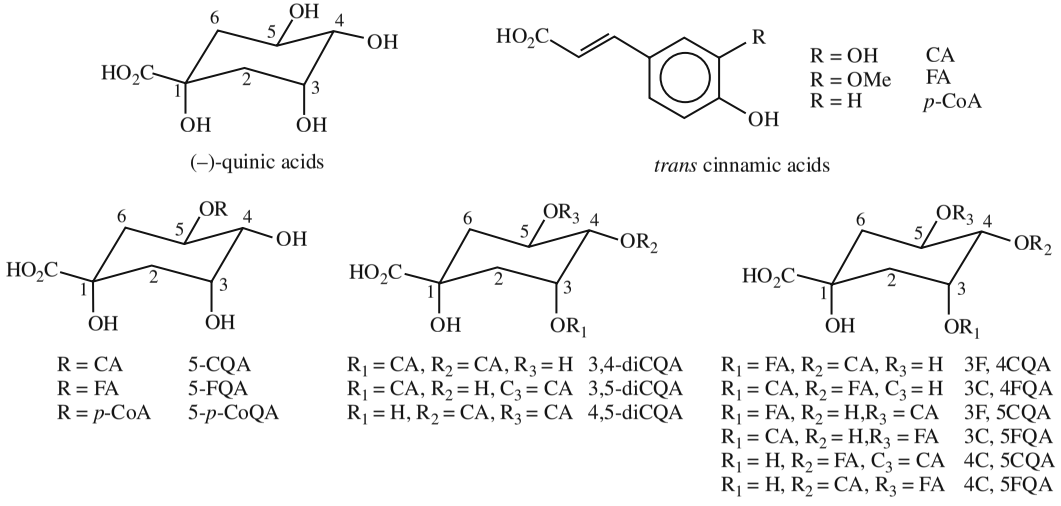

Fig. 2.62 Structure of chlorogenic acids precursors – quinic acid, caffeic acid (CA), ferulic acid (FA), *p*-coumaric acid (*p*-CoA) – followed by CGA main subclasses: caffeoylquinic acids (CQA), feruloylquinic acids (FQA), *p*-coumaroylquinic acids (*p*-CoQA) (example of 5-isomers for CGA monoesters), dicaffeoylquinic acids (diCQA) and caffeoylferuloylquinic acids (CFQA). Reprinted with permission from Perrone et al. (2008)

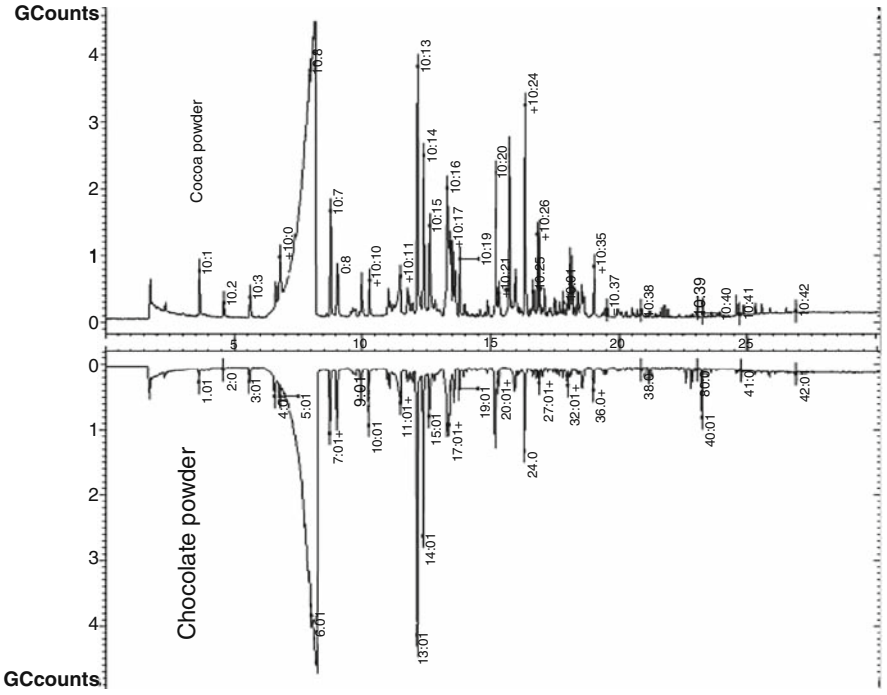

Fig. 2.63 Typical HS-SPME-GC–MS chromatograms of compounds extracted from chocolate (*left*) and cocoa (*right*) powders with DVB/CAR-PDMS fiber. For peak identification see Table 2.63. Reprinted with permission from Ducki et al. (2008)

Analysis time was 100 min; EI source was operated in the negative mode. A typical total ion chromatogram is shown in Fig. 2.63, illustrating the good separation capacity of the RP-HPLC system. Some new derivatives were identified in the samples such as 1-feruloylquinic acid, 1-feruloylquinic lactone, 3,4-diferuloylquinic acid (*C. arabica, C. canephora*), 3- and 4-*p*-coumaroylquinic lactones (*C. canephora*) and 3,4-di-*p*-coumaroylquinic acid (*C. arabica*) (Perrone et al., 2008).

Ion-pair HPLC was also applied for the measurement of nonvolatile free bioactive amines (possible aroma substances) in instant coffee samples. Amines were derivatised with *o*-pthalaldehyde and detected fluorometrically. It was found that the amount and composition of free amines (serotonin, cadaverine, tyramine, spermidine, putrescine, histamine, agmatine, phenylethylamine and spermine) show high variations according to the type of instant coffee (da Silveira et al., 2007).

The volatile components of Kangra orthodox black tea was analysed by GC-MS using various preconcentration techniques such as simultaneous distillation extraction (SDE) and hydrodistillation of Clavenger type and mini distillation apparatus (Babu et al., 2002, 2005). Analytes were separated on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Injector temperature was 220°C.

Column temperature started at 70°C (4 min hold), at 4°C/min to 200°C final hold 5 min. The flow rate was 1.1 ml/min. Analytes were detected by MS. The analytes identified by the different extraction procedures are listed in Table 2.59. The data in Table 2.59 indicate that the efficacy of the various extraction procedures is markedly different, suggesting that the application of more than one extraction technologies enhances considerably the reliability of the measurement. It was found that the best results can be achieved by using SDE (Marcha Agresti et al., 2008).

The impact of heat treatment on the aroma composition of green tea liquor was followed by GC-MS and HLPC. The interest in the aroma composition of teas was motivated by their healthy benefits (Dufresne and Farnworth, 2001) as anticancer agents (Fujiki, 2005; Huang and Xu, 2004; Xu and Huang, 2004).

The influence of the heat treatment of green tea liquor was assessed by the simultaneous using of GC-MS for the analysis of volatile aroma substances and RP-HPLC for the measurement of tea catechins. Volatile compounds were extracted from tea liquor by SDE and were separated in a fused silica capillary column (30 m × 0.22 mm i.d., film thickness 0.5 μm). Injector temperature was 250°C. Initial column temperature was 50°C (5 min hold), ramped at 3°C/min to 210°C, 10 min hold, then raised to 230°C at 3°C/min. The flow rate of helium was 1.0 ml/min. Analytes were detected by MS employing ionisation voltage of 70 eV and ion source temperature of 230°C. RP-HPLC separation was performed on a C18 column (250 mm × 4.6 mm, particle size, 5 μm) at 40°C. The components of the gradient elution were acetonitrile–acetic acid–water (6:1:193 v/v/v) (mobile phase A) and acetonitrile–acetic acid–water (60:1:193 v/v/v) (mobile phase B). Gradient started at 100% mobile phase A decreasing linearly to 0% in 45 min, then to 100% mobile phase B to 60 min. Catechins were detected at 280 nm. The total ion chromatograms of volatiles in green tea liquors after various heat treatments are depicted in Fig. 2.64. The chromatograms demonstrate that the heat treatment exerts a considerable influence on the composition of volatile substances in green tea liquor. The concentrations of the 20 compounds isolated by the method are compiled in Table 2.60, illustrating the impact of heating on the composition of aroma substances. The results indicated that the maximal temperature of heat treatment cannot exceed 85°C (Kim et al., 2007).

Another HPLC and GC-MS method were employed for the assessment of the impact of region of production on black teas. Some data showing the effect of the region of production and that of fermentation times are compiled in Table 2.61. The results demonstrated that the optimal fermentation time can be different in different areas of production. Furthermore, it was established that short fermentation time produces more aromatic black teas (Owuor et al., 2008).

The composition of aroma profile of green mate and mate tea (*Ilex paraguariensis*) infusions was also determined by GC-MS. The measurements were motivated by the healthy effect of the antioxidants present in mate tea (Bracesco et al., 2003; Filip et al., 2000: Schinella et al., 2000). Analytes were concentrated by steam distillation, extracted with dichloromethane and separated in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Injector and detector temperatures were 220°C and 230°C, respectively. Initial column temperature was 60°C ramped

Table 2.59 Tentative identification of compounds that were detected exclusively in the black coffee beans at a specific retention time and roasting degree

Retention time (min)	S/N[a]	Compound	*m/z* of the most intense ions (relative abundance %)
60 min roasting			
15.65	4812	2-Ethyl-5-methylpyrazine	121(100), 122(37), 123(7)
16.28	65	2-Carboxaldehyde-1*H*-pyrrole	94(100), 95(87), 66(42)
16.69	349	3,6-Dimethyl-2(1*H*)-pyridinone	80(100), 94(75), 123(35)
18.15	66	Tricyclo[2.2.1.0.2,6] heptan-3-one, oxime	94(100), 106(95), 123(80)
20.35	433	1-Methyl-3-piperidinone	84(100), 108(71), 113(53)
21.29	790	2,3-Dihydro-1*H*-indole	118(100), 117(37), 91(27)
21.63	387	2-Methyl,5-propyl-pyrazine	108(100), 136(37), 93(31)
25.49	114	2-Pentyl-pyridine	93(100), 106(44), 120(34)
38.05	77	1-(4-Hydroxy-3-methoxyphenyl)-ethanone	151(100), 166(37), 127(23)
120 min roasting			
12.57	59	2-(Methyl amino)-benzoic acid	106(100), 107(81), 77(41)
16.33	88	2-Dodecyl-1-methyl-pyrrolidine	84(100), 94(20), 66(12)
17.62	109	2-Cyclopenten-1-one, 3,4-dimethyl	110(100), 93(67), 95(32)
18.40	381	*n*-Buthylbenzene	91(100), 92(54), 65(21)
19.22	407	Isopropenyl-pirazine	119(100), 78(21), 120(15)
21.61	343	2-Methyl-6-propyl-pirazine	108(100), 136(36), 93(34)
22.20	73	3-(4-Methyl-5-*cis* phenyl-1,3-oxazolidin-2-yl)-furan	122(100), 123(59), 94(25)
22.36	170	(Unidentified)	126(100), 133(57), 98(46)
23.11	72	4-Methyl-5-ethyltiazole	127(100), 112(62), 71(39)
23.22	751	1-Isopropyl-3,4-dimethyl 2-pyrazoline	125(100), 140(14), 69(11)
23.29	182	1,4-Diisopropyl cyclohexane	69(100), 55(79), 83(63)
24.19	247	2-One-5,9-dimethyl-, €-5,8-decadien	107(100), 122(41), 77(41)
24.75	520	1-(2-Furanyl methyl)-1*H*-pyrrole	81(100), 147(83), 53(43)
25.44	389	2-Pentyl-pyridine	93(100), 106(39), 120(29)
26.65	540	3-Methyl, 2-furanylmethyl ester butanoic acid	81(100), 98(57), 53(46)
27.49	126	1,5-Dimethyl-2-pyrrole carbonitrile	119(100), 120(71), 108(23)
27.92	449	2-Buthy-l,3-methylpirazine	108(100), 121(33), 107(31)
34.75	97	3-(3,4-Dihydro-2*H*-pyrrol-5-yl)-pyridine	146(100), 145(56), 104(35)
35.81	178	(Unidentified)	173(100), 174(84), 145(23)
37.79	218	3-Amino-4-methyl-6-methoxyquinoline	145(100), 188(63), 159(16)
46.34	80	(1,1,3,3-Tetramethylbutyl)-phenol	135(100), 107(43), 136(11)
46.99	75	4-Nonylphenol	135(100), 107(77), 212(50)
47.99	50	Hexestrol (phenol,4,4′-[1,2-diethyl-1,2-etanediyl] bis-	134(100), 107(45), 136(10)

[a] S/N = signal-to-noise ratio.

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Table 2.60 Changes in concentrations of volatiles of green tea liquors[a]

Peak no.	Volatile components	Control	85°C	95°C	110°C	120°C
1	Pentanol	0.0733a	0.0420b	0.0389bc	0.0333d	0.0238e
2	*cis*-3-Hexenol	0.0538a	0.0513a	0.0447b	0.0454b	0.0347c
3	1,2-Dimethyl benzene	0.0331a	0.0168d	0.0341ab	0.0277c	0.0317bc
4	Benzaldehyde	0.0328e	0.0587bc	0.0606b	0.0632a	0.0522d
5	Benzyl alcohol	0.119c	0.194a	0.176b	0.165b	0.207a
6	Phenylacetaldehyde	0.193c	0.229bc	0.238b	0.233b	0.2710a
7	Linalool oxide I	0.0669a	0.0571b	0.0592b	0.0562b	0.0486c
8	Linalool oxide II	0.0557a	0.0512b	0.0478c	0.0492bc	0.0452c
9	Linalool	0.248b	0.2742b	0.303a	0.307a	0.324a
10	Nonanal	0.0475d	0.0858c	0.145a	0.0742c	0.111b
11	Phenethyl alcohol	0.0752b	0.112a	0.0666b	0.107a	0.104a
12	Linalool oxide III	0.0706b	0.0884a	0.0816a	0.0831a	0.0869a
13	∗-Terpineol	0.0418c	0.0510b	0.0513b	0.0526b	0.0689a
14	Geraniol	0.133c	0.137b	0.135c	0.138b	0.141ab
15	Indole	0.0497c	0.0586b	0.0520b	0.0553b	0.0968a
16	β-Ionone	0.0652a	0.0622a	0.0625a	0.0592b	0.0503c
17	2,4-Di-*tert*-butylphenol	0.142b	0.148b	0.212a	0.162b	0.214a
18	Diisobutyl phthalate	0.0754a	0.0770a	0.0658ab	0.0655ab	0.0585b
19	Dibutyl phthalate	0.0621c	0.0714bc	0.105a	0.0793b	0.0979a
20	Phytol	0.0444ab	0.0486a	0.0415b	0.0266c	0.0091d
Total		1.68c	1.93ab	2.02a	1.88b	2.08a

[a] The data are presented as the ratio of the peak area of each volatile to the peak area of the internal standard reference decanoic acid ethyl ester, and those marked with different letters in the same row were statistically different at $p = 0.05$.
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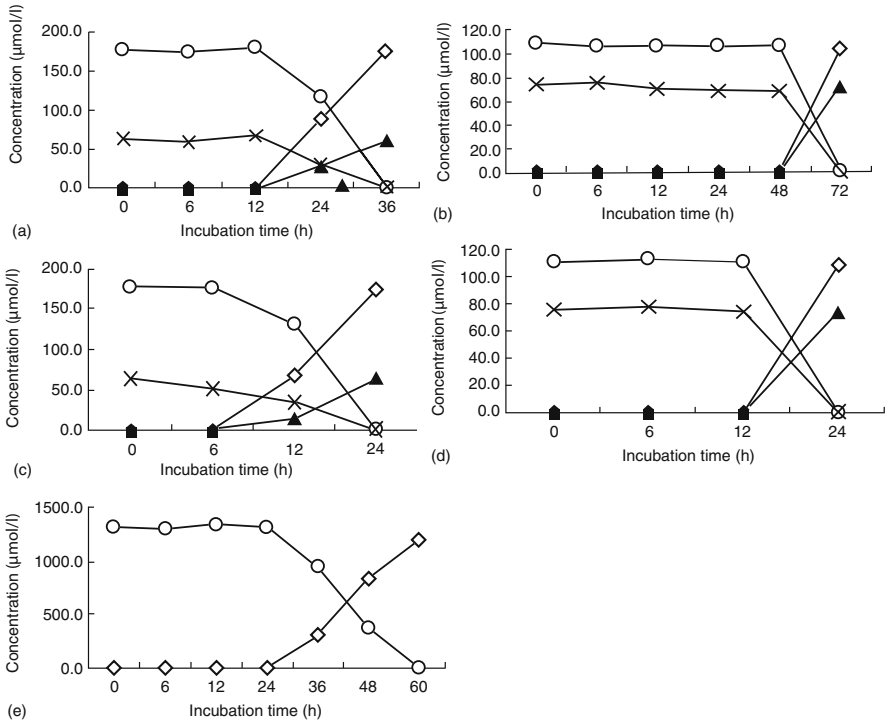

Fig. 2.64 Concentration of four flavour components in (**a**) cocoa B incubated at 25°C; (**b**) cocoa C incubated at 25°C; (**c**) cocoa B incubated at 35°C; (**d**) cocoa C incubated at 35°C; and (**e**) cocoa D incubated at 35°C. *B. firmus* was added at approximately 100 cfu/ml to each cocoa drink. Subsequently, these samples were incubated at 25°C and 35°C. Vanillin and ethylvanillin were originally contained at 177 and 63 μmol/l, respectively, in cocoa B. Vanillin and ethylvanillin were originally contained at 110 and 75 μmol/l, respectively, in cocoa C. Vanillin was originally contained at 1320 μmol/l in cocoa D. (◯) Vanillin; (×) ethylvanillin; (◇) 2-methoxyphenol; (▲) 2-ethoxyphenol. Reprinted with permission from Ohashi et al. (2007)

at 3°C/min to 220°C. The flow rate was 1.0 ml/min. Analytes were detected by MS. Chromatograms showing the baseline separation of volatiles are depicted in Fig. 2.65. The volatile compounds identified in green mate and mate tea infusion are compiled in Table 2.62. It was concluded from the measurements that the roasting process influences markedly the composition of aroma profile of mate tea (Bastos et al., 2006).

The volatile composition of non-alkalised natural cocoa powder and conched chocolate powder was investigated by HS-SPME-GC-MS. The efficacy of the adsorption of various fibres was compared using 100 μm polydimethylsiloxane (PDMS), 65 μm PDMS/divinylbenzene (DVB), 75 μm carboxen/PDMS (CAR) and 30/50 μm DVB/CAR/PDMS coatings. The extraction time was 15 min at 60°C. Desorption time was 5 min and the temperature of the GC liner was 250°C.

Table 2.61 Response of cultivar 6/8 to fermentation times of Kenya and Malawi

	Kenya					Malawi				
Fermentation time (mins)	30	50	70	90	110	30	50	70	90	110
2-Methyl butanal	0.15	0.15	0.14	0.17	0.19	0.13	0.16	0.14	0.14	0.13
Pentanal	0.06	0.05	0.05	0.07	0.06	0.04	0.04	0.04	0.05	0.05
Hexanal	0.23	0.27	0.33	0.32	0.38	0.11	0.12	0.13	0.14	0.14
E-3-Penten-2-one	0.04	0.08	0.07	0.08	0.10	0.03	0.03	0.04	0.03	0.05
Z-2-Penten-3-ol	0.11	0.12	0.10	0.14	0.12	0.07	0.09	0.06	0.09	0.07
Heptanal	0.03	0.02	0.03	0.04	0.03	0.02	0.02	0.02	0.02	0.02
Z-3-Hexenal	0.08	0.13	0.11	0.13	0.12	0.05	0.06	0.08	0.06	0.06
E-2-Hexenal	1.99	2.48	2.55	2.79	2.89	1.19	1.30	1.43	1.57	1.65
n-Pentyl furan	t[a]	t	0.01	0.01	0.02	t	t	t	t	t
n-Pentanol	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.01
3,6,6-Trimethylcyclohexanone	0.01	0.01	0.01	0.02	0.01	t	t	t	0.01	0.01
Z-3-Penten-1-ol	0.07	0.07	0.06	0.08	0.06	0.04	0.05	0.03	0.05	0.03
n-Hexanol	0.03	0.02	0.03	0.02	0.02	0.01	t	t	t	0.01
Z-3-Hexen-1-ol	0.09	0.10	0.09	0.10	0.09	0.03	0.02	0.02	0.01	0.01
Nonanal	0.03	0.04	0.05	0.04	0.05	0.02	0.02	0.02	0.02	0.02
E-2-Hexen-1-ol	0.04	0.05	0.05	0.05	0.06	0.03	0.02	0.01	0.03	0.02
E,Z-2,4-Heptadienal	t	t	0.01	t	0.01	t	t	t	t	t
E,E-2,4-Heptadienal	0.05	0.06	0.06	0.07	0.09	0.03	0.03	0.03	0.03	0.02
Sum of Group 1 VFC	*2.98*	*3.66*	*3.77*	*4.15*	*4.32*	*1.81*	*1.97*	*2.06*	*2.27*	*2.30*
Linalool oxide I	0.07	0.08	0.07	0.06	0.07	0.03	0.03	0.02	0.02	0.02
Linalool oxide II	0.20	0.23	0.22	0.20	0.22	0.06	0.08	0.05	0.07	0.06
Bezaldehyde	0.03	0.05	0.03	0.04	0.04	0.04	0.05	0.05	0.06	0.06
Linalool	0.99	1.10	1.12	1.06	1.03	0.29	0.20	0.19	0.18	0.17
Alpha-Cedrene	0.35	0.35	0.36	0.45	0.56	0.12	0.15	0.18	0.19	0.20
Beta-Cedrene	0.05	0.04	0.04	0.06	0.08	0.01	0.01	0.02	0.02	0.03
3,7-Dimethyloctatrienol	0.03	0.03	0.03	0.03	0.04	0.01	t	0.01	0.01	t

Table 2.61 (continued)

	Kenya					Malawi				
Fermentation time (mins)	30	50	70	90	110	30	50	70	90	110
Beta-Cyclocitral	0.04	0.04	0.04	0.04	0.05	0.02	0.03	0.04	0.03	0.02
Phenyl acetaldehyde	0.32	0.44	0.53	0.57	0.56	0.54	0.59	0.87	0.90	1.00
Neral	0.09	0.06	0.07	0.04	0.05	0.02	0.02	0.02	0.02	0.01
Alpha-Terpineol	0.05	0.05	0.06	0.05	0.06	0.02	0.02	0.02	0.02	0.02
Linalool oxide III	0.02	0.02	0.01	0.01	0.03	0.01	t	t	t	t
Linalool oxide IV	0.02	0.02	0.02	0.03	0.03	0.02	t	t	t	t
Methyl salicylate	0.35	0.41	0.43	0.40	0.41	0.14	0.06	0.09	0.08	0.05
Nerol	0.04	0.05	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02
Geraniol	1.51	1.74	1.67	1.47	1.47	0.51	0.40	0.34	0.37	0.38
Benzyl alcohol	0.04	0.03	0.02	0.05	0.05	0.03	0.05	0.02	0.02	0.02
2-Phenyl ethanol	0.57	0.61	0.66	0.80	0.64	0.36	0.34	0.27	0.37	0.32
Beta-Ionone	0.13	0.13	0.13	0.18	0.15	0.09	0.09	0.06	0.08	0.08
Epoxy-beta-Ionone	0.20	0.21	0.24	0.28	0.24	0.13	0.14	0.10	0.13	0.12
Nerolidol	0.11	0.12	0.12	0.13	0.14	0.06	0.07	0.06	0.06	0.04
Cedrol	0.17	0.10	0.11	0.14	0.21	0.07	0.12	0.11	0.12	0.14
Bovolide	0.07	0.07	0.06	0.07	0.08	0.04	0.05	0.04	0.05	0.08
Methyl palmitate	0.04	0.02	0.05	0.04	0.04	0.01	0.02	0.02	0.03	0.03
Trimethylpentadecan-2-one	0.19	0.15	0.13	0.16	0.13	0.08	0.09	0.07	0.07	0.05
E-Geranic acid	0.41	0.42	0.46	0.40	0.53	0.59	0.08	0.08	0.07	0.06
Sum of group II VFC	*6.10*	*6.61*	*6.67*	*6.94*	*7.02*	*2.67*	*2.71*	*2.74*	*2.98*	*2.90*
Flavour index (group II/I)	*2.05*	*1.81*	*1.78*	*1.67*	*1.63*	*1.48*	*1.38*	*1.33*	*1.31*	*1.26*
Terpene index		*0.40*	*0.40*	*0.41*	*0.40*	*0.40*	*0.41*	*0.39*	*0.39*	*0.39*

[a] t = trace.

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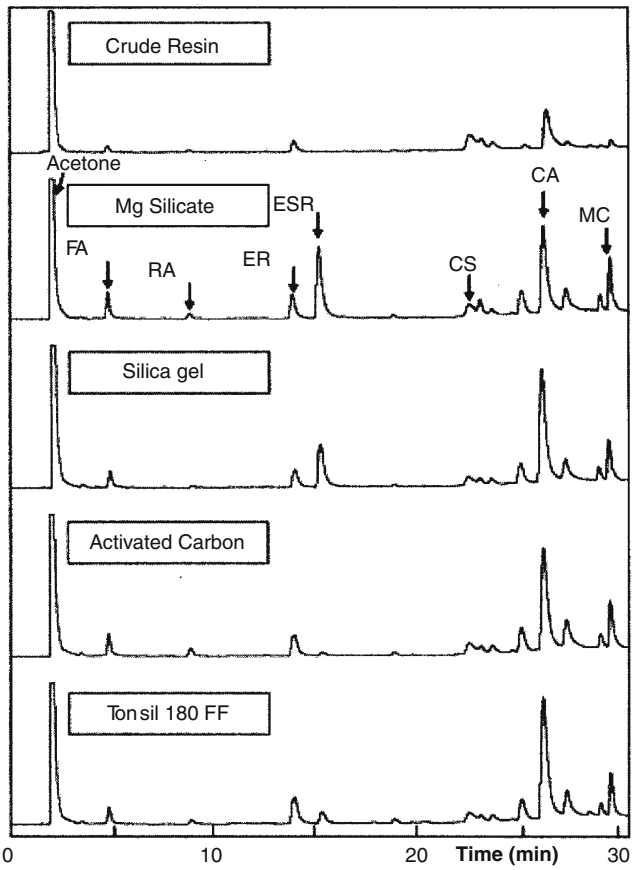

Fig. 2.65 Chromatograms of rosemary crude resin and after adsorption extracts obtained with different adsorbents. CA: carnosic acid, CS: carnosol, RA: rosmarinic acid, ER: epirosmanol, ESR: epiisorosmanol, MC: methyl carnosate, FA: ferrulic acid. Reprinted with permission from Braida et al. (2008)

Separation and quantitative analysis were carried out on a capillary column (30 m × 0.25 mm i.d., film thickness, 1 μm). Starting oven temperature was 30°C (5 min hold), raised to 200°C at 10°C/min, then to 280°C at 25°C/min, final hold 5 min. It was found that the capacity of the extracting fibres showed high variations depending both on the chemical structure of the analyte and on the composition of the fibre. The differences between the volatile profile of chocolate and cocoa powder are demonstrated in the HS-SPME-GC-MS chromatogram (Fig. 2.66). The peak areas obtained by the application of various fibres are compiled in Table 2.63. The data in Table 2.63 entirely support the previous qualitative conclusions that the adsorption capacity of fibres shows considerable differences. It was established that

Table 2.62 Volatiles compounds identified in green mate and mate tea based on Kovats Index and mass spectra

Kovats Index: experimental	Kovats Index: literature[a]	Compound	Green mate: relative (%)	Mate-tea: relative (%)
–	830	Furfural	n.f.	1.69
–	854	Hexenal (*E*)-2	1.50	n.f.
932	939	α-Pinene	0.51	n.f.
955	961	Benzaldehyde	n.f.	0.34
957	962	Methyl-5-furfural	n.f.	1.17
982	985	Hepten-2-one6 methyl-5	0.66	0.41
990	991	Myrcene	1.10	n.f.
995	998	Furfuryl methyl sulphide	1.88	1.80
1001	1001	*n*-Octanal	0.56	n.f.
1008	–	Furfuryl methyl sulphide isomer	4.32	1.92
1028	1031	Limonene	18.22	5.40
1070	1074	Linalool oxide *cis*	n.f.	0.98
1087	1088	Linalool oxide *trans*	n.f.	0.78
1093	1098	Linalool	12.16	n.f.
1104	1098	*n*-Non-anal	1.06	0.45
1189	1189	$*$-Terpineol	2.17	n.f.
1191	1190	Methyl salicylate	n.f.	0.46
1204	1204	*n*-Decanal	0.64	0.44
1253	1255	Geraniol	1.91	n.f.
1259	1261	Decenal (*E*)-2	1.10	n.f.
1313	1314	(*E,E*)-2,4-Decadecenal	n.f.	1.34
1383	1383	β-(*Z*)-Damascone	1.74	0.67
1412	1409	β-(*E*)-Damascona	0.95	0.70
1426	1426	α-(*E*)-Ionone	n.f.	1.51
1452	1453	Geranyl acetone	7.05	11.35
1485	1485	β-(*E*)-Ionone	2.81	4.83
1559	1559	Longicamphenylone	n.f.	0.56
1563	1564	(*E*)-Nerolidol	n.f.	0.67
1700	1700	Heptadecane	0.68	n.f.
1927	1927	Methyl hexadecanoate	2.65	n.f.
2106	2100	*n*-Heneicosane	1.19	n.f.
2302	2300	*n*-Tricosane	1.15	

[a]Refers to Kovats index taken from the literature
n.f., not found.
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the method is sensitive and highly reproducible, therefore, it can be employed for the analysis of chocolate and cocoa products (Ducki et al., 2008).

Vanillin and related compounds (ethylvanillin, 2-methoxyphenol, 2-ethoxyphenol) were separated and quantitated in cocoa drinks using CZE. Measurements were carried out in a fused-silica capillary (total length 37 cm, effective length 30 cm, 100 μm i.d.) at 25°C. Injection was performed in pressure mode (3.56 kPa, 2 s) and analytes were separated at –10 kV. Running electrolyte was the mixture of 50 mM phosphoric acid and 50 mM sodium hydroxide (pH 10) containing 2 mM cetyltrimethylammonium hydroxide (CTAH) and 10% acetonitrile. Sample

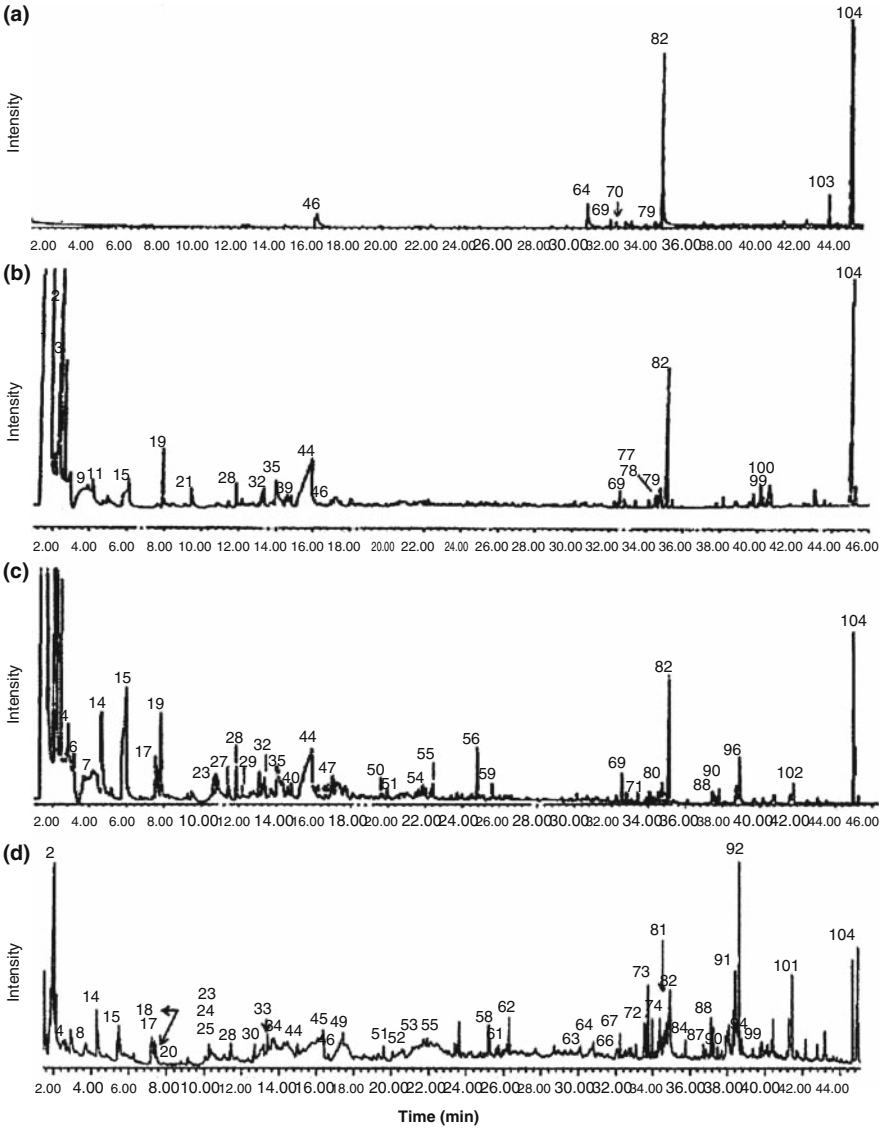

Fig. 2.66 Typical GC–MS chromatograms of volatile flavour components extracted by SFSI from (**A**) blank, (**B**) fresh healthy, (**C**) naturally infected, and (**D**) artificially inoculated peppers. For peak identification see Table 2.64. Reprinted with permission from Kim et al. (2007)

preparation consisted only of the dilution and filtering of the cocoa drinks. Electropherograms of various samples are compiled in Fig. 2.67. The electropherograms illustrate that the aroma compounds are well separated under the CZE conditions in 7 min. LOD was 1.6 μ/ml, the linear range of quantitation varied

Table 2.63 Effect of fiber type on the peak area ($\times 10^8$ area units) of natural cocoa powder (NCP) and conched chocolate powder (CCP) using HS-SPME-GC–MS[a]

			DVB/ CAR-PDMS		DVB-PDMS	CAR-PDMS	DVB/ CAR-PDMS		DVB-PDMS	CAR-PDMS
	DVB-PDMS	CAR-PDMS	NCP				NCP			
					Volatilealdehydesandketones					
1	Acetone[b]	495	65 ± 11	22 ± 8	1246 ± 11	243 ± 61	33,050 ± 3275	6311 ± 945	3073 ± 458	1660 ± 355
2	Methyl acetate[c]	521	18 ± 2	ND	456 ± 2	49 ± 23	14,080 ± 1648	982 ± 145	1416 ± 342	332 ± 82
3	2-Methylpropanal[b]	550	69 ± 12	66 ± 14	916 ± 12	661 ± 128	40,910 ± 5224	15,550 ± 2513	914 ± 245	1719 ± 320
4	2,3-Butanedione[c]	581	1783 ± 251	1292 ± 185	757 ± 251	562 ± 152	13,760 ± 2360	15,970 ± 2458	1908 ± 265	2925 ± 236
5	2-Butanone[c]	586	ND	ND	813 ± 145	224 ± 49	37,620 ± 5124	10,060 ± 1420	3470 ± 521	1567 ± 251
7	3-Methylbutanal[b]	653	139 ± 32	169 ± 29	3401 ± 242	3091 ± 423	77,690 ± 9423	86,430 ± 11,045	4439 ± 623	7179 ± 1366
8	2-Methylbutanal[b]	662	69 ± 14	97 ± 21	1361 ± 142	1476 ± 252	41,520 ± 6210	59,890 ± 8541	2241 ± 252	4231 ± 651
9	Pentanal[c]	696	ND	ND	304 ± 64	86 ± 26	7509 ± 845	3940 ± 687	906 ± 260	544 ± 124
10	3-Hydroxy-2-butanone[c]	707	ND	63 ± 10	1550 ± 194	1610 ± 210	19,490 ± 2180	49,900 ± 8511	1560 ± 310	4926 ± 845
12	35-Dimethyl-dihydro-furan-2-one[c]	766	11 ± 4	ND	610 ± 154	59 ± 24	5700 ± 945	2344 ± 322	583 ± 240	240 ± 62
15	Hexanal[c]	802	ND	ND	403 ± 94	57 ± 21	1221 ± 182	ND	607 ± 124	322 ± 58
					Acidsandalcohols					
6	Acetic acid[c]	630	1612 ± 211	1519 ± 261	29,290 ± 3250	21,460 ± 2854	389,700 ± 40,211	509,000 ± 61,525	78,310 ± 11,254	134,000 ± 21,804
11	Dimethylpropanedioic acid[c]	755	ND	ND	4875 ± 954	3125 ± 487	33,710 ± 4127	ND	2400 ± 325	3256 ± 812
13	2,3-Butanediol[c,d]	782	560 ± 102	670 ± 123	55,860 ± 5821	60,960 ± 6221	170,000 ± 22,943	276,500 ± 36,102	33,990 ± 5442	61,060 ± 12,209
14	2,3-Butanediol[c,d]	792	306 ± 51	314 ± 62	32,670 ± 4151	28,590 ± 3562	89,220 ± 12,452	123,300 ± 19,450	11,990 ± 2112	22,210 ± 3251
17	3-Methyl-butanoic acid[c]	837	185 ± 42	ND	16,180 ± 1822	15,420 ± 2511	35,600 ± 3254	92,010 ± 14,520	9667 ± 1225	14,860 ± 2524
18	2-Methyl-butanoic acid[c]	847	ND	ND	3491 ± 385	3.091 ± 520	7457 ± 1346	15,680 ± 2364	2146 ± 363	2666 ± 326
19	2-Furanmethanol[c]	854	6 ± 3	ND	439 ± 84	203 ± 38	3288 ± 651	3245 ± 458	996 ± 251	860 ± 152
30	Benzyl alcohol[b]	1048	ND	ND	256 ± 62	586 ± 154	234 ± 74	871 ± 210	118 ± 22	344 ± 54
35	Phenylethyl alcohol[c]	1133	78 ± 44	44 ± 9	6248 ± 1114	11,850 ± 1514	3158 ± 845	6628 ± 1125	5645 ± 945	7342 ± 945

Table 2.63 (continued)

			DVB/ CAR-PDMS		DVB-PDMS	CAR-PDMS	DVB/ CAR-PDMS		DVB-PDMS	CAR-PDMS
	DVB-PDMS	CAR-PDMS	NCP				NCP			
36	Benzoic acid[c]	1157	17 ± 5	12 ± 4	157 ± 34	456 ± 86	106 ± 32	796 ± 124	189 ± 34	464 ± 214
39	Isopentyl benzoate[c]	1404	45 ± 11	ND	831 ± 214	166 ± 53	30 ± 15	ND	226 ± 14	61 ± 24
					Pyrazines					
16	Methylpyrazine[c]	832	30 ± 13	18 ± 6	1117 ± 224	551 ± 94	17,480 ± 3254	19,690 ± 3458	3243 ± 432	4249 ± 832
20	2,5-Dimethylpyrazine[c]	921	146 ± 44	116 ± 34	8254 ± 1244	5817 ± 854	30,760 ± 1346	40,710 ± 8245	6987 ± 732	12,760 ± 1532
21	2,3-Dimethylpyrazine[b]	928	14 ± 6	ND	396 ± 85	764 ± 152	705 ± 251	ND	351 ± 122	1175 ± 232
22	Ethylpyrazine[c]	926	ND	ND	ND	ND	603 ± 124	259 ± 51	428 ± 74	1429 ± 645
27	Trimethylpyrazine[b]	1011	75 ± 21	123 ± 42	3517 ± 655	4936 ± 866	2578 ± 345	8396 ± 1257	1693 ± 645	4206 ± 532
28	2-Ethyl-6-methylpyrazine[c]	1013	2 ± 1	ND	143 ± 44	243 ± 62	106 ± 31	850 ± 53	101 ± 32	1489 ± 445
33	3-Ethyl-2,5-dimethylpyrazine[c]	1086	3 ± 1	ND	202 ± 57	824 ± 122	136 ± 42	651 ± 132	160 ± 35	690 ± 232
34	Tetramethylpyrazine[b]	1094	169 ± 54	105 ± 37	2648 ± 454	2484 ± 482	1150 ± 185	1846 ± 324	1714 ± 382	1829 ± 352
					Semivolatilealdehydesandketones					
24	Benzaldehyde[b]	981	75 ± 34	54 ± 25	6138 ± 1032	6617 ± 1102	19,790 ± 3232	34,290 ± 4251	11,790 ± 1845	17,010 ± 2842
29	1*H*-Pyrrole-2-carboxaldehyde[c]	1018	7 ± 4	ND	424 ± 82	654 ± 148	777 ± 151	2653 ± 450	255 ± 82	624 ± 255
31	Benzeneacetaldehyde[c]	1062	33 ± 15	58 ± 34	1567 ± 284	13,780 ± 2152	164 ± 21	626 ± 122	1192 ± 355	5198 ± 1252
32	2-Acetylpyrrole[c]	1072	19 ± 6	19 ± 8	1479 ± 314	4235 ± 651	1829 ± 251	6870 ± 853	925 ± 132	2561 ± 512
37	3,5-Dihydroxy-6-methyl-2,3-dihydro-pyran-4-one[c]	1164	39 ± 21	ND	1341 ± 253	2628 ± 541	ND	ND	339 ± 82	734 ± 252

Table 2.63 (continued)

			DVB/ CAR-PDMS		DVB-PDMS	CAR-PDMS	DVB/ CAR-PDMS		DVB-PDMS	CAR-PDMS
	DVB-PDMS	CAR-PDMS	NCP				NCP			
38	3,5-Dimethyl-benzaldehyde[c]	1250	ND	ND	391 ± 132	147 ± 25	47 ± 25	ND	161 ± 25	69 ± 18
40	Vanillin[b]	1419	7 ± 4	451 ± 148	699 ± 157	11,180 ± 2104	22 ± 9	777 ± 178	135 ± 34	1661 ± 258
					Terpenesandothers					
23	∗-Pinene[c]	949	66 ± 28	ND	7478 ± 1612	ND	20,440 ± 3512	69 ± 22	6259 ± 845	ND
25	β-Pinene[c]	999	ND	ND	544 ± 126	ND	1273 ± 210	ND	287 ± 42	ND
26	35-Dimethyl-octane[c]	1007	ND	ND	4701 ± 745	ND	ND	ND	1340 ± 281	ND
41	8-Hydroxy-3-methyl-iso-chroman-1-one[c]	1565	ND	ND	106 ± 34	112 ± 35	ND	ND	22 ± 12	24 ± 18
42	Caffeine[b]	1783	170 ± 46	101 ± 54	348 ± 72	594 ± 142	ND	ND	107 ± 32	109 ± 34
Total			5816	5311	203,606	209,591	1,122,912	1,397,093	204,283	328,585

ND: compound not detected.

[a] All runs preformed with extraction at 60°C for 15 min under dry conditions.

[b] Compound identified by GC–MS and RI using authentic compounds.

[c] Compound tentatively identified by GC–MS and RI using NIST98 database.

[d] Diastereoisomers.

PDMS=polydimethylsiloxane, DVB=divinylbenzene, CAR=carboxene

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between 5 and 500 μg/ml. Recoveries were 96.3–103.8%. Because of the rapidity, reliability and simplicity, the method was proposed for the analysis of this class of aroma compounds in cocoa drinks (Ohashi et al., 2007).

2.10 Spices

The aroma composition of spices plays a decisive role in the consumer acceptance of the products. The analysis of volatile compounds is of considerable importance because it facilitates the evaluation of the quality of the products and may promote the determination of their authenticity. The extraction methods described and discussed in the previous subchapters have also found application in the prepurification and preconcentration of the aroma substances in spices. The use of SFE for the extraction of bioactive compounds from labiatae family herbs (rosemary, sage, thyme, oregano, etc.) has been investigated in detail. The investigations were motivated by the elevated antioxidant capacity of the members of the labiatae family (Tepe et al., 2006: Yepez et al., 2002; Carvalho et al., 2005; Cavero et al., 2006; Hadolin et al., 2004; Ramirez et al., 2004, 2006) and by their protective power against diseases (Suhaj, 2006; Leal et al., 2003). HPLC analyses were performed in RP separation mode using a linear gradient from 90% A (840 ml of water, 8.5 ml of acetic acid and 150 ml of ACN), 10% B (methanol), to 100% B in 30 min. The chromatograms showing the influence of the different adsorbent on the aroma profile of rosemary crude resin is shown in Fig. 2.68. It was established that the combination of SFE with adsorption and desorption of analytes results in higher yield and lower level of impurities (Braida et al., 2008).

The fruits (pericarp) of the genus *Capsicum* are consumed as vegetable foods, spices, external medicines, etc. Because of their considerable commercial importance, many chromatographic methods were developed and employed for the separation and quantitative determination of the aroma compounds. A solvent-free solid injector combined with GC-FID and GC-MS was applied for the study of the composition of volatile compounds in fresh healthy and diseased peppers (*Capsicum annuum* L). Peppers were inoculated by *Colletotrichum gloesporioides* and *C. acutatum*. The effect of various parameters (injector temperature, preheating times and holding times) on the efficacy of extraction was investigated in detail and the procedure was optimised accordingly. GC-FID used a fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm), the injector and detector temperatures being 250°C and 300°C, respectively. Initial oven temperature was 50°C (5 min hold), raised to 280°C at 5°C/min (final hold 10 min.). Nitrogen was employed as carrier gas. GC-MS measurements were performed as the GC-FID analyses. EI-MS was carried out at 70 eV ionisation energy and at 250°C. Analytes were detected in the scan mode between 10 and 400 *m/z* (3.71 scan/s). The optimal conditions for the analysis were: injection temperature, 250°C; preheating time, 7 min; and holding time, 7 min. Characteristic chromatograms illustrating the good separation capacity of the system are shown in Fig. 2.69. The compounds

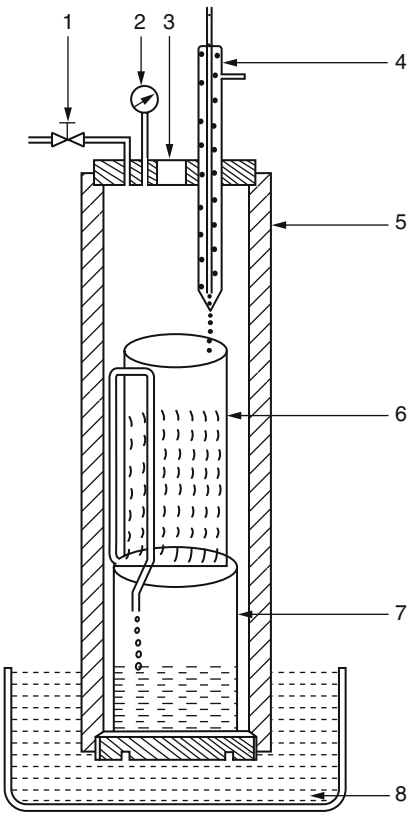

Fig. 2.67 Subcritical CO_2 extraction apparatus. (1) High pressure valve, (2) pressure gauge, (3) sapphire window, (4) cooling finger, (5) cylinder, (6) glass. Reprinted with permission from Rout et al. (2007)

identified by the GC procedures are compiled in Table 2.64. It was found that solvent-free solid injection technique based on direct vaporisation is rapid and simple and can be successfully applied for the analysis of volatiles. It was further suggested that the differences between the volatile profile of healthy and contaminated peppers can facilitate the identification of the disease at its early stage (Kim et al., 2007).

The effect of ripening on the composition of volatile aroma substances in Habanero chile pepper (*Capsicum chinenese* Jack. Cv. Habanero) was also investigated using GC-FID and GC-MS techniques. Samples were preconcentrated in a simultaneous steam-distillation-solvent extraction apparatus. Analytes were separated in a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm), the injector and detector temperatures were 250°C. Initial oven temperature was 50°C (2 min hold), raised to 280°C at 4°C/min (final hold 10 min.). Hydrogen was employed as carrier gas. GC-MS measurements were performed as the GC-FID analyses. EI-MS was carried out at 70 eV ionisation energy and at 230°C. Analytes were detected in the scan mode between 30 and 400 *m/z*. The concentration of identified volatile compounds in green and orange Habanero chile

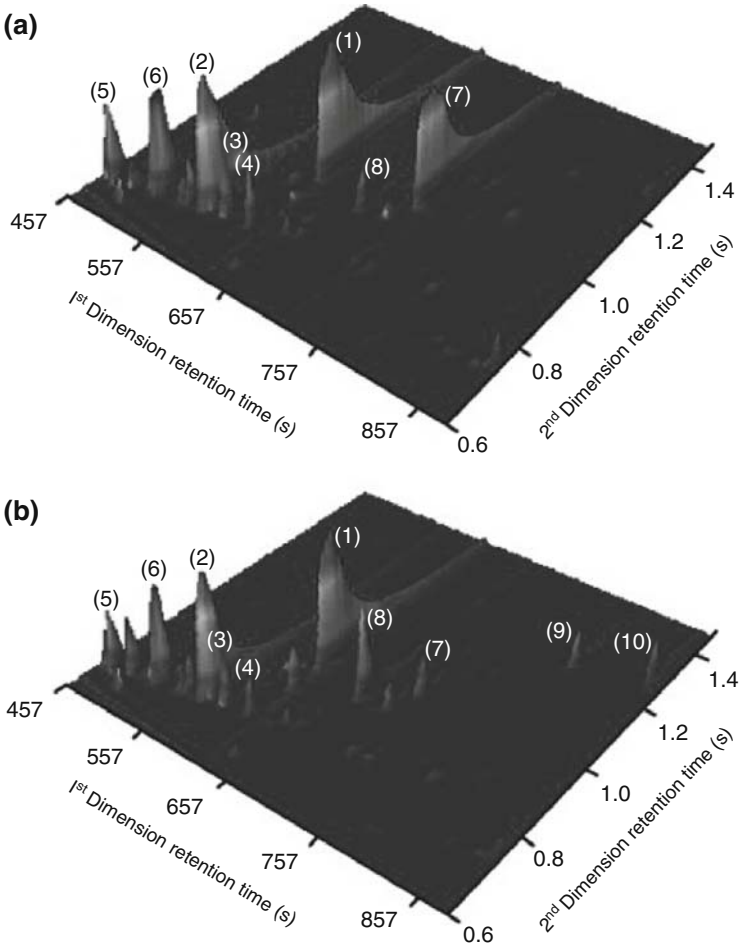

Fig. 2.68 GC×GC–TOFMS chromatograms of volatiles isolated by SPME from (**a**) conventional (cultivar III), and (**b**) ecological (cultivar III). Marked compounds: (1) linalool, (2) 1,8-cineole, (3) γ-terpinene, (4) terpinen-4-acetate, (5) ∗-pinene, (6) d-limonen, (7) methyl chavicol, (8) camphor, (9) and (10) methyl cinnamate isomers. Reprinted with permission from Klimánková et al. (2008)

pepper are compiled in Table 2.65. The data indicated that main constituents of the volatile fraction were (*E*)-2-hexenal, hexyl 3-methylbutanoate, (*Z*)-3-hexenyl-3-methylbutanoate, hexyl pentanoate, 3,3-dimethylcyclohexanol, and hexadecanoic acid. It was further established that the amount of volatile compounds decreases during maturation, influencing the sensory characteristics of the Habanero chile pepper (Pino et al., 2006).

Another study applied an optimised HS-SPME/GC-MS technology for the determination of VOCs in red, yellow and purple varieties of *Capsicum*

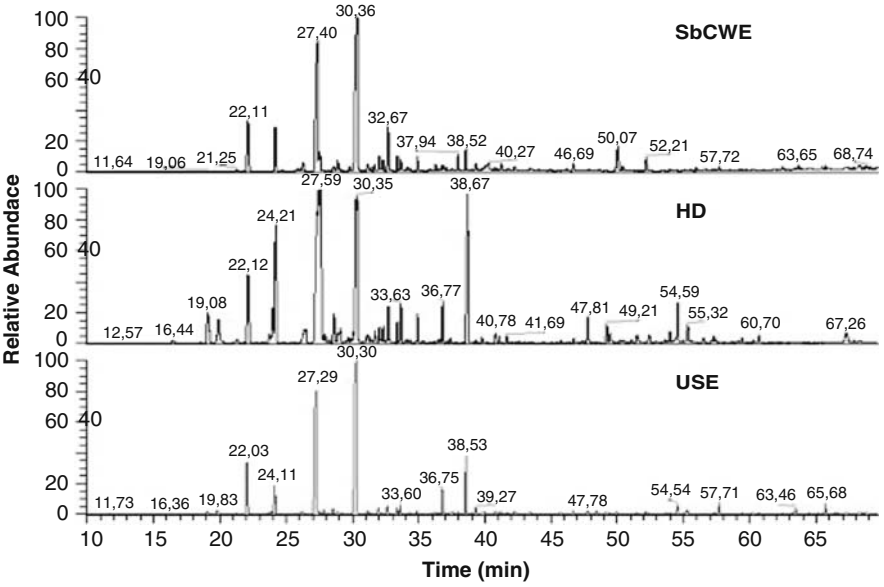

Fig. 2.69 GC–MS chromatograms of essential oils of *Lavandula stoechas* flowers extracted by SbCWE, HD, and USE methods. For peak identification see Table 2.68. Reprinted with permission from Giray et al. (2008)

chinense sp. peppers. The measurements demonstrated that hexyl ester of pentanoic acid, dimethylcyclohexanols, humulene and esters of butanoic acid are the main constituents of the volatile fraction of peppers (Souza et al., 2006).

The extraction of volatiles from the dried fruits of *Zanthoxylum rhesta* DC syn. *Z. budrungawall* syn. *Z. limonella* (Dennst) was carried out by subcritical CO_2, modified methanol-subcritical CO_2, hydrodistillation and traditional solvent extraction procedures, and the components of the extracts were separated and identified by GC-MS and GC-FID. The investigation was motivated by the commercial importance of the fruit as condiment, spice and pharmaceutical (Jain et al., 2007). The subcritical CO_2 extraction apparatus is shown in Fig. 2.70 GC-FID separations were performed on a 30×0.25 mm (film thickness, 0.25 μm) column. Helium was employed as carrier gas. Oven temperature started at 60°C and was raised to 200°C at 2°C/min. GC-MS measurements were carried out under the same conditions, the ionisation voltage being 70 eV, mass range 40–400 *m/z*, detector voltage 1.5 V. The composition of extracts achieved by subcritical CO_2 and methanol-subcritical CO_2 extracting agents is shown in Table 2.66. It was established that the common solvent extraction procedures results in an end product containing a considerable quantity of waxy component while hydrodistillation produces wax-free essential oil, but the yield is relatively low. Subcritical methods were superior to the traditional extraction procedures; however, the composition of the extract depended on the method applied (Rout et al., 2007).

Table 2.64 Identified compounds and their average relative GC–MS peak areas in healthy and diseased peppers

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
1	3-Methylbutanal	2.07	<800	–	2.10	4.55	–
2	Acetic acid	2.37	<800	–	25.02	21.39	22.01
3	2-Propanone	2.42	<800	–	3.24	3.79	–
4	Pyrazine	2.84	<800	–	–	4.98	0.36
5	*N*,*N*-dimethylaminoethanol	2.91	<800	–	–	–	0.61
6	1H-pyrrole	3.16	<800	–	–	2.63	–
7	1,2-Ethanediamine	3.70	<800	–	–	2.05	0.13
8	Ethanamine, *N*-methyl-	3.73	<800	–	–	–	0.97
9	2-Amino-4-hydroxypteridine-6-carboxylic acid	3.89	801.4	–	0.54	–	–
10	1,4-Dideuterio-2-methylbutane	4.02	806.9	–	–	0.91	–
11	Trimethylurea	4.19	814.2	–	0.63	3.04	–
12	2[3*H*]-Furanone	4.37	821.1	–	–	0.89	–
13	Topotecan	4.43	823.6	–	–	0.83	–
14	2-Methyl-pyrazine	4.63	831.2	–	–	3.39	4.86
15	2-Furanmethanol	5.95	874.0	–	7.15	10.26	5.64
16	2-ethylpyrazine	7.31	912.8	–	–	–	0.23
17	2,6-Dimethylpyrazin	7.55	920.6	–	–	1.53	2.07
18	Ethylpyrazine	7.65	923.8	–	–	0.99	0.27
19	Butyrolactone	7.84	929.6	–	3.36	2.37	–
20	Gamma valerolactone	8.78	956.7	–	–	–	0.21
21	2-Furancarboxaldehyde	9.43	973.7	–	1.81	–	–
22	Phenol	10.07	989.5	–	–	–	0.21
23	2-Ethyl-6-methyl-pyrazine	10.62	1003.5	–	–	0.51	0.82

Table 2.64 (continued)

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
24	2-Ethyl-5-methyl-pyrazine	10.73	1006.9	–	–	0.35	0.46
25	2,3,5-Trimethylpyrazine	10.80	1009.5	–	–	1.38	0.06
26	4(*H*)-Pyridine	11.08	1018.6	–	–	–	0.25
27	Endo-2-methyltricyclo [4,10]decane	11.42	1029.5	–	–	0.60	–
28	2-Cyclopenten-1-one	11.83	1041.9	–	1.45	0.85	1.84
29	Benzeneacetaldehyde	12.15	1051.6	–	–	0.46	–
30	1-[1*H*-pyrrol-2-yl]-Ethanone	13.08	1078.0	–	–	0.68	1.56
31	Hydroxy dimethyl furanone	13.19	1080.9	–	–	–	0.80
32	2,5-Dimethyl-4-hydroxy-3[2*H*]-furanone	13.32	1084.5	–	2.84	1.19	0.04
33	Phenol, 2-methoxy-	13.4	1086.6	–	-	–	1.29
34	2-Butanamine, hydrochloride	13.72	1095.1	–	-	–	0.17
35	Cyclobutanol	13.98	1102.6	–	2.65	2.63	–
36	1,2-Propanediol, 3-chloro-	14.02	1103.9	–	-	–	0.19
37	1-Propanol	14.23	1111.3	–	-	0.42	–
38	1-Guanidinosuccinimide	14.31	1114.0	–	-	0.73	–
39	4*H*-Pyran-4-one	14.61	1124.4	–	0.77	–	–
40	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	14.79	1130.5	–	–	0.27	–
41	Erythro-1,2,4-trimethylpnet-4-en-1-ol	15.27	1146.5	–	–	0.46	–
42	β-D_2-γ-picoline	15.36	1149.3	–	–	0.68	–
43	4-Pyridinol	15.56	1155.8	–	–	2.20	0.15
44	3-Hydroxypyridine	15.90	1166.5	–	26.15	7.02	–
45	4-Hydroxypyridine	16.35	1180.3	–	–	–	1.61
46	Decanal	16.55	1186.4	0.74	0.37	0.36	0.38

Table 2.64 (continued)

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
47	6-Methyl-3-pyridinol	17.01	1200.0	–	–	1.00	0.04
48	5-Ethyldihydro-2[3*H*]-furanone	17.26	1209.3	–	–	1.00	–
49	Dianhydromannitol	17.57	1220.9	–	–	–	0.02
50	4-Pyridinamine	19.63	1292.4	–	–	0.68	–
51	1*H*-Indole	19.95	1303.4	–	–	0.43	1.01
52	2-Methoxy-5-vinylphenol	20.11	1309.9	–	–	–	0.51
53	Phenol, 2,6-dimethoxy-	21.15	1350.1	–	–	–	0.25
54	1,3-Benzenediamine	21.86	1376.2	–	–	0.63	–
55	4-Methylindole	22.39	1395.4	–	–	0.43	2.18
56	1-Ethylindole	24.79	1491.6	–	–	1.12	–
57	1,4,8-Dodecatriene, (*E*,*E*,*E*)-	24.89	1495.4	–	–	–	0.30
58	9-Octadecenamide, (*Z*)-	25.22	1507.9	–	–	–	3.19
59	Acetamide	25.59	1525.5	–	–	0.40	–
60	Tetradecanamide	25.60	1525.9	–	–	–	0.55
61	Benzeneacetic acid, 4-hydroxy-3-mehoxy-	25.73	1531.6	–	–	–	0.53
62	(–)-(1*R*,5*S*)-exo-2®-Methylbicyclo [3.2.1.] octan-3-one	26.30	1556.0	–	–	–	1.92
63	3-Pyrrolidin-2-yl-propionic acid	30.10	1724.8	–	–	–	1.25
64	Tetradecanoic acid	30.80	1758.2	1.58	–	–	0.91
65	Pyrrolo[1,2-a]pyrazine- 1,4-dione, hexahydro-	31.26	1779.7	–	–	–	0.15
66	2-Decene, 3-methyl-	32.09	1819.8	–	–	–	1.54
67	Neophytadiene	32.22	1826.3	–	–	–	1.29
68	Cyclododecane	32.36	1833.4	–	–	–	0.13
69	Tetradecanoic acid, 1-methylethyl ester	32.55	1842.6	0.56	0.92	0.61	0.70

Table 2.64 (continued)

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
70	9-Octadecanol	32.81	1855.5	0.30	0.23	0.38	0.97
71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	33.41	1885.1	–	–	0.27	-
72	2-Octylfuran	33.56	1892.3	–	–	–	2.32
73	Cyclohexanol, 1-ethynyl-	33.77	1902.7	–	–	–	3.74
74	Hexadecanoic acid, methyl ester	33.98	1913.8	–	–	–	2.47
75	4a[2*H*] Naphthalenemethanol	34.08	1919.2	–	–	0.21	–
76	Oxacycloheptadecan-2-one	34.16	1923.3	–	–	–	0.44
77	Benzene	34.51	1941.8	–	0.61	0.40	–
78	Pyrrolo[1,2,*a*] pyrazine-1,4-dione	34.52	1941.9	–	0.98	–	–
79	1-Octadecanol	34.66	1949.3	0.47	0.38	0.16	1.27
80	Butanoic acid	34.73	1953.1	–	–	0.29	–
81	Hexadecanoic acid	34.93	1963.3	–	–	–	6.53
82	Dibuthyl phthalate	35.08	1971.2	14.98	6.36	2.37	1.01
83	*d*-Nerolidol	35.97	2017.8	–	–	–	0.15
84	6-C14H26	36.73	2059.3	–	–	–	0.72
85	(1*S*, 15*S*)-Bicyclo[13.1.0]hexadecan-2-one	36.84	2065.2	–	–	–	0.40
86	1-Octadecene	36.93	2070.1	–	–	–	0.30
87	9-Octadecenoic acid (Z)-, methyl ester	37.25	2087.2	–	–	–	1.56
88	9,12-Octadecadienoic acid	37.47	2098.9	–	–	0.23	2.26
89	Octadecanoic acid, methyl ester	37.71	2112.8	–	–	–	0.25

Table 2.64 (continued)

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
90	Phytol	37.81	2118.5	–	–	0.34	0.55
91	9,12-Octadecadienoic acid (*Z*,*Z*)-	37.99	2128.9	–	–	–	1.12
92	9-Octadecanoic acid, -	38.08	2134.0	–	–	–	1.75
93	1-Epoxy-2-methyl-3-isobutenyl-1,4-pentadiene	38.74	2171.4	–	–	0.39	–
94	Dodecanamide	38.76	2172.6	–	–	–	1.12
95	∗-Farnesene	38.82	2175.9	–	–	0.29	–
96	β-Myrcene	38.90	2180.3	–	–	0.95	–
97	2-Cyclohexenecarboxanilide	39.83	2234.6	–	–	–	1.06
98	(*E*,*Z*)-alpha-Farnesene	40.01	2245.2	–	–	–	0.17
99	Camphene	40.17	2254.9	–	1.10	–	0.06
100	7-Propylidene-bicyclo-[4,1,0]heptane	40.64	2281.8	–	1.22	–	–
101	Bicyclo[10.1.0] tridec-1-ene	41.68	2345.5	–	–	–	0.32
102	3-Methyl-thiophene	41.77	2351.3	–	–	0.43	–
103	Tritetracontane	43.85	2481.8	1.64	–	–	–
104	Bis-phthalate	45.00	2557.6	79.73	10.75	3.34	5.79

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Table 2.65 Volatile compounds (ppm) in Habanero chile pepper at the ripening stages green and orange

Compound	RI[a]	Identification[b]	Green	Orange
Hexanal	800	A	0.98	0.25
5-Methyl-2(5*H*)-furanone	815	B	<0.01	<0.01
(*E*)-2-Hexenal	854	A	8.87	4.37
(*Z*)-3-Hexenol	857	A	1.59	0.44
(*E*)-2-Hexenol	861	B	1.87	2.44
Hexanol	867	A	1.16	0.57
2-Heptanone	889	A	0.05	0.04
Tricyclene	926	B	nd[c]	<0.01
α-Pinene	939	A	0.07	0.07
3-Hepten-2-one	942	C	0.05	0.03
Hexyl acetate	1008	A	nd	<0.01
4-Methyl-3-pentenoic acid	1011	C	<0.01	<0.01
Isobutyl 2-methylbutanoate	1015	A	<0.01	<0.01
Isobutyl isopentanoate	1018	A	<0.01	0.04
Isopentyl isobutanoate	1021	A	nd	<0.01
p-Cymene	1026	A	nd	<0.01
Limonene	1031	A	<0.01	<0.01
(*E*)-β-Ocimene	1050	A	nd	<0.01
Isopentyl butanoate	1060	A	<0.01	<0.01
Linalool	1098	A	0.26	0.22
Isopentyl isopentanoate	1103	A	0.20	0.52
2-Methylbutyl isopentanoate	1105	A	<0.01	0.10
Methyl octanoate	1126	A	<0.01	0.05
Pentyl 2-methylbutanoate	1142	A	<0.01	0.08
(*Z*)-3-Hexenyl isobutanoate	1145	A	0.41	1.00
Pentyl isopentanoate	1148	A	0.24	<0.01
Hexyl isobutanoate	1150	A	0.19	0.72
Isoprenyl pentanoate	1152	A	<0.01	0.32
Hexyl butanoate	1184	A	nd	0.06
E-2-Nonenal	1185	C	<0.01	nd
2-Isobutyl-3-methoxypyrazine	1186	B	0.01	<0.01
(*Z*)-3-Hexenyl butanoate	1187	B	nd	<0.01
α-Terpineol	1189	A	0.06	<0.01
Methyl salicylate	1190	A	0.67	0.69
Hexyl 2-methylbutanoate	1234	A	1.43	3.13
Hexyl isopentanoate	1243	A	9.92	25.5
Heptyl isobutanoate	1248	A	nd	0.08
Isopentyl hexanoate	1260	A	nd	<0.01
Heptyl butanoate	1291	A	nd	0.08
β-Cyclocitral	1292	B	<0.01	0.03
(*Z*)-3-Hexenyl 2-methylbutanoate	1293	A	0.98	1.77
(*Z*)-3-Hexenyl isopentanoate	1295	A	7.78	14.6
Hexyl pentanoate	1298	A	9.10	18.5
E-2-Hexenyl pentanoate	1299	A	1.84	2.47
Heptyl isobutanoate	1300	A	0.21	0.50
Pentyl isohexanoate	1303	C	<0.01	0.14
9-Decanolide	1308	C	0.07	0.14

Table 2.65 (continued)

Compound	RI[a]	Identification[b]	Green	Orange
Octyl isobutanoate	1311	B	<0.01	<0.01
(*E*,*E*)-2,4-Decadienal	1313	B	0.46	<0.01
4-Vinylguaiacol	1315	A	<0.01	<0.01
Heptyl 2-methylbutanoate	1332	B	1.53	2.10
Heptyl isopentanoate	1338	B	5.30	11.91
Methyl anisate	1340	B	nd	<0.01
Octyl isobutanoate	1348	A	0.37	0.76
(*Z*)-3-Hexenyl hexanoate	1382	B	0.29	0.78
Hexyl hexanoate	1383	A	0.33	2.62
Decanoic acid	1385	A	<0.01	3.22
β-Cubebene	1390	B	nd	<0.01
3,3-Dimethylcyclohexanol	1392	C	14.3	35.7
Benzyl pentanoate	1396	A	1.22	2.47
Octyl 2-methylbutanoate	1418	C	1.56	4.43
β-Caryophyllene	1420	A	0.74	1.48
E-α-ionone	1426	A	0.22	0.40
Octyl 2-methylbutanoate	1430	B	0.55	0.78
Octyl isopentanoate	1440	B	1.57	5.15
2-Methyl-1-tetradecene	1445	C	0.64	2.64
α-Himachalene	1447	B	0.41	1.08
Heptyl hexanoate	1448	B	<0.01	<0.01
α-Humulene	1454	A	0.05	0.13
E-β-Farnesene	1458	A	0.18	0.55
2-Methyltetradecane	1462	C	1.43	3.86
β-Chamigrene	1475	B	3.95	10.38
Germacrene-D	1480	B	0.65	1.83
E-β-Ionone	1485	A	0.85	1.40
Pentadecane	1500	A	0.14	0.48
α-Muurolene	1502	B	0.16	0.48
γ-Cadinene	1513	A	<0.01	0.09
Cubebol	1515	B	<0.01	0.22
δ-Cadinene	1524	A	0.32	0.78
Cadina-1,4-diene	1531	B	<0.01	0.20
2-Methylpentadecane	1533	C	0.13	0.40
(*Z*)-Nerolidol	1534	A	nd	0.16
Hexyl benzoate	1576	B	0.69	3.18
Phenylacetic acid	1579	B	nd	<0.01
Hexadecane	1600	A	0.12	0.35
Nonyl pentanoate	1610	C	0.07	0.52
Tetradecanal	1613	A	nd	0.31
Cubenol	1642	B	0.20	0.67
Oxacyclopentadecan-2-one	1650	C	0.29	1.04
α-Cadinol	1653	B	0.14	0.52
Pentadecanal	1707	A	0.46	3.50
(*E*)-11-Hexadecenal	1759	C	0.22	2.24
Benzyl benzoate	1762	A	0.32	1.12
Tetradecanoic acid	1782	A	0.18	3.08

Table 2.65 (continued)

Compound	RI[a]	Identification[b]	Green	Orange
Hexadecanal	1811	A	<0.01	0.65
Pentadecanoic acid	1883	A	1.95	2.35
(*Z*)-11-Hexadecenoic acid	1915	C	3.39	9.35
Ethyl (*Z*)-9-hexadecenoate	1975	A	1.48	2.53
Hexadecanoic acid	1983	A	8.35	11.1
Octadecanol	2079	A	nd	0.08
Pentacosane	2500	A	0.07	0.12
Hexacosane	2600	A	<0.01	0.11

[a] Calculated retention indices on HP-5 column.
[b] The reliability of the identification proposal is indicated by the following: A, mass spectrum and Kovats index agreed with standards; B, mass spectrum and Kovats index agreed with database or literature; C, mass spectrum agreed with mass spectral database.
[c] nd: not detected.
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The aroma profile of fice basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions was determined by HS-SPME followed by GC-ion trap MS (GC-ITMS) and by GC × GC-TOFMS. Besides its culinary use, basil can be employed for the treatment of headache, cough, diarrhoea, kidney malfunction, etc. (Grayer et al., 2004; Ozcan et al., 2005; Politeo et al., 2007). The objectives of the measurements were the assessment of the effect of environmental conditions on the aroma profile. The influence of agronomical practices on the concentration of aroma substances has been previously established (Jirovetz et al., 2003; Vina and Murillo, 2003). HS-SPME was performed on various fibres such as PDMS, PDMS/DVB, DVB/CAR/PDMS and CW/DVB. It was found that the best extraction efficacy can be achieved by using DVB/CAR/PDMS fibre at 30°C for 5 min. GC-ITMS system consisted of a capillary column (60 m × 0.2 mm i.d., film thickness, 1.1 μm). Initial oven temperature was 45°C (1 min hold), raised to 200°C at 15°C/min, then to 275°C at 5°C/min (final hold 4 min.). MS detector was operated in electron ionisation mode (70 eV). Volatile compounds were in the segment scan mode 35–70, 71–110, 111–160, 161–220, 221–320, 321–420, 421–520 *m/z*. The dimensions of first and second columns in GC × GC-TOFMS were 30 m × 0.25 mm i.d., film thickness, 0.25 μm and 1.25 m × 0.10 mm i.d., film thickness, 0.10 μm, respectively. Temperature program of the first column started at 45°C (0.2 min hold), ramped to 200°C at 10°C/min, to 245°C at 30°C/min (1.8 min hold). The temperature program of the second column was +20°C above the primary column temperature. Mass range was 25–300 *m/z*. The volatiles identified in fresh basil are compiled in Table 2.67. The investigations established that linalool, methyl clavicol, eugenol, bergamotene, and methyl cinnamate are the main components in basil samples. GC × GC-TOFMS chromatograms are depicted in Fig. 2.71. It was concluded from the results that the higher sensitivity of GC × GC-TOFMS method makes it suitable for the differentiation between basil cultivars (Klimánková et al., 2008).

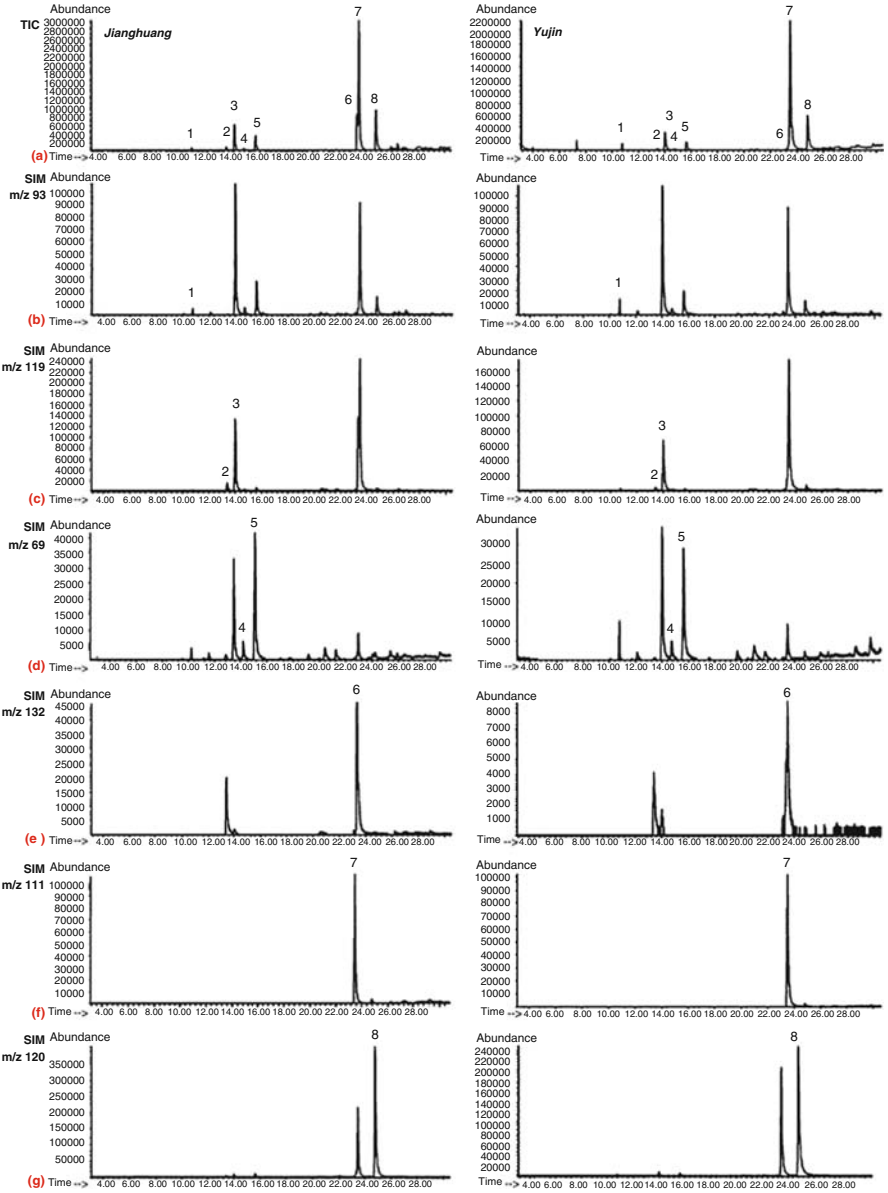

Fig. 2.70 GC–MS total ion chromatograms of (**A**) PLE extract and the selected ion chromatograms for (**B**) β-caryophyllene, (**C**) ar-curcumene + zingiberene, (**D**) β-bisabolene + β-sesquiphellandrene, (**E**) ar-turmerone, (**F**) α-turmerone and (**G**) β-turmerone. Reprinted with permission from Quin et al. (2007)

The aroma substances in the extract of basil and thyme leaves (*Thymus vulgaris* L.) were separated and identified by GC-MS. The measurements indicated that 3,7-dimethyl-1,6-octadien-3-ol (linalool), 1-methoxy-4-(2-propenyl) benzene (estragole), methyl cinnamate, 4-allyl-2-methoxyphenol (eugenol), and 1,4-cineole were the main components of basil extract, while thyme extract contained mainly 2-isopropyl-5-methylphenol (thymol), 4-isopropyl-2-methylphenol (cravacrol), linalool, α-terpineol, and 1,8-cineol (Lee et al., 2005).

Various extraction methods such as hydrodistillation. (HD), subcritical water extraction (SbCWE), and organic solvent extraction under ultrasonic irradiation (USE) were applied for the analysis of the components of *Lavandula stoechas* flowers. The volatile compounds were separated and partially identified by GC-MS and the aroma profiles were compared. GC-MS measurements were carried out in a capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Initial oven temperature was 50°C, raised to 240°C at 3°C/min. MS detector was operated in electron ionisation mode (70 eV). MS detection range was 41–400 *m/z*. The chromatograms of the various extracts are depicted in Fig. 2.72. The chromatograms demonstrate that the method of extraction exerts a considerable effect on the composition of volatile compounds. The volatiles identified by the method are listed in Table 2.68. It was concluded from the data that the efficacy of various extraction methods show considerable variations and the application of SbCWE method carried out at 100°C was proposed for the analysis of the extract of *Lavandula stoechas* leaves (Giray et al., 2008).

Because of their considerable importance as spices and traditional medicines, the properties and composition of *Zingiber* species have been vigorously investigated. The pharmacological activities of *Z. officinale* have been previously reviewed (Afzal et al., 2001). Volatiles from *Z. officinale* and *Z. zerumbei* were determined (Chane-Ming et al., 2003; Pino et al., 2004) and their insecticidal capacity was assessed (Anotious and Kocchar, 2003). Moreover, they show anti-inflammatory and chemopreventive activities (Kitayama et al., 2001; Murakami et al., 2004; Kirana et al., 2003).

The composition of other *Zingiber* species such as *Z. wray* var. *halabala* (Chargulprasert et al., 2005) and *Z. ottensii* (Phetchaburi, Thailand) has also been studied in detail (Thubthimthed et al., 2005). Volatile compounds of *Zingiber nimmonii* were extracted by hydrodistillation and separated by GC-FID and GC-MS. GC-FID measurements were performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Initial oven temperature was 60°C, ramped to 260°C at 5°C/min. Detector temperature was set to 250°C. MS detector was operated in electron ionisation mode (70 eV). The concentration of volatile components in the rhizome of *Zingiber nimmonii* are compiled in Table 2.69. It was found that the extract contains a considerable amount of β- and α-caryophyllene. Furthermore, it was established that the extract showed marked inhibitory activity against *Candida glabrata, C. albicans, Aspergillus niger, Bacillus subtilis* and *Pseudomonas aeruginosa* (Sabulai et al., 2006).

Table 2.66 Chemical composition of subcritical CO_2 and methanol–subcritical CO_2 extracts of pericarp of *Zanthoxylum rhesta*

GC RT	Compound	Subcritical CO_2	0.9% Methanol–subcritical CO_2	3.0% Methanol–subcritical CO_2	RRI cal	RRI lit
7.6	α-Thujene	0.7 ± 0.6	1.4 ± 0.1	0.6 ± 0.2	934	931
8.1	α-Pinene	4.6 ± 0.1	3.1 ± 0.2	0.7 ± 0.3	941	939
8.4	Camphene	0.1	t	t	948	951
10.1	Sabinene	42.5 ± 0.6	34.0 ± 0.1	13.9 ± 1.8	979	976
10.3	β-Pinene	5.5 ± 0.5	4.7 ± 1.2	2.5 ± 1.3	982	980
10.5	Myrcene	2.4 ± 1.4	0.7 ± 0.4	0.3 ± 0.1	988	991
11.4	Octanal	0.8 ± 0.4	0.2 ± 0.1	0.5 ± 0.1	1011	1001
12.0	α-Terpinene	0.3 ± 0.1	0.7 ± 0.4	1.0 ± 0.2	1024	1018
12.5	*p*-Cymeme	0.5 ± 0.1	0.6 ± 0.1	1.3 ± 0.1	1032	1026
13.1	β-Phellandrene	2.4 ± 0.1	2.1 ± 0.2	0.2 ± 0.1	1036	1031
13.6	*E*-β-ocimene	1.2 ± 0.1	0.2 ± 0.1	0.8 ± 0.6	1050	1050
15.4	Terpinolene	0.2 ± 0.1	1.6 ± 0.2	0.5 ± 0.2	1092	1088
16.2	Linalool	2.6 ± 0.1	3.6 ± 0.2	2.6 ± 0.1	1110	1098
17.7	*Z*-pinene hydrate	0.1 ± 0.1	2.6 ± 0.2	1.3 ± 0.1	1121	1121
18.5	*E*-Pinene hydrate	0.2 ± 0.1	1.2 ± 0.2	2.2 ± 0.3	1141	1140
19.3	*E*-β-terpineol	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	1163	1163
21.4	Terpinen-4-ol	3.5 ± 0.1	8.4 ± 0.3	19.2 ± 2.1	1186	1177
21.7	Cryptone	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	1192	–
22.4	α-Terpineol	0.7 ± 0.1	1.5 ± 0.1	3.5 ± 0.5	1204	1189
23.2	*Z*-piperitol	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	1212	1193

Table 2.66 (continued)

GC RT	Compound	Subcritical CO_2	0.9% Methanol–subcritical CO_2	3.0% Methanol–subcritical CO_2	RRI cal	RRI lit
26.1	Piperitone	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	1258	1252
27.0	Nonanoic acid	0.1 ± 0.1	–	0.1 ± 0.1	1272	1280
27.8	3-Thuyl acetate	t	0.1 ± 0.1	0.2 ± 0.1	1289	1291
28.1	Tridecane	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	1294	1299
32.5	α-Cubebene	0.4 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	1350	1351
34.0	α-Copaene	1.3 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1371	1376
35.1	β-Cubebene	4.8 ± 0.7	5.7 ± 0.2	6.0 ± 0.5	1387	1390
35.7	β-Elemene	0.3 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	1390	1391
GC RT	Compound	Subcritical CO_2	0.9% Methanol–subcritical CO_2	3.0% Methanol–subcritical CO_2	RRI cal	RRI lit
36.1	*Z*-caryophyllene	0.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	1404	1404
36.4	*E*-caryophyllene	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	1418	1418
37.6	γ-Elemene	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	1436	1433
38.5	Fatty acid[a]	1.0 ± 0.1	1.9 ± 0.1	0.4 ± 0.1	1460	–
40.4	Germacrene-d	1.0 ± 0.3	1.6 ± 0.1	1.8 ± 0.2	1481	1480
41.3	Bicyclogermacrene	0.7 ± 0.4	0.6 ± 0.2	1.7 ± 0.5	1494	1494
42.5	(*E*,*E*)-α-farnesene	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	1502	1508
43.2	γ-Cadinene	0.7 ± 0.1	0.6 ± 0.4	1.1 ± 0.5	1515	1513
43.5	δ-Cadinene	0.2 ± 0.1	t	1.0 ± 0.5	1521	1524
45.1	Cadina-1,4-diene	t	t	0.1	1534	1532
46.0	*Z*-isoeugnol acetae	t	–	t	1558	1563
46.3	*E*-nerolidol	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1563	1564
47.0	Globulol	t	0.1 ± 0.1	0.1 ± 0.1	1580	1583
48.0	Spathulenol	t	0.1 ± 0.1	0.1 ± 0.1	1588	1576

Table 2.66 (continued)

GC RT	Compound	Subcritical CO_2	0.9% Methanol–subcritical CO_2	3.0% Methanol–subcritical CO_2	RRI cal	RRI lit
49.2	α-Muurolol	t	0.1 ± 0.1	0.4 ± 0.2	1656	1645
50.3	α-Cadinol	0.4 ± 0.1	0.2 ± 0.1	1.3 ± 1.0	1669	1653
51.5	α-Bisabolol	t	0.3 ± 0.1	0.1 ± 0.1	1671	1671
54.1	(*Z*,*E*)-farnesol	0.8 ± 0.1	0.4 ± 0.1	1.1 ± 0.5	1705	1697
55.3	(*E*,*E*)-farnesol	0.1	0.3 ± 0.1	0.5 ± 0.1	1725	1722
56.8	Oplopanone	t	0.1 ± 0.1	t	1734	1733
60.5	Octadecane	0.2 ± 0.1	t	0.1 ± 0.1	1796	1800
61.2	Myristic acid	t	0.1 ± 0.1	0.1 ± 0.1	1826	–
65.0	Nonadecane	t	–	t	1894	1900
66.1	Methyl palmitate	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	1928	1927
68.0	1-Eicosene	0.7 ± 0.2	–	0.9 ± 0.5	1993	1994
68.8	Palmitic acid	1.2 ± 0.2	0.7 ± 0.1	1.3 ± 0.4	2012	–
72.8	Methyl oleate	0.5 ± 0.4	0.1 ± 0.1	1.0 ± 0.1	2102	2105
73.5	Methyl stearate	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	2115	2128
77.5	9,12-Octadecadienol[a]	3.0 ± 1.1	0.8 ± 0.1	1.0 ± 0.2	2165	–
78.6	Stearic acid	0.5 ± 0.2	0.3 ± 0.1	2.0 ± 0.1	2186	–
83.0	Mixed hydrocarbons[a]	0.5 ± 0.2	2.6 ± 0.1	4.5 ± 1.0	–	–

[a] Tentative identification.

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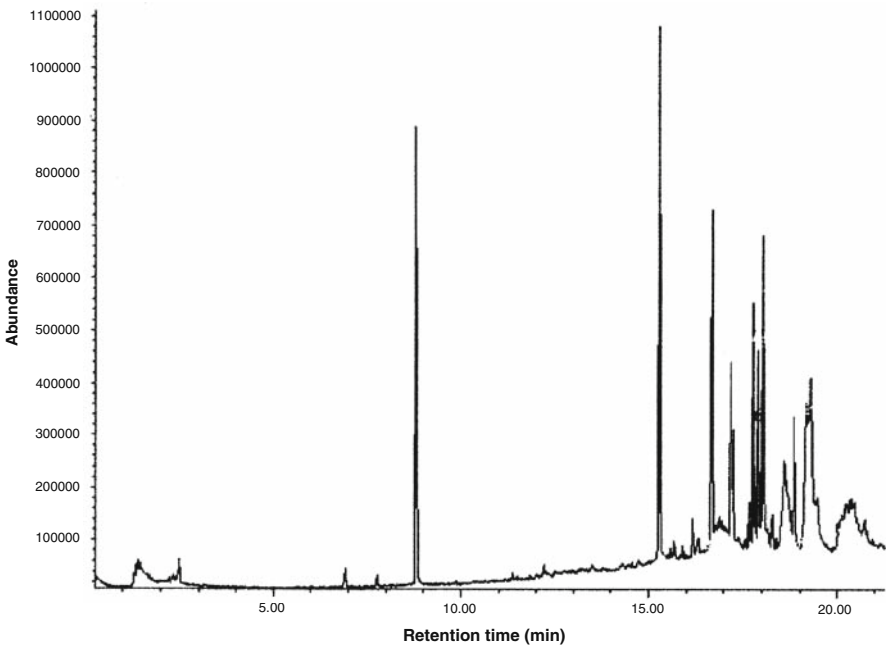

Fig. 2.71 Typical gas chromatograms of galangal extract: 1,8-cineole (1), β-caryophyllene (2), farnesene (3), α-humulene (4), β-selinene (5), pentadecane (6), α-selinene (7), β-bisabolene (8), germacrene-B (9) and 1,2-benzenedicarboxylic acid (10). Reprinted with permission from Mayachiew and Devahastin (2008)

The composition of rhizome (*Jianghuang*) and tuberous root (*Yujin*) of the plant *Curcuma longa* was investigated by using pressurised liquid extraction (PLE) and GC-MS. PLE was performed with methanol at 140°C, static extraction time, 5 min; pressure, 1,000 p.s.i. Volatiles were separated in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Initial oven temperature was 80°C, ramped to 120°C at 20°C/min, then to 130°C at 1°C/min (5 min hold), to 160°C at 4°C/min, finally to 280°C at 20°C/min. MS detector was operated in electron ionisation mode (70 eV). The mass range varied between 40 and 550 *m/z*. Typical chromatograms showing the separation of the analytes are depicted in Fig. 2.73. The concentrations of volatile components in the rhizome (*Jianghuang)*and tuberous root (*Yujin*) from *Curcuma longa* are compiled in Table 2.70. Hierarchical cluster analysis demonstrated that ar-curcumene, ar-turmerone, α-turmerone, and β-turmerone can be employed for the discrimination between rhizome and tuberous root and can be used for the quality control of these products (Quin et al., 2007).

Table 2.67 Volatile compounds identified in fresh basil expressed as a relative percentage ($n = 3$); 100% is equivalent to the sum of all 23 identified compounds

Cultivar	t_R (min)	Cultivar I	Cultivar II	Cultivar III	Cultivar IV	Cultivar V	Cultivar	t_R (min)	Cultivar I	Cultivar II	Cultivar III
Farming[a] Compound	Relative percentage %	E	C	E	C	E	Farming[a] Compound	Relative percentage %	E	C	E
4-Hexen-1-ol	10.26	t	t	t	t	1.2	t	0.2	0.4	0.2	0.4
2-Hexenal	10.28	0.3	0.4	t	t	1.1	t	t	t	1.0	1.0
∗-Pinene	11.30	0.6	0.4	1	t	t	t	0.5	0.5	1.1	1.3
Camphene	11.52	t	t	t	t	t	t	t	t	t	t
β-Myrcene	11.99	5.1	4.4	1.1	1.2	1.0	1.2	5.1	5.3	7.0	5.2
β-Pinene	12.05	t	t	1.0	t	t	t	t	t	2.2	2.1
∗-Phellandrene	12.35	t	t	t	t	t	t	t	t	t	t
3-Carene	12.48	1.5	1.2	t	t	t	t	t	t	t	t
d-Limonene	12.80	**11.1**	**11.1**	**15.0**	**12.2**	**11.2**	**7.3**	**3.1**	**3.1**	**2.0**	**1.3**
1,8-Cineole	12.98	**15.6**	**16.9**	**7.1**	**9.2**	**3.1**	**8.2**	**18.1**	**17.3**	**20.0**	**20.2**
Terpinen-4-acetat	13.03	t	t	3.1	4.2	1.0	1.0	t	t	t	t
Linalool	13.61	**15.6**	**18.2**	**24.8**	**32.2**	**21.1**	**17.2**	**23.1**	**26.2**	**21.0**	**19.3**
Camphor	14.93	2.3	t	2.0	t	2.0	1.6	4.2	t	t	t
γ-Terpinene	15.03	0.9	t	2.1	2.0	1.1	1.2	t	t	t	t
Methyl chavicol	15.32	**0.9**	**1.2**	t	t	**10.1**	**44.2**	**2.1**	**1.4**	**1.2**	**1.4**
Bornyl acetate	16.81	**3.2**	**2.2**	**8.2**	**6.2**	**1.2**	**1.1**	t	t	**12.1**	**9.3**
Methyl cinnamate	18.09	ND	ND	ND	ND	**10.2**	ND	ND	ND	ND	ND
Eugenol	19.29	**10.8**	**13.1**	**13.1**	**12.2**	t	t	**22.2**	**17.1**	**12.2**	**9.1**
Bergamotene	19.56	**14.0**	**13.2**	**1.1**	**1.2**	**6.2**	**1.0**	**4.2**	**6.1**	**17.1**	**20.2**
β-Caryophyllene	20.48	2.6	2.8	8.8	10.1	3.2	4.4	6.2	9.1	2.3	3.0
∗-Humulene	20.98	t	t	t	t	t	t	t	t	t	t
β-Muurolene	21.16	7.2	6.8	t	t	6.1	6.0	4.3	6.1	3.2	4.0
Cadina-3,9-dien	23.75	t	t	t	t	t	t	t	t	t	t

t – traces.
ND – not detected.
[a] E – ecological cultivation, C – conventional cultivation.
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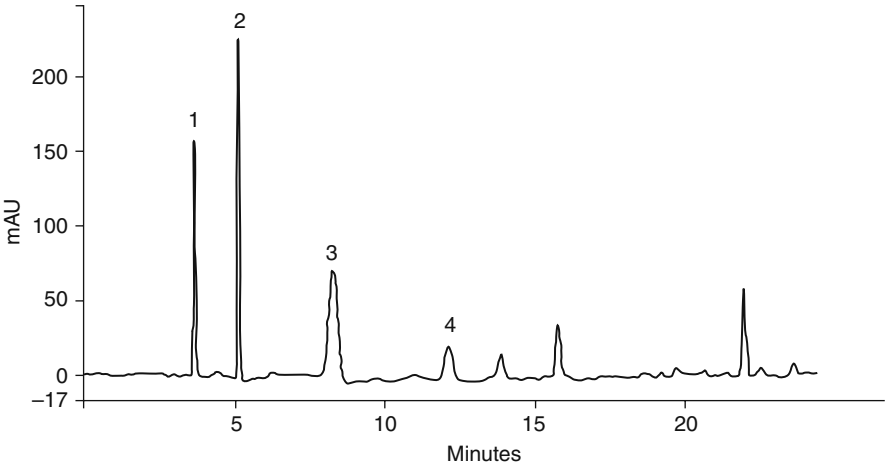

Fig. 2.72 HPLC chromatograms of Indian gooseberry extract at wavelength 220 nm: ascorbic acid (1), hydrolysable tannins (2, 3) and gallic acid (4). Reprinted with permission from Mayachiew and Devahastin (2008)

The composition of the ethanolic extracts of Indian gooseberry (*Phyllantus emblica*) and galangal (*Alpinia galanga*) was investigated by using GC-MS and RP-HPLC. The measurements were motivated by the antioxidant properties of the galangal extract (Juntachote and Berghofer, 2005) and by the antiproliferative capacity of the gooeseberry extract (Khan et al., 2002). The components of galangal extract were separated in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Starting oven temperature was 40°C (2 min hold), increased to 250°C at 10°C/min (5 min hold). MS detector was operated in electron ionisation mode (70 eV). The mass range varied between 40 and 550 *m/z*. Typical gas chromatogram illustrating the good separation of the components of galangal extract is shown in Fig. 2.74. RP-HPLC analysis of gooseberry extract was performed on a C18 column (250 mm × 4.6 mm, particle size, 5 μm). Solvent A and B were 0.05% aqueous H_3PO_4 and ACN, respectively. Gradient elution started at 5% B (0–6 min), increased to 15% (6–15 min), to 20% B (15–35 min), to 40% B (35–40 min). Detection wavelength was set to 220 nm. A HPLC chromatogram is shown in Fig. 2.75. The concentrations of the components of galanga extract are compiled in Table 2.71. It was concluded from the results of the analyses that both Indian gooseberry extract and galangal extract can be applied as natural antimicrobial and antioxidant agents (Mayachiew and Devahastin, 2008).

The effect of radiation on the concentration and composition of aroma glycosides in nutmeg was followed by traditional column chromatography, analytical and preparative TLC. Samples were extracted with hexane, chloroform:methanol (2:1), methanol:water (80:20), consecutively. The water solution was further extracted with *n*-butanol and then separated on an Amberlite XAD-2 column using methanol

Table 2.68 Essential oil constituents of Lavandula stoechas determined by GC-MS

Component	Type	Area (%) SbCWE	HD	USE
α-Pinene	M	t	2.94	0.41
Camphene	M	t	2.08	0.62
Limonen	M	t	2.52	0.31
1,8-Cineol	LOC	4.38	7.67	3.06
Epoxy linalool	LOC	1.25	0.78	0.31
Fenchon	LOC	26.93	32.03	34.23
Linalool	LOC			
β-Pinene	M	0.96	1.64	–
Camphor	LOC	29.64	14.71	41.09
p-Mentha-1,5-dien-8-ol	LOC	0.53	0.25	0.31
Isoborneol	LOC	0.52	0.25	–
4-Terpineol	LOC	0.70	0.55	
p-Cymen-8-ol	LOC	1.42	0.61	0.65
α-Terpineol	LOC	1.05	0.62	
Myrtenol	LOC	3.82	1.95	0.84
Verbenone	LOC	1.18	0.10	0.64
trans-Carveol	LOC	0.87	0.17	0.78
Carvon	LOC	1.08	1.24	0.26
Bornyl acetate	LOC	0.31	1.68	1.97
Myrtenyl acetate	LOC	1.66	11.70	4.97
Terpendiol	LOC	0.72	0.13	–
-Campholenic acid	LOC	t		0.63
Myrtensaeure	LOC	1.53	t	t
(+) Cycloisosativene	S	0.23	0.62	0.25
2-4-Methyl-3-penteylidene- Butadienal	LOC	0.77	–	–
Sesquisabinenhydrate	HOC	0.16	1.68	0.48
δ-Cadinol	HOC	0.14	1.17	0.24
δ-Cadinene	S	–	0.58	0.14
3-Caren-10-al	LOC	3.16	0.20	0.30
α-Cetone	HOC	0.51	0.20	
$C_{15}H_{22}O$	HOC	t	0.65	
Nopyl acetate	LOC	1.55	0.49	0.21
Caryophyllene oxide	HOC	0.21	0.56	0.16
Viridiflorol	HOC	0.16	2.32	1.17
Epiglobulol	HOC	0.13	0.12	0.62
$C_{15}H_{22}O$	HOC	0.31	0.97	0.17
$C_{17}H_{24}O$	HOC	0.85	0.19	0.14
Izovelleral	HOC	0.54	–	–

[$Y\,\% =$ g of extract/g of dried material $\times$ 100]. The yield of essential oil is highest in the subcritical water extract. The overall yields of essential oil of *L. stoechas* obtained by HD, USE and SbCWE techniques were 1.61 $\pm$ 0.03; 3.92 $\pm$ 0.03 and 4.19 $\pm$ 0.05 g/100 g dried flower, respectively.
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Table 2.69 Chemical composition of the rhizome oil of *Zingiber nimmonii*

Constituent	RR_t	%
n-Nonane	898	t
Tricyclene	923	t
α-Thujene	927	t
α-Pinene[c]	936	t
Camphene	951	0.08
Sabinene	974	0.41
β-Pinene[c]	979	0.68
Myrcene[c]	991	4.18
δ-2-Carene	1003	0.06
α-Phellandrene[c]	1010	0.74
α-Terpinene[c]	1018	1.11
p-Cymene[c]	1025	0.64
o-Cymene	1028	0.19
Limonene[c]	1033	0.44
(*E*)-β-ocimene	1049	0.07
γ-Terpinene[c]	1058	0.10
Terpinolene	1088	0.13
trans-Sabinene hydrate	1104	0.09
2-Nonen-1-ol	1145	0.10
Camphor	1151	1.00
Camphene hydrate	1154	0.25
α-Phellandren-8-ol	1166	0.06
Borneol[c]	1172	0.08
Terpinen-4-ol	1182	0.22
α-Terpineol[c]	1195	0.07
Myrtenal	1203	0.10
n-Decanal	1207	0.09
t-Piperitol	1214	t
Bornyl acetate	1291	t
6-Tridecene	1307	t
α-Copaene	1384	t
β-Elemene	1399	0.14
Isocaryophyllene	1418	t
β-Caryophyllene[c]	1441	42.15
α-Humulene (α-caryophyllene)[c]	1474	27.68
γ-Muurolene	1486	0.15
2-Nonyn-1-ol	1501	0.07
α-Muurolene	1510	0.14
β-Bisabolene	1514	0.06
γ-Cadinene	1525	0.08
δ-Cadinene	1534	0.25
Zonarene	1537	0.25
10-*epi*-Cubebol	1545	1.42
Germacrene B	1554	0.23
Nerolidol[a]	1570	0.14
trans-Sesquisabinene hydrate	1590	0.55
Caryophyllene oxide[c]	1604	1.68
Globulol	1610	0.33
(Z)-Bisabol-11-ol	1616	t

Table 2.69 (continued)

Constituent	RR_t	%
TMCD[b]	1618	0.33
3-Octadecyne	1629	1.05
cis-Cadin-4-en-7-ol	1643	0.19
Epoxy-*allo*-alloaromadendrene	1650	0.68
τ-Muurolol	1657	2.06
α-Muurolol	1660	0.68
Cubenol	1664	0.09
α-Cadinol	1670	2.72
14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1674	1.15
β-Bisabolol	1680	1.29
α-Bisabolol	1695	0.19
cis-*Z*–bisabolene epoxide	1704	0.08
(*Z*)–*trans*-bergamotol	1707	0.06
(2*Z*,6*Z*)-Farnesol	1723	0.10
(2*E*,6*E*)-Farnesol	1729	0.06
(2*E*,6*Z*)-Farnesol	1750	0.36
Total number of constituents		81
Number of constituents identified		65
% Identified		97.5%
Monoterpene hydrocarbons		8.87%
Oxygenated monoterpenes		1.91%
Sesquiterpene hydrocarbons		71.19%
Oxygenated sesquiterpenes		14.19%
Other constituents		1.34%

RR_t – relative retention time (calculated); t – trace, <0.05%.
[a] Correct isomer not identified.
[b] TMCD – 1,5,8,8-tetramethyl-cycloundeca-5,9-dien-1-ol. All oil constituents identified by (i) mass spectral database match, (ii) comparison of mass spectrum with literature data and (iii) RR_t.
[c] Constituents identified by (i), (ii), (iii) and (iv) co-injection.
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as eluting solvent. Analytical TLC was performed on an ammonium sulphate impregnated silicagel G plates using ethylacetate:isopropanol:water (65:30:15, v/v/v) as mobile phase. Preparative TLC was carried out on silicagel plates of 0.5 mm thickness using the same eluent system. Running time was 2.5 h at 28°C. The extract was loaded on a silica column and eluted by chloroform containing increasing amount of methanol as additive. After acid hydrolysis, the aglycones were separated by GC-MS on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Starting oven temperature was 60°C, increased to 200°C at 4°C/min (5 min hold), to 280°C at 10°C, final hold 20 min. MS detector was operated in electron ionisation mode (70 eV). The aroma glycosides found in butanol extract and XAD-2 are compiled in Table 2.72. The data demonstrated that radiation decreases the concentration of aroma glycosides in nutmeg (Ananthakumar et al., 2006).

Table 2.70 The contents (mg/g) of eight investigated compounds in rhizome (*Jianghuang*) and tuberous root (*Yujin*) from *Curcuma longa*

Sample	β-caryophyllene	ar-curcumene	Zingiberene	β-Bisabolene	B -Sesqui-phellandrene	Ar -Turmerone	α-Turmerone	β-Turmerone	Total
J1	+[b]	3.19[c]	3.63	3.43	2.81	9.02	14.58	17.27	53.93
J2	+	3.32	8.67	1.49	4.78	12.55	18.14	28.33	77.27
J3	+	3.48	11.86	1.57	5.92	12.63	21.85	31.43	88.74
J4	+	2.67	13.19	1.59	5.17	8.52	21.87	27.70	80.71
J5	+	2.55	12.84	1.52	5.47	7.55	21.31	26.49	77.73
H1	+	+	1.94	+	1.02	+	4.27	5.86	13.09
H2	0.75	0.85	4.02	0.78	1.59	+	9.91	11.14	29.02
H3	0.86	+	6.30	0.89	2.21	1.03	10.86	9.53	31.66
H4	0.88	+	5.17	0.95	2.41	0.92	10.01	8.26	28.61
H5	0.85	+	6.61	0.79	2.15	+	9.32	11.23	30.94

[a] *Jianghuang* (J1–J5) and *Yujin* (H1–H5) are rhizome and tuberous root of *C. longa*, which were collected from Wuning, Jiangxi Province; Quanzhou, Fujian Province; Shuangliu, Sichuan Province; Qianwei, Sichuan Province; Chongzhou, Sichuan Province, respectively.
[b] Below the limit of quantitation.
[c] The data were presented as average of three replicates (R.S.D.% < 4%).
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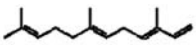
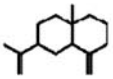
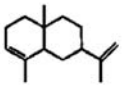
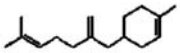
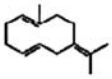
Table 2.71 Chemical compositions of *Alpinia galanga* essential oil

No.	Composition	% Composition	RT
	1,8-cineole		
1		20.95	8.81
	β-caryophyllene		
2		13.16	16.68
	farnesene		
3		7.47	17.19
	α-humulene		
4		5.02	17.24
	β-selinene		
5		10.56	17.77
	pentadecane		
6		2.70	17.82
	α-selinene		
7		9.67	17.90
	β-bisabolene		
8		17.95	18.04
	germacrene B		
9		6.10	18.86
	1,2-benzenedicarboxylic acid		
10		6.42	19.24
	Total	100	

RT – retention time.
Reprinted with permission from Mayachiewa and Derahastin (2008).

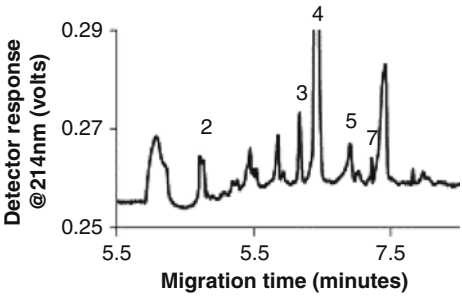


Fig. 2.73 Electropherogram of an Indonesian extract, buffer, 10 mM boric acid, 10 mM sodium tetraborate, 100 mM SDS, 40 mM SC (pH 7.0); voltage, 18 kV. Peak identification: 1: 4-hydroxybenzyl alcohol; 2: 4-hydroxy-3-methoxybenzyl alcohol; 3: 4-hydroxybenzaldehyde; 4: vanillin; 5: vanillic acid; 6: ethyl vanillin; 7: 4-hydroxybenzoic acid. Reprinted with permission from Boyce et al. (2003)

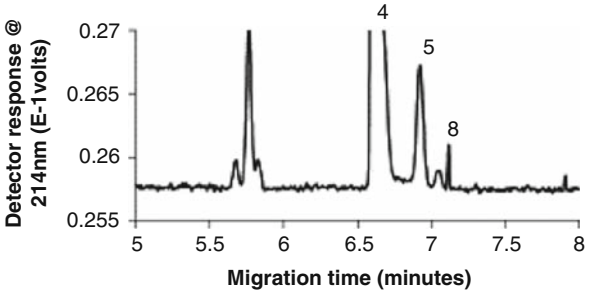


Fig. 2.74 Electropherogram of a nature identical extract. Peak identification : vanillin (4), vanillic acid (5), piperonal (8). Reprinted with permission from Boyce et al. (2003)

Table 2.72 Quantitative distribution (mean ± SD, $n = 9$) of aroma glycosides in BuOH extract and XAD-2

Compound	XAD-2 (mg/100 g)	Butanol (mg/100 g)
p-Cymene-7-ol	3.10 ± 0.12	3.15 ± 0.10
Eugenol	0.48 ± 0.02	0.50 ± 0.03
Methoxy eugenol	0.58 ± 0.03	0.61 ± 0.01
α-Terpineol	0.50 ± 0.02	0.51 ± 0.02

Means are not significantly different at 5% level of confidence.
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Micellar electrokinetic capillary chromatography (MECC), SPME-GC-MS and HPLC were employed for the separation and quantitative determination of the main aroma substances in vanilla extracts and synthetic flavourings. MECC analyses were performed in a fused silica capillary (60 cm total length, 52 cm effective length,

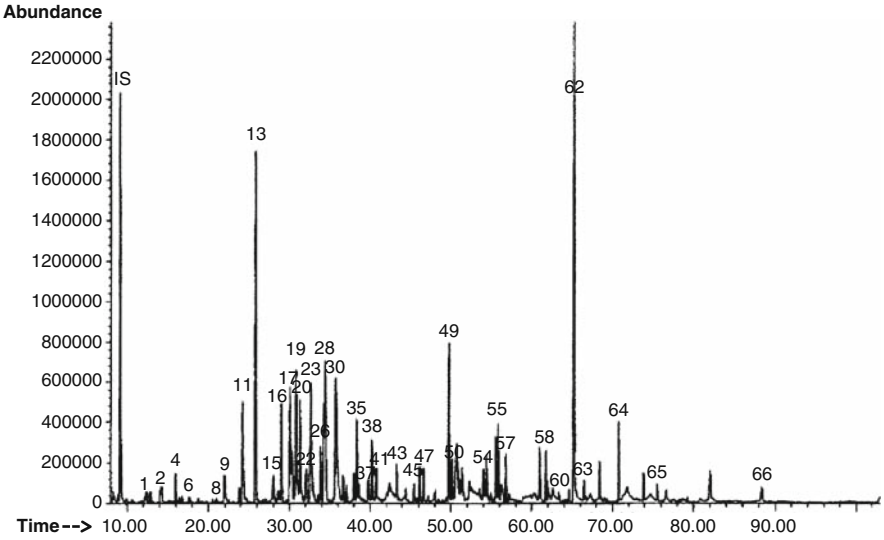

Fig. 2.75 GC–EM profile of SDE extract from Spanish citrus honey. Peak identification are in Table 2.75. Reprinted with permission from Castro-Vázquez et al. (2007)

75 μm i.d.). Samples were injected by hydrostatic injection. Measurements were run at 25°C; applied voltage was 18 kV. Analytes were detected at 214 nm. Running buffers consisted of 10 mM sodium tetraborate, 10 mM boric acid, 100 mM SDS and 0–50 mM sodium cholate at various pH values. RP-HPLC separations were carried out in a C18 column (250 mm × 4.6 mm, particle size, 5 μm). Mobile-phase components were methanol (A) and water–acetic acid 95:5 (B). Gradient started at 18% A (0–1 min), 18–50% A (1–8 min), 50–75% A (8–20 min), 75% A (20–30 min). Analytes were detected at 280 nm. PA fibre was applied for HS-SPME preconcentration of analytes for 40 min at ambient temperature. GC-MS measurements were performed in a capillary column (30 m × 0.2 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Starting oven temperature was set to 40°C (2 min hold), increased to 200°C at 8°C/min, to 250°C at 50°C. MS detector was operated in electron ionisation mode (70 eV). The elctropherogram of an Indonesian extract is depicted in Fig. 2.76. It was found that the RSD value of the migration times was less than 1% and the theoretical plate number varied between 130.000 and 200.000. The electrophoregram of a nature identical extract is shown in Fig. 2.77, illustrating again the good separation power of the MECC method. The results obtained by MECC and HPLC are compiled in Table 2.73. It was concluded from the data that both MECC and HPLC are suitable for the separation and quantitative determination of the aroma substances in vanilla extracts, MECC being more rapid and reproducible (Boyce et al., 2003).

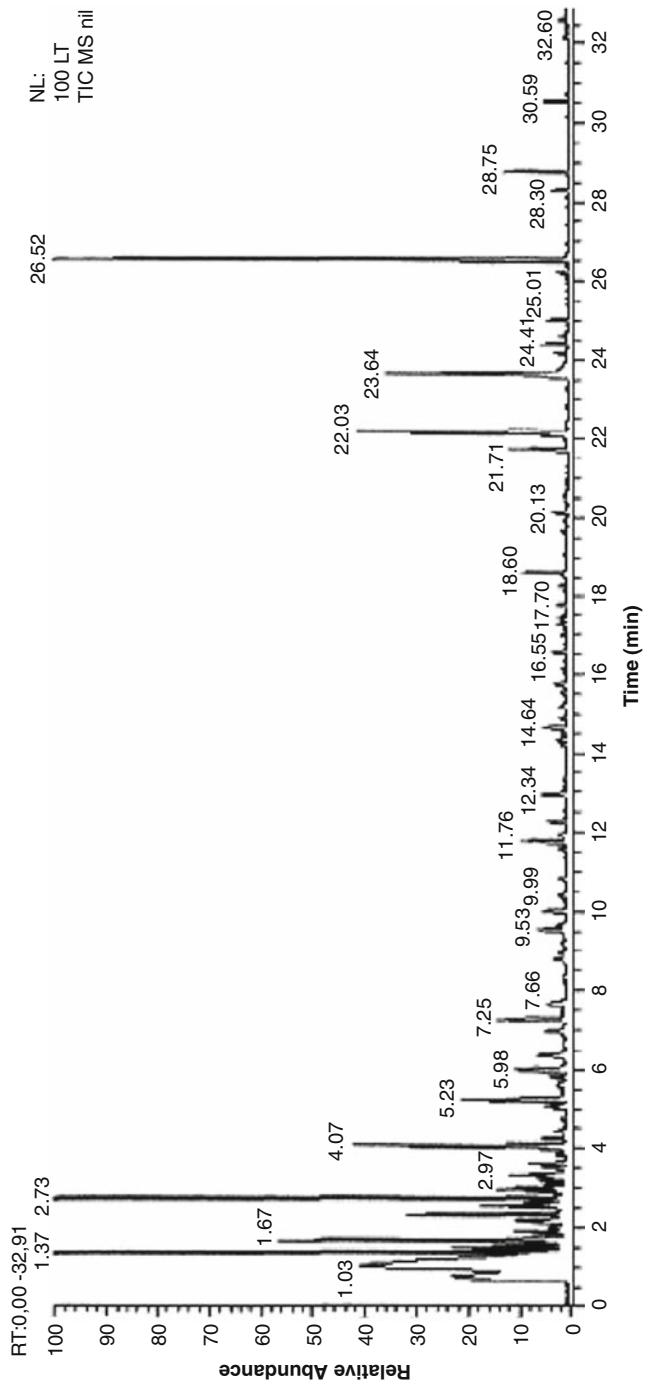

Fig. 2.76 Total ion current GC–MS chromatogram of the volatile fraction of strawberry tree (*Arbutus unedo* L.) honey extracted by the DHS technique. See Table 2.75 for peak identification. Reprinted with permission from Bianchi et al. (2005)

Table 2.73 Quantitative determination of key components in natural vanilla extracts by MECC and HPLC

	Concentration (mg/ml)[2]											
	Indonesian A		Tongan		Madagascan		Tahetian		Mexican		Indonesian B	
Components	MECC	HPLC	MECC	HPLC	MECC	HPLC	MECC	HPLC	MECC	HPLC	MECC	HPLC
4-hydrOxy benzaldehyde	0.18	0.21	0.16	0.14	0.34	0.35	0.40	0.39	0.24	0.27	0.17	0.19
4-Hydroxy benzoic acid	0.16	–	0.05	–	0.81	–	0.81	–	0.05	–	0.05	–
Vanillic acid	0.06	0.07	0.03	0.03	0.08	0.09	0.14	0.17	0.08	0.08	0.05	–
Vanillin	2.04	2.00	1.76	1.64	3.62	3.81	1.16	1.18	1.23	1.26	2.62	2.79
Vanillin/4-hydroxy-benzaldehyde ratio	11.3	9.5	11.0	11.7	10.6	11.2	2.9	3.1	5.0	4.7	15.4	14.7

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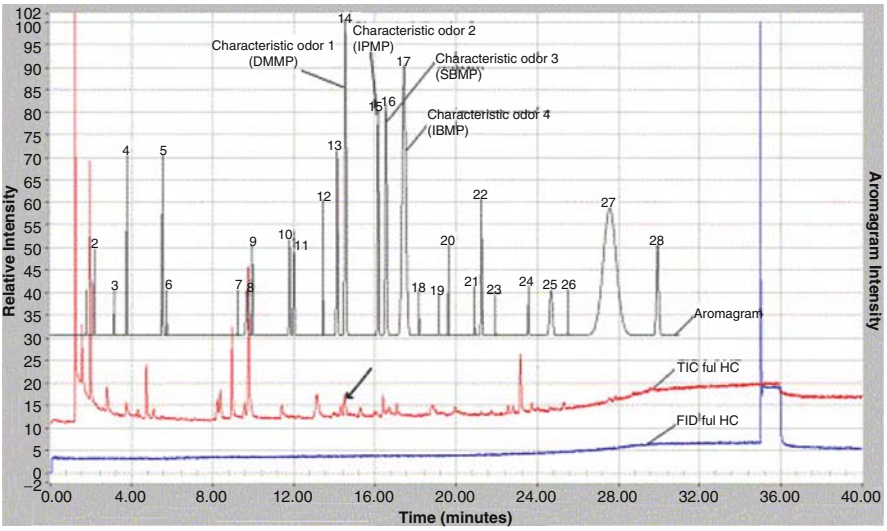

Fig. 2.77 Comparison of total ion chromatogram and aromagram with full heart-cut mode of headspace gases released by live *H. axyridis* in September (2005) and collected with 50/30 μm DVB/Carboxen/PDMS SPME using 24 h sampling time. Peak identification are in Table 2.78. DMMP = 2,5-dimethyl-3-methoxypyrazine; IPMP = 2-isopropyl-3-methoxypyrazine; SBMP = 2-*sec*-butyl-3-methoxypyrazine; IBMP = 2-isobutyl-3-methoxypyrazine. Arrow marks odorous 2-ethyl-1-hexanol co-eluting with IPMP in this GC–MS–O mode. Reprinted with permission from Cai et al. (2007)

2.11 Other Food Products

Besides the determination of the aroma compounds in foods and food products discussed above, the aroma substances and fragrances were analysed in a wide variety of other commercial products. Thus, the composition of honeys of various origins has been vigorously investigated. The method of preference used for the analysis of honey includes various preconcentration techniques (Alissandrakis et al., 2005) mainly SPME (Alissandrakis et al., 2007; de la Fuente et al., 2005; Pena et al., 2004), dynamic SPME (Radovic et al., 2001) combined by GC-MS (Pérez et al., 2002; Soria et al., 2003; Verzera et al., 2001). Many honey varieties were investigated by the method mentioned above such as buckwheat honey (Zhou et al., 2002), cashew (*Anacardium occidentale*) and Marmeleiro (*Croton species*) honeys (Moreira et al., 2002), rosemary honey (Castro-Vázquez et al., 2003), honeys of *Lavandula angustifolia* and *Lavandula angustifoliaaxlatifolia* (Guyot-Declerck et al., 2002), cambara (*Gochnatia Velutina*) honey (Moreira et al., 2005), etc. Earlier results in the analysis of volatiles in honey were previously reviewed (Cuevas-Glory

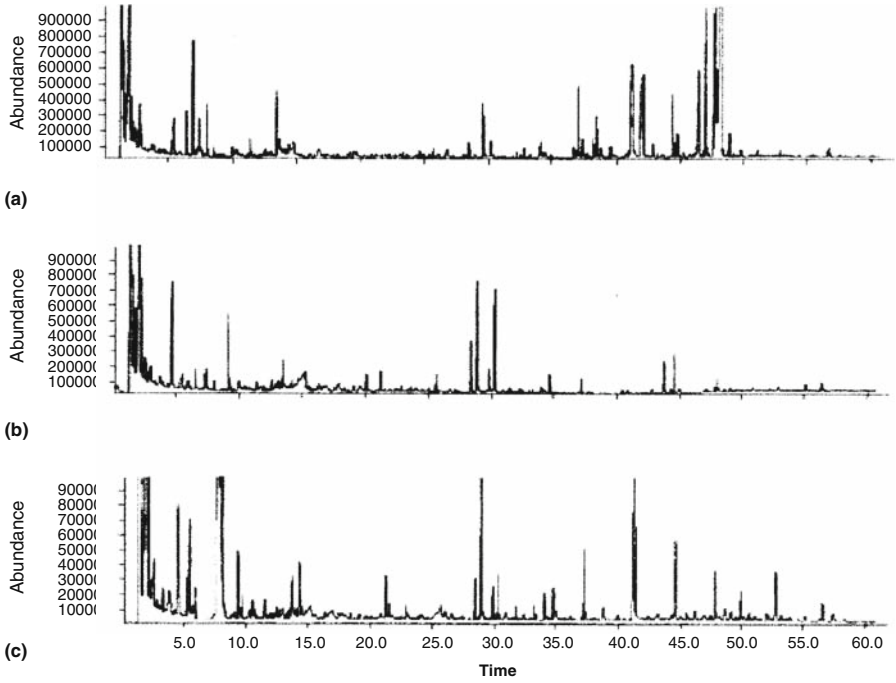

Fig. 2.78 Typical GC chromatograms of volatile flavour components extracted by SFSI from (**A**) Korean (**B**) Chinese and (**C**) Japanese danggui. Reprinted with permission from Kim et al. (2006)

et al., 2007). Simultaneous extraction and distillation (SDE) coupled to GC-MS was applied for the separation of volatile compounds in Spanish citrus honey. SDE was carried out by extracting the aqueous solution of honey (15 g honey in 40 ml of deionised water) with dichloromethane for 2 hours. Analytes were separated in a capillary column (60 m × 0.32 mm i.d., film thickness, 0.32 μm). Starting oven temperature was set to 60°C (3 min hold), increased to 200°C at 2°C/min. MS detector was operated in electron ionisation mode (70 eV), mass acquisition range was 40–450 *m/z*. The method allowed the separation and identification of 66 volatile compounds in unifloral Spanish citrus honey. A typical chromatogram depicting the aroma profile of a honey sample is shown in Fig. 2.78. AZONOSITAS. The aroma-active compounds found in citrus honey are compiled in Table 2.74. It was established that the high concentrations of (*Z*) (*E*)-linalool oxide, α-terpineol, terpineol and isomers of lilac aldehyde and lilac alcohol are characteristic of this floral source. Furthermore, it was found that sinensal isomers can be used as new chemical markers for this type of honey (Castro-Vázquez et al., 2007).

Table 2.74 Aroma-active compounds found in citrus honey

Compounds	OAV		Odour descriptor	Odour threshold (ppb)
	Min	Max		
(*Z*)-Linalool oxide	29.4	86.1	Fresh, sweet, floral	6.0–7.0
(*E*)-Linalool oxide	19.1	38.0	Fresh, sweet, floral	6.0–7.0
Furfural	1.1	3.1	Almod, sweet, bread	776
Benzaldehyde	1.6	6.3	Almod, sweet, fruit	41.7
Linalool	9.8	30.1	Fresh, floral	6
α-Terpineol	0.4	1.6	Green, floral	46
Lilac aldehyde (*isomer I*)	4.3	23.4	Fresh, flowery	0.2–20
Lilac aldehyde (*isomer II*)	12.9	858	Fresh, flowery	0.2–20
Lilac aldehyde (*isomer III*)	73.8	26.8	Fresh, flowery	0.2–20
Lilac aldehyde (*isomer IV*)	5.5	36.6	Fresh, flowery	0.2–20
Lilac alcohol (*isomer I*)	0.5	3.3	Fresh,sweet, flowery	4.0–74
Lilac alcohol (*isomer II*)	1.2	4.1	Fresh,sweet, flowery	4.0–74
Lilac alcohol (*isomer III*)	1.3	4.2	Fresh,sweet, flowery	4.0–74
Lilac alcohol (*isomer IV*)	0.3	1.1	Fresh,sweet, flowery	4.0–74
Hotrienol	0.7	1.7	Fresh, floral, fruity	110
Phenylacetaldehyde	151	339	Honey-like	4
β-damascenone	575	1800	Fruity, Sweet, honey	0.004
Sinensal (*isomer I*)	1346	5042	Sweet, orange	0.05
Sinensal (*isomer II*)	2352	7810	Sweet, orange	0.05
Methyl anthranilate	53.7	232	Grape, fruity	10

Odour activity values (OAV): compound concentrations (μg/l) divided by odour threshold.
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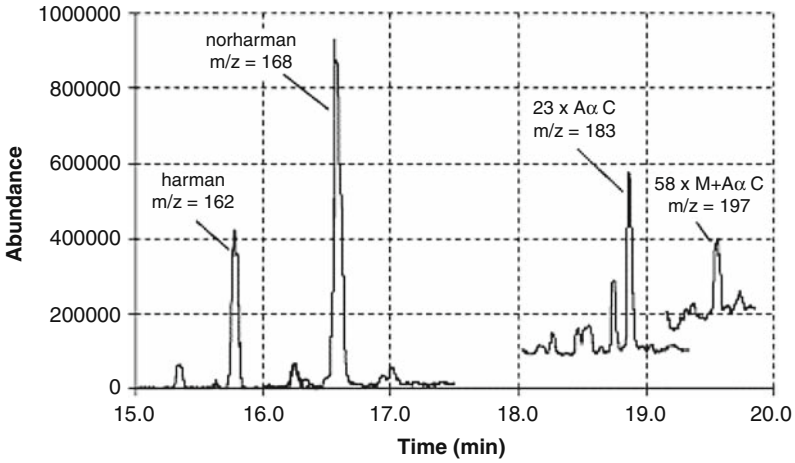

Fig. 2.79 Overlay of the extracted analyte ion signals from a 2R4F sample. Each signal is scaled for viewing. Reprinted with permission from Smith et al. (2004)

Dynamic headspace extraction (DHS) combined with GC-MS was employed or the analysis of the volatile compounds in Sardinien strawberry tree (*Arbutus unedo* L.) honey. Volatiles were separated on a fused-silica bonded-phase capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas. Starting oven temperature was set to 35°C (8 min hold), increased to 60°C at 4°C/min, to 160°C at 6°C, to 220°C at 20°C/min (final hold 1 min). MS detector was operated in electron ionisation mode (70 eV), the mass range of detection was 35–350 *m/z*. TIC chromatogram of a honey sample is shown in Fig. 2.79. The volatile compounds identified by the method are compiled in Table 2.75. It was concluded from the data that only α-isophorone, β-isophorone and 4-oxoisophorone were characteristics for the honey of strawberry tree. These compounds were proposed as markers for the authenticity of the honey (Bianchi et al., 2005).

Treacle (black honey) is prepared by heating sugar cane juice obtained from matured sugar can stalks. The aroma profile and the concentration of 5-hydroxymethylfurfural (HMF) in treacle were investigated by HS-SPME and HPLC, respectively. The measurement of the amount of HMF was motivated by the suspected health hazards (Sommer et al., 2003). Volatiles were preconcentrated on a DVB/CAR/PDMS fibre at 35°C for 20 min. Aroma substances were separated in a capillary column (30 m × 0.25 mm i.d., film thickness, 1 μm). Helium was used as carrier gas. Starting oven temperature was –10°C (1 min hold), increased to 250°C at 12°C/min (final hold 1 min). MS detector was operated in electron ionisation mode (70 eV), the mass range of detection was 20–350 *m/z*. The concentrations of volatile compounds are compiled in Table 2.76. The results demonstrated that aliphatic short chain acids, alcohols, aldehydes, ketones and furan derivatives were the main constituents of the volatile fraction. The amount of HMF was measured by HPLC using a RP column (55 mm × 2 mm, particle size, 3 μm). HMF was separated by an isocratic mobile phase (methanol–water, 5:95, v/v). HMF was detected at 280 nm, the

Table 2.75 Volatile compounds identified in Sardinian strawberry tree (*Arbutus unedo* L.) honey

No.	Compound	RT(min)	KI_{calc}	ID[3]	Occurrences
1	Acetone	2.32	n.c.	MS, RT	9
2	2-Butanone	3.30	905	MS, RT, KI	6
3	Ethanol	3.98	939	MS, RT, KI	6
4	2,5-Dimethylfuran	4.43	965	MS, KI	10
5	2,3-Butanedione	5.22	989	MS, RT, KI	10
6	2,3-Pentanedione	8.80	1047	MS	9
7	Hexanal	9.53	1088	MS, RT, KI	10
8	Methyl-2-butenal	9.99	1103	MS	5
9	2-Methyl-1-propanol	10.39	1106	MS, RT, KI	5
10	1-Butanol	12.34	1155	MS, RT, KI	4
11	Heptanal	14.64	1183	MS, RT, KI	10
12	2,4,4-Trimethylcyclopentanone	15.62	1211	MS	7
13	3-Methyl-1-butanol	15.75	1214	MS, RT, KI	4
14	Octanal	18.60	1291	MS, RT, KI	10
15	2,3,4-Trimethyl-2-cyclopentene-1-one	19.28	1311	MS	10
16	3-(1-Methylethyl)-2-cyclopenten-1-one	18.61	1322	MS	10
17	5-Hepten-2-one-6-methyl	20.13	1343	MS, RT, KI	9
18	3,3,5-Trimethylcyclohexanone (ihydroisophorone)	21.02	1368	MS	4
19	Nonanal	21.71	1397	MS, RT, KI	10
20	3,5,5-Trimethyl-3-cyclohexen-1-one(β-isophorene)	22.03	1407	MS	10
21	3-Furancarboxaldehyde	22.79	1441	MS	7
22	Furfural	23.64	1447	MS, RT, KI	10
23	Decanal	24.41	1503	MS, RT, KI	10
24	1-(2-furanyl)ethanone	24.60	1512	MS	9
25	Benzaldehyde	25.01	1528	MS, RT, KI	10
26	3,5,5-Trimethyl-2-cyclohexen-1-one(α-isophorene)	26.52	1591	MS, RT	10
27	3,5,5-Trimethylcyclohex-2-ene-1,4-dione(4-oxoisophorone)	28.75	1698	MS, RT	10
28	3,5,5-Trimethylcyclohexan-1,4-dione	30.53	1768	MS	10

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Table 2.76 Volatile constituents of three different commercial treacle samples as determined by HS-SPME

Compound	RT (min)	RI[a]	Sample A		Sample B		Sample C	
			Area %[b]	RSD %[g]	Area %[b]	RSD %[g]	Area %[b]	RSD %[g]
Ethanol[c]	4.19	<600	0.4	6.1	1.0	3.1	31.7	3.3
Acetone[c]	4.83	<600	0.7	4.2	0.2	2.7	0.2	3.5
Dimethylsulfide[c]	5.37	<600	12.8	5.1	8.1	5.3	1.9	12.4
2-Methylpropanal[d]	6.19	552	4.7	0.6	2.3	1.1	0.2	5.7
2-Butanone[d]	6.96	597	0.6	1.7	0.2	0.9	0.2	6.9
2-Methyl-3-buten-2-ol[d]	7.27	620	0.7	6.1	nd[f]		nd[f]	
2-Methyl-1-propanol[d]	7.61	626	nd[f]		nd[f]		2.5	11.1
Acetic acid[e]	7.65	–	22.5	2.1	40.1	1.7	26.3	0.5
3-Methylbutanal[d]	8.13	648	6.4	0.4	5.3	1.9	0.5	6.3
2-Methylbutanal[d]	8.33	658	12.7	1.8	6.3	1.7	0.4	7.8
1-Hydroxy-2-propanone[c]	8.47	–	4.4	5.4	2.6	4.0	3.1	2.5
Propanoic acid[e]	8.8	–	1.9	1.9	1.4	0.3	0.9	6.5
3-Hydroxy-2-butanone[d]	9.26	707	0.9	0.0	0.7	0.9	0.9	5.1
3-Methyl-1-butanol[d]	9.63	730	nd[f]		nd[f]		5.8	3.5
2-Methyl-1-butanol[d]	9.71	733	nd[f]		nd[f]		7.6	0.4
Butanoic acid[e]	10.38	–	1.3	7.0	1.1	1.3	2.8	1.5
2,3-Butanediol[c]	10.43	–	0.0		1.0	2.8	0.0	
Dihydro-2-methyl-3(2H)-furanone[c]	10.95	–	8.2	1.4	4.7	2.1	4.2	0.2
2-Furfural[d]	11.53	830	5.7	2.5	10.8	1.7	0.4	1.8
2-Furfuryl alcohol[d]	11.75	852	1.8	4.7	0.9	14.3	2.4	0.2

Table 2.76 (continued)

Compound	RT (min)	RI[a]	Sample A		Sample B		Sample C	
			Area %[b]	RSD %[g]	Area %[b]	RSD %[g]	Area %[b]	RSD %[g]
2-Acetylfuran[d]	12.73	910	3.5	0.9	2.4	1.3	2.4	2.3
5-Methyl-2-furancarboxaldehyde[d]	13.58	962	1.2	1.7	1.3	4.8	nd[f]	
Aliphatic acids			25.7		42.6		30.0	
Aliphatic alcohols			1.1		2.0		47.6	
Aliphatic aldehydes			23.8		13.9		1.1	
Aliphatic ketones			6.9		3.7		4.4	
Sulfur compounds			12.8		8.1		1.9	
Furan derivatives			20.4		20.1		9.4	
Amount of the total area (%)			90.7		90.4		94.4	

[a] The retention indices given (on an HPS column) are those, where the measured values are in good accordance with the tabulated values in a retention index database.
[b] The given values are the arithmetic means from duplicate analysis. Differences between the single measurements were not higher than 10% for the whole procedure. Areas were normalized to a sample weight of exactly 200 mg.
[c] Tentatively identified; the identification was based on the mass spectra and comparison of the spectra with those from a mass spectra library. The accordance of the spectra with the mass spectra from the MS database was very high (>90%).
[d] The identification was based on the mass spectra and comparison of the spectra with those from an MS library. In addition the obtained retention indices were compared with those from a retention index database.
[e] The organic acids acetic acid, propionic acid and butyric acid were identified based on their retention behaviour, the typical peak shape of the polar compounds on the non-polar stationary phase of the analytical column and the mass spectra in comparison with those from the MS database. Due to the strong fronting of the peak, no retention index could be calculated.
[f] Not detected.
[g] Relative standard deviation.
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Table 2.77 Content of 5-hydroxymethylfurfural (HMF) in three different commercial treacle samples

Sample	HMF content (mg/kg sample)	SD[a] (mg/kg)
A	66.1	0.9
B	179.0	2.5
C	92.4	1.3

[a] Standard deviation.
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LOD being 7 ng/ml. The HMF concentration in different samples are compiled in Table 2.77. It was stated that the methods applied are suitable for both the determination of the aroma profile of treacle and the assessment of the amount of HMF in the treacle samples (Edris et al., 2007).

The odorants of Asian ladybird beetles (*Harmonia axiridis*, Coleoptera: Coccinellidae) were concentrated by HS-SPME and separated and identified by GC-MS-O. HS-SPME was carried out at 25°C for 24 h. Multidimensional GC system applied a nonpolar pre-column (12 m × 0.53 mm i.d., film thickness, 1 μm) and a polar analytical column (25 m × 0.53 mm i.d., film thickness, 1 μm). Injector and detector (FID) temperatures were 260°C and 280°C, respectively. Helium was used as carrier gas. Initial oven temperature was 40°C (3 min hold), increased to 220°C at 7°C/min (final hold 10 min). MS detection was in the range 33–280*m/z*. A characteristic TIC and aromagram are depicted in Fig. 2.80. It was established that the extract contained alkanes and alkenes, alcohols, aldehydes, aromatic hydrocarbons, acids, halogenated hydrocarbons, ketones, pyrazines, N- and S-containing compounds and terpenes. The volatile organic compounds (VOCs) are compiled in Table 2.78. It was found that 2,5-dimethyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine are responsible for the characteristic odour of Asian lady bird beetles (Cai et al., 2007).

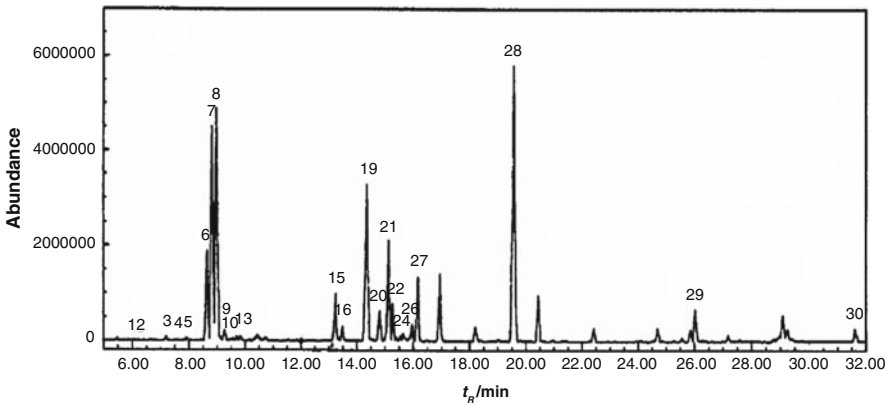

Fig. 2.80 Total ion current chromatogram of a real sample of tobacco flavour. For peak identification see on Table 2.81. Reprinted with permission from Ying et al. (2006)

Table 2.78 Identification of VOCs released from live *H. axiridis*

Compound #	Aromagram peak #	Retention time (min)	Compound	CAS	MW	Odor threshold (ppb)	Odor character	Flavornet
1		1.51	1,4-Pentadiene[a]	591-93-5	68.12			
	1	1.75					Sewer, skunky, fecal	
2	2	1.93	Acetone[a]	67-64-1	58.08	14,454	Ketone	
3		2.53	Heptane[a]	142-82-5	100.21	9,772		Alkane
4		2.75	2-Butanone[a]	78-93-3	72.11	7,762		Ether
	3	3.13					Foul	
5	4	3.68	Diacetyl[a]	431-03-8	86.09	4.4	Buttery	
6		4.16	2-Pentanone[a]	107-87-9	86.14	1,548		
7		4.41	Octane[a]	111-65-9	114.23	5,754		Alkane
8		4.66	2-Methyl-3-buten-2-ol[a]	115-18-4	86.14			
	5	5.48					Sweet, flora	
9	6	5.68	Methyl-benzene[a]	108-88-3	92.14	1,549	Sweet	Paint
10		5.86	2-Ethyl-5-methylthiopene	40323-88-4	126.05			
11		7.05	Nonane[a]	111-84-2	128.26	1,259		Alkane
12		8.18	Isoamyl alcohol[a]	123-51-3	88.15	44.7		Whiskey, malt, burnt
13		8.3	Alpha-Pinene[a]	80-56-8	136.24	692		Pine, turpentine
14	7	8.86	Camphene[a]	79-92-5	136.24		Peanut	Camphor
15	8	9.7	3-Hydroxy-2-butanone[a]	513-86-0	88.11		Buttery, nut	Butter, cream
16	9	9.75	3,4-Dimethyl-2-hexanol	19550-05-1	130.23		Sweet, flora	
17	10	11.36	Limonene[a]	138-86-3	136.24	437	Milky, citrus	Lemon, orange

Table 2.78 (continued)

Compound #	Aromagram peak #	Retention time (min)	Compound	CAS	MW	Odor threshold (ppb)	Odor character	Flavornet
18		11.78	Octanal[a]	124-13-0	128.22	1.35		Fat, soap, lemon, green
	11	11.95					Mushroom, mouldy	
19		12.16	6-Methyl-5-hepten-2-one[a]	110-93-0	126.2	38		
20	12	13.05	Acetic acid[a]	64-19-7	60.05	144	Acidic, sour	
21		13.9	1,3-Dichloro-benzene[a]	541-73-1	147			
22	13	14.03	2,5-Dimethyl-3-methoxypyrazine	19846-22-1	138.08	0.002	Characteristic, mouldy, earthy	
23		14.16	Nonanal[a]	124-19-6	142.24	2.24		Fat, citrus, green
24	14	14.43	2-Isopropyl-3-methoxypyrazine[a]	25773-40-4	152.2	0.002	Characteristic, peanut, potato	Peas, earth
25		14.45	2-Ethyl-1-hexanol[a]	104-76-7	130.23	245		
26		14.68	Propanoic acid[a]	79-09-4	74.08	35.5	Fatty acid	
27		14.9	Benzaldehyde[a]	100-52-7	106.13	417		Almond, burnt sugar
28		15.93	Dihydro-3-methyl-2[3*H*]-furanone[a]	1679-47-6	100.05			
29	15	16.05	2-*sec*-Butyl-3-methoxypyrazine[a]	24168-70-5	166.11	0.002	Characteristic, nutty, potato, peanut	

Table 2.78 (continued)

Compound #	Aromagram peak #	Retention time (min)	Compound	CAS	MW	Odor threshold (ppb)	Odor character	Flavornet
30	16	16.35	2-Isobutyl-3-methoxypyrazine[a]	24683-00-9	166.22	0.002	Characteristic, peanut, potato	Earth, spice, green pepper
31		16.37	Dihydro-4-methyl-2[3*H*]-furanone	1679-49-8	100.05			
32	17	17.01	Isovaleric acid[a]	503-74-2	102.13	2.45	Body odour, fatty acid	Sweat, acid, rancid
33		18.2	5-*Ethyldihydro-5-methyl-2[3H]*-furanone	2865-82-9	128.08			
	18	18.14					Burnt	
34	19	18.33	1-Borneol[a]	464-45-9	154.3	2.09	Burnt, plastic	Camphor
	20	19.60					Earthy, mouldy	
35	21	20.56	Benzenemethanol[a]	100-51-6	108.14		Sweet, flora	
	22	21.20					Mouldy, musty	
	23	21.94					Herbaceous	
36	24	22.5	Phenol[a]	108-95-2	94.11	110	Phenolic, medicinal	Phenol
37	25	23.66	Ionol	4130-42-1	234.39		Phenolic	
	26	25.52					Solvent	
	27	26.81					Musty, mouldy	
38	28	28.65	Indole[a]	120-72-9	117.15	0.032	Barnyard	Mothball, burnt

Odour character refers to the descriptors used by panelists in this study. Flavornet database summarizes odour descriptors.
[a] Confirmed with pure standard.
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Angelica roots such as *Angelica sinensis* (Chinese danggui, CDG), *Angelica acutiloba* (Japanese danggui, JDG) and *Angelica gigas* (Korean danggui, KDG) are important traditional medicines. Because of their importance, a considerable number of analytical methods were developed and applied for the separation and quantitative determination of volatile compounds in *Angelica* roots. The application of solvent-free injection and hydrodistillation coupled to GC-MS for the analysis of the flavour substances in danggui cultivars was reported. Solvent-free solid injector (SFSI) used 1 mg of samples in a glass capillary tube, which was heated about 5 min and then crushed and the volatile analytes were introduced to the GC column. Hydrodistillation (HD) was performed for 4 h at 70°C. VOCs were separated in a capillary column (30 m × 25 mm i.d., film thickness, 0.25 μm). Injector port and interface temperatures were 250 and 300°C, respectively. Helium was used as carrier gas. Starting oven temperature was 50°C (4 min hold), increased to 280°C at 5°C/min (final hold 10 min). Ionising energy was 70 eV, MS detection was in the range of 10–650 *m/z*. Typical chromatograms showing the differences between the aroma profile of danggui samples are depicted in Fig. 2.81. The concentrations of volatile compounds in the danggui extracts are compiled in Table 2.79. The analytes identified were terpenes, aldehydes, alcohols, coumarins, acids, pthalides?? and sterols the amount of decursinol angelate and decursion being the highest. It was further established that the efficacy of SFSI was higher than that of HD (Kim et al., 2006).

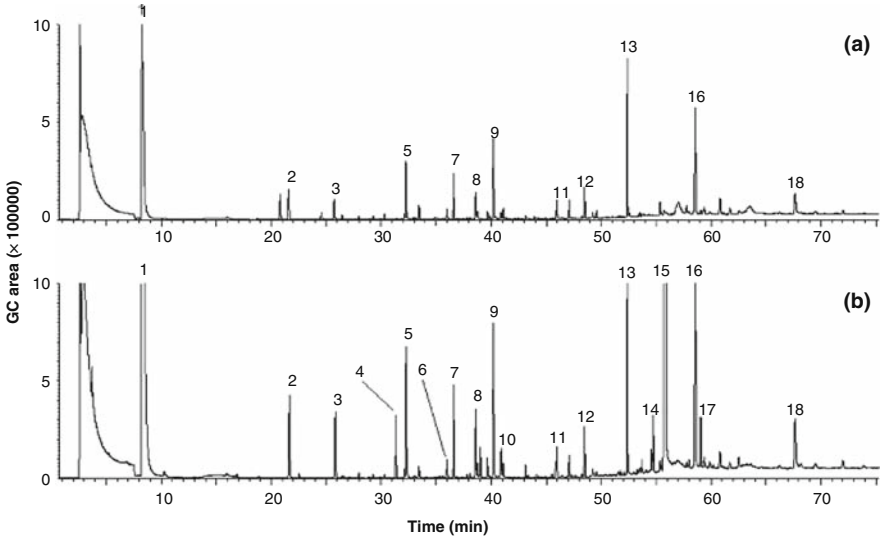

Fig. 2.81 SPME profiles of the coculture of *S. cerevisiae*, *C. milleri*, *L. sanfranciscensis* in the CMs of *L. sanfranciscensis* without (**a**) and with (**b**) starch. (1) ethanol; (2) isoamyl alcohol; (3) acetoin; (4) ethyl octanoate; (5) acetic acid; (6) 1-octanol; (7) isobutyric acid; (8) butyric acid; (9) isovaleric acid; (10) ethyl-9-decenoate; (11) hexanoic acid; (12) phenylethanol; (13) octanoic acid; (14) γ-octalactone; (15) γ-decalactone; (16) decanoic acid; (17) ethyl-9-hexadecenoate; and (18) dodecanoic acid. Reprinted with permission from Vernocci et al. (2008)

Table 2.79 Volatile flavour components (area%) identified in danggui cultivars by GC–MS–SFSI

Components	RT[a]	RI[b]	Area (%)		
			Korean	Chinese	Japan
Furfural	4.5	829	–	16.00	13.67
2-Furanmethanol	5.3	857	3.04	3.18	11.97
Acetol acetate; acetoxyacetone	5.8	876	–	–	3.27
Nonane	6.8	900	3.41	–	–
2-Methyl-2-cyclopentenone	7.1	914	0.25	–	–
Butyrolactone	7.3	917	1.87	2.30	–
Alpha-Pinene	7.9	935	1.52	1.23	–
Campene	8.5	953	0.29	10.66	–
5-Methyl furfural	9.1	969	0.06	–	8.50
4-Octanone	9.6	984	–	–	1.70
2-Furanmethanol, acetate	10.3	998	0.25	–	–
1*H*-pyrrole-2-carboxaldehyde	10.8	1013	–	0.69	–
o-Cymene	11.3	1029	–	–	0.54
2-Cyclopenten-1-one	11.3	1029	–	1.19	2.18
Limonene	11.4	1032	0.62	–	–
Benzeneacetaldehyde	11.9	1051	–	0.69	–
γ-Terpinene	12.4	1063	–	–	1.50
2-Acetylpyrrole	12.5	1065	0.27	1.88	1.36
Guaiacol; 2-methoxyphenol	13.4	1090	2.08	3.80	1.29
Maltol	14.2	1118	–	1.80	7.28
4-Pyridinol	15.3	1154	–	7.17	–
2-Methoxy-4-vinylphenol	20.1	1318	–	2.45	–
1-Phenyl-1-pentanone	21.3	1364	–	2.38	2.99
Methylphthalimide	22.9	1425	–	0.73	–
Isoeugenol	23.7	1457	–	0.58	–
Butylphthalide	28.5	1656	–	5.75	3.67
β-Eudesmol	28.5	1656	0.44	–	–
α-Eudesmol	28.5	1656	0.31	–	–
Butylidene phthalide	28.9	1673	–	14.27	17.82
Butylidene dihydro-phthalide	30.3	1739	–	15.23	5.78
1-Tetraldecene	31.7	1810	–	–	0.95
Angelicin	32.2	1835	0.27	–	–
1-Octadecanol	34.0	1925	0.67	–	2.04
Hexadecanoic acid	34.1	1931	–	0.84	1.63
Methoxsalen	36.2	2034	–	–	0.41
Seselin	37.0	2085	2.73	–	–
1-Heptadecrnol	37.1	2090	0.17	–	–
9,12-Octadecanoic acid	37.3	2101	0.54	1.42	4.76
2-Isopropylpsoralen	37.7	2124	0.17	–	–
2-Isopropenyl-2,3-dihydrofuro [3,2-g]chromen-7-one	38.8	2186	0.37	–	–

Table 2.79 (continued)

Components	RT[a]	RI[b]	Area (%)		
			Korean	Chinese	Japan
Columbianetin	39.5	2227	0.27	–	–
Marmesin	41.1	2322	9.33	–	–
Lomatin	41.8	2365	10.25	–	–
Unknown	46.5	2673	4.52	–	–
Unknown	47.1	2714	7.96	–	–
Decursinol angelate	47.7	2757	16.83	–	–
Decursin	48.2	2792	29.34	–	–
Squalene	48.8	2823	–	–	5.17
Stigmasterol	55.4	>3000	–	1.23	–
γ-Sitosterol	56.7	>3000	0.69	2.22	–
Total			100	100	100

[a] Retention time.
[b] Retention index.
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The heterocyclic aromatic amines such as 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC or 2-amino-α-carboline), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC or 2-amino-3-methyl-α-carboline), 9*H*-pyrido[3,4-*b*]indole (norharman), and 1-methyl-pyrido[3,4-*b*]indole (harma) were determined in the mainstream of reference cigarettes using SPE-GC-MS. The high level of mutagenicity of aromatic amines motivated the investigation. Analytes were separated on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Injector temperature was 310°C. Helium was used as carrier gas. Starting oven temperature was 50°C (2 min hold), ramped to 190°C at 30°C/min (0.5 min hold), to 240°C at 5°C/min, to 335°C at 20°C/min. Ionising energy was 70 eV, MS detection was in the range of 45–430 *m/z*. The average amount of analytes are compiled in Table 2.80. It was assessed that the recovery of the method varied between 79.9% and 102.5%. The method was proposed for the quantitative determination of these analytes in cigarettes (Smith et al., 2004).

Table 2.80 Average levels and standard deviation (S.D.; $n = 5$) of harman, norharman, A∗C and MeA*C in three reference cigarettes

	Harman ng/cigarette		Norharman ng/cigarette		A∗C ng/cigarette		MeA*C ng/cigarette	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
1R5F	254	17.1	676	46.3	29.9	2.3	4.9	0.5
2R4F	668	33.7	1731	78.4	60.4	1.8	9.5	0.3
CM4	1026	38.9	2534	139.5	45.8	3.2	10.3	1.2

Five separate analyses for each cigarette were spread over 2 weeks. The values are given in ng/cigarette.
Reprinted with permission from Smith et al. (2004).

Another study investigated the composition of the components in tobacco flavours using SBSE-GC-MS. The optimum conditions for SBSE were 1,100 r/min for 60 min at room temperature. GC measurements were performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Analytes were injected by a PTV injector. Helium was used as carrier gas. Initial oven temperature was 50°C (1 min hold), raised to 150°C at 10°C/min, then to 250°C at 5°C/min (final hold, 3 min). MS detection was in the range of 35–400 *m/z*. The components of a sample of tobacco flavour are listed in Table 2.81. Because of the low RSD value (less than 10%), the method was proposed for the routine quality control of tobacco flavour (Ying et al., 2006).

Table 2.81 Chemical components in a real sample of tobacco flavour and their relative peak areas

Peak No.	T_R	Compound	Similarity %	Relative peak area[1]
1	6.11	Benzaldehyde		0.096
2	6.27	Pyrazine, trimethyl	80	0.065
3	7.25	Nonanal	87	0.770
4	7.60	Hexanoic acid, 2-methylpropyl ester	83	0.065
5	7.96	Hexanethioic acid, S-heptyl ester	81	0.150
6	8.68	Hexanoic acid, 2-methylbutyl ester	84	20.435
7	8.91	Isophenyl hexanoate	82	60.185
8	9.05	Hexanoic acid, pentyl ester	80	37.656
9	9.32	Methyl salicylate	94	1.279
10	9.5	Nonanoic acid	83	0.260
11	9.61	Pentanoic acid, 4-methyl pentyl ester	82	0.208
12	9.77	Bornyl acetate	85	0.686
13	9.89	Isobornyl acetate	91	0.478
14	13.15	Phenol, 2,4-bis(1,1-dimethylethyl)	90	0.250
15	13.29	Vanillin	97	8.122
16	13.52	2-propenoic acid, 3-phenyl-, ethyl ester	98	2.247
17	13.59	1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl	81	0.356
18	13.96	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	80	1.411
19	14.44	2H-1-benzopyran-2-one	94	43.127
20	14.88	Megastigmatrienone-1[2]	96	4.673
21	15.20	Megastigmatrienone-2	98	14.246
22	15.32	Ethanone, 1-[5-(furanylmethyl)-2-furanyl]	83	5.978
23	15.45	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)	85	0.901

Table 2.81 (continued)

Peak No.	T_R	Compound	Similarity %	Relative peak area[1]
24	15.71	Benzoic acid, 2-hydroxy-4-methoxy-6-methyl, methyl ester	96	1.414
25	15.88	3-Butene-2-one,1-(2,3,6-trimerhyl phenyl)	81	0.718
26	16.01	Megastigmatrienone-3	96	2.463
27	16.22	Megastigmatrienone-4	99	9.498
28	19.67	Benzyl benzoate	97	78.530
29	26.06	Benzyl cinnamate	99	5.230
30	31.66	Cinnamyl cinnamate	91	2.146

[1]ratios of peak areas of compounds to internal standards.
[2]isomers were marked with 1, 2, 3 and 4 based on the retention time.
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Various chromatographic technologies have been also applied for the study of the microcomponents in infant formulas. The use of HS-SPME-GC-MS for the analysis of volatile profile of infant formulas has been previously reported (Romeu-Nadal et al., 2004). The oxidation process of formulas (Fenaille et al., 2003) and the measurement of furfural compounds have also been assessed (Ferrer et al., 2005). The aroma profile of infant formulas was investigated by GC-MS. Thirteen infant formulas were included in the experiments varying in brand, type and physical form. Separation of volatiles was performed in a capillary column (60 m × 0.32 mm i.d., film thickness, 1.0 μm). Helium was used as carrier gas. Starting oven temperature was 40°C (4 min hold), raised to 90°C at 2°C/min, then to 130°C at 4°C/min, to 250°C at 8°C/min. MS detection was in the range of 25–400 *m/z*. The volatile compounds identified in the infant formulas are compiled in Table 2.82. It was established that the brand, type and physical form equally influences the composition of volatile substances (Ruth et al., 2006).

The composition of various mushrooms were investigated by chromatographic methods too. Thus the nutrients of mushrooms (*Lentinus edodes*, *Pleurotus ostratetus* and *Pleroutus sajor-caju*) were determined by GC-MS. The measurements indicated that the mushrooms contain various esters, hydrocarbons and fatty acid derivatives (Caglarirmak, 2007). Another study investigated the optical purity of *R*-(–)-1-octene-3-ol in the aroma of different edible mushroom using chiral GC column. *Agaricus bisporus, Pleurotus ostreatus, Hericium erinaceum, Pholiota nameco, Lentinus edodes, Boletus edulis, Xerocomus badius* and *Macrolepiota procera* were included in the experiments. The optical purity was high in each mushroom, suggesting that the determination of the optical purity of this aroma substance can be used for the authenticity test of mushroom-like aromas (Zawirska-Wojtasiak, 2004). SBSE-GC-MS was employed for the analysis of VOCs in truffle species. The

Table 2.82 Volatile compounds identified in the headspace of infant formulas by gas chromatography–mass spectrometry analysis, their retention indices (RI) and expected parent mass or fragment in proton transfer reaction mass spectrometry analysis

Compound	RI	Expected major mass fragment
Acetic acid	<600	61[a]
Acetone	<600	59[a]
2-Methylpropanal	<600	55[c]
Dimethyl sulphide	<600	63[a]
2-Butenal	600	71[c]
2-Butanone	600	73[b]
Butanal	614	55[b]
2-Methyl-3-buten-2-ol	629	69[a]
3-Methylbutanal	644	69[a]
3-Methyl-3-buten-1-ol	671	69[a]
2-Methylbutanal	682	87[a]
1-Penten-3-ol	689	–[d]
3-Methyl-2-butanone	691	–
Ethyl cyclopentane	696	–
Pentanal	705	69[b]
Methyl propanoate	716	75[b]
3-Methyl-1-butanol	741	43[c]
3,4-Dihydro-2*H*-pyran	741	–
2-Methyl-1-butanol	747	43[c]
trans-2-Pentenal	751	67
Dimethyl disulphide	756	95[a]
1-Octene	767	–
Hexanal	812	83[b]
cis-3-Octen-1-ol	838	69[c]
trans-3-Nonene	850	–
Ethyl benzene	871	–
1-Hexanol	878	43[b]
Heptanal	914	97[b]

[a] Fragmentation patterns.
[b] Fragmentation patterns reported.
[c] Fragmentation expected from patterns of homologous compounds.
[d] Fragmentation pattern unknown.
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results demonstrated the high intra- and interspecific variability of the aroma profiles. The main components were alcoholic and sulphur compounds (*Tuber borchii*), alcohols, aldehydes and aromatic compounds (*T. melanosporum* and *T. indicum*) (Splivallo et al., 2007).

The interaction of aroma substances with other components of foods and food products was extensively investigated. These interactions can modify the sensorial characteristics and acceptance of the products and can influence their self-life. The binding of aroma substances to the pea proteins, legumin and vicillin was studied by using HS-GC measurements, HPLC-MS and gel filtration chromatography

(GFC). It was established that environmental conditions such as pH and heating influence considerably the binding of aroma substances to pea legumin and vicillin. The chemical structure of aroma compounds exerts also a marked influence on the protein–flavour compound interaction (Heng et al., 2004). The binding of aroma substances to active packaging material was investigated by multisensor system or electronic nose and GC-MS. The results indicated that electronic nose measurements can be applied for the monitoring the ageing of certain food products (Strathmann et al., 2005).

The transfer of strawberry flavour substances between a pectin and a dairy gel phase was investigated by HS-SPME-GC-FID method. The behaviour of ethyl acetate, ethyl isobutanoate, ethyl butanoate, ethyl hexanoate and ethyl octanoate was studied under different experimental conditions. Volatiles were extracted on a CAR/PDMS fibre and separated on a capillary column (30 m × 0.32 mm i.d., film thickness, 0.25 μm). Helium was used as carrier gas. Starting oven temperature was 50°C (4 min hold), raised to 180°C at 5°C/min (final hold, 17 min). The results demonstrated that the composition of the accompanying matrices and the storage temperature influence markedly the distribution modifying the sensory characteristics of the samples (Nongonierma et al., 2007).

The diffusion of four aroma substances (ethyl butyrate, 1-hexanol, heptanal and limonene) in latex coatings with different vinyl acid content was investigated by GC-FID using a capillary column (50 m × 32 mm i.d., film thickness, 1.05 μm). Helium was used as carrier gas. Starting oven temperature was 25°C (5 min hold), raised to 100°C at 4°C/min, to 220°C at 50°C/min (final hold, 5 min). It was established that the temperature, the chemical structure of the aroma substances and that of the latex coatings equally influence the mass transport of the aroma compounds (Nestorson et al., 2007).

The retention behaviour of various aroma substances in the presence of different macromolecules was also studied in detail (Guichard, 2002). The effect of polysaccharide solutions (Terta et al., 2006), the influence of lactobacilli–yeasts interactions (Guerzoni et al., 2007) and the formation of starch inclusion complexes (Heinemann et al., 2001; Heinemann et al., 2003) have been recently investigated.

The impact of the addition of starch on the fermentation aroma production by yeasts and lactobacilli in simulated sourdough systems was followed by SPME-GC-MS. Separation of aroma substances was performed in a capillary column (50 m × 0.32 mm i.d). Helium was used as carrier gas. Starting oven temperature was 50°C (2 min hold), raised to 65°C at 1°C/min, to 220°C at 5°C/min (final hold, 22 min).

The data illustrating the effect of starch addition on selected metabolites are compiled in Table 2.83. The results demonstrated that the addition of starch to the liquid fermentation systems increased the production of some metabolites when the fermentation system was inoculated with pure and mixed population of *Saccharomyces cerevisiae, Candida milleri* and *Lactobacillus sanfranciscensis* (Vernocchi et al., 2008).

Another study investigated the effect of fat content in strawberry-flavoured custard cream on the partition of some aroma substances. The measurement was

Table 2.83 Effect of starch addition on selected metabolites released (after 10 h of incubation) by pure cultures of *L. sanfranciscensis* in WFH and by mixed cultures of *S. cerevisiae*, *C. milleri*, and *L. sanfranciscensis* or *S. cerevisiae* and *C. milleri* after 4 h of incubation in the CMs of *L. sanfranciscensis* (data are expressed as mg/l)

	L. sanfranciscensis inoculated in WFH				*L. sanfranciscensis*, *S. cerevisiae*, and *C. milleri* inoculated in the CMs of *L. sanfranciscensis*				*S. cerevisiae* and C. *milleri* inoculated in the CMs of *L. sanfranciscensis*			
	WFH[a]	WFHS[b]	WFHO[c]	WFHOS[d]	CM[e]	CMS[f]	CMO[g]	CMOS[h]	CM	CMS	CMO	CMOS
Ethanol	n.d[i]	n.d	n.d	n.d	3952 ± 345	4680 ± 421	6147 ± 593	6728 ± 622	814 ± 74	5225 ± 503	1947 ± 185	7745 ± 764
Isoamyl alcohol	n.d	n.d	n.d	n.d	453 ± 41	344 ± 31	226 ± 20	310 ± 27	179 ± 18	843 ± 85	364 ± 37	952 ± 96
Acetic acid	89 ± 8	56 ± 4	104 ± 10	65 ± 6	152 ± 13	312 ± 27	414 ± 38	462 ± 40	22 ± 2	149 ± 12	66 ± 5	147 ± 12
Isobutyric acid	40 ± 4	29 ± 3	151 ± 15	147 ± 15	362 ± 35	248 ± 22	271 ± 24	273 ± 24	47 ± 5	111 ± 10	165 ± 16	187 ± 17
Isovaleric acid	46 ± 5	32 ± 3	290 ± 26	300 ± 30	780 ± 72	824 ± 81	539 ± 51	743 ± 72	80 ± 6	200 ± 23	358 ± 36	385 ± 32
Hexanoic acid	n.d	n.d	36 ± 4	35 ± 4	120 ± 11	70 ± 7	61 ± 6	60 ± 6	n.d	27 ± 3	47 ± 5	171 ± 16
Phenylethanol	116 ± 30	88 ± 9	81 ± 7	85 ± 9	n.d	763 ± 75	662 ± 57	715 ± 70	147 ± 15	324 ± 33	129 ± 19	516 ± 50
Octanoic acid	33 ± 3	n.d	141 ± 14	145 ± 14	1024 ± 99	765 ± 75	539 ± 49	663 ± 64	97 ± 10	453 ± 41	453 ± 40	694 ± 64
γ-Octalactone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	19 ± 2	n.d	n.d	n.d	n.d
γ-Decalactone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	14422 ± 1435	n.d	n.d	n.d	n.d
Ethyl-9-hexa-decenoate	n.d	n.d	n.d	n.d	18 ± 2	39 ± 3	27 ± 3	13 ± 1	n.d	152 ± 15	n.d	486 ± 41
Decanoic acid	59 ± 6	62 ± 6	178 ± 18	99 ± 8	540 ± 48	843 ± 82	826 ± 81	785 ± 76	119 ± 12	210 ± 22	258 ± 21	1720 ± 155

[a] WFH: control.
[b] WFHS: control added with starch.
[c] WFHO: WFH added with sucrose 40%.
[d] WFHOS: WFH added with sucrose 40% and starch.
[e] CM: conditioned medium of *L. sanfranciscensis* grown in WFH.
[f] CMS: conditioned medium of *L. sanfranciscensis* grown in WFH added with starch.
[g] CMO: conditioned medium of *L. sanfranciscensis* grown in WFH added with sucrose 40%.
[h] CMOS: conditioned medium of *L. sanfranciscensis* grown in WFH added with sucrose 40% and starch.
[i] Under the detection limit.
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motivated by the fact that the nature of fat influences the release of aroma substances from food emulsions. The aroma substances were separated and quantitatively determined by HSSPME-GC-FID using a capillary column (30 m × 0.32 mm i.d, film thickness, 0.5 μm). Helium was used as carrier gas. Starting oven temperature was 40°C (5 min hold), raised to 240°C at 5°C/min, (final hold, 10 min). The temperatures of injector and detector were 240°C and 250°C, respectively. The strawberry flavour mixture consisted of ethyl hexanoate, ethyl butanoate, benzyl acetate, methyl benzyl acetate (styrallyl acetate), ethyl 3-methyl-butanoate (ethyl isopentanoate), *cis*-3-hexen-1-yl acetate, methyl anthranilate, methyl cinnamate, methyl dihydrojasmonate, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol), γ-decalactone, *cis*-3-hexen-1-ol, 4-hydroxy-3-methoxy-benzaldehyde (vanillin), hexanal and β-ionone. Custard prepared from full fat milk and skimmed milk served as model food products. The results demonstrated that the fat content of the custard influences markedly the release of flavour substances (Martuscelli et al., 2008).

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Chapter 3
Essential Oils

3.1 General Considerations

The separation and quantitation of the odorant molecules in essential oils is of paramount importance for the perfume and flavour industry. As the concentration and odorant capacity of an analyte are generally not correlated, the employment of human assessors is required for the evaluation of the volatile analytes. GC-olfactometric (GC-O) methods represent a valuable tool for this purpose. The principle of the techniques is that a split column divides the separated analytes between the chemical detector and the sniffing port, making possible the simultaneous sensory evaluation and analytical signal of the analyte. One of the most frequently employed GC-O methods uses a dilution series, and the analyte is assessed until no odour is perceived. Other method constructs a chromatogram (aromagram) where the peaks are proportional to the odour concentration of the analyte. The application possibility of various physical and physicochemical extraction and concentration methods in the preparation and analysis of essential oils has been vigorously investigated. The use of SFE for the extraction of essential oils from plant materials has been previously reviewed. It was established that SFE makes possible the on-line coupling with GC, HPLC and SFC. It was further stated that the selectivity, rapidity and cleanliness of the extract are comparable with the results obtained by other traditional extraction methods such as liquid–liquid and liquid–solid extraction (Pourmortazavi and Hajimirsadeghi, 2007). The CO_2 extraction of essential oils of *Elettaria cardamomum* (Marongio et al., 2004), coriander seed (Illés et al., 2000), the pungent component of pepper (Daood et al., 2002), and black pepper (Catchpole et al., 2003) was previously reported. The application of triple-dimensional analysis for the separation of the volatile substances using GC × GC-TOFMS and GC × GC-FID has also been reported. The good separation power of the system is shown in the chromatograms depicted in Fig. 3.1. It was found that the triple-dimensional method (GC × GC-TOFMS) shows a higher separation power and more accurate peak assignment than the traditional single-column systems (Shellie et al., 2001).

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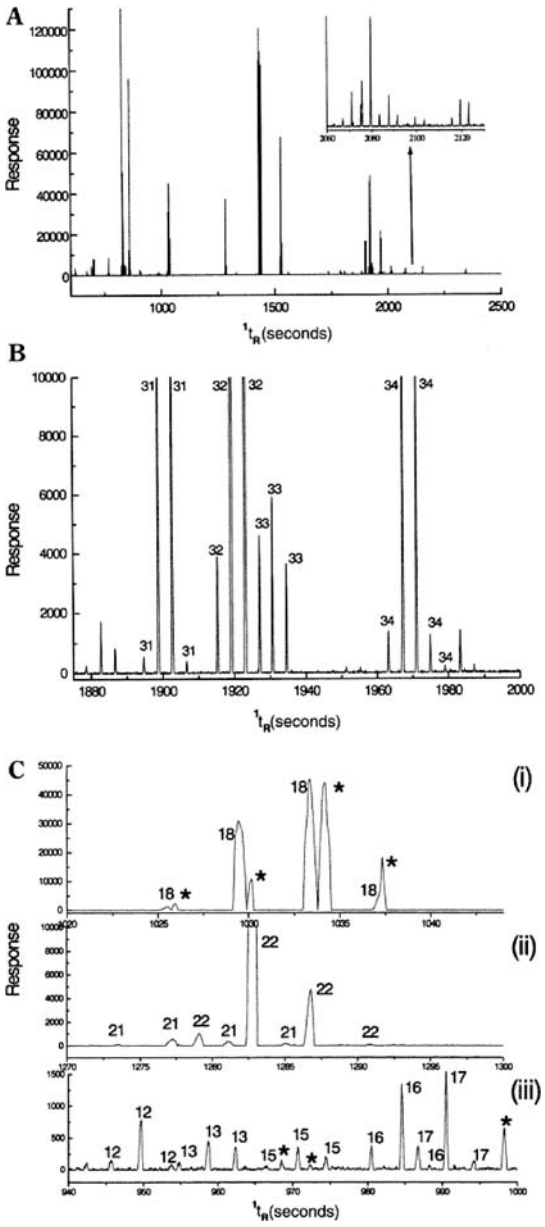

Fig. 3.1 A: GC×GC-TOFMS chromatogram of the extracted ion chromatogram of *m*/*z* 93 ion for French lavender essential oil, with an inset of expanded peaks from $^1t_R = 2060–2130$ s. B: Expanded region of $^1t_R = 1878–2000$ s of the chromatogram shown in part A, with two components, 32 and 33, overlapping on the first column. C: Further expansions of the chromatogram shown in part A. In part I, a selection of related pulses of peaks that are close to detection limit (as defined by low signal-to-noise ratio) is given. Note that peaks 15 and 18 will overlap with unidentified components [labelled with an asterisk (∗)] on the first column because their peak pulses are interleaved. (18: linalool, 21: borneol, 22: terpinen-4-ol, 31: 1,7-dimethyl-7-(4-methyl-3-pentenyl)-tricyclo [2.1.1.0(2,6)]heptane, 32: *cis*-caryophyllene, 33: β-farnesene, 34: α-farnesene. Reprinted with permission from Shellie et al. (2001)

3.2 Essential Oils with Favourable Biological Actions

Traditional medicines including various essential oils are extensively used in primary health care in various countries such as South Africa (van Zyl and Viljoen, 2003). Because of their considerable importance, many chromatographic methods were developed and successfully applied for their separation and identification. Both HPLC and GC-MS were employed for the analysis of the nonvolatile and volatile components of the essential oil of *Salvia stenophylla, Salvia runcinata* and *Salvia repens*. The anti-inflammatory, antimalarial and antimicrobial activities of the essential oils were also determined. HPLC measurements were performed on a C18 column (250 × 2.1 mm i.d.) using photodiode array (PDA) detector and a thermobeam mass selective detector (TMD). TMD operated in electron impact mode (70 eV), the mass range being 50–550 *m/z*. Volatile substances were analysed in a capillary column (60 × 0.25 mm). Initial column temperature was 60°C for 10 min, then ramped to 220°C at 4°C/min. The mass range of detection was 35–425 *m/z*. The compositions of the essential oils extracted from various *Salvia* species are compiled in Table 3.1. The data in Table 3.1 demonstrated that the oils were qualitatively and quantitatively different. HPLC measurements prove that rosmaniric acid is present in each essential oil, while carnosic acid is present only in *S. repens* and *S. stenophylla* as demonstrated in Fig. 3.2 (Kamatou et al., 2005). Similar results were achieved by the investigation of other *Salvia* species such as *Salvia cryptantha* (Montbret et Aucher ex Berth) and *Salvia multicaulis* (Vahl) (Tepe et al., 2004).

Table 3.1 Percentage composition of the essential oil of *Salvia stenophylla*, *Salvia runcinat a* and *Salvia repens* with their relative retention times (RRI)

RRI	Compounds	Salvia stenophylla	Salvia runcinata	Salvia repens
1000	Decane	–	0.1	–
1014	Tricyclene	0.1	tr	0.2
1032	α-Pinene	2.7	1.8	6.6
1035	α-Thujene	tr	–	–
1072	α-Fenchene	tr	–	–
1076	Camphene	3.3	0.6	4.0
1100	Undecane	–	tr	–
1118	β-Pinene	0.7	0.8	3.0
1132	Sabinene	0.1	tr	0.2
1145	Ethylbenzene	–	tr	–
1146	δ-2-Carene	tr	–	–
1159	δ-3-Carene	18.4	0.3	0.1
1174	Myrcene	1.7	0.2	2.3
1176	α-Phellandrene	tr	–	–
1187	*o*-Cymene	0.1	–	–
1188	α-Terpinene	0.3	tr	0.2
1203	Limonene	5.3	0.6	9.8

Table 3.1 (continued)

RRI	Compounds	Salvia stenophylla	Salvia runcinata	Salvia repens
1205	Sylvestrene	1.3	–	–
1213	1,8-Cineole	–	2.0	–
1218	β-Phellandrene	2.9	tr	22.2
1244	Amylfuran	–	tr	–
1246	(*Z*)-β-Ocimene	tr	0.4	2.7
1255	γ-Terpinene	0.5	0.1	0.5
1265	5-Methyl-1,3-heptanone	–	tr	–
1266	*E*-β-Ocimene	–	0.8	1.5
1278	*m*-Cymene	0.2	–	–
1280	*p*-Cymene	tr	0.2	0.7
1282	*cis*-Allo ocimene	–	–	0.1
1286	Isoterpinolene	0.8	–	–
1290	Terpinolene	0.5	0.1	0.3
1327	(*Z*)-3-hexenylacetate	–	–	0.1
1348	6-Methyl-5-hepten-2-one	–	tr	–
1360	Hexanol	–	tr	–
1382	*cis*-Allo ocimene	–	tr	–
1393	3-Octanol	–	0.1	0.1
1400	Nonanal	tr	tr	–
1443	2,5-Dimethylstyrene	0.1	–	–
1450	*trans*-Linalool-oxide	–	tr	–
1452	1-Octen-3-ol	0.2	0.4	0.5
1467	6-Methyl-1,5-hepten-2-ol	–	–	0.1
1474	*trans*-Sabinene hydrate	0.1	0.1	0.3
1478	*cis*-Linalool oxide	–	0.1	–
1497	α-Copaene	–	0.1	–
1532	Camphor	6.0	2.0	6.9
1544	α-Gurjunene	–	–	0.2
1553	Linalool	0.1	0.3	0.2
1556	*cis*-Sabinene hydrate	–	–	0.2
1562	Octanol	–	tr	–
1568	1-Methyl-1,4-acetyl-cyclo-hex-1-ene	–	0.2	–
1571	*trans-p*-menth-2-en-1-ol	0.2	–	0.2
1586	Pinocarvone	–	–	0.1
1589	*iso*-Caryophyllene	–	0.6	–
1594	*trans*-β-Bergamotene	–	0.6	–
1597	Bornyl acetate	–	–	0.5
1612	β-Caryophyllene	7.3	11.4	12.4
1628	Aromadendrene	1.2	–	0.9
1638	*cis-p*-menth-2-en-1-ol	–	–	0.2
1638	β-Cyclocitral	–	0.1	–
1650	γ-Elemene	–	–	0.5
1661	Alloaromadendrene	0.1		0.5
1668	(*Z*)-β-Farnesene	tr	tr	–
1687	α-Humulene	1.7	2.7	3.2

Table 3.1 (continued)

RRI	Compounds	Salvia stenophylla	Salvia runcinata	Salvia repens
1695	E-β-Farnesene	–	0.2	–
1706	α-Terpineol	1.0	0.2	–
1719	Borneol	4.8	0.3	1.5
1741	β-Bisabolene	0.2	0.9	–
1755	Bicyclogermacrene	–	–	0.8
1755	Sesquicineole	–	0.5	–
1766	1-Decanol	–	tr	–
1773	δ-Cadinene	0.5	tr	0.3
1776	γ-Cadinene	–	tr	–
1783	β-Sesquiphellandrene	–	0.3	–
1804	Myrtenol	1.0	–	–
1805	α-Campholene-alcohol	–	–	2.3
1838	*E*-β-Damascenone	–	tr	–
1845	*trans*-Carveol	tr	tr	0.2
1853	*cis*-Calamenene	–	tr	–
1854	Germacrene B	–	–	0.6
1864	*p*-Cymen-8-ol	0.2	tr	–
1868	*E*-Geranylacetone	tr	tr	–
1900	*epi*-Cubelol	–	tr	–
1941	α-Calacorene-I	tr	tr	–
1949	*trans*-Jasmone	–	0.1	–
1969	*cis*-Jasmone	–	0.2	–
1984	α-Calacorene-II	–	tr	–
2008	Caryophyllene-oxide	1.2	6.7	2.0
2045	Humulene-epoxide I	tr	–	–
2050	*E*-Nerolidol	0.3	6.8	–
2057	Ledol	–	–	0.6
2071	Humulene-epoxide-II	0.4	1.4	0.5
2098	Globulol	–	–	0.2
2103	Guaiol	3.3	–	–
2104	Viridiflorol	0.3	–	3.5
2131	Hexahydrofarnesyl acetone	–	0.1	–
2144	Spathulenol	0.3	–	1.0
2156	α-Bisabolol oxide B	–	1.7	–
2162	Bisabolol oxide	0.1	1.8	–
2185	γ-Eudesmol	–	0.1	–
2187	τ-Cadinol	0.3	–	0.2
2209	τ-Muurolol	–	–	0.1
2232	α-Bisabolol	8.2	41.1	0.7
2250	α-Eudesmol	0.8	–	0.1
2255	α-Cadinol	–	–	0.3
2256	*epi*–Bisabolol	–	2.1	–
2257	β-Eudesmol	0.9	–	–
2324	Caryophylladienol-	0.7	1.4	0.3
2389	Caryophyllenol-I	–	0.8	0.9

Table 3.1 (continued)

RRI	Compounds	Salvia stenophylla	Salvia runcinata	Salvia repens
2392	Caryophyllenol-II	0.7	1.1	0.1
2518	*cis*-Lanceol	3.6	1.5	0.3
2676	Manool	10.1	–	–
Total		94.8	96.0	98.0

tr: Traces: <0.1%.
Reprinted with permission from Kamatou et al. (2005).

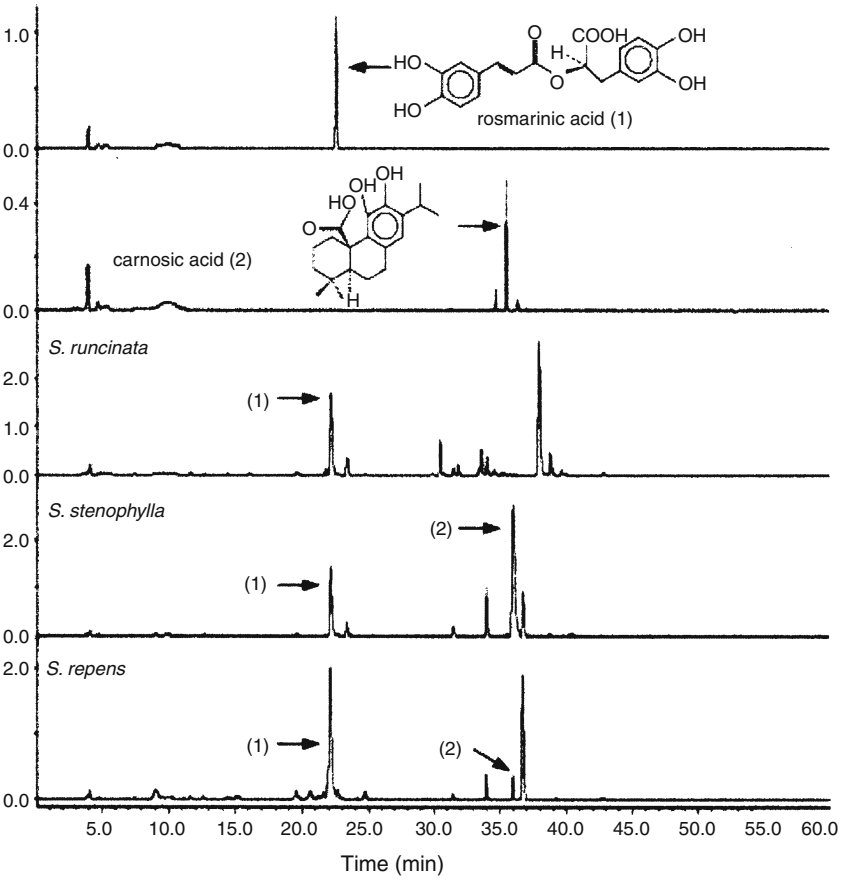

Fig. 3.2 HPLC, chromatograms of *Salvia runcinata, Salvia stenophylla* and *Salvia repens* and standards of rosmarinic and carnosic acids. Reprinted with permission from Kamatou et al. (2005)

The composition and antiplasmodial activities of essential oils extracted from some Cameroonian medicinal plants were investigated by traditional microbiological methods and GC-MS. The species included in the experiments were *Xylopia phloiodora* (stem bark), *Pachypodhantium confine* (stem bark), *Antidesma laciniatum* (leaves), *Xylopia aethiopica* (stem bark) and *Hexalobus crispiflorus* (stem bark). GC separations were performed on a capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Oven temperature was raised from 70°C to 200°C at 10°C/min. The volatile compositions of the essential oils are compiled in Table 3.2. It was found that the main components of the aroma substances are terpenoids (α-copaene, γ-cadinene, δ-cadinene, α-cadinol, spathulenol and caryophyllene oxide). It was further demonstrated that each oil showed marked activity against *Plasmodium falciparum* (Boyom et al., 2003).

Table 3.2 Chemical composition of essential oils of five Cameroonian plants

		%			
RI[a]	Compounds	Xylopia phloiodora	Pachypodhantium confine	Anitdesma laciniatum	Xylopia acthiopica	Hexalobus crispiflorus
	Monoterpene hydrocarbons	2.99	5.41		24.29	
913	α-Thujene		0.59		0.61	
920	α-Pinene	0.58			4.05	
933	Camphene	1.38			4.87	0.13
955	Sabinene		0.59		0.46	
959	-Pinene	0.68			10.07	0.12
999	δ-3-carene		1.1			
1006	α-terpinene		0.48		0.43	
1011	p-cymene	0.35	0.32		1.72	
1038	*E*-β-ocimene		1.93		1.13	
1058	γ-terpinene				0.58	
1068	Terpinolene		0.4		0.37	
	Oxygenated monoterpenes	6.28	4.03	24.8	30.85	0.13
1072	Linalool	0.31	0.22	9.4	1.58	
1095	Nopinone	0.65			2.53	
1096	Fenchol		0.18			
1112	*E*-pinocarveol	1.23	0.31		5.42	
1117	Camphor				1.4	
1147	*p*-cymen-8-ol	1.58				
1151	Pinocarvone		3.16		1.84	
1161	Terpinen-4-ol		0.16		0.49	0.13
1166	Myrtenal	0.28			2.85	
1172	α-terpineol				4.99	
1181	Myrtenol				6.4	
1192	Verbenone	2.23			2.68	
1220	Geraniol			0.5		
1256	Thymol	0.28				
1263	Bornyl acetate				0.67	
1345	Geranyl acetate			14.9		

Table 3.2 (continued)

RI[a]	Compounds	Xylopia phloiodora	Pachypodhantium confine	% Anitdesma laciniatum	Xylopia acthiopica	Hexalobus crispiflorus
	Sesquiterpene hydrocarbons	69.56	60.61	23.4	33.1	75.54
1334	δ-elemene	3.29	0.53		0.44	0.21
1347	α-cubebene		0.52		1.04	0.36
1356	α-ylangene				5.32	0.33
1361	α-copaene	0.53	7.06	2.2	4.07	13.27
1372	β-bourbonene			0.5		
1373	Cyclosativene	2.07				
1374	β-elemene	0.58	0.73		1.34	1.92
1387	cyperene	0.34	15.54		3.95	110.53
1397	α-cedrene		6.02			
1401	Isocaryophillene					0.75
1404	α-gurjunene		1.86		0.64	
1413	β-caryophillene		0.28	5.2	1.67	1.33
1414	β-copaene			0.3		
1423	*E*-α-bergamotene	0.46	0.18			
1447	Aromadendrene					1.08
1453	α-humulene		5.04	2.1	1.09	1.76
1455	Alloaromadendrene					8.52
1455	γ-Muurolene		0.35	0.7	2.64	1.93
1458	Epi-bicyclo-sesquiphellandrene	3				
1461	Germacrene-D	1.02	0.84	8.5	0.94	2.62
1472	α-Muurolene			1.5	1.84	1.29
1492	γ-Cadinene	11.27	3.51	0.3		2.53
1494	Bicyclogermacrene	1.43	2.41			
1497	α-Selinene		0.28			2.2
1499	(*E*,*E*)-α-Farnesene		2.66			
1501	α-Selinene	21.92	1.41	0.5	0.56	
1505	δ-Cadinene	15.11	8.06		0.82	3.37
1506	Cadina-1,4-diene			1.3	4.3	10.07
1507	Calacorene	0.89			0.67	
1513	(*Z*)-Calamenene		2.32		0.84	7.82
1519	α-Cadinene		1.01	0.3	0.93	1.09
1636	Cadalene	7.65				
	Oxygenated sesquiterpenes	18.24	27.34	23.8	11.56	23.91
1518	Elemol	2.04	1.24		1.09	
1537	€-nerolidol	0.64	2.42			2.82
1543	Germacrene-D-4-ol			0.4		
1551	Spathulenol	1.02	2.16	1.4	6.33	1.97
1555	Caryophyllene oxide	5.07	7.24	8.5	1.99	2.54
1567	Fonenol	0.76				
1568	γ-Eudesmol					0.99
1574	Globulol	1.93	2.38			
1578	Humulene oxide	0.68	3.17	3.5		1.38
1595	Cubenol		3.23			

Table 3.2 (continued)

RI[a]	Compounds	Xylopia phloiodora	Pachypodhantium confine	% Anitdesma laciniatum	Xylopia acthiopica	Hexalobus crispiflorus
1597	T-Muurolol+torreyol	3.7	2.1			
1602	Epi-α-cadinol	0.95	1.25	0.2	1	7.34
1607	Epi-α-muurolol			2.5		
1611	1,10-Di-epi-cubenol					1.3
1619	β-Eudesmol				1.15	1.08
1635	α-Muurolol	0.58	0.64	1		
1638	α-Cadinol	0.5	1.16	3		1.41
1643	α-Cadinol					1.61
1680	(*E*,*E*)-α-Farnesol			2		
1682	Farnesol	0.37	0.35			
1687	Epi-α-bisabolol					1.47
1789	(*E*,*E*)-α-Farnesil acetate			1.3		
	Aromatic compounds	2.55	2.42	27.3		
989	*p*-Methyl anisole			2.1		
1062	Methyl benzoate			0.5		
1127	Benzyl acetate			1.5		
1138	Ethyl benzoate	0.25				
1253	(*E*)-Anethole			0.5		
1400	(*E*)-Cynnamyl acetate			0.6		
1474	Eugenyl acetate		1.78			
1512	Methoxy cinnamaldehyde	1.47				
1621	2,4,5-Trimethoxy-styrene		0.43			
1719	Benzyl benzoate	0.83	0.21	19.1		
1826	Benzylsilicylate			3		

Reprinted with permission from Boyom et al. (2003).

The composition of the essential oil and the antimicrobial activity of *Osmitopsis asteriscoides* (Ateraceae) were determined by using GC-MS, GC-FID, disc diffusion assay, microplate bioassay and time-kill measurement. GC-MS separations were carried out on a capillary column (60 m × 0.25 mm, film thickness, 0.25 μm). Temperature program initiated at 60°C (10 min hold), then raised to 220°C at 4°C/min (10 min hold), to 240°C at 1°C/min. Detector temperature was 250°C. The mass range of the MS detection was 35–425 *m/z*. The volatile compounds found in the essential oil of *Osmitopsis asteriscoides* are compiled in Table 3.3. It was established that (–)-camphor and 1,8-cineole were the main constituents of the essential oil and they show synergetic antimicrobial effect (Viljoen et al., 2003).

The activation of prohaptenes to sensitisers has been studied in detail. The chemical structures of the compounds investigated are listed in Fig. 3.3. The purity of the compounds was tested by TLC, HPLC-MS and GC-MS. It was assessed that naturally occurring monoterpenes (α-phellandrene, β-phellandrene and α-terpinene)

Table 3.3 Essential oil composition of *Osmitopsis asteriscoides*

RRI	Compound name	Column A (%)	Column B (%)
1014	Tricyclene	tr	tr
1032	α-Pinene	0.8	3.0
1035	α-Thujene	tr	0.1
1048	2-Metyl-3-buten-2-o1	tr	–
1076	Camphene	1.4	1.8
1118	β-Pinene	0.2	0.6
1132	Sabinene	1.1	1.4
1174	Myrcene	tr	tr
1188	α-Terpinene	tr	tr
1195	Dehydro-l,8-cineole	0.2	0.3
1203	Limonene	0.1	tr
1213	1,8-Cineole	56.0	59.9
1224	o-Mentha-l(7),5,8 -triene	tr	
1255	γ-Terpinene	tr	0.3
1280	*p*-Cymene	0.9	0.9
1290	Terpinolene	tr	tr
1348	6-Methyl-5- hepten-2-one	tr	–
1360	Hexanol	tr	–
1384	α-Pinene oxide	0.2	–
1391	(*Z*)-3-Hexen-l-o1	0.2	tr
1450	*trans*-linalool oxide (furanoid)	0.1	tr
1451	β-Thujone	tr	–
1458	*cis*-l,2-limonene epoxide	tr	–
1474	*trans*-sabinene hydrate	1.3	0.1
1482	Longipinene	2.4	2.9
1493	α-Ylangene	0.1	tr
1499	α-Campholenal	0.1	tr
1522	Chrisanthenone	0.2	tr
1532	(–)-Camphor	14.8	12.4
1553	Linalool	tr	0.3
1556	*cis*-sabinene hydrate	1.1	0.1
1571	*trans-p*-menth-2-en-l-ol	0.1	0.1
1586	Pinocarvone	0.1	0.1
1611	Terpinen-4-ol	0.4	2.3
1638	*cis-p*-menth-2-en-l-ol	tr	tr
1642	Thuj-3-en-l0-al	tr	–
1648	Myrtenal	tr	tr
1651	Sabinaketone	0,1	tr
1657	Umbellulone	tr	–
1664	*trans*-pinocarvenol	0.1	0.1
1682	*o*-Terpineol	0.5	0.4
1683	*trans*-verbenol	0.2	–
1706	α-Terpineol	3.9	7.8
1719	Borneol	06	4.8
1725	Verbenone	0.2	–
1729	*cis*-l,2-epoxy-terpin-4-ol	0.3	–

Table 3.3 (continued)

RRI	Compound name	Column A (%)	Column B (%)
1748	Piperitone	tr	–
1751	Carvone	tr	–
1758	*cis*-pperitol	tr	tr
1786	Aar-curcumene	0.1	–
1798	Methyl salycilate	0.2	0.1
1802	Cumin aldehyde	tr	–
1804	Myrtenol	0.1	tr
1831	2-Hydroxypiperitone	0.3	–
1845	*trans*-carveol	0.1	–
1864	*p*-Cymen-8-ol	0.3	tr
1875	*trans*-2-hydroxy-l,8-Cineole	0.7	–
1889	Ascaridole	tr	–
1946	4-Hydroxypiperitone	tr	–
2008	Caryophyllene oxide	1.1	0.4
2008	*p*-Menta-l,8-dien-l0-ol	–	0.1
2074	Caryophylla-2(12),6(13)-diene-5-one	0.3	–
1957	Cubenol	0.2	–
2113	Cumin alcohol	0.2	tr
2144	Spathulenol	0.3	–
2256	Longiverbenone	0.5	0.2
Total		92.1	96.1

RRI: relative retention indices calculated against *n*-alkanes. Percentage calculated from TIC data; tr: trace «0.1%).
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present in the essential oil of tea tree are prohaptenes and they are able to induce contact allergy (Bergström et al., 2006).

Clausena lansium Skeels (wampee) has found manyfold application. The fruit is eaten, the juice is fermented to produce a carbonated beverage, and the essential oil shows marked antifungal (Ng et al., 2003) and skin tumour inhibitory effect (Johnson et al., 2001; Natarajan et al., 2003; Dwivedi et al., 2003). The various samples (leaf, flower and sarcocarp together with seeds) were steam-distilled for 3 h. GC measurements were carried out in a capillary column (30 mm × 0.25 mm, film thickness 0.25 μm), helium being the carrier gas. Initial oven temperature was 60°C for 2 min, raised to 250°C at 10°C/min, final hold 10 min. MS was operated in EI mode (70 eV), the detection range was set to 41–450 *m/z*. The volatiles separated and identified by GC-MS are compiled in Table 3.4. The results demonstrated that the composition of essential oils extracted from different parts of *Clausena lansium* shows considerable differences; therefore, their potential medicinal applications can also be different (Zhao et al., 2004).

GC-FID, GC-MS and in vitro antidermatophytic assay were employed for the investigation of the volatile fractions of hexane extract from leaves of *Cupressus lusitanica* Mill. The measurements were motivated by the anticancer activity of the leaf extract (Lopez et al., 2002). The leaves of *Cupressus lusitanica* were extracted

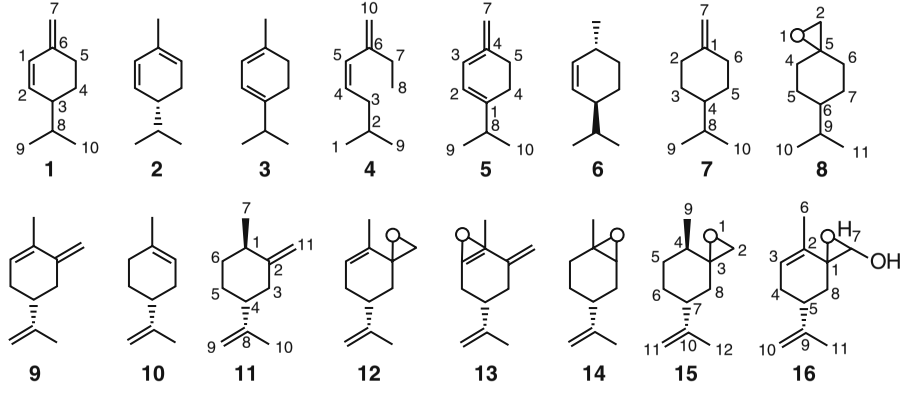

Fig. 3.3 Structures of compounds studied. Reprinted with permission from Bergström et al. (2006)

Table 3.4 Constituents of the essential oils of different parts of *Clausena lansium*

Compound	Leaf %	Flower (%)	Sarcocarp (%)	Seed (%)
n-Caproaldehyde	0.1			
2-Hexenal	0.7			
3-Hexenal	0.4			
Thujene				0.3
α-Pinene			0.1	1.3
Phellandrene			0.1	54.8
β-Pinene				0.8
Myrcene			0.2	Tr
Carene				0.4
Cymene				0.5
Limonene	Tr		0.2	23.6
Ocimene	0.1			
Dodecane	Tr			
Terpinen				1.2
Acetophenone		0.1		
Terpineol				2.9
Unidentified		0.1		
n-Dodecane		0.1		
Methyl isopropenyl-cyclohexen-1-ol				0.8
Borneol			0.1	
p-Menth-1-en-4-ol		0.1	0.3	7.5
1-(3-methylphenyl)-ethanone		tr		
p-Menth-1-en-8-ol		tr	0.1	0.5
Unidentified		0.1		
Linalool	0.1	0.1		
n-Pentadecane		0.1		
Butyl octanol		tr		
Nerol acetate				0.1
Zingiberene	0.1			
Geraniol acetate				0.1
Unidentified		0.3		0.2
Santalene	0.1			0.1
Sesquiphellandrene	0.1			
Caryophyllene	0.6	0.2		0.7
β-caryophyllene	0.2	tr		0.1
Farnesene	1.1	0.3		
Liongipinene	0.8			
Cadinene				0.1
Germacrene-D				0.4
Unidentified	0.4	1.2		0.2
Cadina-1(10),4-diene				0.1
Epiglobulol	2.0			
Unidentified		0.5		0.6
Nerolidol	5.0	0.5	0.9	
Denderalasin	0.8	0.2		
Ledol	6.5	0.5		
Spathulenol	1.3	0.1	0.3	

Table 3.4 (continued)

Compound	Leaf %	Flower (%)	Sarcocarp (%)	Seed (%)
Caryophyllene oxide	1.1	0.1	2.2	
Unidentified	0.6		8.3	
Cadinol		0.2		
Bisabolol	13.7			
Farnesal	0.2			
α-Santalol		1.6	15.5	1.8
Bergamotol	4.4	3.2		0.1
Sinensal	5.6	4.1	4.0	
β-Santalol	35.2	50.6	52.0	0.2
Farnesol	2.7		5.2	
Unidentified		6.7	3.5	
Methyl lanceol	0.6			
Lanceol		0.7		
Methyl santalol	6.9			
Unidentified		1.3		
Palmitic acid	0.4			
Hexadecanoic acid		1.2	3.9	
Phytol	0.3		0.6	
Linoenic acid methyl ester	0.2			
Octadecadienoic acid		0.2		
9-Octadecenamide	3.8	17.2		
Stearic acid			0.7	
Unidentified	0.3		2.1	
Palmitamide		1.9		
Stearamine		1.0		

% percentage of the content of each constituent in total essential oil.
tr: Trace quantities (<0.1%).
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by hexane and the extract was fractionated on a silica column using hexane, ethyl acetate and acetonitrile as mobile phases. GC-FID measurements were carried out on a capillary column (30 m × 0.25 mm i.d). Oven temperature was ramped from 50°C to 280°C at 4°C/min. Injector and detector temperatures were 220°C and 250°C, respectively. Nitrogen was employed as carrier gas. GC-MS separations were performed on another capillary column (30 m × 0.20 mm i.d.). Helium was used as carrier gas. Oven temperature was raised from 50°C to 220°C at 4°C/min. For MS 70 eV was employed. GC methods separated 104 identified and 5 non-identified volatile compounds in the methanolic extract as demonstrated in Table 3.5. It was established that the fractions showed antidermatophytic activities against *Microsporum audouinii, M. Langeroni, M. canis, Trichophyton rubrum* and *T. tonsurans* (Kuiate et al., 2006).

The composition and biological activities of *Chaerophyllum* species have been frequently investigated. The interest in these species is motivated by the fact that they are consumed as food and they are used for flavouring (Coruh et al., 2007). The composition of the essential oils of various species such as *C. prescotti* (Letchamo

Table 3.5 Chemical composition of the volatile column fractions of hexanic extract of *Cupressus lusitanica* leaves

No.	constituents	IR	F_1	F_2	F_3	F_4	F_5
	Monoterpene hydrocarbone		99.0	23	1.1		
1	α-Pinène	939	80.0				
2	β-Pinène	980	2.0				
3	Sabinene	976	8.0		0.1		
4	Myrcene	991	2.0	0.2	0.2		
5	δ^3-Carene	1011		0.1	0.1		
6	*p*-Cymene	1026		0.4	0.3		
7	Limonene	1031	2.0	0.7	0.1		
8	γ-Terpinene	1062	3.0	0.6	0.2		
9	Terpinolene	1088	2.0	0.3	0.1		
	Oxygen containing monoterpenes				3.5		
10	1,8-Cineole	1033			0.2		
11	*cis*-*p*-Menth-2-en-l-ol	1121			0.2		
12	*cis*-Pinene hydrate	1121			0.1		
13	α-Campholenal	1125			0.8		
14	Camphene hydrate	1148			0.2		
15	Thymol meihyl ester	1238			0.1		
16	Linalyl acetate	1249			0.6		
17	Bornyl acetate	1285			0.1		
18	α-Terpinyl acetate	1350			1.2		
	Sesquiterpenes hydrocarbone			87.7	42.4		
19	α-Copaene	1377		0.8	0.3		
20	Farnesene[b]	1389		0.4			
21	α-Cubebene	1395		0.2	0.1		
22	α-Bergamotene	1397		0.5			
23	α-Longipiene	1407			0.1		
24	α-Cedrene				0.4		
25	β-Caryophyllene	1418		4.7	0.9	0.1	
26	γ-Muurolene	1427			0.3		
27	*cis*-Muurola-3,5-diene	1446		4.5			
28	α-Humulene	1454			1.8		
29	*epi*-Bicyclosesquiphellandrene	1476		35.3	14.3	0.8	
30	δ-Cadinene				0.6		
31	α-Amorphene	1477			0.3	3.7	
32	ar-Curcumene	1487		5.2	4.4	1.2	
33	Aromadendrene	1492			0.2		
34	Cadina-1,4-diene	1495			0.2		
35	*epi*-zonarene	1497		10.3	3.3	0.1	
36	Alloaromadendrene	1505			0.3		
37	γ-Curcumene	1515			2.4		
38	β-Himachalene	1517		10.4	1.0		
39	α-Amorphene	1518			1.7		
40	*cis*-Calamenene	1521		13.1	3.7		
41	Cadina-1,5,3-triene	1527			3.9		
42	α-Cadienene	1540		2.1	0.9		
43	α-Colacorene	1544			1.3		
44	Valencene	1559		0.2			

Table 3.5 (continued)

No.	constituents	IR	F_1	F_2	F_3	F_4	F_5
	Oxygen containing sesquiterpenes			2.4	6.3		
45	Caryophyllene oxide	1581			0.5		
46	Cedrol	1596		0.2			
47	β-Oplopenone	1606			0.2		
48	1,10-di-*epi*-Cubenol	1614			0.5		
49	β-Ionone	1625			0.1		
50	1-*epi*-Cubenol	1627		0.2			
51	di-*epi*-α-Cedrene	1634			1.5		
52	*epi*-α-Cadinol	1640		0.5	0.1		
53	*epi*-α-Muurolol	1642			0.8		
54	α-Muurolol	1645			0.5		
55	α-Cadinol	1653		0.3			
56	Cadalin	1674		0.4	0.5		
57	*cis*-14-Normuurol-5-en-4-one	1682			0.4		
58	14-Norcadin-5-en-4-one	1697		0.8	0.2		
59	Oplopanonyl acetate	1681			0.3		
60	Cinnamyl cinnamate	2341			0.7		
	Lipidic derivatives				6.7		
61	Butanoic acid, 1-methylhexyl ester	1210			0.3		
62	Methyl hexadecanoate	1922			1.0		
63	Hexadecanoic acid	1957			0.2		
64	9,12-Octadecadienoic acid (*Z,Z*), methyl ester	2071			1.8		
65	9,12,15-Octadecatrienoic acid (*Z,Z,Z*), methyl ester	2077			1.5		
66	Octadecanoic acid, methyl ester	2100			0.1		
67	Ethyl linoleate	2133			0.2		
68	Ethyl linoleolate	2134			0.5		
69	Octadecanoic acid, ethyl ester	2161			1.1		
	Diterpenes			0.2	32.1		
70	*ent*-Pimara-8(14),15-diene	1963		0.1			
71	Kaur-15-ene	1975		0.1	0.5		
73	1,3-Epimanoyl oxide	1986			1.8		
74	Manoyl oxide	1990			0.1		
75	*epi*-13-Manoyl oxide	2010			0.1		
76	Phyllocladene	2011			0.1		
77	Dehydroab ietadiene	2037			0.2		
78	Abietatriene	2054			0.7		
79	Isopimaradien-3-one[a]						
80	8.β-Hydroxysandaracopimarane	2111			3.5		
81	Nezukol	1126			0.4		
82	4,4-Dimethyl-13α-androst-5-ene	2156			1.0		
83	Neophytadiene	2180			1.6		
84	*cis*-Totarol	2278			2.0		
85	*trans*-Totarol	2303			3.2		

Table 3.5 (continued)

No.	constituents	IR	F_1	F_2	F_3	F_4	F_5
86	Ferruginol	2325			1.6		
87	Pimaric acid[a]				7.5		
88	Kaurenoic acid[a]				6.9		
89	5,8-Epoxy-5,8-dihydroetinoic acid[a]				0.9		
	Aliphatic hydrocarbons				0.1	90.8	99.9
90	Decane	1000			0.1		
91	Hexadecane[a]					1.6	
92	Hexatricontane[a]					2.9	
93	Octacosane[a]					3.5	2.1
94	Nonacosane[a]					5.6	4.8
95	Triacontane[a]					4.14	4.3
96	Henlriacontane[a]					6.8	7.7
97	Dotriacontane[a]					3.3	3.9
98	Eicosane[a]					41.1	
99	Tricosane[a]						37.3
100	Hexadecane[a]						6.3
101	Pentacosane[a]						6.6
102	Tétratricontane[a]					3.75	4.8
103	Dotricontane[a]					18.2	
104	Heptacosane[a]						22.1
	Unidentified compounds			3.9	1.9		
105	M+221(100), 105(21), 121(21), 91(16), 205(11)	1558		1.7	1.2		
106	M+236(18), 235(100), 205(15), 89(17), 174(21), 159(27), 119(49), 105(24), 91(21)	1578		1.9	0.1		
107	M+204(16)185(100), 200(70), 177(28), 143(29), 105(21), 91(20), 77(18)	1592			0.2		
108	M+218(18), 161(100), 131(97), 147(55), 130(44), 240(43), 91(27), 77(41)	1747			0.3		
109	M+316(100), 301(35), 259(16), 33(48), 219(16), 189(13), 121(12), 69(29)			0.3	0.1		

[a] Identified using only their mass spectra.
[b] Isomer not identified.
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et al., 2005), *C. byzantinum* (Kürkcüoglu et al., 2006) and *C. macrospermum* (Sefidkon and Abdoli, 2005) has been previously determined. The presence of lignans, phenylpropionoids and polyacetylenes (Rollinger et al., 2003), phenolic acids (Dall'Acqua et al., 2004) in the essential oils was demonstrated. The antimicrobial (Durmaz et al., 2006) and antioxidant activities of the essential oils have also been reported (Dall'Acqua and Innocenti, 2004). The composition, antimicrobial and antioxidant activities of the essential oil of *Chaerophyllum libanoticum* Boiss. *et* Kotschy were assessed by GC-FID, GC-MS, microdilution assay and visible spectrometry. Dried fruits were crushed and hydrodistillated for 3 h. GC-MS and

GC-FID measurements were performed on the same capillary column (60 m × 0.25 mm, film thickness 0.25 μm). Oven temperature started at 60°C (10 min hold), increased to 220°C at 4°C/min (10 min hold) to 240°C at 1°C/min. Injector and FID temperatures were 250°C and 300°C, respectively. Helium was the carrier gas. MS were recorded at 70 eV, mass range being 35–450 *m/z*. The identified components of the essential oil are compiled in Table 3.6 (Demirci et al., 2007).

Table 3.6 Essential oil composition of *Chaerophyllum libanoticum*

RRI	Compound	Percent
1032	α-Pinene	2.9
1035	α-Thujene	0.2
1076	Camphene	0.1
1118	β-Pinene	8.8
1132	Sabinene	8.5
1159	δ-3-Carene	0.2
1174	Myrcene	1.5
1176	α-Phellandrene	0.2
1183	*p*-Mentha-1,7(8)-diene (=*Pseudolimonene*)	0.1
1188	α-Terpinene	0.2
1203	Limonene	15.9
1218	β-Phellandrene	17.6
1246	(*Z*)-β-Ocimene	1.1
1255	γ-Terpinene	9.9
1266	(*E*)-β-Ocimene	0.3
1280	*p*-Cymene	1.9
1290	Terpinolene	2.4
1296	Octanal	0.2
1468	*trans*-1,2-Limonene epoxide	0.1
1474	*trans*-Sabinene hydrate	0.1
1497	α-Copaene	0.1
1553	Linalool	0.1
1556	*cis*-Sabinene hydrate	0.1
1594	*trans*-β-Bergamotene	0.2
1597	β-Copaene	0.1
1600	β-Elemene	0.1
1611	Terpinen-4-ol	0.3
1612	β-Caryophyllene	0.1
1650	γ-Elemene	0.4
1668	(*Z*)-β-Farnesene	0.2
1687	α-Humulene	0.1
1690	Cryptone	0.1
1726	α-Zingiberene	2.7
1726	Germacrene-D	4.3
1741	β-Bisabolene	1.4
1755	Bicyclogermacrene	0.6
1773	δ-Cadinene	0.5
1783	β-Sesquiphellandrene	7.9
1854	Germacrene-B	3.1
1864	*p*-Cymen-8-ol	0.3

Table 3.6 (continued)

RRI	Compound	Percent
2008	Caryophyllene oxide	0.1
2053	Germacrene-D-1,10-epoxide	0.1
2069	Germacrene-D-4β-ol	1.5
2144	Spathulenol	0.2
2187	T-Cadinol	0.1
2209	T-Muurolol	0.2
2219	δ-Cadinol (=∗-Muurolol)	0.1
2255	α-Cadinol	0.7
2296	Myristicine	0.1
2931	Hexadecanoic acid	0.3
Total		98.3

RRI: Relative retention indices calculated against *n*-alkanes on the HP Innowax column.
%: Percentages were calculated from Flame Ionization Detector (FID) data.
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GC × GC-TOFMS was employed for the separation and identification of the volatile components in the essential oil of *Artemisia annua* L. prepared by steam distillation. The main parameters of the GC × GC-TOFMS system are listed in Table 3.7. Helium was applied as carrier gas, the MS range was 35–400 *m/z*. It was established that the method separated 303 components, terpene derivatives being the main components of the essential oil as illustrated in Table 3.8. It was stated that the method can be employed for the study of the metabolic pathway of artemisnin (Ma et al., 2007).

A different GC-MS strategy was applied for the analysis of the composition of volatile compounds of the essential oil hydrodistilled from the aerial part of Tunisian *Thymus capitatus* Hoff. Et Link. It was found that carvacrol (62–83%), *p*-cymene

Table 3.7 GC × GC experimental conditions

Set	First column	Second column
Length (m)	50	2.6
Diameter (mm)	0.2	0.1
Stationary phase	DB-Petro[a]	DB-17ht[b]
Film thickness (μm)	0.5	0.1
Temperature program	60–260°C at 3°C/min (held 15 min)	60–260°C at 3°C/min (held 15 min)

[a] DB-Petro (J&W Scientific, Folsom, CA, USA), a 100% dimethylpolysiloxane.
[b] DB-17ht (J&W), a 50% phenyl-methylpolysiloxane.
Carrier gas in all column systems: helium, constant pressure: 600 kPa.
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Table 3.8 Thirty-three main components, Artemisia ketone and Arteannuic acid identified in this paper

Peak	t_1^R(s)	t_2^R(s)	Name	Formula	Weight	Similarity	Reverse	Probability	Cas	Content %
1*	1810	2.48	Borneol	$C_{10}H_{18}O$	154	945	945	4499	10385-78-1	15.903
2*	2655	1.48	(*Z*)-β-Farnesene	$C_{15}H_{24}$	204	939	939	5128	28973-97-9	12.920
3*	2770	1.48	Germacrene D	$C_{15}H_{24}$	204	927	927	6378	23986-74-5	10.900
4*	2615	1.55	β-*cis*-Caryophyllene	$C_{15}H_{24}$	204	921	921	2991	118-65-0	5.984
5	1205	2.38	Sabinene	$C_{10}H_{16}$	136	916	916	3230	3387-41-5	3.213
6	2795	1.45	β-Eudesmene	$C_{15}H_{24}$	204	916	916	1398	17066-67-0	1.254
7	1345	2.37	β-Cymene	$C_{10}H_{14}$	134	958	958	4290	535-77-3	1.224
8*	2490	1.55	Copaene	$C_{15}H_{24}$	204	932	932	5090	3856-25-5	1.209
9*	2170	1.98	Acetic acid, bornyl ester	$C_{12}H_{20}O_2$	196	949	949	4392	92618-89-8	1.139
10	3125	1.44	*cis*-*Z*-α-Bisabolene epoxide	$C_{15}H_{24}O$	220	808	857	1092	0-00-0	1.010
11*	2810	1.45	γ-Elemene	$C_{15}H_{24}$	204	870	884	1385	30824-67-0	0.991
12*	3005	1.55	(−)-Spathulenol	$C_{15}H_{24}O$	220	929	929	4092	77171-55-2	0.979
13	1175	2.64	Morillol	$C_8H_{16}O$	128	940	940	6225	3391-86-4	0.912
14*	1145	2.39	Camphene	$C_{10}H_{16}$	136	954	954	4352	79-92-5	0.840
15*	1570	2.37	Linalool	$C_{10}H_{18}O$	154	916	916	4708	78-70-6	0.823
16	2515	1.57	(−)-β-Elemene	$C_{15}H_{24}$	204	919	919	3543	515-13-9	0.730
17*	1380	2.26	Limonene	$C_{10}H_{16}$	136	900	900	2949	138-86-3	0.720
18	3185	1.46	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220	826	826	1309	0-00-0	0.656
19*	775	2.93	2-Hexenal	$C_6H_{10}O$	98	941	941	6362	505-57-7	0.550
20	3020	1.42	3-Methyl-but-2-enoic acid, 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl ester	$C_{15}H_{24}O_2$	236	874	909	6475	91404-82-9	0.538
21*	1840	2.22	4-Terpinenol	$C_{10}H_{18}O$	154	926	926	7454	562-74-3	0.471

Table 3.8 (continued)

Peak	t_1^R(s)	t_2^R(s)	Name	Formula	Weight	Similarity	Reverse	Probability	Cas	Content %
22	2365	1.85	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, (1*R-trans*)-	$C_{12}H_{18}O_2$	194	936	936	3725	7053-79-4	0.437
23	1555	2.45	2-Methyl-6-methylene-oct-3,7-dien-2-ol	$C_{10}H_{16}O$	152	889	950	8713	0-00-0	0.301
24	2830	1.49	Bornyl ester of *n*-pentanoic acid	$C_{15}H_{26}O_2$	238	912	912	3089	7549-41-9	0.293
25*	4125	3.32	Phytol	$C_{20}H_{40}O$	296	877	877	4082	150-86-7	0.287
26	1585	2.23	Butyric acid, 2-methyl-, 2-methylbutyl ester	$C_{10}H_{20}O_2$	172	927	927	6935	2445-78-5	0.266
27	2365	1.64	Elixene	$C_{15}H_{24}$	204	887	894	2497	3242-08-8	0.263
28	2135	2.40	Carvacrol	$C_{10}H_{14}O$	150	929	929	5622	499-75-2	0.257
29*	2790	1.43	γ-Gurjunene	$C_{15}H_{24}$	204	912	912	1255	22567-17-5	0.254
30*	2860	1.35	δ-Cadinene	$C_{15}H_{24}$	204	908	912	5755	483-76-1	0.253
31	3230	1.30	α-Bisabolol	$C_{15}H_{26}O$	222	944	944	7674	515-69-5	0.250
32	3200	1.30	Cyclopentanecarboxylic acid, 3-methylene-, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	$C_{17}H_{26}O_2$	262	891	891	5036	74793-59-2	0.231
33	1590	2.26	(1*E*)-1-Octenyl acetate	$C_{10}H_{18}O_2$	170	881	881	5175	32717-31-0	0.209
	2855	2.35	Artemisia ketone	$C_{10}H_{16}O$	152	823	943	3802	546-49-6	0.069
	3565	3.55	Arteannuic acid	$C_{15}H_{22}O_2$	234	802	805	2646	80286-58-4	0.044

Note: t_1^R and t_2^R retention times of peaks on first and second dimension, respectively. The peaks with (*) mean that they were reported earlier in other papers. The complete information on all identified compounds is available for those investigators that are specially interested by e-mail.
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(5–17%), γ-terpinene (2–14%) and β-caryophyllene (1–4%) were the main constituents of the essential oil. It was further established that the essential oil shows marked antioxidant and antibacterial activities against *Bacillus cereus, Salmonella*sp., *Listeria innocua* and four strains of *Staphylococcus aureus* (Bounatirou et al., 2007).

The antiulcer and anti-inflammatory activities of the essential oil of *Caesaria sylvestris* Sw. leaves were assessed, and the composition of the volatile compounds in the essential oil was investigated by GC-MS method. Essential oil was hydrodistillated and the components were separated in a capillary column (30 m × 0.25 mm). Oven temperature started at 60°C and was increased to 240°C. The essential oil contained 13.8% caryophyllene, 5.2% thujopsene, 3.7% α-humulene, 20.8% β-acoradiene, 1.9% germacrene-D, 40.9% bicyclogermacrene, 1.5% calamenene, 3.9% germacrene-B, 12.6% spathulenol and 2.2% globulol. The anti-inflammatory and antiulcer effects of the essential oil were also demonstrated (Esteves et al., 2005).

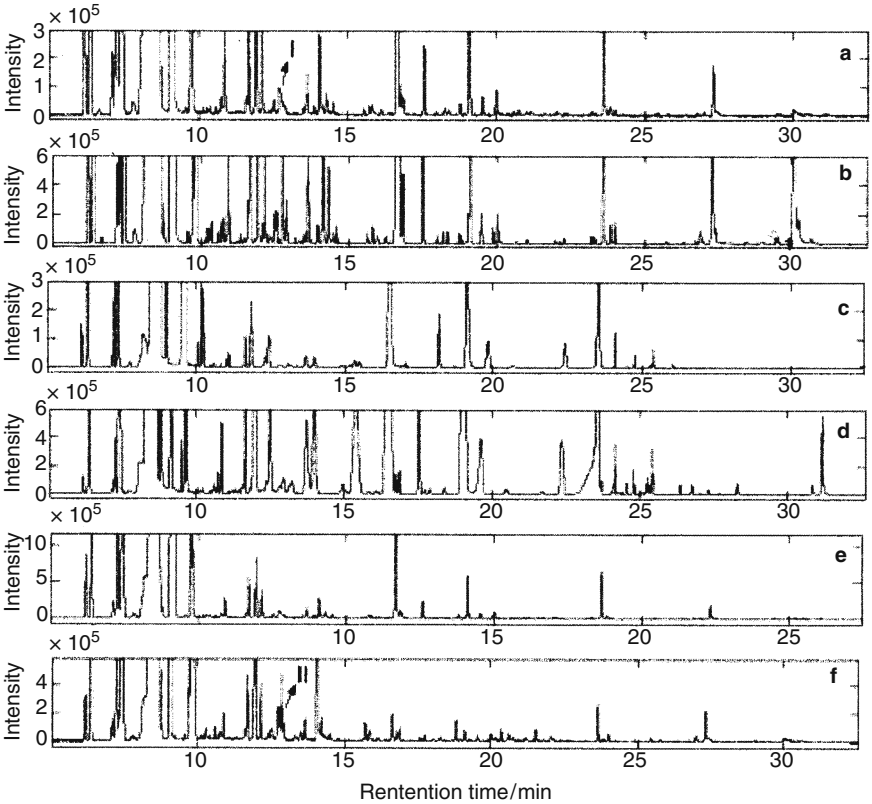

Fig. 3.4 GC–MS TIC of PCRV and PCR. (**a**) PCRV of *Citrus reticulata* "*chazhi*"; (**b**) PCR of *Citrus reticulata* "*chachi*"; (**c**) PCRV of *Citrus erythrosa tanaka*; (**d**) PCR of *Citrus erythrosa tanaka*; (**e**) PCRV of *Citrus reticulate* "*dahongpao*"; (**f**) PCR of *Citrus reticulate* "*dahongpao*". Reprinted with permission from Wang et al. (2008)

Table 3.9 The quantitative and qualitative results of the essential oils in PCRV and PCR

Peak number	compounds	Molecular form	Relative content %						I
			Citrus chachi PCRV	Citrus reticulate PCR	Citrus "Dahongpao" PCRV	Citrus reticulate PCR	Citrus Tanaka PCRV	Citrus erythrosa PCR	
1	α-Thujene	$C_{10}H_{16}$	0.43	0.33	0.09	0.13	0.15	0.07	931
2	α-Pinene	$C_{10}H_{16}$	1.67	1.05	0.44	0.82	0.77	0.35	940
3	Camphene	$C_{10}H_{16}$	tr	0.01	0.01	nd	nd	nd	981
4	Sabinene	$C_{10}H_{16}$	0.14	0.14	0.07	0.09	0.1	0.06	986
5	β-Pinene	$C_{10}H_{16}$	1.5	1.02	0.34	0.09	0.42	0.28	987
6	Octanal	$C_8H_{16}O$	0.12	tr	0.1	tr	0.02	0.1	994
7	β-Myrcene	$C_{10}H_{16}$	1.2	1.6	1.72	0.59	1.8	1.75	1003
8	α-Phellandrene	$C_{10}H_{16}$	0.05	0.05	0.01	0.02	0.02	0.01	1012
9	*p*-Cymene	$C_{10}H_{14}$	1.43	0.1	0.4	nd	0.57	0.41	1019
10	. . .. .d-Limonene	$C_{10}H_{16}$	65.61	73.39	67.83	82.2	83.14	77.19	1035
11	β-*cis*-Ocimene	$C_{10}H_{16}$	0.04	0.03	0.19	0.08	0.42	0.38	1051
12	γ-Terpinen	$C_{10}H_{16}$	22.38	10.78	5.46	7.05	5.53	4.65	1065
13	1-Octanol	$C_8H_{18}O$	nd	nd	nd	tr	nd	nd	1077
14	Isopropenyltoluene	$C_{10}H_{12}$	0.01	0.02	0.03	nd	0.09	0.14	1094
15	(+)-4-Carene	$C_{10}H_{16}$	1.19	0.87	0.32	0.25	0.33	0.32	1085
16	Nonanal	$C_9H_{18}O$	nd	tr	0.01	0.01	0.1	0.36	1104
17	Linalool	$C_{10}H_{18}O$	0.35	0.7	15.99	5.77	0.27	0.78	1106
18	Linderol	$C_{10}H_{18}O$	tr	nd	tr	nd	nd	nd	1124
19	(*R*)-3,7-Dimethyl-6-octenal	$C_{10}H_{18}O$	nd	0.02	0.05	0.01	nd	nd	1138
20	Camphor	$C_{10}H_{16}$	nd	0.01	tr	0.01	0.03	0.02	1149
21	Citronellal	$C_{10}H_{18}O$	0.1	0.1	0.09	0.14	0.05	0.18	1153
22	Nonanol	$C_9H_{20}O$	0.01	0.02	nd	0.01	0.01	tr	1168
23	1-Terpinen-4-ol	$C_{10}H_{18}O$	0.2	0.78	0.45	0.1	0.14	0.35	1182
24	α-Terpineol	$C_{10}H_{18}O$	0.33	1.45	0.98	0.22	0.28	0.85	1196

Table 3.9 (continued)

Peak number	compounds	Molecular form	Relative content %						I
			Citrus chachi PCRV	Citrus reticulate PCR	Citrus "Dahongpao" PCRV	Citrus reticulate PCR	Citrus Tanaka PCRV	Citrus erythrosa PCR	
25	*n*-Decanal	$C_{10}H_{20}O$	0.13	0.24	0.23	0.24	0.11	0.3	1207
26	2,5,5-Trimethyl-1,6-Heptadiene	$C_{10}H_{18}$	0.08	0.05	tr	0.13	0.01	0.01	1211
27	Citronellol	$C_{10}H_{20}O$	0.04	0.02	0.02	0.07	tr	tr	1225
28	*cis*-Citral	$C_{10}H_{16}O$	0.02	0.01	tr	tr	tr	tr	1227
29	(+)-Carvone	$C_{10}H_{14}O$	nd	0.01	tr	0.17	tr	0.01	1228
30	2,5-Dimethyl-1,6-heptadiene	C_9H_{16}	0.01	nd	tr	nd	tr	nd	1228
31	4-Acetylbenzoic acid	$C9H_8O_3$	0.02	tr	tr	0.02	tr	tr	1236
32	β-Citronellol	$C_{10}H_{20}O$	0.01	0.44	0.26	0.12	0.06	0.27	1243
33	Nerol	$C_{10}H_{18}O$	0.07	nd	nd	nd	nd	nd	1248
34	Thymol methyl ether	$C_{11}H_{16}O$	0.01	nd	0.48	0.09	nd	nd	1255
35	Neral	$C_{10}H_{16}O$	nd	nd	nd	tr	0.04	0.3	1262
36	2-Decenal	$C_{10}H_{18}O$	nd	tr	nd	tr	0.01	0.01	1272
37	Geranial	$C_{10}H_{16}O$	tr	0.05	0.27	0.44	0.03	0.08	1279
38	Perillaldehyde	$C_{10}H_{14}O$	0.04	0.24	0.16	nd	0.03	0.59	1294
39	Decanol	$C_{10}H_{22}O$	0.01	0.02	0.01	nd	nd	nd	1303
40	Thymol	$C_{10}H_{14}O$	0.1	0.31	1.93	0.28	2.12	4.86	1308
41	Bornyl acetate	$C_{12}H_{20}O_2$	tr	tr	nd	0.01	tr	0.05	1318
42	Carvacrol	$C_{10}H_{14}O$	0.02	0.1	tr	nd	0.01	0.06	1341
43	*n*-Undecanal	$C_{11}H_{22}O$	tr	0.02	tr	0.01	0.02	0.05	1354
44	Cavrbenol	$C_{10}H_{16}O$	nd	nd	nd	0.01	tr	nd	1365
45	Citronellyl butyrate	$C_{14}H_{26}O_2$	tr	tr	tr	0.02	tr	tr	1380
46	δ-Elemene	$C_{15}H_{24}$	nd	nd	0.07	0.04	0.17	0.2	1381
47	Neryl acetate	$C_{12}H_{20}O_2$	0.01	0.02	0.01	0.03	tr	0.01	1396

Table 3.9 (continued)

Peak number	compounds	Molecular form	Relative content %						I
			Citrus chachi PCRV	Citrus reticulate PCR	Citrus "Dahongpao" PCRV	Citrus reticulate PCR	Citrus Tanaka PCRV	Citrus erythrosa PCR	
48	Geraniol acetate	$C_{12}H_{20}O_2$	nd	0.01	nd	0.01	0.01	0.02	1409
49	Decanoic acid	$C_{10}H_{20}O_2$	nd	0.01	0.1	0.01	nd	0.01	1437
50	Copaene	$C_{15}H_{24}$	tr	0.01	nd	0.03	0.01	0.02	1458
54	γ-Muurolene	$C_{15}H_{24}$	tr	0.01	tr	0.03	tr	tr	1527
55	Undecyl acetate	$C_{13}H_{26}O_2$	tr	0.01	tr	nd	0.12	0.13	1565
56	α-Farnesene	$C_{15}H_{24}$	0.19	0.36	0.01	0.1	nd	nd	1583
57	δ-Cadinene	$C_{15}H_{24}$	0.01	0.04	0.01	tr	0.01	tr	1588
58	Elemol	$C_{15}H_{26}O$	0.02	0.02	0.01	0.01	nd	tr	1649
59	Germacrene	$C_{15}H_{24}$	nd	0.01	0.06	0.02	0.13	0.12	1715
60	α-Sinensal	$C_{15}H_{22}O$	0.26	0.7	0.23	0.14	nd	nd	1746
61	Hexadecanoic acid	$C_{16}H_{32}O_2$	0.06	0.01	tr	0.09	0.01	0.02	1772
Total			99.66	99.17	98.48	99.84	98.15	97.6	

Notes: nd, not detected; tr (trace), relative content <0.01%.
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Table 3.10 Chemical composition of *Cumminum cyminum* essential oil

No.	Compounds	RI	%
1	Isobutyl isobutyrate	892	0.8
2	α-Thujene	922	0.3
3	α-Pinene	931	29.1
4	Sabinene	971	0.6
5	Myrcene	981	0.2
6	δ-3-Carene	998	0.2
7	*p*-Cymene	1013	0.3
8	Limonene	1025	21.5
9	1,8-Cineole	1028	17.9
10	€-Ocimene	1038	0.1
11	γ-Terpinene	1051	0.6
12	Terpinolene	1082	0.3
13	Linalool	1089	10.4
14	α-Campholenal	1122	0.03
15	*trans*-Pinocarveole	1130	0.07
16	δ-Terpineole	1154	0.09
17	Terpinene-4-ol	1169	0.5
18	α-Terpineole	1180	3.17
19	*trans*-Carveole	1213	0.4
20	*cis*-Carveole	1217	0.07
21	Geraniol	1242	1.1
22	Linalyl acetate	1248	4.8
23	Methyl geranate	1310	0.2
24	α-Terpinyl acetate	1342	1.3
25	Neryl acetate	1351	0.09
26	Methyl eugenol	1369	1.6
27	β-Caryophyllene	1430	0.2
28	α-Humulene	1463	0.2
28	α-Humulene	1463	0.2
29	Spathulenol	1562	0.07
30	Caryophylleneb epoxide	1586	0.1
31	Humulene epoxide II	1608	0.08
32	Acetocyclohexane dione (2)	1704	0.4

Chemical composition of *Rosmarinus officinalis* L. essential oil

No.	Compounds	RI	%
1	α-Pinene	934	14.9
2	Camphene	945	3.33
3	3-Octanone	966	1.61
4	Sabinene	972	0.56
5	Myrcene	982	2.07
6	*O*-Cymene	1013	0.71
7	1,8-Cineole	1024	7.43
8	Linalool	1089	14.9
9	Myrcenol	1104	0.75
10	Camphor	1127	4.97

Table 3.10 (continued)

No.	Compounds	RI	%
11	Borneol	1155	3.68
12	Terpinen-4-ol	1166	1.70
13	α-Terpineol	1177	0.83
14	Verbinone	1187	1.94
15	Piperitone	1246	23.7
16	Bornyl acetate	1274	3.08
17	β-Caryophyllene	1424	2.68
18	*cis*-β-Farnesene	1448	1.26
19	Germacrene-D	1481	0.52
20	α-Bisabolol	1673	1.01

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GC-MS method combined with moving window factor analysis was employed for the separation and quantitative determination of volatile compounds in the essential oil of *Pericarpium Citri Reticulatae Viride* (PCRV) and *Pericarpium Citri Reticulatae* (PCR). These essential oils are extensively employed in traditional Chinese medicines facilitating expectoration and showing anticancer activity. Volatiles were separated in a capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Oven temperature started at 65°C, increased to 260°C at 6°C/min. Helium was the carrier gas. MS were recorded at 70 eV, mass range being 30–500 *m/z*. Some chromatograms showing the separation of volatiles are depicted in Fig. 3.4. The quantitative results are compiled in Table 3.9. The data demonstrated that the composition of PCRV and PCR essential oils differs considerably; however, the main component was D-limonene in both essential oils (Wang et al., 2008).

The composition and various biological activities of the essential oils extracted by steam distillation from *Cuminum cyminum* and *Rosmarinus officinalis* were investigated by GC-FID and GC-MS. The bactericidal effect and radical-scavenging capacity of oils were also determined. GC-FID was carried out in a fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Oven temperature started at 40°C, increased to 250°C at 4°C/min. Helium was the carrier gas. Injector and detector temperatures were 250°C and 265°C, respectively. GC-MS was performed under identical GC conditions. The volatile compounds identified in *Cuminum cyminum* and *Rosmarinus officinalis* are compiled in Table 3.10. It was found that both essential oils showed marked antimicrobial activity against *E. coli, S. aureus* and *L. monocytogenes*. Because of the biological activities, the oils were proposed as agents in food preservation (Gachkar et al., 2007).

3.3 Other Essential Oils

The composition of essential oils showing no beneficial health effect has also been frequently investigated. The objectives of these measurements were the elucidation of the composition of essential oils not analysed before, the study of the influence of seasonal variation and that of chemotype on the GC profile of essential oils.

A complex chromatographic system was developed and applied for the separation, identification and quantification of the component in the essential oils of coriander leaf (*Coriandrum sativum*) and hop (*Humulus lupulus*). Measurements were performed by GC-O, comprehensive two-dimensional GC (GC × GC) followed with TOFMS and heart-cut multidimensional GC-O (MDGC-O). GC-O measurements were performed on a capillary column (25 m × 0.32 mm, film thickness 0.5 μm). Oven temperature was raised from 60°C to 210°C at 6°C/min, then to 290°C at 10°C/min, final hold 20 min. GC × GC-FID analyses were carried out on a capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) coupled in series with a column of 1.1 m × 0.1 mm i.d., film thickness 0.25 μm. Temperature program started from 60°C to 210°C at 3°C/min, then raised to 240°C at 10°C/min, final hold 10 min. Injector and detector temperatures were 210°C and 260°C, respectively. The first column for GC × GC-TOFMS was the same as for GC × GC-FID; the second column was 0.8 m × 0.1 mm i.d., film thickness 0.1 μm. MDGC conditions were different for coriander leaf and hop essential oils. The flow diagram of the methodology is shown in Fig. 3.5. It was established that *E*-2-dodecenal is the main odorant in coriander while the essential oil of hop contains eight peaks in the odorant region (Eyres et al. 2007).

GC-O using incremental dilution technique and GC-MS were employed for the analysis of the composition of the essential oil of *Clinopodium tomentosum* (Kunth)

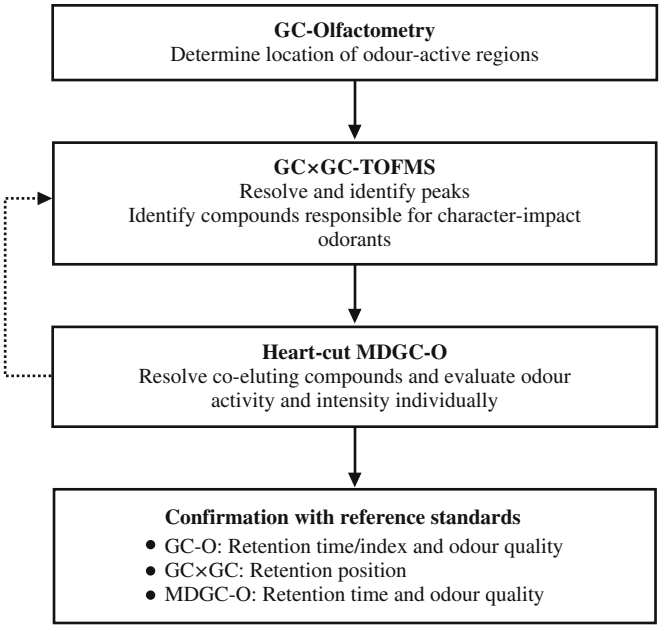

Fig. 3.5 Flow diagram of the methodology used to identify character-impact odorants in essential oils. Reprinted with permission from Eyres et al. (2007)

Govaerts. GC-MS was carried out on a capillary column (30 mm × 0.53 mm, film thickness 2.65 μm), helium being the carrier gas. Initial oven temperature was 60°C for 1 min, raised to 100°C at 10°C/min, then to 200°C at 5°C/min, to 250°C at 30°C, final hold 10 min. Injector and detector temperatures were 250°C and 200°C, respectively. GC-MS measurements were performed in a capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Initial column temperature was 40°C for 1 min, raised 250°C at 5°C/min, final hold 10 min. The analytes separated and identified by GC-MS are compiled in Table 3.11. It was stated that the method is suitable for the separation, identification and the determination of the odorant capacity of the individual analytes (Benzo et al. 2007).

A complex GC system was developed and applied for the determination of the composition of essential oils employed for the formulation of gin. Two-dimensional GC × GC-MS with heart cutting was applied and sample specific libraries were made. It was established that with the help of the library, one-dimensional GC with the ion fingerprint deconvolution algorithm can be successfully used for the identification of the components of complicated mixtures of essential oils. One-dimensional GC was performed in a capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Temperature program started at 60°C, raised to 260°C at 10°C/s, final hold 5 min. Helium was employed as carrier gas. The dimensions of the second column were the same as the first column. The analytes separated by the method are compiled in Table 3.12. Because of the high separation capacity of the system, its application for the analysis of essential oils was proposed (Mac Namara et al. 2007).

A comprehensive two-dimensional GC × GC-TOFMS and GC × GC-FID methods were developed and applied for the separation, identification and quantitation of tobacco essential oils. The composition of the GC × GC column sets are shown in Fig. 3.6. The results obtained by the different column sets are shown in Fig. 3.7.

Table 3.11 GC–MS analysis of the essential oil of *Clinopodium tomentosum* (Kunth) Govaerts

Calculated linear Retention index	Retention index from Adams	Attribution	Percent
934	930	Alpha-tujene	0.06
940	927	Alpha-pinene	1.10
953	954	Camphene	0.04
970	975	Sabinene	0.32
974	979	Beta-pinene	0.74
977	979	1-Octen-3-ol	0.04
987	–	6-Methyl-5-hepten-2-one	0.03
990	991	Beta-myrcene	0.37
1016	1017	Alpha-terpinene	0.05
1024	1025	*p*-Cymene	0.08
1028	1029	Limonene	0.76
1030	1031	1,8-Cineole	0.80
1048	1050	Beta-(*E*)-ocimene	0.11
1058	1060	Gamma-terpinene	0.06

Table 3.11 (continued)

Calculated linear Retention index	Retention index from Adams	Attribution	Percent
1112	1113	1-Octen-3-ol acetate	0.30
1124	1123	Octan-3-ol acetate	0.11
1155	1153	Menthone	6.60
1170	1163	Isomenthone	41.72
1173	1172	Menthol	0.10
1177	–	Isopulegone	1.97
1191	1189	Alpha-terpineol	0.28
1231	1226	Citronellol	0.33
1244	1237	Pulegone	29.94
1256	1254	*cis*-Piperitone epoxide	7.54
1261	1261	Methyl citronellate	0.17
1271	1267	Geranial	0.49
1287	1286	Isobornyl acetate	0.11
1291	1290	Thymol	0.10
1338	1342	*trans*-Carvyl acetate	0.02
1343	1343	Piperitenone	1.83
1353	1353	Citronellyl acetate	0.04
1364	1362	Neryl acetate	0.02
1368	1369	Piperitenone epoxide	0.98
1383	1381	Geranyl acetate	0.09
1387	1388	Beta-bourbonene	0.03
1423	1419	*trans*-Caryophyllene	0.26
1453	1455	Geranyl acetone	0.02
1484	1485	Germacrene-D	0.11
1527	1530	Zonarene	0.03
1994	1998	Manoyl oxide	0.19

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Table 3.12 Lists the 20 compounds found in nutmeg oil with their retention times, target ions and relative abundances

No.	Compound	t_R (min)	Main ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)
1	15	13.71	167	81 (60)	93 (50)	139 (45)
2	Solanone	16.70	93	121 (70)	136 (65)	
3	Linalyl acetate[a]	16.89	93	80 (36)	121 (34)	
4	Safrole	18.43	162	104 (39)	131 (35)	135 (31)
5	Eugenol	21.51	164	103 (25)	77 (24)	149 (32)
6	Methyl eugenol	23.55	178	163 (27)	147 (28)	103 (23)
7	α-Bergamotene	24.65	119	93 (101)	105 (28)	77 (30)
8	(*E*)-Isoeugenol	25.50	91	77 (100)	121 (95)	
9	Methyl isoeugenol	27.41	91	79 (60)	147 (42)	
10	Elemicin	29.85	208	193 (55)	209 (13)	91 (10)
11	Spathulenol[a]	30.60	205	119 (70)	105 (59)	159 (55)
12	Caryophyllene oxide[a]	30.77	79	93 (100)	109 (50)	121 (40)

Table 3.12 (continued)

No.	Compound	t_R (min)	Main ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)
13	Ethyl laurate	31.33	88	101 (55)	157 (25)	
14	Methoxyeugenol	31.82	194	133 (22)	131 (22)	
15	B-Eudesmol	33.47	93	91 (100)	149 (100)	59 (100)
16	Myristic acid	38.20	73	129 (70)	185 (60)	228 (50)
17	Ethyl myristate	38.88	88	101 (47)	43 (20)	41 (16)
18	Isopropyl myristate	40.00	228	211 (60)	185 (60)	102 (60)
19	Palmitic acid	45.00	73	129 (60)	256 (49)	
20	Ethyl palmitate	45.76	88	101 (60)	43 (27)	41 (21)

[a] Denotes compounds found in the initial test mixture.
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The contour plots demonstrate that column set 1 is suitable for the group-type separation of analytes, but the overall separation capacity of column set 2 was markedly higher. The one-dimensional GC-MC chromatogram (TIC) of tobacco essential oils is depicted in Fig. 3.8. It was concluded from the results that the separation efficacy of this comprehensive two-dimensional GC is higher than that of one-dimensional GC and, consequently, its application for the analysis of the composition of tobacco essential oils is advocated (Zhu et al., 2005).

The essential oil extracted from the different parts of *Eryngium bourgati* Guan was investigated by GC-FID and GC-MS. Stems + leaves, inflorescences and roots were extracted by steam distillation for 8 h. GC-FID measurements were performed in a capillary column (50 m × 0.25 mm i.d., film thickness 0.25 μm). Initial column temperature was 95°C, raised 240°C at 4°C/min. FID temperature was 250°C. Nitrogen was the carrier gas. GC-MS investigations were carried out in two different systems. System 1: Column parameters were 50 m × 0.22 mm i.d., film thickness 0.25 μm. Initial column temperature was 70°C, raised 220°C at 4°C/min. System 2: Column parameters were 60 m × 0.32 mm i.d., film thickness 0.25 μm. Initial column temperature was 35°C, raised 220°C at 3°C/min. Helium was used as carrier gas in both instances. The analytes separated and identified in Table 3.13. It was established that the composition of the essential oils in the different parts was similar but its amount showed marked variations (Palá-Paúl et al., 2005).

GC-FID and GC-MS were employed for the analysis of the essential oils of palmarosa (*Cymbopogon martinii* (Roxb.) Wats var. *motia* Burk. Family Poaceae). Essential oil was extracted from the crops using steam distillation (primary oil). As a considerable part of the essential oil was dissolved in the condensate or distillation water, it was re-extracted by hexane (secondary oil). GC-FID measurements were performed in a capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Nitrogen was the carrier gas. Initial column temperature was 60°C, ramped 245°C at 5°C/min (final hold 10 min). Injector and detector temperatures were 250°C and 300°C, respectively. GC-MS used a longer capillary column (50 m × 0.2 mm i.d.,

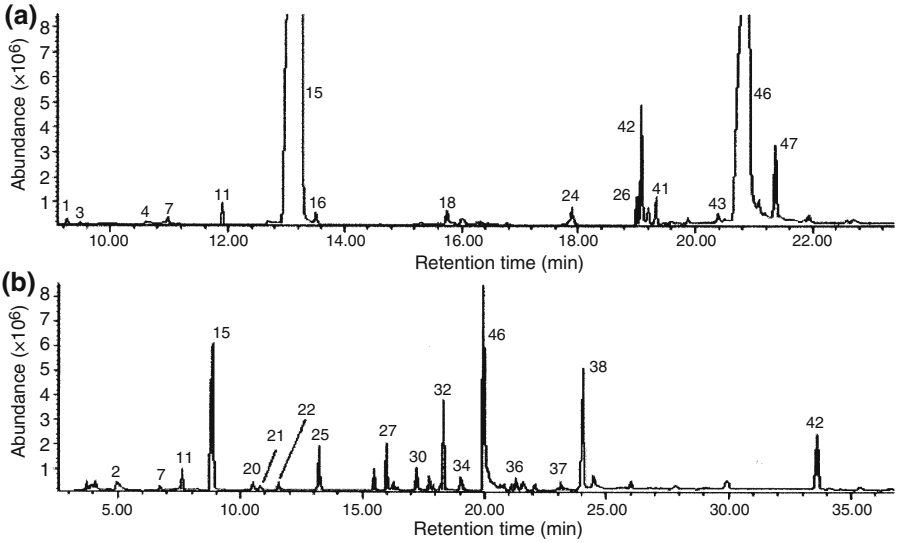

Fig. 3.6 The GC/MS total ion current (TIC) chromatograms of the essential oil sample obtained under the optimised separation conditions of two different coupled columns in series. (**A**) HP-INNOWAX (30 m × 0.25 mm × 0.25 μm) + HP-1 (30 m × 0.25 mm × 0.25 μm); (B) SUPELCOWAX-10 (30 m × 0.32 mm × 0.25 μm) + HP-5 (30 m × 0.32 mm × 0.25 μm). (Peak identifications: 1: ethyl acetate, 2: 1-propanol, 3: 2-butanone, 3-hydroxy-, 4: caryophyllene, 7: pyrazine, trimethyl-, 11: pyrazine, tetramethyl-, 15: propylene glycol, 16: bicyclo[2.2.1]heptan-2-ol, -1,7,7-trimethyl-,acetate, 18: 2-propanol, 1,1-oxybis-, 20: furfural, 21: 2,3-butanedione, 22: acetic acid, hydroxy-, ethyl ester, 24: 3,7,11-trimethyl-3-hydroxy-6, 10-dodecadien-1-yl acetate, 25: 2-propenoic acid, 3-phenyl-, (*e*), 26: (–)-spathulenol, 27: 4-cyanobenzophenone, 30: *n*-hexadecanoic acid, 32: 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 34: 2-propanone,1,3-dihydroxy-, 36: 1,3-propanediol, 37: 3,4-dihydroxy-5-methyl-dihydrofuran-2-one, 38: 4*H*-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, 41: pyrazine, 3,5-diethyl-2-methyl-, 42:eugenol, 43: benzyl cinnamate, 46: glycerine, 47: benzyl benzoate). Reprinted with permission from Zhu et al. (2005)

film thickness 0.25 μm). Helium was used as the carrier gas. Initial column temperature was 60°C, ramped 245°C at 5°C/min (final hold 10 min). MS detection was performed with the ionisation energy of 70 eV, the detection range being 40–400 *m/z*. Typical gas chromatograms of the primary (upper lane) and secondary essential oils (lower lane) are depicted in Fig. 3.9. The chromatograms illustrate that the composition of the primary and secondary oils show marked differences. The identified constituents of the essential oils are compiled in Table 3.14. It was assessed that the concentration of linalool, geraniol and geranial was higher in the secondary essential oil of palmarosa (Rajeswara et al., 2005).

The impact of the seasonal variation and that of chemotype on the composition of volatiles of *Lantan camara* L. essential oils from Madagascar. The volatiles were separated and identified by GC-FID and GC-MS methods. GC-FID measurements were carried out in two different capillary columns A (30 m × 0.25 mm i.d.,

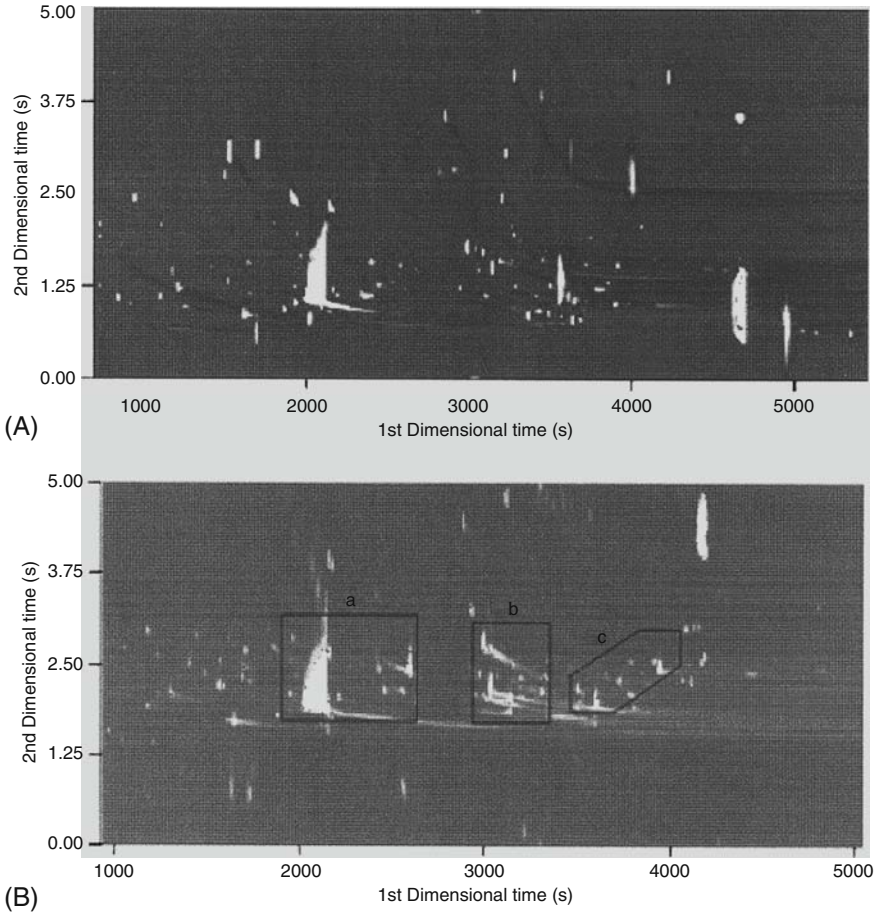

Fig. 3.7 The GC × GC contour plots of essential oil under different column systems and the optimised separation conditions. (**A**) Column set 1; (**B**) column set 2. Zones a–c are mainly alcohols, ketones and pyrazines, respectively. Reprinted with permission from Zhu et al. (2005)

film thickness 0.10 μm) and B (30 m × 0.25 mm i.d., film thickness 0.20 μm). Temperature of column A changed from 70°C to 220°C at 3°C/min, temperature of column B increased from 80°C to 240°C at 3°C/min. GC-MS measurements were carried out in a fused silica capillary column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature changed from 60°C to 240°C at 4°C/min. Ionisation energy was 70 eV. The compositions of the volatile compounds extracted from pink-violet and yellow-orange *L. camara* aerial parts in rainy and dry season are compiled in Table 3.15. The data indicated that the composition of the essential oils extracted from pink-violet and yellow-orange flowers showed marked differences while the effect of the season was negligible (Randrianalijaona et al., 2005).

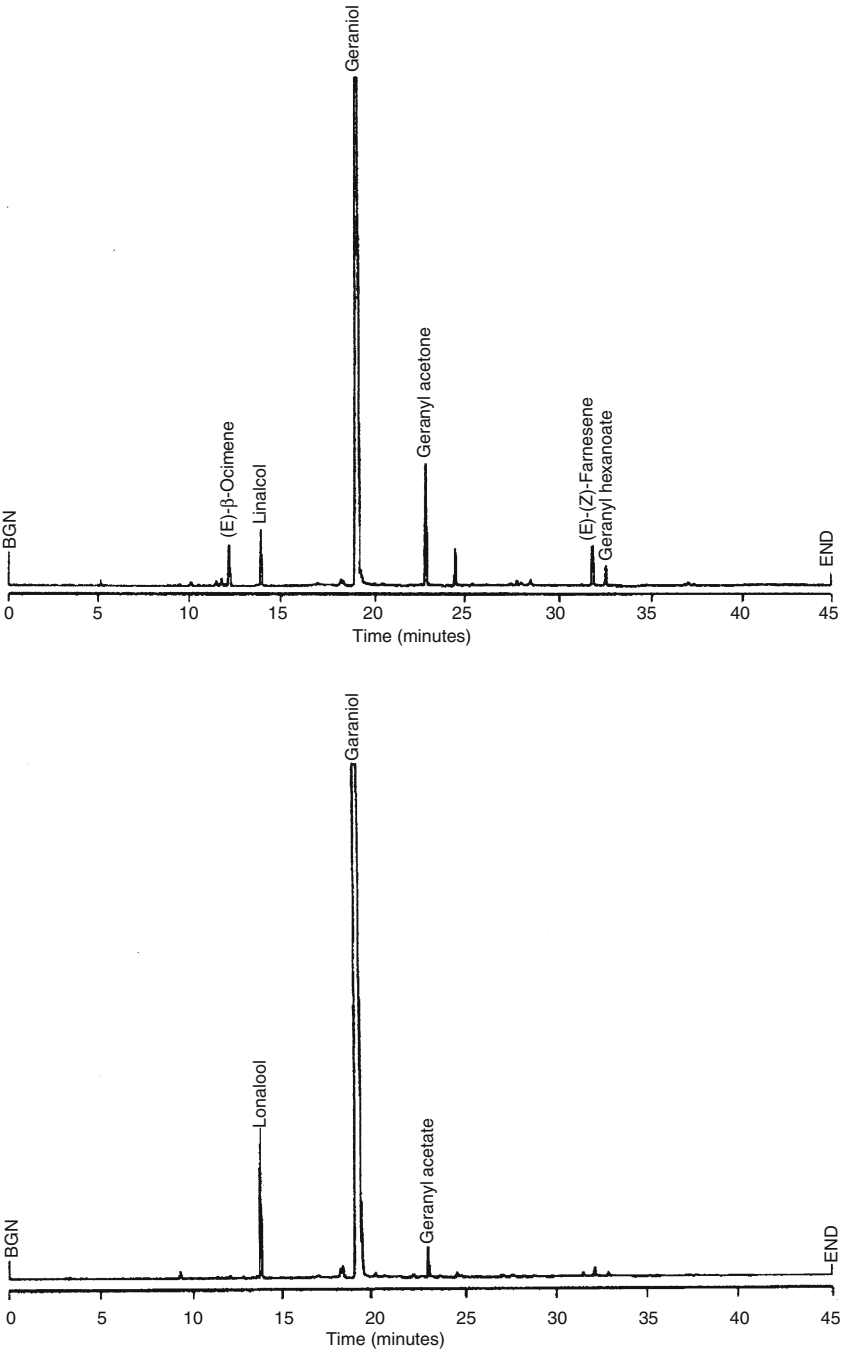

Fig. 3.8 Gas chromatogram of the primary essential oil of palmarosa (*upper lane*). Gas chromatogram of the secondary essential oil of palmarosa (*lower lane*). Reprinted with permission from Rajeswara et al. (2005)

Table 3.13 Essential oil composition of the different parts of *E. bourgatii* from Spain

Compound	I	E.b.I	E.b.SL	E.b.R
α-Pinene	932 (1012)	1.0	1.5	0.2
Sabinene	963 (1113)	t	t	t
β-Pinene	970 (1097)	t	0.4	0.3
3-*p*-Menthene	976	t	1.3	1.1
Myrcene	985 (1160)	0.1	0.1	0.0
Mesitylene	989	t	t	0.1
n-Decane	1000	t	t	t
α-Phellandrene	1005 (1157)	t	t	t
1,2,4-Trimethyl benzene	1021 (1275)	t	–	–
p-Cymene	1023 (1264)	t	–	–
Limonene	1026 (1191)	t	0.1	0.2
β-Phellandrene	1027 (1201)	t	–	–
1,8-Cineole	1029 (1204)	t	–	–
(*Z*)-β-Ocimene	1031 (1232)	t	–	–
(*E*)-β-Ocimene	1041 (1249)	t	–	–
γ-Terpinene	1058 (1240)	t	–	–
Cryptone	1087 (1669)	0.1	0.1	0.5
Linalool	1096 (1549)	0.4	0.9	0.8
6-Camphenol	1106	t	t	0.1
trans-3-Caren-2-ol	1111	t	0.2	t
Chrysanthenone	1122	t	–	–
α-Terpineol	1183 (1700)	t	t	0.2
iso-Pinocampheol	1190	t	0.1	–
(*E*)-Ocimenone	1252	t	t	0.2
(*E*)-Anethol	1300	t	–	–
δ-Elemene	1333 (1468)	0.2	t	0.1
α-Cubebene	1345 (1455)	t	0.1	0.1
β-Ylangene	1350 (1573)	t	t	t
α-Copaene	1366 (1480)	t	0.1	0.1
Daucene	1370	t	0.7	0.4
E-β-Damascenone	1371	t	–	–
β-Bourbonene	1376 (1515)	0.7	0.2	–
β-Elemene	1387 (1587)	1.1	5.2	0.6
α-Gurjunene	1406 (1528)	0.1	–	–
E-Caryophyllene	1410 (1594)	8.3	10.1	1.6
β-Gurjunene	1426 (1595)	t	0.2	0.2
γ-Elemene	1429 (1636)	t	0.1	6.0
Aromadendrene	1433 (1605)	0.4	0.3	0.1
-Guaiene	1434	t	–	–
α-Patchoulene	1443	t	–	–
α-*neo*-Clovene	1445	3.4	0.2	t
α-Humulene	1447 (1667)	0.5	0.9	0.2
E-β-Farnesene	1452 (1770)	1.0	1.2	0.9
cis-Muurola-4(14)-5-diene	1454	t	–	–
γ-Himachalene	1461	t	–	–
γ-Gurjunene	1463	t	–	–
γ-Muurolene	1465 (1675)	4.4	11.8	15.4
Germacrene-D	1476 (1713)	0.7	0.8	0.4
Viridiflorene	1487 (1695)	t	0.4	0.4

Table 3.13 (continued)

Compound	I	E.b.I	E.b.SL	E.b.R
Bicyclogermacrene	1493 (1750)	15.1	1.8	5.4
α-Bulnesene	1498 (1642)	t	–	–
α-Muurolene	1499 (1724)	0.4	1.0	0.6
Sesquicineole	1509	t	–	–
β-Bisabolene	1513 (1727)	0.3	0.2	0.4
(*Z*)-γ-Bisabolene	1514	t	–	–
δ-Cadinene	1522 (1760)	1.6	2.0	7.4
Cadina-1,4-diene	1528 (1783)	t	0.1	0.1
α-Calacorene	1538	t	0.2	0.1
α-Cadinene	1539	0.2	0.6	0.4
1-*nor*-Bourbonanone	1548	t	–	–
β-Calacorene	1553	t	0.3	–
Eremophyllene	1554	1.0	1.9	0.2
cis-Muurol-5-en-4-∗-ol	1556	0.1	0.4	–
n.i. 1 ($C_{15}H_{24}O$)	1557	0.3	1.7	0.8
n.i. 2 ($C_{15}H_{24}O$)	1559	0.4	t	5.3
Germacrene-B	1563	0.1	0.2	–
Ledol	1570	t	–	–
Spathulenol	1576 (2133)	1.6	2.3	2.1
Germacrene-D-4-ol	1578	t	t	0.3
Palustrol	1579 (1931)	t	–	–
Caryophyllene oxide	1580 (1987)	1.7	4.6	1.0
Globulol	1583 (2064)	1.3	0.2	0.6
Viridiflorol	1590 (2091)	1.1	t	1.1
Carotol	1594 (2026)	6.0	0.7	0.6
Guaiol	1595	t	–	–
β-Oploplenone	1597	t	–	–
n.i. 3 ($C_{15}H_{24}O$)	1602	t	0.5	1.2
1,10-Di-*epi*-cubenol	1611	t	–	–
10-*epi*-γ-Eudesmol	1623	0.3	1.3	0.8
Cedr-8-(15)-en-9-α-ol	1625	0.1	0.1	0.2
epi-α-Cadinol	1630 (2177)	t	–	–
epi-α-Muurolol	1631 (1890)	0.4	0.3	0.3
α-Muurolol	1650 (2246)	0.1	0.3	0.1
α –Cadinol	1651 (2243)	1.3	2.1	2.1
14-Hydroxy-9-*epi*-E-caryophyllene	1662 (1924)	1.5	2.1	3.1
Kusinol	1665	0.3	0.5	0.3
n.i. 4 ($C_{15}H_{26}O$)	1674	0.7	3.4	1.7
(*E*)-Nerolidolol acetate	1693	0.2	0.2	0.5
14-Hydroxy-α-muurolene	1757 (2026)	0.1	0.9	0.1
n.i. 5 ($C_{15}H_{24}O$)	1770	0.3	1.6	3.3
14-Hydroxy-δ-cadinene	1788	0.3	0.4	t
iso-Acorenone	1812	0.2	2.6	0.4
Sclarene	1935	1.3	0.2	2.0
Phyllocladene	1985	37.6	20.4	15.0
Monoterpene hydrocarbons		1.1	3.4	1.8
Oxygenated monoterpenes		0.4	1.2	1.3
Sesquiterpene hydrocarbons		38.5	38.7	40.9

Table 3.13 (continued)

Compound	I	E.b.I	E.b.SL	E.b.R
Oxygenated sesquiterpenes		16.7	19.1	14.1
Diterpene hydrocarbons		38.9	20.6	17.0
Oxygenated diterpenes		0.0	0.0	0.0
Total		95.6	83.0	75.10

I=Kováts retention indices on DB-1 column on DB-wax in parenthesis; t=traces (%<0.1); n.i.=not identified; E.b.=*E. bourgatii*; I=inflorescences; SL=stems and leaves; R=roots; n.i. 1 K.I.=1557 ($C_{15}H_{24}O$), 220[M^+](10), 123(100), 131(75), 109(43), 91(40), 146(39), 163(27), 187(9), 202(5); n.i. 2 K.I.=1559 ($C_{15}H_{24}O$), 220[M^+](35), 135(100), 107(88), 159(85), 91(83), 121(81), 177(79), 81(60), 41(45), 55(40), 137(39), 69(30), 161(23), 205(20), 189(10); n.i. 3 K.I.=1602 ($C_{15}H_{24}O$), 220[M^+](5), 161(100), 105(65), 43(60), 107(50), 93(45), 119(40), 204(38), 69(35), 138(30), 189(25), 177(18); n.i. 4 K.I.=1674 ($C_{15}H_{26}O$), 222[M^+](10), 84(100), 81(65), 109(50), 41(43), 55(42), 121(40), 69(39), 95(28), 161(25), 137(30), 204(10), 189(8); n.i. 5 K.I.=1770 ($C_{15}H_{24}O$), 220[M^+](10), 159(100), 93(52), 79(46), 105(35), 177(30), 121(25), 135(20), 43(20), 207(20), 187(8).

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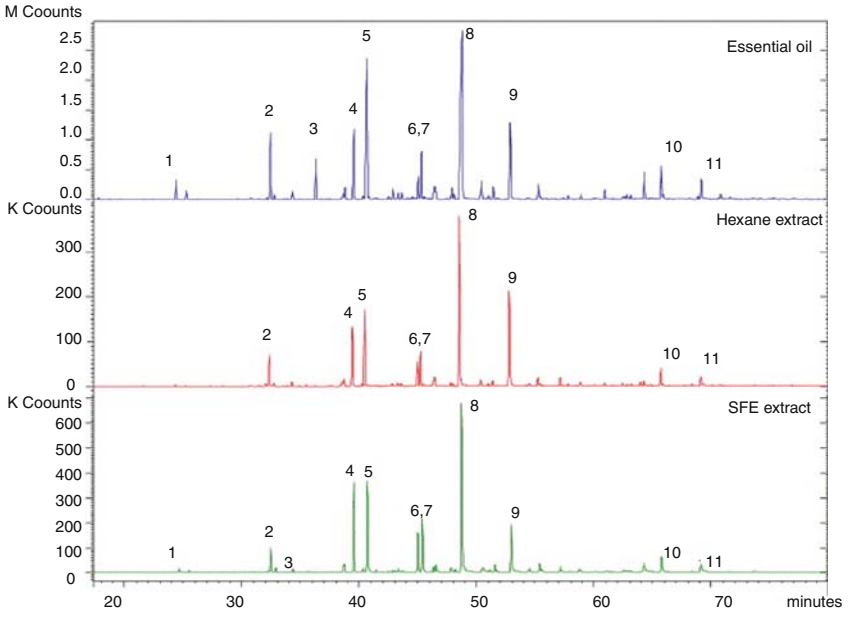

Fig. 3.9 GC/MS chromatograms of geranium products using different techniques: essential oil by hydrodistillation, hexane extract by extraction with organic solvents and SFE extract by SFE, at the reference operating conditions Components present in geranium products: (1) rose oxide; (2) isomenthone; (3) linalool; (4) guaia-6,9-diene; (5) citronellyl formate; (6) germacrene-D; (7) geranyl formate; (8) citronellol; (9) geraniol; (10) geranyl tiglate; (11) 2-phenylethyl tiglate. Reprinted with permission from Gomes et al. (2007)

Table 3.14 Volatile constituents (%) of Clevenger distilled, primary and secondary essential oils of palmarosa

Constituent	Retention index	Clevenger distilled oil	Primary oil			Secondary oil		
			1	2	3	1	2	3
Sabinene	967		0.1	0.1	0.1	–	0	–
Myrcene	983	0.1	0.2	0.1	0.2	t	t	t
Limonene	1022	0.1	0.3	0.1	0.5	0.1	t	0.2
(*Z*)-β-octimene	1031	0.1	0.3	0.3	0.4	t	t	t
E-β-octimene	1042	0.7	1.5	1.3	1.6	0.1	t	t
Linaool	1085	2.3	2.2	2.4	2.3	3.1	3.8	2.6
Citronellol	1211	0.1	0.1	0.1	0.2	0.1	0.1	0.3
Geraniol	1238	84.0	83.8	78.0	85.7	92.1	92.8	91.8
Geranial	1243	–	–	–	–	1.8	1.8	2.0
Geranyl acetate	1356	5.3	4.7	12.0	2.9	0.6	0.4	0.3
Geranyl butirate	1531	0.2	0.2	0.2	0.1	–	–	–
Geranyl isovalerate	1582	0.1	0.1	0.1	t	–	–	–
(*E,Z*)-Farnesol	1693	1.9	1.8	1.5	0.8	0.2	0.2	0.1
Geranyl hexanoate	1723	0.1	0.8	0.7	0.4	0.1	0.1	–

1, 2, 3: distillation batch numbers, t: traces (<0.1%).
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The composition of the essential oils from *Lavandula* species and the efficacy of various extraction methods on the yield of *Lavandula* essential oils have also been vigorously investigated. Thus, the influence of the variation in morphology and in the composition of essential oil in phenotypic regenerated plantlets of *Lavandula vera* was studied in detail. It was concluded from the measurements that sonaclonal variation may facilitate the production of variance with different fragrance in *L. vera* (Tsuro et al., 2001). SFE has also been employed for the fractionation of Lavandin essential oil, and the influence of various technological steps on the composition of the efficacy of fractionation was followed by GC-FID. The components of the essential oil of *Lavandula hybrida* are compiled in Table 3.16. It was found that that high temperature and high operating pressure increase the performance of the continuous-operating high-pressure counter-current packed column (Varona et al., 2008).

The extracting capacity of hydrodistillation (HD), subcritical water extraction (SbCWE) and organic solvent extraction under ultrasonic irradiation (USE) was compared using *Lavandula stoechas* flowers as model analytes. Volatiles were separated by GC-MS. It was established that the main components of the extracts were fenchon, camphor, myrtenyl acetate, mytenol and 1,8-cineol. It was further observed that both the quality and amount of the extract depended on the method of extraction. Because of its rapidity, SbCWE was proposed for the extraction of essential oil from *Lavandula stoechas* (Giray et al., 2008).

Table 3.15a Chemical composition of essential oils of pink-violet and yellow-orange *L. camara* aerial part collected during the rainy season in Madagascar

		Pink-violet[a]		Yellow-orange[b]	
Peak No	Component	Mean	S.D.	**Mean**	S.D.
4	**Sabinene**	**9.385**	**3.132**	**14.27**	**3.24**
7	α-Phellandrene	0.087	0.223	0.146	0.194
10	1,8-Cineole	3.897	0.873	0.828	0.304
13	*p*-Cymene	0.036	0.093	0.310	0.406
14	α-Cubebene	0.307	0.551	0.315	0.555
15	δ-Elemene	0.768	0.506	1.552	0.582
17	Camphor	0.587	0.148	0.685	0.197
18	**Linalool**	**5.028**	**1.59**	**0.377**	**0.371**
19	β-Elemene	0.031	0.144	0.872	0.318
20	β-Caryophyllene	13.64	1.91	30.85	4.05
21	Aromadendrene	0.663	0.362	1.413	0.687
24	γ-Muurolene	1.455	0.290	0.000	0.000
25	α-Terpineol	0.273	0.260	0.352	0.218
26	**β-Bisabolene**	**2.300**	**0.485**	**14.68**	**1.72**
27	α-Selinene	0.480	0.278	0.116	0.153
28	Germacrene-D	4.351	0.933	5.247	1.135
30	γ-Cadinene	0.160	0.218	0.673	0.248
32	Not identified	0.331	0.249	0.000	0.000
33	Not identified	0.475	0.296	0.028	0.077
35	Caryophyllene oxide	0.705	0.306	0.405	0.273
36	*cis*-Nerolidol	0.206	0.260	0.318	0.362
37	**Davanone**	**22.93**	**4.24**	**0.000**	**0.000**
38	Not identified	0.187	0.273	0.136	0.283
40	Cubenol	0.354	0.364	0.060	0.134
41	Not identified	0.410	0.317	0.063	0.109
42	Not identified	0.663	0.309	0.161	0.187
43	Epicubenol	0.719	0.385	0.047	0.101
44	Viridiflorol	0.579	0.460	0.064	0.131
45	Zingiberenol	0.522	0.496	0.089	0.199
48	δ-Cadinol	0.415	0.570	0.017	0.062
49	α-Cadinol	0.268	0.448	0.105	0.277

[a] 22 samples.
[b] 15 samples.
Bold values have been used for showing the main representative compounds.

Because of its importance in perfumery and cosmetics, the extraction methods (Babau and Kaul, 2005) and composition of geranium essential oils have been extensively investigated (Gomes et al., 2004). SFE has also found application for the extraction of the natural essential oil from Portuguese-grown rose geranium and the yield and composition of the essential oil were compared with those obtained by hydrodistillation and hexane extraction. GC-FID and GC-MS were performed in a fused silica capillary column (50 m × 0.25 mm i.d., film thickness 0.20 μm). Initial column temperature was set to 50°C for 5 min, then ramped to 200°C at 2°C/min (final hold 40 min). Carrier gas was helium, FID temperature was 250°C.

Table 3.15b Chemical composition of essential oils of pink-violet and yellow-orange *L. camara* aerial part collected during the dry season in Madagascar

		Pink-violet[a]		Yellow-orange[b]	
Peak No	Component	Mean	S.D.	**Mean**	S.D.
2	Camphene	1.047	0.495	0.000	0.000
4	**Sabinene**	**11.23**	**2.63**	**13.79**	**2.55**
5	Δ^3-Carene	1.596	0.629	1.118	0.320
7	α-Phellandrene	0.000	0.000	1.032	0.319
8	Limonene	1.339	0.334	0.497	0.162
10	1,8-Cineole	3.713	0.799	0.973	0.362
12	*E*-β-ocimene	0.768	0.262	1.988	0.297
14	δ-Cubebene	0.498	0.290	0.000	0.000
15	β-Elemene	0.812	0.217	1.123	0.339
17	Camphor	0.677	0.175	1.583	0.289
18	**Linalool**	**4.810**	**1.814**	**0.580**	**0.215**
20	**β-Caryophyllene**	**11.28**	**2.43**	**29.84**	**3.05**
21	Aromadendrene	0.797	0.312	1.534	0.239
23	α-Humulene	4.405	1.093	2.377	0.359
25	α-Terpineol	0.682	0.576	0.363	0.301
26	**β-Bisabolene**	**1.821**	**0.517**	**14.93**	**1.66**
27	α-Selinene	0.509	0.249	0.405	0.497
29	δ-Cadinene	0.807	0.283	2.118	0.156
30	γ-Cadinene	0.342	0.258	1.378	0.673
32	Not identified	0.831	0.719	0.000	0.000
33	Not identified	0.962	0.592	0.000	0.000
34	*ar*-Curcumene	1.605	0.787	0.688	0.131
35	Caryophyllene oxide	1.208	0.929	0.127	0.179
36	*cis*-Nerolidol	0.264	0.372	0.245	0.279
37	**Davanone**	**22.57**	**6.16**	**0.000**	**0.000**
39	Humulene oxide	2.261	0.920	0.000	0.000
40	Cubenol	0.182	0.212	0.000	0.000
41	Not identified	0.292	0.274	0.000	0.000
42	Not identified	0.966	0.550	0.000	0.000
43	Epicubenol	0.877	0.492	0.000	0.000
45	Zingiberenol	0.813	0.557	0.000	0.000
46	Spathulenol	1.171	0.373	0.297	0.509
48	δ-Cadinol	0.851	0.508	0.000	0.000
49	α-Cadinol	0.937	0.519	0.475	0.283

[a] 18 samples.
[b] 6 samples.
Bold values have been used for showing the main representative compounds.
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Characteristic chromatograms are depicted in Fig. 3.9. The chromatograms illustrate that the composition of the essential oil depends on the method of extraction. The concentrations of the analytes are compiled in Table 3.17. The data demonstrated that, except linalool, the composition of the essential oils extracted by different methods are similar but not identical. It was established that SFE method produces

Table 3.16 Chemical composition and retention times (RT, min) of the essential oil of *Lavandula hybrida*

Component	RT (min)	wt.%
Linalool	10.7	33.2
Linalyl acetate	14.7	29.7
Camphor	11.9	7.1
1,8-Cineole	7.9	7.6
Terpinen-4-ol	13.0	3.3
Lavandulyl acetate	16.5	2.6
Endo-borneol	12.6	2.7
β-Farnesene	20.1	1.9
β-Caryophyllene	21.1	1.4
α-Terpineol	13.4	1.5
Limonene	6.5	0.9
trans-β-Ocimene	8.2	0.4
cis-β-Ocimene	8.5	0.4
Germacrene-*d*	21.7	0.7
β-Myrcene	5.9	0.5
α-Bisabolol	26.7	0.4

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a organoleptically superior essential oil; therefore, its application for the extraction of geranium essential oil is highly advocated (Gomes et al., 2007).

Supercritical CO_2 and subcritical propane were applied for the extraction of cardamom oil, and the extraction procedures were optimised. The composition of the extracts was investigated by HPLC and GC-MS. Pigments (chlorophylls and

Table 3.17 Percentage composition of geranium essential oil and extracts obtained with different extraction techniques

		Traditional techniques		Control sample	Clean technology
No.	Component	Hydrodistillation	Solvent extraction (hexane)	Hydrodistillation	Supercritical extraction (CO_2)
1	Rose oxide	0.5	0.0	0.5	0.4
2	Isomenthone	5.6	2.1	4.8	3.5
3	Linalool	2.7	0.0	4.4	0.1
4	Guaia-6,9-diene	5.9	5.5	7.2	8.8
5	Citronellyl formate	13.2	6.0	11.1	10.2
6	Germacrene-D	2.4	3.2	2.5	4.6
7	Geranyl formate	5.5	4.7	3.7	7.9
8	Citronellol	26.9	21.3	26.5	24.8
9	Geraniol	8.1	10.8	8.4	8.5
10	Geranyl tiglate	3.3	3.3	3.1	3.3
11	2-Phenylethyl tiglate	1.8	2.2	1.9	1.8
	Total	75.9	59.2	74.2	73.8

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carotenoids) were separated by RP-HPLC using a C18 column (250 × 46 mm, particle size, 5 μm). Solvents A and B were methanol–water (90:10 v/v) and acetonitrile–isopropanol–methanol (35:55:10 v/v/v), respectively. Gradient started at 100% A changed to 100% B in 20 min, final hold 5 min. Fatty acids were measured by GC. Volatile components were extracted by a PDMS fibre and separated in a capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Initial column temperature was 60°C for 10 min, then ramped to 200°C at 10°C/min (final hold 5 min). Carrier gas was helium. Tocopherols were separated by normal-phase HPLC using a 240 × 4.6 mm column and *n*-hexane–absolute alcohol 99.6:0.4 (v/v) mobile phase. Excitation and emission wavelengths of the fluorescence detector were 295 and 330, respectively. The amount of the main aroma substances extracted under various conditions are compiled in Table 3.18. It was concluded from the results that both extraction procedures are suitable for the extraction of essential oils from cardamom seeds, the yield achieved by the use of subcritical propane being higher than that of CO_2 extraction (Hamdan et al., 2008).

Cold-pressing method was applied for the isolation of the essential oil of *Citrus aurantifolia* Persa (lime) from Vietnam and the volatiles were separated and identified by GC-MS and GCO. GC-MS separated 96 and identified 92 volatile compounds in the essential oil. The main constituents of the essential oil were limonene (73.5%), geranial (8.4%), neral (4.9%), myrcene (2.1%) and β-bisabolene. GCO results suggested that neryl acetate, β-bisabolene, 1-carvone, geranyl acetate, α- and β-cironellol, cumin aldehyde, perillaldehyde, nerol, tridecanal, germacrene-B, geraniol, dodecyl acetate, caryophyllene oxide and perillyl alcohol contribute to the aroma of *C. aurantifolia* (Phi et al., 2006).

Microwave "dry" distillation or microwave accelerated distillation (MAD) was applied for the extraction of essential oils. The method combines the microwave heating with dry distillation. The extraction is performed at atmospheric pressure without water or organic solvent. The efficacy of MAD procedure was compared with that of hydrodistillation. The components of the essential oil of Rosemary were analysed by GC-FID and GC-MS. It was established that MAD is an energy-saving method, the extraction time is shorter and the yield and product quality is superior to hydrodistillation (Tigrine-Korjani et al., 2006).

The essential oil of fresh plant, fresh and dry fruit of *Peucedanum verticillare* was extracted by hydrodistillation (5 h) and the volatile substances were separated and identified by GC-FID and GC-MS. GC-FID was performed in a capillary column (30 m × 0.2 mm i.d., film thickness 0.2 μm). Initial column temperature was 80°C (3 min hold), then ramped to 300°C at 5°C/min. Carrier gas was helium. Injector and detector temperatures were 200°C and 300°C, respectively. GC-MS was performed under identical conditions, ionisation voltage being 70 eV. The results of GC separations are compiled in Table 3.19. The data illustrated that the composition of essential oils obtained from the various parts of *Peucedanum verticillare* show marked differences. The main components in leaf and branch were sabinene and (*E*)-anethol, sabinene in fresh fruit, and β-caryophyllene, phellandrene, (*Z*)-β-farnesene and β-bisabolene in dried fruit (Fraternale et al., 2000).

Table 3.18 Effect of extraction conditions on the content of the major aroma components in the extract of cardamom seeds

SFE conditions		Integrated peak area (10 μl extract $\times 10^3$)				
Pressure (MPa)	Temperature (K)	β-Pinene	Cineole	Linalool	α-Terpineol	Terpinyl acetate
Extraction with CO_2						
30	308	17.4 ± 1.04	341 ± 14	32.7 ± 1.6	46.4 ± 2.3	340 ± 20
20	308	20.2 ± 1.16	362 ± 21	55.0 ± 3.8	62.5 ± 4.4	450 ± 36
10	308	27.6 ± 2.21	450 ± 27	73.5 ± 5.8	91.2 ± 5.1	579 ± 47
10	298	18.6 ± 1.67	336 ± 23	42.2 ± 2.5	52 6 ± 3.9	406 ± 29
8	298	16.1 ± 1.13	295 ± 22	34.8 ± 2.1	47.8 ± 3.7	356 ± 25
Extraction with propane						
5	298	26.9 ± 1.72	386 ± 25	72.1 ± 4.3	82.7 ± 3.6	521 ± 21
2	298	15.5 ± 0.92	286 ± 19	25.6 ± 1.4	36.9 ± 1.8	304 ± 18
Extraction with Co_2+ethanol						
	298	6.5 ± 0.26	198 ± 8	5.8 ± 0.4	8.9 ± 0.6	112 ± 7

The values are the averages of two replications±S.D.
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Table 3.19 Composition of the essential oil of *Peucedanum verticillare*

Compound	Retention indices	Fresh plant	Fresh fruits	Dried fruits
α-pInene	938	6.3	–	–
α-Fenchene	952	–	0.2	–
Camphene	954	0.7	1.2	–
Sabinene	977	39.6	63.0	–
β-Pinene	981	–	1.6	–
β-Myrcene	993	4.7	8.1	–
α-Phellandrene	1007	5.6	9.3	20.8
(*Z*)-β-Ocimene	1041	0.8	∗–	2.7–
(*E*)-β-Ocimene	1052	–	1.6	3.8
γ-Terpinene	1063	0.8	0.9	–
Fenchone	1088	–	0.3	–
Undecane	1103	–	0.2	–
Epicamphor	1145	7.8	–	–
cis-*p*-2-menthen-1-ol	1172	–	0.2	–
Nerol	1229	–	3.5	–
(*E*)-Anethole	1285	29.5	1.8	–
Lavandulyl acetate	1291	0.2	–	–
Geranyl acetate	1385	–	0.4	5.0
β-Cubebene	1390	–	–	7.5
β-Elemene	1391	0.3	0.6	–
Linalyl-3-methylbutanoatecis	1395	–	0.4	–
cis-α-bergamotene	1416	–	0.4	–
β-caryophyllene	1418	0.3	2.0	24.2
trans-α-bergamotene	1437	–	0.4	5.3
(*Z*)-β-Farnesene	1444	–	1.1	12.8
α-Humulene	1455	–	0.4	–
(*E*)-β-Farnesene	1459	0.4	0.9	–
cis-caryophyllene	1465	–	–	2.0
β-Bisabolene	1510	–	0.8	9.0
γ-Cadinene	1513	1.9	–	–
Germacrene-B	1557	–	0.3	–
Caryophyllene oxide	1582	–	–	6.7

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Chapter 4
Biological Effect

As it has been indicated in Chaps. 2 and 3, aroma substances and their natural mixtures have marked beneficial biological activity and can be successfully used in human health care as ingredients of traditional medicines. In order to elucidate the biophysical and biochemical aspects of the formation of natural aroma compounds, the biosynthesis, the enzymatic procedures resulting in the development of volatile compounds, the artefact formation during extraction, the adsorption of fragrances on various complex matrices and the determination of various emission patterns have also been investigated using various chromatographic technologies.

4.1 Biochemistry and Biophysics

The biosynthesis of mono- and sesquiterpenes in carrot roots and leaves (*Daucus carota* L.) was studied by using stir bar adsorption on PDMS fibre and the following separation by GC-MS and TD-MDGC-MS (Kreck et al., 2001). The analysis of aroma compounds in the essential oil of carrots (*Dausus carota* L. ssp. *sativus*) and the comparison of the aroma composition of leaves and roots (Habegger and Schnitzler, 2000), the use of large volume injection for the GC analysis of aroma substances in carrot cultivars (Kjeldsen et al., 2001) and the effect of refrigeration and frozen storage on the aroma profile of carrots were previously reported (Kjeldsen et al., 2003). GC-MS analysis of synthesised terpinolene and caryophyllene was performed on a capillary column (30 m × 0.25 mm i.d.; film thickness, 0.23 μm). Helium was the carrier gas. Oven temperature started at 40°C (5 min hold), then increased to 260°C at 5°C/min (final hold 20 min). Mass range was 40–300 *m/z*. TD program started at 10°C, ramped to 250°C at 60°C/min. Chiral separations were carried out on a capillary column (30 m × 0.25 mm i.d.) coated with heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butylmethylsilyl)- β-cyclodextrin in SE-52. The film thickness was 0.23 μm. A chiral chromatogram is depicted in Fig. 4.1, showing the separation of unlabelled and labelled terpinolene. The results suggested that the biosynthesis of terpenes is mainly localised in phloem (Hampel et al., 2005).

The influence of (*E*)-β-caryophyllene synthase (OsTPS3) for the production of volatile sesquiterpenes in rice was investigated applying GC-MS for the identification of enzymatic products, RNA analysis and bioassays The concentrations of

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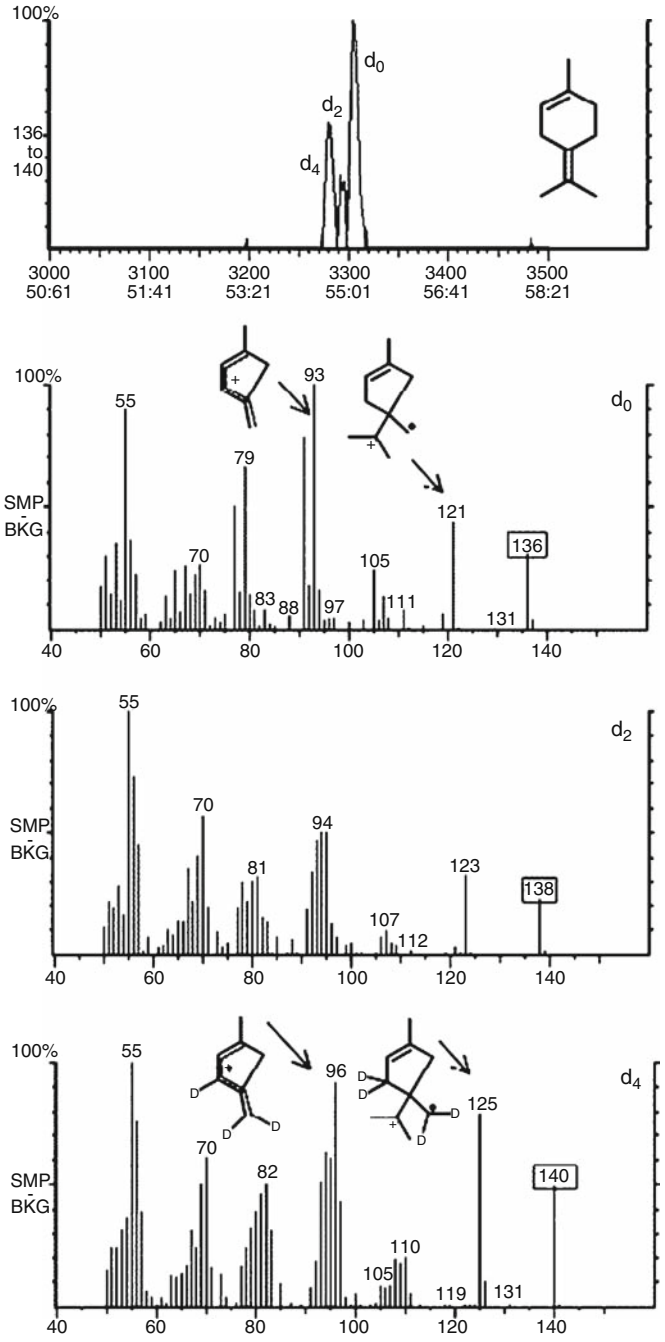

Fig. 4.1 Chiral main column chromatogram and MS spectra of unlabelled and labelled terpinolene obtained from a SBSE–MDGC–MS-analysis of *Daucus carota* L. cv. Kazan when [5,5-$^{2}H_2$]-DOX is administered to root phloem. Reprinted with permission from Hampel et al. (2005)

Table 4.1 Volatile sesquiterpenes of the transgenic (O5 and O7) and the wild-type (WT) rice seedlings (24 h after MeJA treatment)

	WT		05		07	
Compounds	ng/g FW[a]	%	ng/g FW[a]	%	ng/g FW[a]	%
β-Elemene	9.8 ± 1.7	6.1	15.1 ± 3.2	8.9	12.1 ± 2.6	7.2
(E)-β-Caryophyllene	28.7 ± 5.2	17.9	52.9 ± 9.6	31.1	45.7 ± 9.4	27.2
β-Farnesene	12.0 ± 2.9	7.5	12.2 ± 2.1	7.2	14.8 ± 2.7	8.8
α-Humulene	5.0 ± 1.3	3.1	3.9 ± 0.6	2.3	3.2 ± 0.6	1.9
α-Longipinene	17.4 ± 3.6	10.9	8.3 ± 1.4	4.9	8.4 ± 1.4	5
α-Curcumene	27.4 ± 6.1	17.1	28.5 ± 4.9	16.8	32.3 ± 6.1	19.2
Zingiberene	28.8 ± 5.4	18	26.7 ± 5.1	15.7	27.4 ± 4.9	16.3
β-Bisabolene	11.5 ± 2.5	7.2	7.5 ± 1.2	4.4	8.4 ± 1.8	5
γ-Curcumene	5.3 ± 1.2	3.3	4.6 ± 0.8	2.7	5.4 ± 1.2	3.2
β-Sesquiphellandrene	8.6 ± 1.9	5.4	6.6 ± 1.4	3.9	6.5 ± 1.6	3.9
γ-Bisabolene	5.6 ± 1.0	3.5	3.7 ± 0.6	2.2	3.7 ± 0.7	2.2

[a] Mean ± standard error from three independent experiments.
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volatile sesquiterpenes are compiled in Table 4.1. It was concluded from the results that the rice sesquiterpene synthase catalyses the formation of (*E*)-β-caryophyllene and other sesquiterpenes such as β-elemene and α-humulene (Cheng et al., 2007).

The effect of extraction conditions on the composition of indole alkaloids in the mushroom *Cortinarius infractus* was investigated by employing HPLC-MS technique. Separations were carried out in a RP column (150 mm × 4.6 mm i.d.; particle size, 3 μm). Isocratic mobile phase consisted of acetonitrile–0.1 N ammonium hydroxide–500 μM phosphate buffer, pH 2.5 (60:20:20 v/v/v). MS spectra were detected at 50–1,000 *m/z*. A TIC chromatogram is depicted in Fig. 4.2. It was established that β-carboline-1-propionic acid is the main indole alkaloid in *C. infractus* (Brondz et al., 2007).

The composition of volatile compounds emitted from different parts of *Citrus limon* (Rutaceae) was analysed by HS-SPME-GC-MS and HS-SPME-GC-FID. The volatile profile of flower buds, mature flowers, petals, stamens, pollen, gynaeceum, pericarp of unripe fruits, pericarp of ripe fruits, adult leaves and essential oil from the expression of ripe pericarp was determined. Volatiles were extracted by PDMS fibres. GC-FID was performed in a capillary column (30 m × 0.25 mm i.d.; film thickness, 0.23 μm). Nitrogen was the carrier gas. Oven temperature started at 60°C (10 min hold), then increased to 220°C at 5°C/min. Injector and detector temperatures were 250°C. GC-MS was accomplished in a capillary column of similar dimensions. Oven temperature started at 60°C and ramped to 240°C at 3°C/min. Carrier gas was helium. The volatiles emitted by the different parts of *C. limon* are compiled in Table 4.2. The data demonstrated that the emission profile of various parts shows marked differences. It was further established that these HS-SPME-GC-FID and HS-SPME-GC-MS techniques are suitable for the investigation of pollination chemistry and animal–plant relationships (Flamini et al., 2007).

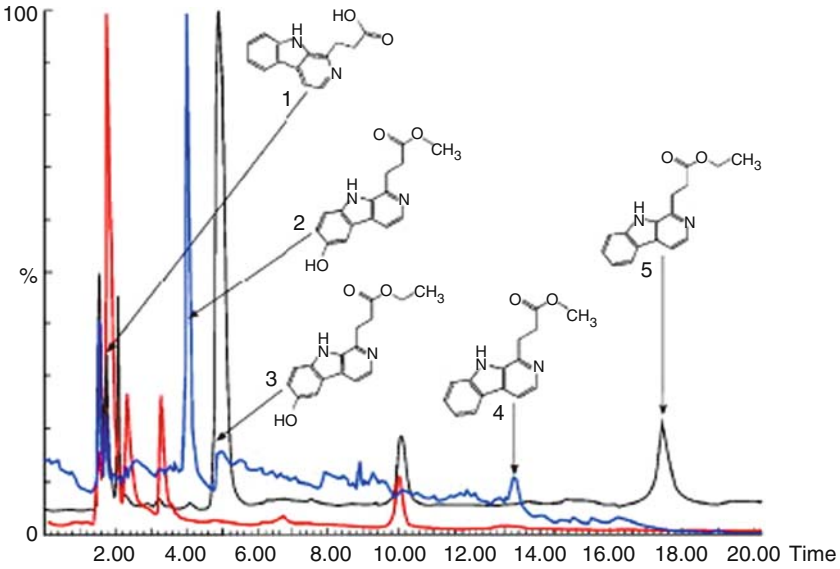

Fig. 4.2 Total ion current (TIC) recorded for HPLC–MS analysis of extracts of *Cortinarius infractus*. The structures of substances resolved are (1) β-carboline-1-propionic acid; (2) 6-hydroxyinfractine; (3) ethyl analogue of 6-hydroxyinfractine; (4) infractine; and (5) ethyl analogue of infractine. Reprinted with permission from Brondz et al. (2007)

GC-FID and electroantennographic detection (EAD) were applied for the separation and identification of active components of the abdominal sex-attracting secretion of the male dung beetle, *Kheper bonelli*. Active compounds were extracted with dichloromethane and used for GC-FID/EAD measurements. Separations were carried out in a capillary column (40 m × 0.3 mm i.d.; film thickness, 0.38 μm). Helium was the carrier gas. Oven temperature started at 40°C, then increased to 250°C at 2 or 4°C/min. Enantioselective separation was performed in a capillary column (30 m × 0.3 mm i.d.; film thickness, 0.38 μm) using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butylmethylsilyl)-β-cyclodextrin as chiral selector. Hydrogen was the carrier gas. Oven temperature started at 40°C, then increased to 90°C at 10°C/min (1 min hold), to 120°C at 10°C/min (10 min hold), to 160°C at 10°C/min. MS measurements were accomplished using the capillary columns listed above. TIC chromatogram of the abdominal sex pheromone glands is shown in Fig. 4.3. The main components were the branched and unbranched chain fatty acids, alcohols and esters, which did not elicit EAD responses. The FID/EAD chromatogram is depicted in Fig. 4.4.

It was established that the main components of the secretion were propanoic acid, butanoic acid, indole, 3-methylindole (skatole) and methyl *cis*-cascarillate (methyl *cis*-2,2′-hexylcyclopropylacetate) (Burger et al., 2008).

The interaction of aroma substances with each other and with the other components in foods and food products and in living organisms can influence considerably

Table 4.2 In vivo volatile emission of different parts of *Citrus limon*

Constituents[a]	I.r.i.[b]	Flower buds (1)	Mature flowers (2)	Petals (3)	Stamens (4)	Pollen (5)	Gynaec. (6)	Unripe peric. (7)	Ripe peric. (8)	Young leaves (9)	Adult leaves (10)	Express. ess. oil (11)
α-Thujene	932	–[c]	–	–	–	–	0.2	0.2	0.2	–	–	0.4
α-Pinene	942	2.4	0.5	tr[d]	0.5	–	0.9	1.0	1.6	1.0	0.6	1.9
Camphene	955	–	–	–	–	–	–	–	–	–	–	0.1
Sabinene	978	3.4	1.6	–	1.4	–	1.5	1.5	1.8	2.4	1.8	2.4
β-Pinene	982	24.0	7.8	0.9	5.8	–	7.9	9.2	12.0	6.3	6.2	13.7
Myrcene	992	0.4	1.4	–	0.7	–	1.4	2.2	1.5	2.7	–	1.6
Octanal	1002	–	–	–	–	–	–	–	–	–	–	tr
(*E*)-3-Hexen-1-ol acetate	1004	–	–	–	–	–	–	–	–	–	1.4	–
α-Phellandrene	1008	–	–	–	–	–	–	–	–	–	–	tr
3-Carene	1013	–	–	–	–	–	0.1	–	–	2.1	0.2	–
α-Terpinene	1020	–	0.1	–	0.2	–	0.3	0.3	0.3	–	–	0.3
p-Cymene	1028	–	0.2	–	0.4	–	0.4	0.1	–	–	0.2	0.1
Limonene	1033	38.9	44.3	3.1	22.9	–	62.5	65.3	68.3	65.3	30.1	62.5
1,8-Cineole	1035	–	6.7	10.2	23.1	–	1.7	–	tr	–	–	–
(*E*)-β-Ocimene	1051	7.0	5.4	3.9	3.1	–	3.1	0.1	0.1	3.0	5.4	tr
γ-Terpinene	1064	6.0	6.7	0.7	4.5	–	9.2	11.5	11.4	0.3	0.3	11.6
cis-Sabinene hydrate	1070	–	–	–	–	–	0.2	0.1	–	–	–	–
Terpinolene	1088	0.4	0.5	–	0.3	–	0.8	0.7	0.6	0.5	–	0.6
Linalool	1101	0.2	0.2	2.7	0.2	–	1.5	0.2	–	0.5	–	tr
trans-Sabinene hydrate	1103	–	–	–	0.2	–	–	0.3	–	–	–	–
Nonanal	1106	–	–	–	–	6.7	–	–	–	–	1.0	tr
allo-Ocimene	1131	tr	0.2	–	–	–	0.1	–	–	–	–	–
Limonene oxide	1136	–	–	–	–	–	0.2	–	–	–	–	–

Table 4.2 (continued)

Constituents[a]	I.r.i.[b]	Flower buds (1)	Mature flowers (2)	Petals (3)	Stamens (4)	Pollen (5)	Gynaec. (6)	Unripe peric. (7)	Ripe peric. (8)	Young leaves (9)	Adult leaves (10)	Express. ess. oil (11)
Citronellal	1155	–	–	–	–	–	–	–	–	0.2	–	0.2
4-Terpineol	1179	–	–	–	–	–	0.1	–	–	–	–	–
α-Terpineol	1192	tr	–	–	–	tr	0.5	0.2	0.2	0.2	–	tr
Decanal	1202	tr	–	–	–	tr	–	–	–	–	1.5	tr
Nerol	1228	–	–	–	–	–	0.1	1.3	0.2	0.1	1.3	–
Neral	1243	–	–	–	–	tr	–	0.2	tr	6.4	0.8	1.0
Geraniol	1256	–	–	–	–	–	–	0.4	0.2	0.1	0.7	–
Geranial	1272	–	–	–	–	–	–	0.2	tr	5.5	1.3	1.4
Indole	1289	–	–	4.8	2.5	tr	–	–	–	–	1.0	–
Tridecane	1300	–	–	0.1	0.2	–	–	–	–	–	–	–
Methyl anthranylate	1338	–	0.7	8.4	0.2	–	–	–	–	–	–	–
δ-Elemene	1340	0.3	0.2	–	–	–	0.1	–	–	–	0.8	–
Citronellyl acetate	1356	–	–	–	–	–	–	–	–	–	–	tr
Eugenol	1358	–	–	–	–	–	–	–	–	–	–	tr
Neryl acetate	1365	–	–	–	–	–	0.1	0.3	0.2	0.1	–	0.6
Geranyl acetate	1385	–	–	–	–	–	–	0.5	0.2	–	–	0.6
1-Tetradecene	1393	–	–	–	0.2	–	–	–	–	–	–	–
Tetradecane	1400	–	–	0.2	0.2	tr	–	–	–	–	–	–
Italicene	1404	–	–	–	–	–	–	0.4	–	–	–	–
Dodecanal	1411	–	–	–	–	–	–	–	–	–	1.2	–
cis-α-Bergamotene	1415	tr	0.1	–	–	–	0.1	–	–	–	–	–
β-Caryophyllene	1419	11.3	9.5	2.5	–	14.5	3.7	1.1	0.1	2.3	25.1	0.3
trans-α-Bergamotene	1436	1.2	1.4	0.2	–	3.2	0.7	–	–	0.2	2.8	0.2
(E)-Geranyl acetone	1453	–	–	0.2	1.9	9.0	–	–	–	–	1.9	–

Table 4.2 (continued)

Constituents[a]	I.r.i.[b]	Flower buds (1)	Mature flowers (2)	Petals (3)	Stamens (4)	Pollen (5)	Gynaec. (6)	Unripe peric. (7)	Ripe peric. (8)	Young leaves (9)	Adult leaves (10)	Express. ess. oil (11)
α-Humulene	1456	0.6	0.6	0.2	–	–	0.3	–	–	0.1	1.0	–
(*E*)-β-Farnesene	1458	–	0.1	0.2	–	–	–	–	–	–	–	–
β-Santalene	1463	–	–	–	–	–	0.1	–	–	–	–	–
(*Z*,*E*)-α -Farnesene	1491	–	–	0.5	0.2	–	0.2	–	–	–	–	–
Valencene	1493	tr	–	–	–	–	–	0.1	–	–	–	–
Bicyclogermacrene	1496	1.3	0.9	0.2	–	4.4	0.5	–	–	0.1	3.5	tr
Pentadecane	1500	–	0.9	4.2	2.0	2.2	–	–	–	–	–	–
(*E*,*E*)-α -Farnesene	1507	–	0.3	9.1	2.9	–	tr	–	–	–	4.4	–
β-Bisabolene	1509	1.6	1.1	0.2	–	5.2	1.0	0.4	–	0.1	1.8	0.3
trans-Nerolidol	1565	–	4.5	27.3	14.2	30.7	tr	–	–	–	–	–
Caryophyllene oxide	1583	tr	–	–	–	–	tr	–	–	–	–	–
Germacrene D-4-ol	1578	tr	tr	–	–	–	tr	–	–	–	0.7	–
Hexadecane	1600	–	–	–	–	1.8	–	–	–	–	–	–
Isolongifolan-7-α-ol	1621	–	–	13.6	6.9	10.0	–	–	–	–	–	–
1-Heptadecene	1673	–	–	–	0.3	–	–	–	–	–	–	–
(*Z*,*E*)-α -Farnesol	1697	–	0.2	–	–	–	–	–	–	–	–	–
Heptadecane	1700	–	0.1	0.2	0.2	–	–	–	–	–	–	
(*E*,*E*)-α -Farnesol	1792	–	0.3	–	–	–	–	–	–	–	–	–
Total identified		99.0	96.5	93.6	95.2	87.7	99.5	97.8	98.9	99.5		

[a] Percentages (by weight) obtained by FID peak-area normalisation.
[b] Linear retention indices (HP-5 column).
[c] Not detected.
[d] tr < 0.1%.
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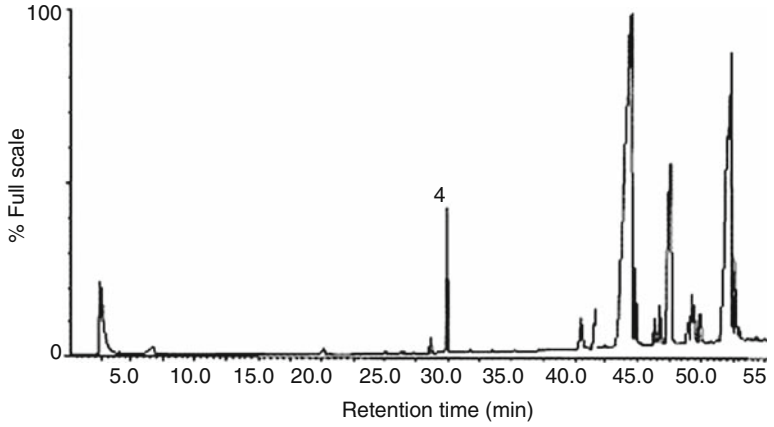

Fig. 4.3 Total ion chromatogram (TIC) of an extract of the abdominal sex pheromone glands of the dung beetle, *Kheper bonelli*. Reprinted with permission from Burger et al. (2008)

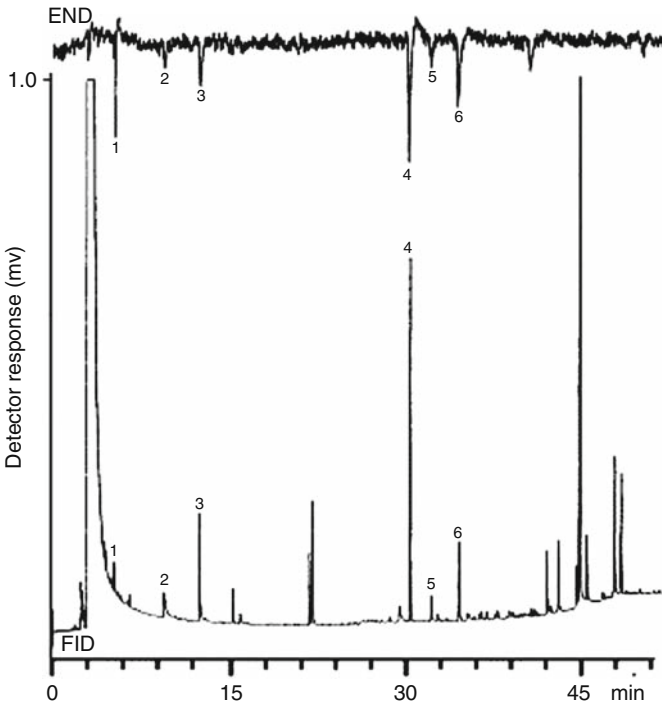

Fig. 4.4 Gas chromatographic analysis with FID and EAD recording in parallel of an extract of the abdominal sex pheromone glands of the dung beetle, *Kheper bonelli*. Peaks are numbered to indicate simultaneous detector responses. Reprinted with permission from Burger et al. (2008)

the sensory characteristics and the stability of the aroma compounds. Moreover, the binding of fragrance molecules to bioactive macromolecules may have a marked impact on the various biochemical functions of the fragrance–macromolecule complexes. Because of the commercial and scientific importance of such interactions, they were vigorously investigated by using the same GC methods as used for the separation and quantitative determination of the individual not-bonded aroma substances. Thus, inverse GC (IGC) was applied for the measurement of the interaction of cotton fabrics with various aroma compounds. Dimetol, decanal, phenylethanol and benzyl acetate were included in the experiments using a packed column of 1.8 m × 3 mm i.d. The column was packed by small pieces of a cotton towel, employing a vacuum pump. The surface of the cotton sample was characterised by the determination of the components of surface energy. The retention time in infinite dilution of hexane, heptane, nonane and decane was measured. These data were employed for the determination of the dispersive component of the surface energy. The retention time in infinite dilution of acetone, tetrahydrofuran, chloroform and ethyl acetate was applied for the calculation of the specific components of surface energy. The enthalpy and entropy of the adsorption of fragrances were calculated from their retention time at infinite dilution. The Henry's constant and the enthalpy and entropy of the adsorption of undecane and dimetol are compiled in Table 4.3. It was found that the adsorption enthalpies are higher for polar molecules, suggesting the decisive role of hydrogen bond in the adsorption of fragrances on cotton surface (Cantergiani and Benczédi, 2002).

TLC and RP-HPLC were employed for the investigation of the in vivo dermal adsorption and metabolism of [4-^{14}C]coumarin in rats and human volunteers. TLC measurements were accomplished on C18 plates using various mobile phases such as acetonitrile–water (80:20,v/v), hexane–ethyl acetate–acetic acid (80:20:1,v/v/v), dichloromethane–hexane (80:20,v/v) and chloroform–methanol–acetic acid (90:10:5,v/v/v). *O*-Hydroxyphenylacetic

Table 4.3 Henry's constant, enthalpy of adsorption (dH) and entropy of adsorption (dS) of undecane and dimetol

Relative humidity (%)	Henry's constant (nmol/Pa g)	dH (kJ/mol)	dS (J/mol K)
Undecane			
0	54	–33	–11
20	40	–54	–84
50	34	–56	–92
80	26	–56	–91
Dimetol			
0	350	–70	–112
20	210	–71	–119
50	160	–81	–148
80	96	–78	–147

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acid, 4-hydroxycoumarin, 7-hydroxycoumarin, 2-hydroxycinnamic acid, 3-hydroxycoumarin, 6,7-dihydrLoxycoumarin and coumarin served as reference compounds. RP-HPLC measurements were performed in two different systems. System 1 employed a C18 column (250 mm × 4.6 mm i.d.; particle size, 5 μm) using mobile phases consisting of 0.5% aqueous acetic acid and methanol. Analytes were detected by a radioactive detector and at 254 nm. System 2 applied a similar C18 column, the mobile phase and detection methods being identical as in system 1. Some HPLC radiochromatograms are depicted in Fig. 4.5. The presence of 7-hydroxycoumarin and its β-glucuronide and sulphate conjugates, *o*-hydroxyphenylacetic acid, was

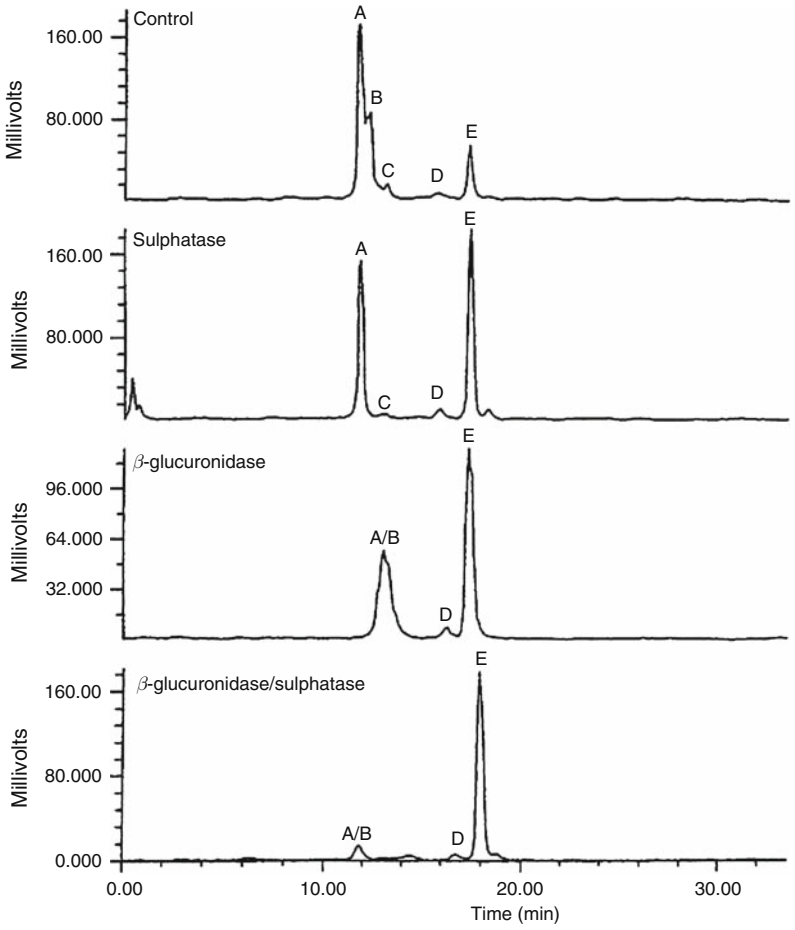

Fig. 4.5 HPLC radiochromatograms of 0–2 h urine after dermal application of ^{14}C-coumarin to volunteer 1. The top graph shows component metabolites before treatment with enzymes. Metabolites are 7-hydroxycoumarin glucuronide (**A**), 7-hydroxycoumarin sulphate (**B**), an unidentified metabolite (**C**),*O*-hydroxyphenylacetic acid (**D**) and 7-hydroxycoumarin (**E**). Reprinted with permission from Ford et al. (2001)

demonstrated. The distribution of ^{14}C-coumarin after various exposure times is compiled in Table 4.4. The results indicated that the metabolism of coumarin is markedly different in rats and humans; therefore, the data obtained in rats cannot be extrapolated to humans (Ford et al., 2001).

The effect of diet on the boar taint, an objectionable odour in meat of some male pigs, has been investigated many times. It was found the dietary ingredients might influence the indole level in the intestine (Knarreborg et al., 2002; Willig et al., 2005; Zamaratskaia et al., 2005a, b, 2006). The impact of the genetic background on the androstenone level has also been demonstrated (Lee et al., 2005; Babol et al., 2004). The influence of raw potato starch and live weight on the concentration of skatole, indole and androstenone in the plasma and fat of entire male pigs was assessed by using ELISA test and HPLC. A C18 column (60 × 4.6 mm; particle size, 3 μm) was employed for the separation and quantitation of androstenone, skatole and indole in fat. Measurements were performed at 40°C. Mobile phase for the analysis of androstenone consisted of tetrahydrofuran–acetonitrile–25 mM sodium phosphate buffer–acetic acid (34:23.8:41.4:0.8). The excitation and emission wavelengths of the fluorescence detector were 346 and 521 nm, respectively. Skatole and indole were separated using a different mobile phase (tetrahydrofuran–25 mM sodium phosphate buffer–acetic acid, 31:67.6:1.4). Fluorescence detection was employed with the excitation and emission wavelengths being 285 and 340 nm, respectively. The determination of skatole and indole in plasma was accomplished in a C18 column (250 × 4 mm; particle size, 5 μm) using gradient elution. Solvents A and B were 10 μM potassium dihydrogen phosphate (pH 3.9)–acetonitrile (9:1) and acetonitrile–water (9:1), respectively. Gradient profile was 0–5 min, 75% A; 5–7 min, 20% A; 7–12 min, 0% A; 12–17 min, 75% A. Also in this case fluorescence detection was employed. The levels of androstenone, skatole and indole found in the plasma and fat of entire pigs are compiled in Table 4.5. It was concluded from the results that the dietary raw potato starch decreases skatole levels in fat and plasma

Table 4.4 Fate of ^{14}C-coumarin after dermal application of 1 mg/kg to male rats

	Time (h after dose application)								
Sample	0.5	1	3	6	12	24	48	72	120
Urine	NC	NC	NC	32.2	38.2	44.3	48.2	48.2	49.7
Faeces	NC	NC	NC	0.09	7.23	17.5	14.08	18.9	21.2
Air	NC	NC	NC	0.17	0.15	0.07	0.10	0.14	0.08
Tissues	16.2	26.5	25.2	31.9	19.5	6.6	2.4	1.8	1.3
Total absorbed	64.4	65.1	68.5	64.8	69.0	72.3			
Treated skin	13.4	13.8	9.7	5.4	4.0	2.91	3.58	4.09	1.08
Skin washings	47.6	34.1	26.9	13.8	16.1	13.4	14.0	11.2	10.1
Gause dressing	NC	NC	NC	0.33	0.26	0.16	0.15	0.13	
Cage wash	NC	NC	NC	0.94	1.02	1.73	0.82	0.31	0.34
Total recovery	–	–	–	84.5	86.5	86.8	83.3	84.7	83.9

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Table 4.5a Least-squares means and 95% confidence intervals (within brackets) of skatole, androstenone and indole levels in plasma from entire male pigs with different live weights and diets

	Live weight and diet				*P*-value	
Plasma (ng/ml)	90 kg	100 kg	115 kg no RPS	115 kg+RPS	Weight and diet	Sire
Skatole	4.67[a] (3.90–5.59)	4.50[a] (3.70–5.48)	3.75[a] (2.93–4.79)	0.70[b] (0.55–0.89)	0.001	0.004
Indole	1.41 (1.21–1.64)	1.50 (1.27–1.77)	1.62 (1.31–2.00)	1.67 (1.36–2.05)	0.303	0.001
Androstenone (direct ELISA)	16.00 (13.46–19.02)	18.10 (14.85–22.07)	16.58 (12.96–21.21)	18.55 (14.63–23.52)	0.479	0.107
Androstenone (after extraction)	4.29[a] (3.49–5.27)	4.16[a] (3.25–5.33)	4.46[a] (3.24–6.12)	2.20[b] (1.62–2.98)	0.001	0.122

Least-squares means with different subscript differ ($p < 0.05$)

Table 4.5b Least-squares means and 95% confidence intervals (within brackets) of skatole, androstenone and indole levels in fat from entire male pigs with different live weights and diets

	Live weight and diet			*P*-value	
Fat (μg/g)	90 kg	115 kg no RPS	115 kg+RPS	Weight and diet	Sire
Skatole	0.06[a] (0.04–0.09)	0.05[a] (0.04–0.08)	0.01[b] (0.01–0.01)	0.001	0.026
Indole	0.02 (0.01–0.03)	0.02 (0.01–0.03)	0.02 (0.01–0.03)	0.644	0.003
Androstenone	0.44[a] (0.27–0.71)	0.90[b] (0.59–1.38)	0.64[a,b] (0.42–0.96)	0.073	0.006

Least-squares means with different superscripts differ ($P < 0.05$).
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and the androstenone level in plasma, but did not influence the indole concentration neither in fat nor in plasma. The living weight did not affect the skatole levels (Chen et al., 2007).

4.2 Toxicity Studies

The considerable price of natural fragrances and aroma compounds made necessary the development of new synthetic pathways for the industrial-scale production of synthetic aroma substances (Joss et al., 2005). While the overwhelming majority of natural fragrances do not show any toxicity, it was many times illustrated that the synthetic products can have noxious side effects (Schreurs et al., 2004; Sköld et al., 2002). The allergy to perfumes has been recently discussed in detail (Pons-Guiraud, 2007). Because of their wide-spread application in perfumery, a considerable number of chromatographic systems were developed for their separation and quantitative determination. Thus, a sequential dual-column GC-MS method was developed and successfully applied for the analysis of 24 raw materials. Measurements were performed on two capillary columns (column 1: 50 m × 0.25 mm i.d.; film thickness, 0.25 μm; column 2: 0 m × 0.25 mm i.d.; film thickness, 0.25 μm). Helium was the carrier gas. Oven temperature of column 1 started at 50°C (1 min hold), then increased to 250°C at 12°C/min (11 min hold), cooled at –40°C to 120°C (final hold 3 min). Oven temperature of the second column started at 120°C (3 min hold), then increased to 216°C at 4°C/min, to 250°C at 10°C/min (final hold 13 min). Helium was used as carrier gas. Injector temperature was 250°°C. MS detection range was 30–372 *m/z*. The following fragrances were included in the experiments: amylcinnamyl alcohol, amyl cinnamal, anisyl alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, cinnamyl alcohol, cinnamal, citral (mixture of neral and geranial), citronellol, coumarin, eugenol, farnesol (main isomers, *ZE* and *EE*), geraniol, hexyl cinnamic aldehyde, hydroxy-citronellal, isoeugenol, 2-(4-*tert*-butylbenzyl) propionaldehyde, *d*-limonene, linalool, hydroxy-methylpentyl-cyclohexenecarboxaldehyde, methyl heptin carbonate and 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-buten-2-one. The measurements indicated that quantification limit for the allergens in mixture was lower than 4 mg/kg. The method was proposed for the determination of allergens in fragrance raw material and perfume oils (Leis et al., 2005).

Another study applied various capillary columns for the separation of allergens. Column length varied between 20 and 60 m, i.d. was 0.18 or 0.25 mm, film thickness 0.18 and 0.25 μm. The ionisation energy of the MS detector was 70 eV. The fragrances investigated are listed below: benzyl alcohol, phenylacetaldehyde, limonene, linalool, methyl-2-octynoate, estragol, citronellol, citral (neral), geraniol, cinnamic aldehyde, citral, geranial, anisic alcohol, hydroxycitronellal, methyl-2-nonynoate, cinnamic alcohol, eugenol, methyleugenol, coumarin, isoeugenol, α-isomethyllionone, butylphenyl methyl-propional, amyl cinnamic aldehyde, hydroxyphenylpentyl cyclohexene-carbaldehyde, amyl cinnamic alcohol, franesol, hexyl cinnamic aldehyde, benzyl benzoate, benzyl salicylate, benzyl cinnamate. It was

found that the GC-MS method is suitable for the separation and quantitation of fragrance compounds suspected to cause skin reactions (Chaintreau et al., 2003).

The skin absorption and metabolism of cinnamic compounds (cinnamic aldehyde and cinnamic alcohol) was measured by HPLC. The results are listed in Table 4.6. It was suggested that the skin absorption and metabolism of these compounds may play a considerable role in the manifestation of allergic contact dermatitis (Smith et al., 2000).

Synthetic musk fragrances such as nitro and polycyclic musks have been extensively applied in perfumes and other personal care products (PCP). It was established that synthetic musks can cause health risks (Schmeiser et al., 2001) by accumulating in human body (Liebl et al., 2000; Kafferlein and Angerer, 2001; Eisenhardt et al., 2001) and showing estrogenic activity (Schreurs et al., 2002; Bitsch et al., 2002). The concentration of polycyclic musks in healthy young adults was measured by GC-MS technique. Musk fragrances were separated in a capillary column (60 m × 0.25 mm i.d., film thickness, 1.4 μm). Oven temperature started at 110°C, increased to 210°C at 15°C/min, to 270°C at 6°C/min (final hold 18 min). Injector temperature was 260°C. Analytes were detected in selected ion-monitoring mode (SIM). The *m/z* values are listed in Table 4.7. The good separation capacity of the GC-MS procedures is visualised in Fig. 4.6. It was found that the concentrations of galaxolide and tonalide were the highest in plasma, the concentration was significantly higher in woman than in men (Hutter et al., 2005).

GC × GC employing the traditional non-polar/polar column combination, the inverse column set (polar/nonpolar) and targeted multidimensional GC (MDGC) was applied for the analysis of the suspected allergens in fragrance products. A GC × GC contour chromatogram is shown in Fig. 4.7. The 24 suspected allergens were as follows: α-isomethylonone, amyl cinnamaldehyde, amyl cinnamic alcohol, anisyl alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, butylphenyl methylpropional, cinnamaldehyde, cinnamic alcohol, citral, citronellol, coumarin, eugenol, farnesol, geraniol, hexylcinnamaldehyde, hydroxycitronellal, hydroxyisohexyl-3-cyclohexene carboxaldehyde, isoeugenol, limonene, linalool and methyl-2-octynoate. The results prove that both methods are suitable for the analysis of allergens and the quantitation is simple in targeted MDGC (Dunn et al., 2006).

Not only various GC technologies but also HPLC found application in the separation of the 24 fragrance allergens. The chemical structure, common name and CAS number of the fragrance allergens are listed in Table 4.8. Analytes were analysed in a C18 column (250 mm × 4.6 mm i.d.; particle size, 5 μm) using gradient elution.

A typical chromatogram is depicted in Fig. 4.8. Some quantitative data are compiled in Table 4.9. It was stated that the method is simple, rapid and suitable for the determination of suspected allergens in complex matrices (Villa et al., 2007).

The capacity of nanofiltration (NF) and ultrafiltration (UF) to remove endocrine-disrupting compounds such as pharmaceuticals and personal care products (PCP) from water was investigated. The recoveries were assessed by HPLC and GC methods. Musk ketone and galaxolide were included in the experiments. It was

Table 4.6 Cumulative levels of penetrated cinnamic compounds detected in receptor fluid 24 h after 78 μmol of neat cinnamaldehyde or cinnamic alcohol application to human skin following various pre-applications of pyrazole/vehicle

	78 μmol (N)[a]	C[a]	80 μmol PYR[a]	160 μmol PYR	320 μmol PYR	80 μmol MePYR
CAld applied						
CAld	2.62±0.98	5.25±0.84[b]	4.68±2.43	ND	3.11±1.28	ND
CAlc	2.44±1.01	1.92±0.77	0.85±0.12[c]	ND	0.52±0.05[c]	ND
CAcid	4.43±1.93	2.62±0.69	1.62±0.34[c]	ND	1.31±0.17[c]	ND
CAlc applied						
CAld	0	0	0	0	0	0
CAlc	1.30±0.14	4.84±2.59	3.12±1.03	3.58±2.51	2.86±1.45	3.32±1.33
CAcid	0.56±0.8	0.66±0.24	0.42±0.21	0.27±0.71	0.18±0.08[c]	0.42±0.17

Values are given as mean percentage of initial applied dose ± SD.
CAld, cinnamaldehyde, CAlc: cinnamic alcohol , CAcid: cinnamic acid, ND: not determined, N: neat, C: vehicle control, 20 μl water preapplied, PYR: pyrazole, MePYR:4-metylpyrazole.
[a]n=4 humans, otherwise data are for n=3 humans.
[b]Significantly different from N only, P<0.05.
[c]Significantly different from both N and C, P<0,05.
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Table 4.7 Ions used for SIM (selected ion monitoring) recording and quantification, recovery rates and standard deviations for single musk compounds ($n = 11$), LODs and LOQs for single musk compounds (values referred to a sample volume of 9 ml)

	m/z	Recovery rates (%)	Standard deviation (%)	LOD (ng/l)	LOQ (ng/l)
Cashmeran	205.3	97.7	2.7	12.0	24.0
Celestolide	243.3	99.4	3.7	25.0	50.0
Phantolide	243.3	97.5	3.5	12.0	24.0
Galaxolide	257.3	95.1	5.0	62.0	124.0
Traesolide	257.3	97.2	5.9	25.0	50.0
Tonalide	257.3	96.4	5.0	31.0	62.0
HCB $^{13}C_6$	290.0	–	–	–	–
Tonalide-D3	260.3	94.3	3.8	–	–

LOD: limit of detection; LOQ: limit of quantification; *m/z*: mass-to-charge ratio.
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established that NF retains the analytes according to their hydrophobicity and dimension while UF retains mainly the hydrophobic aroma substances (Yoon et al., 2006).

The effect of autooxidation on the sensitising capacity of fragrances has been frequently investigated. Thus, the autooxidation of linalyl acetate (Sköld et al.,

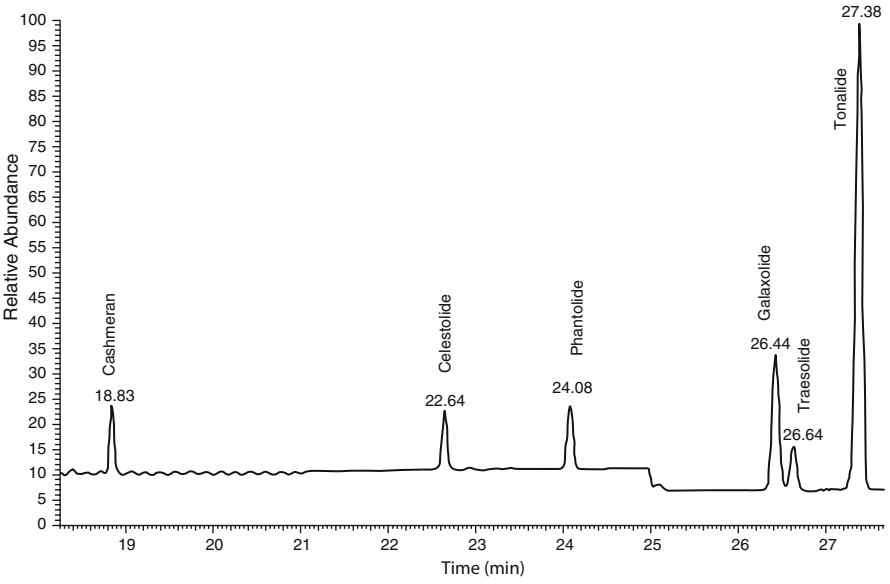

Fig. 4.6 SIM chromatogram of six polycyclic musk compounds (*m/z* 205.3 RT: 18.0–21.0 min; *m/z* 243.3 RT: 21.0–25.0 min; *m/z* 257.3 RT: 25.0–28.0 min). *m/z*: mass to charge ratio. Reprinted with permission from Hutter et al. (2005)

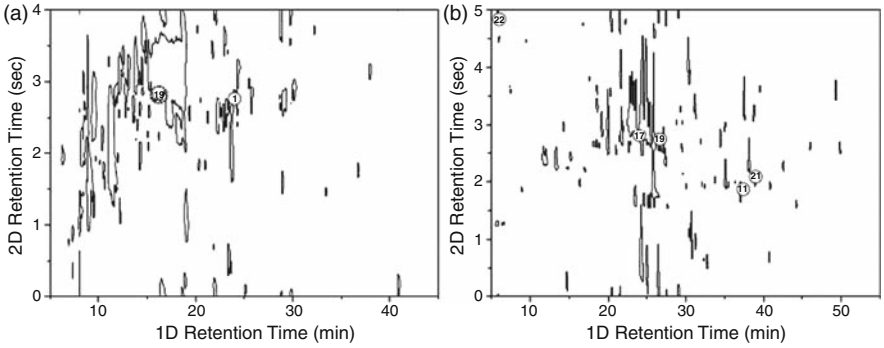

Fig. 4.7 GC × GC contour chromatograms of a commercially available air freshener on a (**A**) conventional non-polar/polar and (**B**) an inverse polarity (polar/non-polar) column set. Resolved target SAs are indicated by circled numbers. For (A), only allergen 1 was clearly resolved from matrix. Allergen 19 (hydroxycitronellal) is shown in a dotted circle at its anticipated position, overlapping matrix components. Reprinted with permission from Dunn et al. (2006)

Table 4.8 A gradient elution was carried out with a mobile phase of acetonitrile (MeCN) and water (H_2O). The best chromatographic assays were performed at room temperature at the following conditions

Time (min)	Flow (ml/min)	MeCN (%)	H_2O (%)
0	0.7	50	50
5	0.7	50	50
15	1.0	60	40
24	1.0	60	40
40	1.0	90	10

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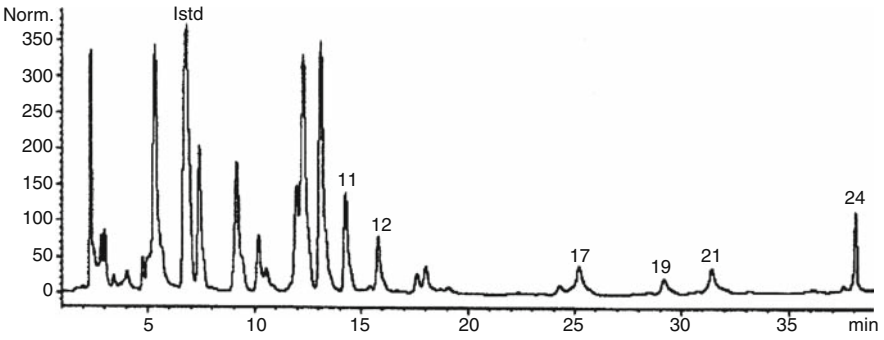

Fig. 4.8 Typical chromatographic acquisition of sample C (all-purpose moisturising cream) at 210 nm. Peak identifications: linalool (11), citronellol (12), benzyl salicylate (17), lilial (19), α-isomethyl ionone (21), limonene (24). Reprinted with permission from Villa et al. (2007)

Table 4.9 Quantitative assay in commercial scented products: sample B – massage oil

Compound	Peak	Content %[a] ± S.D.[a]	R.S.D. (%)
Geraniol	10	$0.11 \pm 0.71 \times 10^{-2}$	6.37
Linalool	11	$0.73 \pm 4.20 \times 10^{-2}$	5.75
Citronellol	12	$0.26 \pm 0.70 \times 10^{-2}$	2.69
Citral	13	$1.56 \times 10^{-2} \pm 0.60 \times 10^{-2}$	3.84
Benzyl benzoate	16	$2.10 \times 10^{-3} \pm 1.41 \times 10^{-4}$	6.71

[a] Mean of five analytical results.
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2008), limonene (Matura et al., 2003; Matura et al., 2006), various hydroperoxides (Christensson et al., 2006), fragrance terpenes (Matura et al., 2005), lavender oil (Prashar et al., 2004) and ethoxylated surfactant was studied in detail (Karlberg et al., 2003). GC, traditional column chromatography, preparative and analytical HPLC were employed for the identification and quantification of primary and secondary oxidation products of linalool exposed to air. GC-MS measurements were carried out on a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Oven temperature started at 35°C (2 min hold), increased to 185°C at 5°C/min (final hold 5 min). Detection range of MS was 50–500 *m/z*. The main oxidation products of linalool were separated on a silica column using various mixtures of hexane–ethylacetate. The fractions obtained on the silica column were further purified by preparative HPLC using a column of 20 × 250 mm, particle size, 7 μm. Mobile phase consisted of 5% 2-propanol, 35% *tert*-butyl methyl ether and 60% *n*-hexane. Analytes were detected at 205 and 230 nm. Analytical HPLC employed a column of 250 × 46 mm i.d. (particle size, 5 μm). Oven was thermostated at 20°C. Initial mobile-phase composition was 40% *tert*-butyl methyl ether and 60% *n*-hexane held for 10 min, then a linear gradient reaching in 5 min 60% *tert*-butyl methyl ether and 40% *n*-hexane (final hold 15 min). Chromatograms depicting the separation of the autooxidation products of linalool are shown in Fig. 4.9. The results illustrated that linalool does not have any allergenic effects while the autooxidation products of linalool show marked sensitisation activity (Sköld et al., 2004). The influence of the air-oxidation of β-caryophyllene on its skin sensitisation capacity has also been elucidated. The oxidation products were analysed by both GC-MS and HPLC-DAD. GC measurements were carried out in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Oven temperature started at 35°C (2 min hold), increased to 180°C at 20°C/min, then to 240°C at 5°C/min (final hold 5 min). Nitrogen was the carrier gas; detector temperature was set to 250°C. Detection range of MS was 50–500 *m/z*. HPLC was performed in a silica column (250 mm × 4.6 mm; particle size, 5 μm) thermostated at 20°C. Mobile phase was *tert*-butyl methyl ether-*n*-hexane (10:90, v/v). The data demonstrated that the main oxidation product of β-caryophyllene was caryophyllene oxide, showing weak allergenic activity (Sköld et al., 2006).

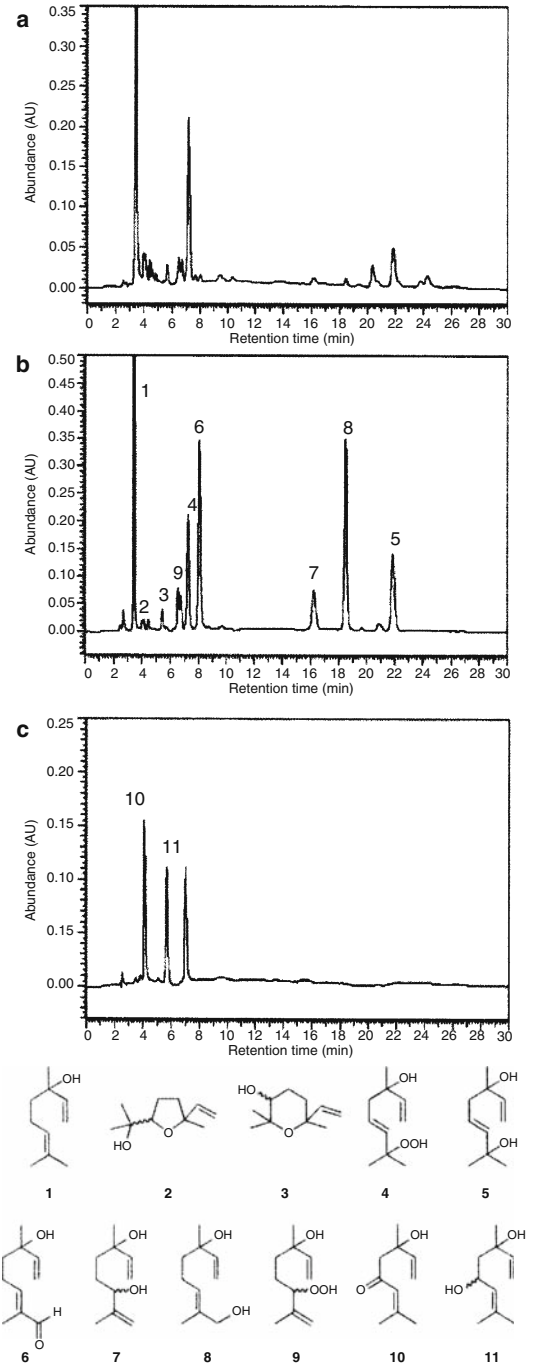

Fig. 4.9 HPLC chromatograms of a sample of oxidised linalool (**a**), a mixture of synthesised reference compounds that are present in oxidised linalool (**b**) and two synthesised reference compounds that were not found in oxidised linalool (**c**). The separations were monitored at 205 nm, and blank subtractions were performed to get a straight baseline. Reprinted with permission from Sköld et al. (2004)

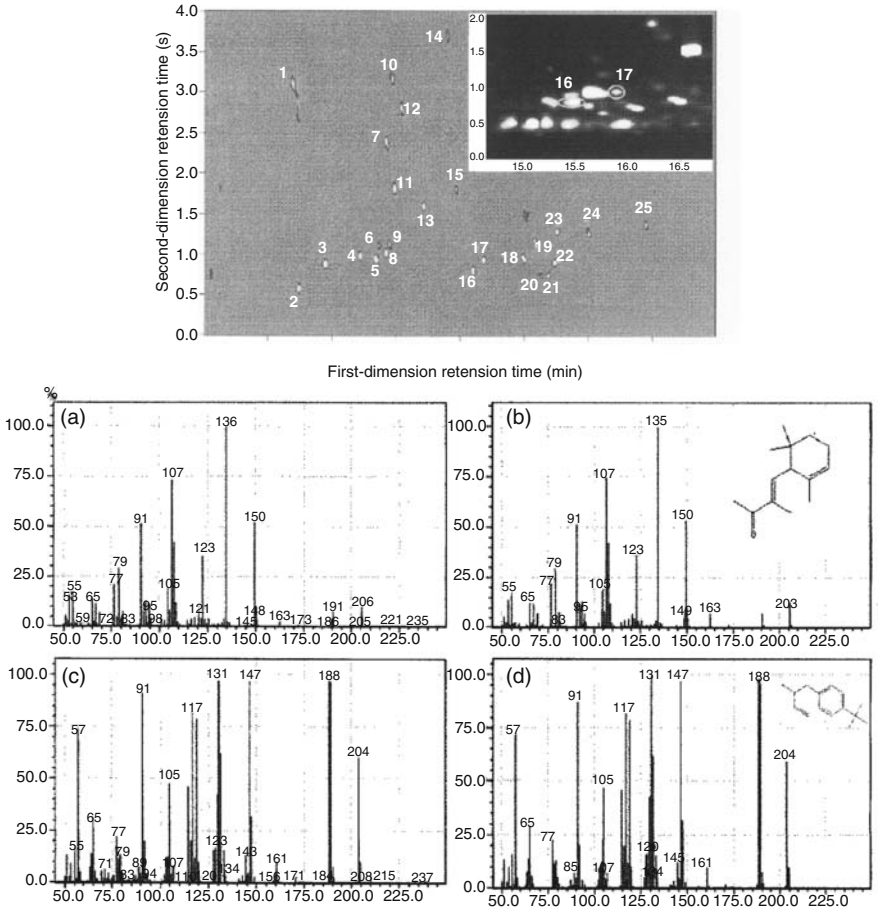

Fig. 4.10 GC × GC–qMS TIC chromatogram (*m*/*z* 50–245) of the allergen standard mixture (for peak designations, see Table 4.10). The insert shows part of the GC × GC–qMS TIC chromatogram of a perfume sample. Mass spectra (compared to the NIST) demonstrate identification of the circled α-isomethylionone (no. 16: mass spectrum, **A**; library spectrum, **B**) and lilial (no. 17: mass spectrum, **C**; library spectrum, **D**). Reprinted with permission from Adahchour et al. (2005)

The application possibilities of comprehensive GC coupled to a rapid-scanning quadrupole mass spectrometer (GC × GC qMS) in the analysis of flavour compounds in olive oil, allergens in fragrances (Cadby et al., 2003; Debonneville and Chaintray, 2004) and polychlorinated biphenyls were elucidated. A typical chromatogram showing the good separation capacity of the system is depicted in Fig. 4.10. The quantification mass and retention times of flavours and allergens are compiled in Table 4.10. The correlation between chromatographic signals and concentration of analytes was linear in the range of 0.05–5 ng/μL for flavours and 2–50 ng/μL for allergens, the regression coefficient being always higher than 0.995.

Table 4.10 Retention time data of the flavour and allergen standard compounds

Flavours	Quant. mass (amu)	^1t_R(min)	^2t_R(s)	Allergents	Quant. mass (amu)	^1t_R (min)	^2t_R (s)
2-Methyl-1-butanol	70	6.0	1.39	Benzyl alcohol	108	8.5	3.17
Ethyl isobutyrate	116	6.2	0.76	Limonene	68	8.7	0.64
Butanoic acid	60	6.7	5.15[a]	Linalool	93	9.8	0.92
Hexanal	72	6.7	1.33	Methyl-2-octynoate	95	11.1	1.07
Ethyl butyrate	88	6.8	0.88	Citronellol	69	11.7	0.93
trans-2-Hexenal	98	7.8	1.79	Citral (neral)	69	11.8	1.18
Isovaleric acid	60	7.9	4.82[a]	Geraniol	69	12.1	2.45
Ethyl-2-methylbutyrate	102	7.9	0.82	Cinnamic aldehyde	131	12.1	1.06
trans-2-Hexenol	82	8.4	2.00	Citral (geranial)	69	12.2	1.13
1-Hexanol	69	8.6	1.67	Anisyl alcohol	108	12.4	3.26
Pentanoic acid	60	8.8	5.33[a]	Hydroxycitronellal	59	12.4	1.83
Heptanal	70	9.0	1.18	Cinnamyl alcohol	92	12.7	2.82
trans-2-Heptenal	83	10.5	1.70	Eugenol	164	13.6	1.64
1-Octen-3-one	70	11.2	1.24	Coumarine	146	14.5	3.72
Octanal	84	11.9	1.09	Isoeugenol	164	14.8	1.86
trans-2,4-Heptadienal	81	11.9	2.52	α-Isomethylionone	135	15.5	0.81
Hexylacetate	84	12.3	0.85	Lilial	189	15.9	0.92
3-Octen-2-one	111	12.9	1.58	Amyl cinnamic aldehyde	202	17.5	0.92
trans-2-Octenal	70	13.5	1.58	Amyl cinnamic alcohol	133	18.0	1.13
1-Octanol	70	14.4	1.33	Farnesol 1	81	18.2	0.74
Nonanal	98	14.9	0.97	Farnesol 2	93	18.4	0.77
β-Phenylethyl alcohol	122	15.4	0.85	Hexyl cinnamic aldehyde	216	18.7	0.92
Ethyl cyclohexanoate	101	15.9	0.88	Benzyl benzoate	105	18.8	1.35
trans-2-Nonenal	83	16.6	1.39	Benzyl salicylate	91	20.0	1.26
1-Nonanol	70	17.4	1.12	Benzyl cinnamate	131	22.2	1.43

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The detection limit varied between 1 and 20 pg for flavours, 2 and 10 pg for allergens and 1 and 2 pg for polychlorinated biphenyls (PCBs). It was found that the baseline separation of these model compounds can be obtained in less than 30 min (Adahchour et al., 2005).

Other comprehensive two-dimensional GC-qMS and GC-FID methods were applied for the separation and quantitation of suspected allergens. The dimensions of the capillary columns were 30 m × 0.25 mm i.d., film thickness, 0.25 μm and 1 mm × 0.10 mm i.d.; film thickness 0.10 μm. Oven temperature initiated at 60°C (1 min hold), ramped to 250°C at 3°C/min. The chemical names, CAS numbers, purity index (%) and *m/z* target ions of the suspected allergens are compiled in Table 4.11. Contour plots of GC × GC-SIM/qMS analyses are depicted in Fig. 4.11.

Table 4.11 List of chemical names, CAS numbers, purity index (%) and *m/z* target ions used for SIM acquisition mode of the analytes under study

Name [CAS registry no.]	Purity (%)	SIM *m/z* ions
Amylcinnamic alcohol [101-85-9]	92.0+4.5 *Z*+*E*	**133**, 115, 204
Amylcinnamic aldehyde [122-40-7]	93.7+4.7 *Z*+*E*	**202**, 201, 129
Anisyl alcohol [105-13-5]	97.2	**138**, 137, 109
Benzyl alcohol [100-51-6]	97.7	**108**, 79, 107
Benzyl benzoate [120-51-4]	>99.9	**105**, 212, 194
Benzyl cinnamate [103-41-3]	98.3	**131**, 192, 193
Benzyl salicylate [118-58-1]	99.6	**91**, 228, 65
Cinnamic alcohol [104-54-1]	98.4	**92**, 134, 115
Cinnamic aldehyde [104-55-2]	1.8+93.63 *Z*+*E*	**131**, 132, 103
Citral [5392-40-5]	37.3−62.6 *Z*+*E*	Neral: **69**, 94, 109 Geranial: **69**, 84, 94
Citronellol [106-22-9]	99.2	**69**, 95, 81
Coumarine [91-64-5]	98.5	**146**, 118, 89
Estragole [140-67-0]	98.9	**148**, 147, 117
Eugenol [97-53-0]	99.7	**164**, 103, 149
Farnesol [106-28-5]	45.9+53.6 ZE+EE	**69**, 93, 81
Geraniol [106-24-1]	95.5	**69**, 123, 93
Hexylcinnamic aldehyde [101-86-0]	94.0+4.0 Z+E	**216**, 215, 129
Hydroxycitronellal [107-75-75]	97.9	**59**, 71, 43
Isoeugenol [97-54-1]	7.8+92.2 Z+E	**164**, 149, 131
Butylphenyl methylpropional [80-54-6]	2.4+96.5 Z+E	**189**, 147, 204
Limonene [5989-27-5]	97.1	**68**, 93, 67
Linalool [78-70-6]	97.9	**93**, 71, 121
Hydroxyisohexyl-3-cyclohexene Carboxaldehyde [31906-04-4]	27.5+72.5 (3-)*c*+(4-)*c*	**136**, 192, 149
Methyl 2-nonynoate [111-80-8]	99.9	**79**, 137, 123
Methyl 2-octynoate [111-12-6]	97.6	**95**, 123, 79
Methyleugenol [93-15-2]	99.3	**178**, 163, 147
Phenylacetaldehyde [122-78-1]	98.2	**91**, 120, 92
∗-Isomethylionone [127-51-5]	85.7	**135**, 206, 150
1,4-Dibromobenzene	100.0	**236**, 234, 238
4,4′-Dibromobiphenyl	98.7	**312**, 310, 314

m/z target ion in bold.
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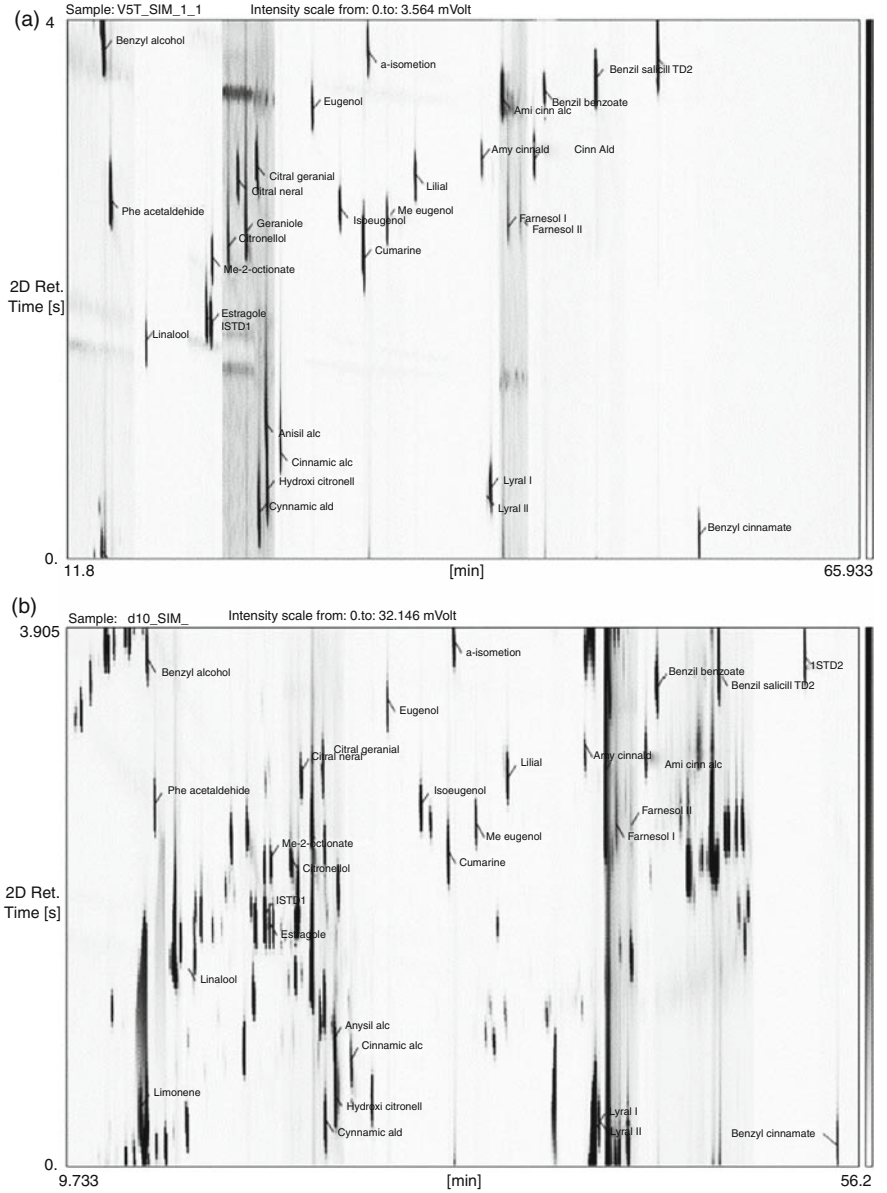

Fig. 4.11 (**a**) Contour plot of a GC × GC-SIM/qMS analysis of allergen standard mixture (5 ppm). (**b**) Contour plot of a GC × GC-SIM/qMS analysis of a QC sample spiked with 10 ppm of allergen standard mixture (CC2dD10). Reprinted with permission from Cordero et al. (2007)

The following validation parameters of the method were determined: confirmation of identity, selectivity, specificity, LOD, LOQ, linearity, repeatability, precision and intermediate precision, accuracy and uncertainty. It was established that the method is reliable and can be applied for the qualitative and quantitative analyses of allergens in complex matrices (Cordero et al., 2007).

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Chapter 5
Environmental Pollution

Various chromatographic technologies are rapid, reliable, versatile and powerful methods for the separation and quantitative determination of environmental pollutants in a wide variety of complicated accompanying matrices such as air, ground and surface waters, sewage, sludges, soils, food and food products, pharmaceutical preparations, personal care products. Natural and mainly synthetic fragrances can occur in various environmental matrices exerting adverse impact on humans and wildlife. Some of these pollutants are persistent, liable to bioaccumulation and show marked toxicity. The occurrence of synthetic fragrances in the environment may increase environmental loading (Tanabe, 2005), and they can concentrate in the blood of healthy young adults (Hutter et al., 2005).

5.1 Ground and Surface Water

The occurrence of natural and synthetic fragrances in ground and surface waters (Aschmann et al., 2001; Heberer, 2002; Garcia-Jares et al., 2002) and treated wastewaters has been extensively investigated (Simonich et al., 2000; Llompart et al., 2003). Because of their very low concentration, the selectivity and performance of the different preconcentration methods have been extensively investigated. The newest results obtained by the application of various GC-MS and GC-MS^2 systems for the determination of various PCP products such as nitro and polycyclic musks, antimicrobial compounds, ultraviolet blockers, antioxidants and insect repellents in water have been recently reviewed (Pietrogrande and Basaglia, 2007). The optimisation of SPME coupled to GC-MS for the analysis of synthetic musk fragrances (galaxolide, tonalide, celestolide, musk ketone) was reported. The chemical structures of the fragrances included in the experiments are listed in Fig. 5.1. It was found that the best recoveries were achieved by employing PDMS/DVB fibres; the efficacy of PDMS, PA and carboxen fibres was lower. It was further established that using extraction times of 45 min at 30°C resulted in recovery in the range of 45–50%; the equilibrium state was achieved only after 2 h. GC separations were performed in a capillary column (25 m × 0.22 mm i.d., film thickness, 0.25 μm). Initial oven temperature was 60°C for 5 min, ramped to

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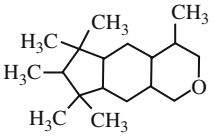
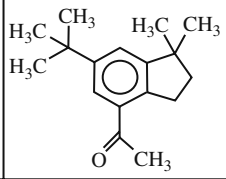
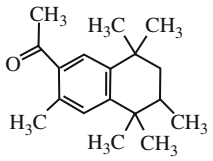
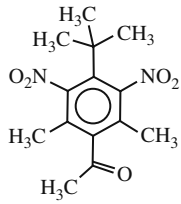
IUPAC-Name Tradename CAS Molecular Mass	Structure	IUPAC-Name Tradename CAS Molecular Mass	Structure
1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyrane Galaxolide®, Abbalide® 1222-05-5 258,4 g/mol		4-acetyl-1,1-dimethyl-6-*tert*-butylindan (ADBI) Celestolide®, Crysolide® 13171-00-1 244,38 g/mol	
7- acetyl-1,1,3,4,4,6-hexamethyltetraline Tonalide®, Fixolide® 1506-02-1 258,4 g/mol		1-*tert*-buty1-3,5-dimethyl-2,6-dinitro-4-acetylbenzene Musk ketone 81-14-1 294,3 g/mol	

Fig. 5.1 Chemical structure of musk fragrances investigated. Reprinted with permission from Winkler et al. (2000)

190°C at 30°C/min (9 min hold), to 250°C at 20°C/min (final hold 3.67 min). MS used positive-ion electron impact conditions (70 eV), mass range being 50–350 *m/z*. Chromatograms showing the separation of musk fragrances are depicted in Fig. 5.2. The dependence of the efficacy of extraction on the experimental conditions (salt content, injection depth in the GC injector, stirring velocity, injection temperature of PDMS/DVB and PA) is compiled in Table 5.1. It was assessed that the RSD varied between 11% and 18% in the concentration range of 25–260 ng/l (Winkler et al., 2000).

An SPME-GC-MS method was developed for the separation and quantitation of "earthy-musty" odourous compounds in water, using a programmable temperature vapouriser. Analytes including in the measurement were 2-methylisoborneol (MIB), geosmin, 2,4,6-trichloroanisole (2,4,6-TCA), 2,3,6-trichloroanisole (2,3,6-TCA), 2,3,4-trichloroanisole (2,3,4-TCA) and 2,4,6-tribromoanisole (2,4,6-TBA). It was established that the best preconcentration efficacy can be achieved by DVB/CAR/PDMS fibres. Earthy-musty compounds were well separated by GC as illustrated in Fig. 5.3. The parameters of method validation are compiled in Table 5.2. Because of the good validation parameters, the optimised method was proposed for the analysis of these odorants in different water samples (Zhang et al., 2005).

Besides SPME and HS-SPME, the application possibility of membrane-assisted liquid–liquid extraction for the preconcentration of polycyclic musk fragrances has also been studied. The method used polyethylene membrane material, water–chloroform extraction system and continuous stirring (250 rpm). GC-MS measurements were carried out in a capillary column (30 m × 0.25 mm i.d., film thickness,

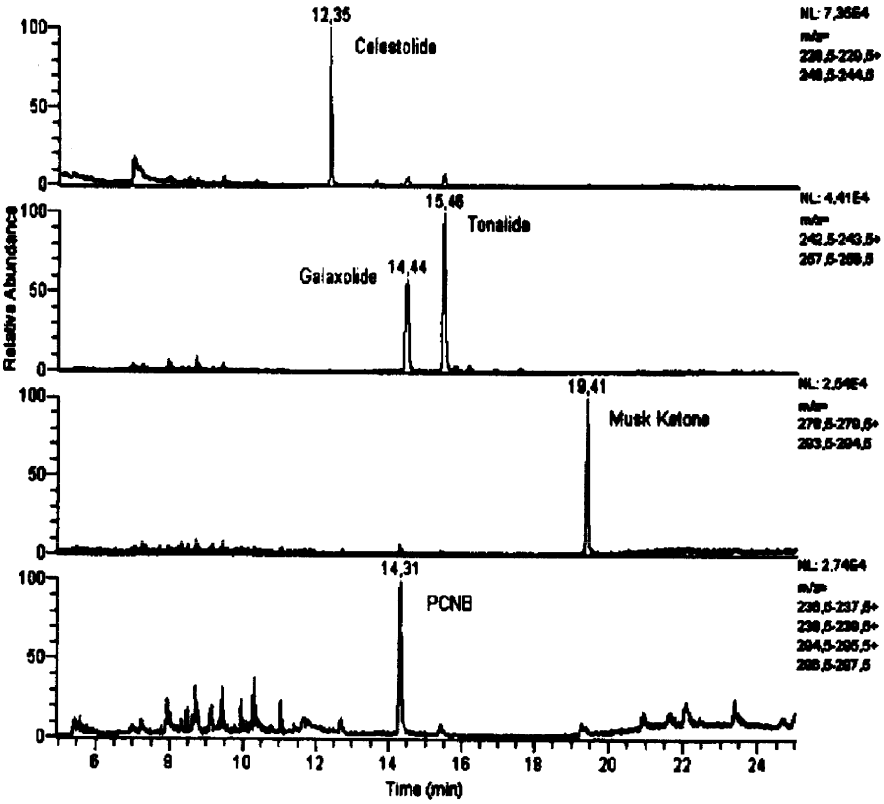


Fig. 5.2 Extracted chromatograms obtained by GC-MS under full scan conditions of the four musk fragrances (286 ng/l) and PCNB (internal standard) after SPME of Nanopure water. Reprinted with permission from Winkler et al. (2000)

0.25 μm). Starting column temperature was 50°C for 1 min, raised to 210°C at 9°C/min. MS used electron impact conditions (70 eV). Chromatograms showing the separation of the analytes are depicted in Fig. 5.4. Comparing this new technology with the standard analytical methods such as SPE-GC-MS and LC-MS-MS, it was found that the method is rapid and reliable and can be employed for the analysis of wastewater samples (Einsle et al., 2006).

The occurrence and removal of 2-methylisoborneol from waters have been many times investigated (Bruce et al., 2002). The fate of MIB and geosmin in surface water supply reservoirs was followed by HS-SPME coupled to GC-MS. Extraction was performed at 50°C for 30 min. It was found that the concentration of MIB was always higher than that of geosmin. The amount of synthetic fragrances depended on both the seasonal variation and the depth of the water column (Westerhoff et al., 2005a).

The occurrence of PCPs and pharmaceuticals was determined in Romanian rivers using SPE coupled to GC-MS. The polycyclic synthetic musks galaxolide and

Table 5.1 Results of the experiments for the dependencies on salt content, injection depth, stirring velocity and injection temperature

	Area counts			
Experiment	Celestolide	Galaxolide	Tonalide	Musk ketone
Salt content				
0 g/1 NaCl	530,028	429,564	575,061	585,501
20 g/1 NaCl	265,344	220,183	296,600	322,208
200 g/1 NaCl	195,580	170,641	227,712	289,937
Injection depth in the GC injector				
3 cm	1,289,480	2,127,382	2,610,470	1,387,997
4 cm	2,615,231	5,381,359	6,629,687	2,945,289
4,5 cm	3,002,549	7,787,926	9,736,782	5,602,342
Stirring velocity 750 rpm				
750 rpm	2,631,571	2,336,758	2,478,872	2,129,701
1,000 rpm	2,820,864	2,748,037	2,726,814	1,692,159
1,250 rpm	3,716,014	3,564,712	3,884,021	2,174,221
Injection temperature PDMS-DVB				
250°C	2,420,116	6,480,091	8,522,614	5,257,918
270°C	2,391,417	5,457,379	6,703,830	3,612,586
Injection temperature polyacrylate				
250°C	2,728,876	6,625,467	7,717,285	2,424,478
270°C	2,576,578	6,402,947	8,191,781	2,245,305
290°C	1,730,750	4,189,661	5,477,845	2,268,739

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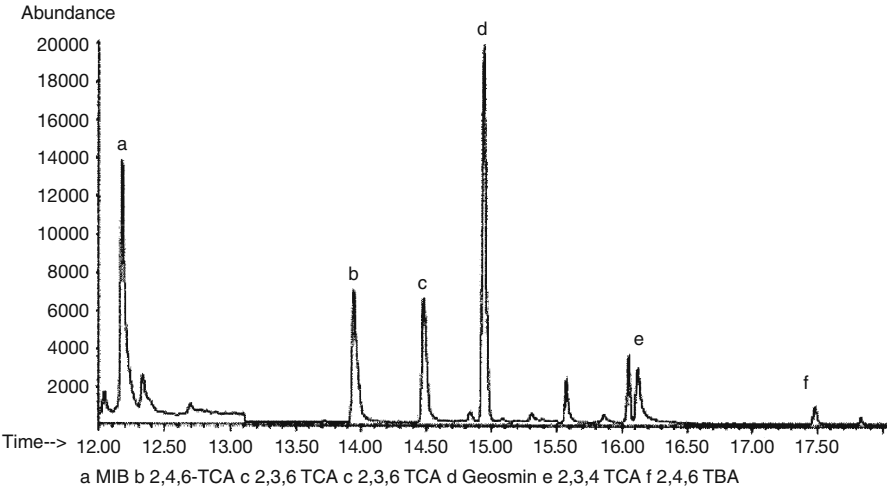

Fig. 5.3 Chromatograms of 50 ng/l earthy-musty compounds. (a: 2-methylisoborneol, b: 2,4,6-trichloroanisole, c: 2,3,6- trichloroanisole, d: geosmin, e: 2,3,4-trichloroanisole, f: 2,4,6-tribromoanisole). Reprinted with permission from Zhang et al. (2005)

Table 5.2 Protocols for method validation

	MIB	2,4,6-TCA	2,3,6-TCA	Geosmin	2,3,4-TCA	2,4,6-TBA
Linear range (μg/l)	0.5–50	0.5–50	0.5–50	0.5–50	0.5–50	0.5–50
Linear regression (r^2)	0.993	0.997	0.998	0.994	0.999	0.996
Within-batch RSD (%)	3.91	4.1	2.3	1	2.6	1
Between-batch RSD (%)	3.3	6.9	4.7	5.5	8.1	6.3
MDL (ng/L)	0.15	0.32	0.14	0.16	0.16	0.38

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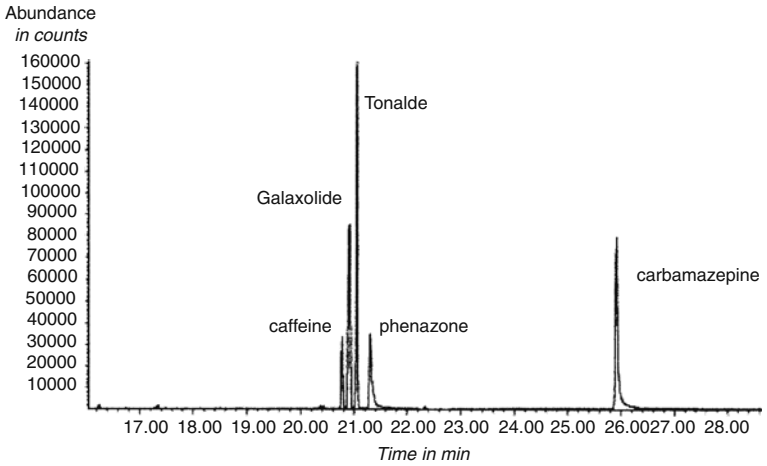

Fig. 5.4 Total ion chromatogram of the GC–MS (SIM) analysis of a chloroform extract obtained at optimum extraction conditions (12.5 g NaCl, 50 °C, pH 7) of analytes spiked in model wastewater at concentrations of 10 μg/l each. Reprinted with permission from Einsle et al. (2006)

tonalide were included in the investigations. Their inclusion was motivated by the fact that they are present in aquatic food chain and other living organisms (Fromme et al., 2001; Kannan et al., 2005). The concentration of musk fragrances in rivers was measured by GC-MS using a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Starting oven temperature was 90°C for 1 min, increased to 120°C at 10°C/min, then to 200°C at 3.5°C/min, to 315°C at 5°C/min (final hold 11 min). MS used electron impact conditions (70 eV). Chromatograms showing the separation of the analytes are depicted in Figs. 5.5, 5.6 and 5.7. The average RSD value of the measurements was 11.5%, the recoveries were varying between 64.1 and 109.7%. LOQ was 30 ng/l (Moldovan, 2006).

The occurrence and fate of pharmaceuticals and PCPs in urban receiving waters were studied by employing HPLC/ESI/MS technology (Ahrer et al., 2001; Ellis, 2006).

The removal of pharmaceuticals, contrast media, endocrine-disrupting compounds and fragrances has been extensively investigated. Thus, the effect of

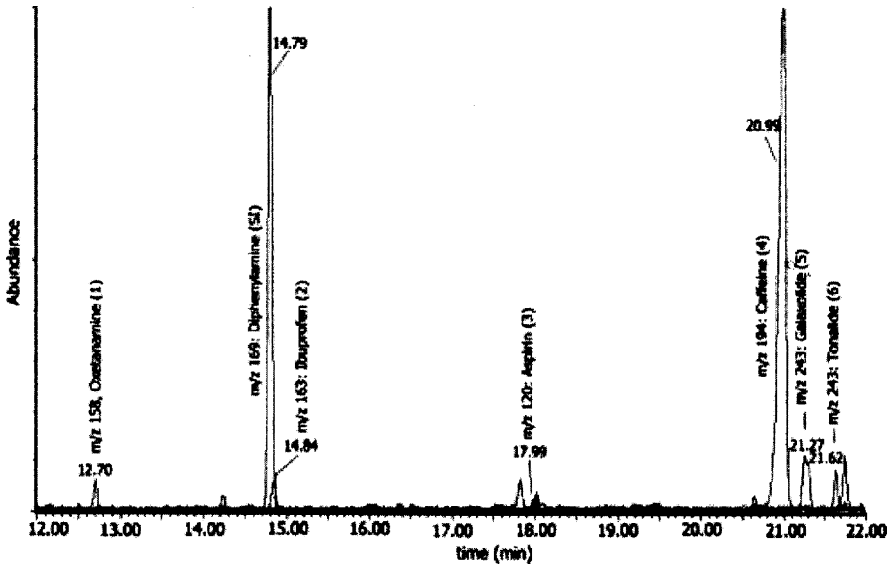

Fig. 5.5 Overlay chromatograms (time range 12–22 min) to diagnostic ions *m*/*z* 120, 158, 163, 169, 194 and 243 corresponding to a water sample. Reprinted with permission from Moldovan (2006)

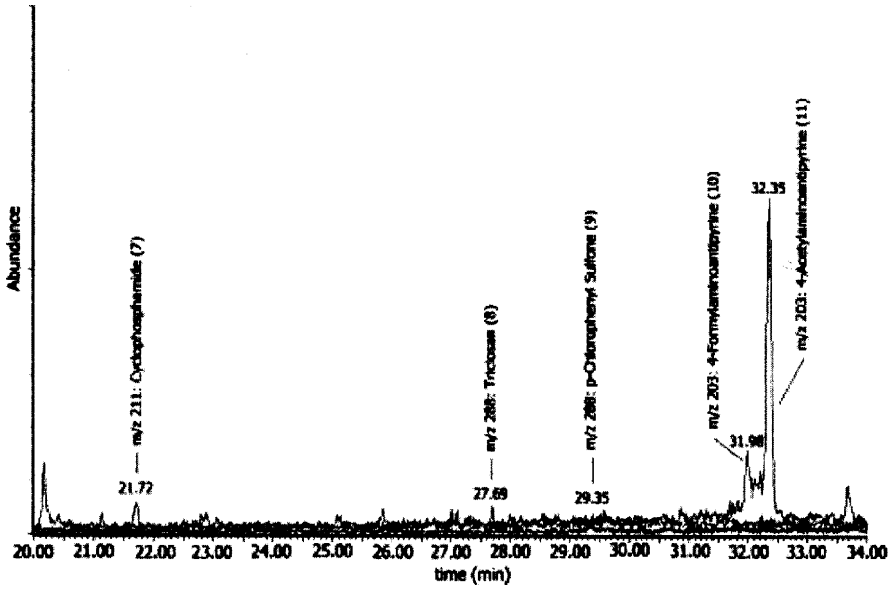

Fig. 5.6 Overlay chromatograms (time range 20–34 min) to diagnostic ions *m*/*z* 203, 211, and 288 corresponding to a water sample. Reprinted with permission from Moldovan (2006)

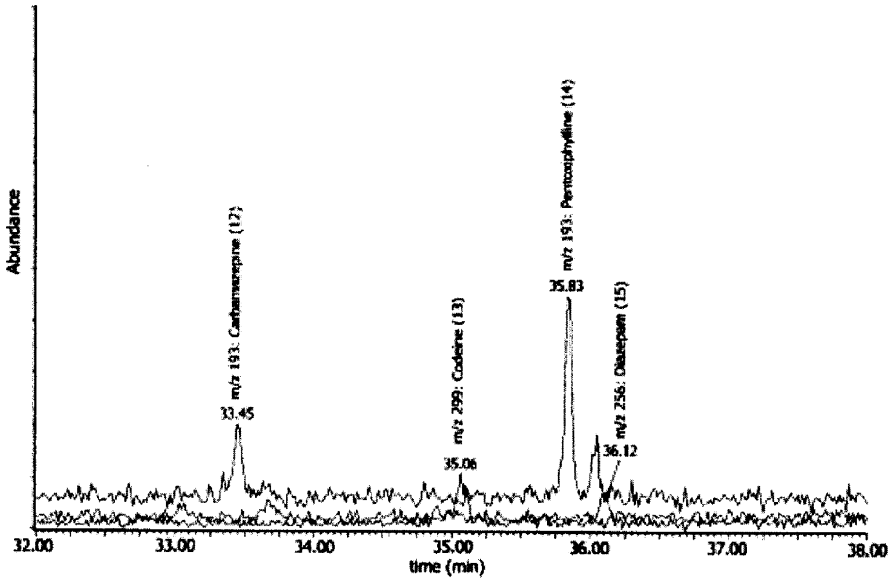

Fig. 5.7 Overlay chromatograms (time range 32–38 min) to diagnostic ions *m*/*z* 193, 256, and 299 corresponding to a water sample. Reprinted with permission from Moldovan (2006)

ozonationon the removal of various pollutants was measured by GC-MS-MS and LC-electrospray tandem MS. The concentration of fragrances markedly decreased after ozonation. Tonalide: (0.10±0.03) μg/l and <LOQ(>50) μg/l before and after ozonation; galaxolide: (0.73±0.14) μg/l and 0.090 μg/l(>93) before and after ozonation (Ternes et al., 2003).

Another study employed SPME-GC-MS for the assessment of the aqueous photodegradation of nitro musks (xylene, moskene, tibetene and ketone). Analytes were separated on a capillary column (25 m × 0.25 mm i.d., film thickness, 0.25 μm). Starting oven temperature was 40°C for 2 min, increased to 280°C at 15°C/min (final hold 5 min). Helium was used as carrier gas. Ion chromatograms showing the separation of the analytes are depicted in Fig. 5.8. The parameters of the photodegradation kinetics are compiled in Table 5.3. The data indicated that the apparent rate constants were considerably different, demonstrating the different stability of nitro musks towards UV radiation (Sanchez-Prado et al., 2004). Similar results were achieved by measuring the solid–water distribution coefficient (K_d) for pharmaceuticals and musk fragrances in sewage sludge (Ternes et al., 2004).

The efficacy of biological wastewater treatment on the decomposition of pharmaceuticals and synthetic fragrances has also been extensively investigated (Joss et al., 2005). The performance of a membrane bioreactor and conventional wastewater treatment plants (WWTP) was compared using pharmaceuticals, fragrances (tonalide, AHTN and galaxolide, HHCB) and other endocrine-disrupting compounds as model analytes. Synthetic fragrances were preconcentrated on a C18

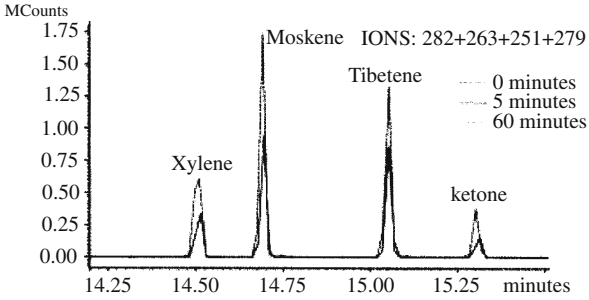

Fig. 5.8 Ion chromatograms showing the nitro musks "on-fibre" photodegradation. Reprinted with permission from Sanchez-Prado et al. (2004)

Table 5.3 Experimental values of the apparent first-order rate constants, half-life times and reaction orders for the nitro musks studied

	"On-fibre" experiments			Aqueous photodegradation		
Compound	C_1	C_2	n^a	C_1	C_2	n^a
Xylene						
k_{ap} (s^{-1})	0.0007	0.0008		0.0030	0.0032	
$t_{1/2}$ (s)	985	923	0.98	230	218	0.98
R^2	0.9939	0.9718		0.9930	0.9943	
Moskene						
k_{ap} (s^{-1})	0.0006	0.0007		0.0017	0.0024	
$t_{1/2}$ (s)	1249	1051	0.93	418	284	0.87
R^2	0.9873	0.9859		0.9893	0.9844	
Tibetenee						
k_{ap} (s^{-1})	0.0004	0.0005		0.0014	0.0014	
$t_{1/2}$ (s)	1924	1496	0.93	497	485	0.99
R^2	0.9964	0.9675		0.9956	0.9728	
Ketone						
k_{ap} (s^{-1})	0.0006	0.0006		0.0026	0.0030	
$t_{1/2}$ (s)	1127	1257	1.03	271	231	0.94
R^2	0.9883	0.9793		0.9943	0.9908	

[a] n, total reaction order.
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sorbent and measured by GC-ESI-MS. The LOD, LOQ and recovery values for tonalide were 10 ng/l, 20 ng/l and 83%, respectively. The same parameters for galaxolide were 20 ng/l, 40 ng/l and 88%, respectively. The efficacy of the removal of fragrances and other pollutants is shown in Fig. 5.9. It was established that both methods are suitable for the removal of synthetic fragrances, but the application of membrane bioreactor was proposed because it is suitable for the detention of particulate matter too (Clara et al., 2005).

The behaviour of the synthetic polycyclic fragrances HHCB and AHTN in lakes was assessed by GC-SIM-MS. Analytes were enriched by a macroporous polystyrene-divinylbenzene adsorbent and separated in a capillary column (25 m × 0.32 mm i.d., film thickness, 0.25 μm). Starting oven temperature was

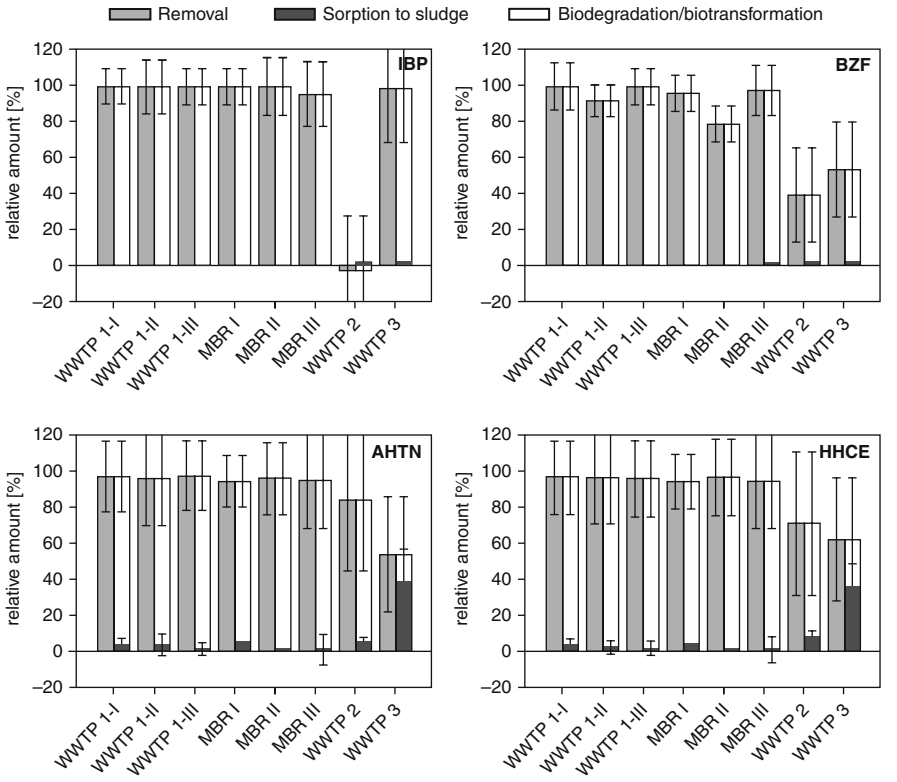

Fig. 5.9 Removal of ibuprofen (IBP), bezafibrate (IBZE), tonalide(AHTN) and galaxolide (HHCE) by adsorption to the sludge and biodegradation/biotransformation in the investigated wastewater treatment facilities (□ removal, ■ sorption, □ biodegradation/biotransformation). Reprinted with permission from Clara et al. (2005)

70°C for 1 min, raised to 180°C at 25°C/min, then to 240°C at 5°C/min (final hold 3 min). MS used electron impact conditions (70 eV), mass range being 45–300 *m/z*. Chromatograms showing the good separation capacity of the GC-MS system are depicted in Fig. 5.10. Some of the results are compiled in Table 5.4. It was concluded from the data that direct photolysis contributes to the removal of AHTN in summer while the photochemical decomposition of HHCB is negligible (Buerge et al., 2003).

The distribution of polycyclic musks in water and particulate matter in river Lippe (Germany) was measured by GC-FID and GC-MS. The concentrations of HHCB, AHTN, ABDI (celestolide) and AHMI (phantolide) were determined using a fused silica capillary column (25 m × 0.25 mm i.d., film thickness, 0.25 μm). Initial column temperature was 60°C for 3 min, increased to 300°C at 3°C/min (final hold 20 min). FID temperature was 300°C. Hydrogen was the carrier gas. GC-MS measurements was performed in a capillary column (30 m × 0.25 mm

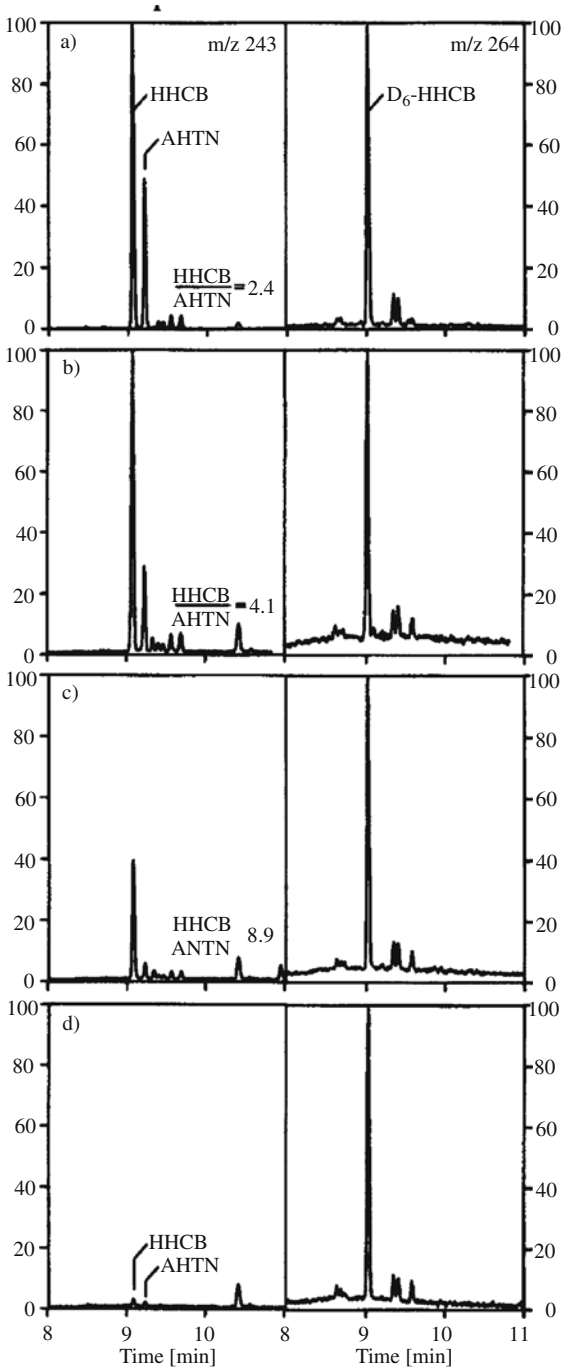

Fig. 5.10 SIM chromatograms of (**a**) a WWTP effluent water samples from Zürichsee (1 m), (**b**) March 7, 2001, (**c**) July 4, 2001 and (**d**) "fossil" groundwater (blanc sample). Panels on the left show elution of HHCB and AHTN, panels on the right show elution of D6-HHCB. Signal intensities of HHCB and D6-HHCB are normalized to 100% (panels a, b) or relative to panel b (panel c, d). Note that the WWTP sample contained more D6-HHCB than the other samples. Reprinted with permission from Buerge et al. (2003)

Table 5.4 Concentrations and Loads of HHCB and AHTN in Effluents of WWTPs, Canton of Zürich, Switzerland

WWTP	Population serviced	Sampling date	Throughput [m^3/d]	HHCB concn [μg/l]	Load per capita [mg/person/d]	AHTN concn [μg/l]	Load per capita [mg/person/d]	Ratio HHCB/AHTN
Gossau	11,000	Jan 25, 2001	3,456	1.95	0.61	0.76	0.24	2.58
Uster	36,000	Feb 14, 2001	14,250	1.93	0.76	0.71	0.28	2.73
Pfäffikon	9,200	Feb 14, 2001	3,177	1.72	0.59	0.63	0.22	2.71
Bubikon-Dürnten	5,650	Jul 17, 2001	5,322	1.21	1.14	0.51	0.48	2.39
Knonau	5,000	Jul 17, 2001	6,028	0.72	0.87	0.31	0.37	2.37
Mean ± SD					0.80 ± 0.22		0.32 ± 0.11	2.56 ± 0.17
Median					0.76		0.28	

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Table 5.5 Concentrations of HHCB, AHTN, ADBI and AHMI and HHCB/AHTN ratios in water samples from the Lippe River and in two samples from Hamm sewage treatment plant (STP)

Ste No.	HHCB (ng/1)	AJHTN (ng/1)	ADBI (ng/1)	AHMI (ng/1)	HHCB/AHTN ratio
1	80	40	<10	<10	2.0
2	70	40	<10	<10	1.8
3	120	50	<10	<10	2.4
4	60	30	<10	<10	2.0
5	160	50	<10	<10	3.2
6	170	60	<10	<10	2.8
7	170	70	<10	<10	2.4
8	180	70	<10	<10	2.6
9	100	30	<10	<10	3.3
10	110	50	<10	<10	2.2
11	120	30	<10	<10	4.0
12	90	20	<10	<10	4.5
13	100	20	<10	<10	5.0
14	50	20	<10	<10	2.5
15	50	20	<10	<10	2.5
16	90	40	<10	<10	2.3
17	50	10	<10	<10	5.0
18	140	60	<10	<10	2.3
19	<10	<10	<10	<10	–
STP influent	970	320	20	20	3.0
STP effluent	1400	360	<10	60	3.9

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i.d., film thickness, 0.25 μm). Temperature program was the same as applied in GC-FID. Helium was the carrier gas. MS used electron impact conditions (70 eV) and a mass range of 35–700 *m/z*. The concentrations of synthetic fragrances are compiled in Table 5.5. The results indicated that the decomposition rate of HHCB is considerably higher than that of AHTN (Dsikowitzky et al., 2002).

A wide variety of pharmaceuticals and endocrine disruptors such as galaxolide were measured in different water samples by SPE-GC-MS-MS and LC-MS-MS. The residue aqueous phase was extracted by dichloromethane/hexane. GC-MS-MS measurements were performed using a fused silica capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Initial column temperature was 60°C for 2 min, increased to 150°C at 20°C/min, to 280C at 3°C/min, 5 min hold, to 315°C at 30°C/min, (final hold 2.5 min). Helium was the carrier gas. HPLC investigations were carried out in a C12 column (250 × 4.6 mm; particle size, 4 μm). Components of binary gradients were 0.1% aqueous formic acid (A) and methanol (B). Separation started at 5% B (3.5 min), increased linearly to 80% by 10 min (3 min hold), raised to 100%, held 8 min. The parameters of galaxolide were 1.2 pg of instrument detection limit, 5.75 pg method detection limit, 10 pg reporting limit,

30% recovery and 12% relative standard deviation. It was found that the method is rapid and sensitive and can be applied for the measurement of a wide variety of environmental pollutants in water samples (Trenholm et al., 2006).

The occurrence of synthetic fragrances in drinking water has also been frequently investigated (Watson et al., 2000; Lin et al., 2002). A separate study was devoted for the determination of the influence of residual chlorine on the measurement of geosmin, MIB and methyl-*tert*-butyl ether (MTBE) in drinking water. DVB/CAR/PDMS, PDMS/DVB, CAR/PDMS fibres were included in the experiments. The measurements indicated that the concentration of each synthetic fragrance was lower in the presence of chlorine in the water (Lin et al., 2003).

The adsorption and degradation of galaxolide, musk ketone and other endocrine-disruptor pharmaceuticals and PCPs have been investigated by GC-MS-MS. The results indicated that 65% of galaxolide can be removed by powder-activated carbon (Westerhoff et al., 2005).

HS-SPME coupled with GC-MS was successfully applied for the analysis of 2-methylisoborneol (MIB) and geosmin (GSM) in environmental waters. HS-SPME was carried out employing PDMS, CAR/PDMS, DVB/CAR/PDMS, PDMS/DVB, PA, and CW/DVB fibres. Extraction time was 30 min. Measurements were performed in a capillary column (60 m × 0.25 mm i.d., film thickness, 1.0 μm). Temperature program started at 190°C for 2 min, increased to 270°C at 10°C/min. Helium was the carrier gas. Ionising voltage was set to 70 eV, the mass range was

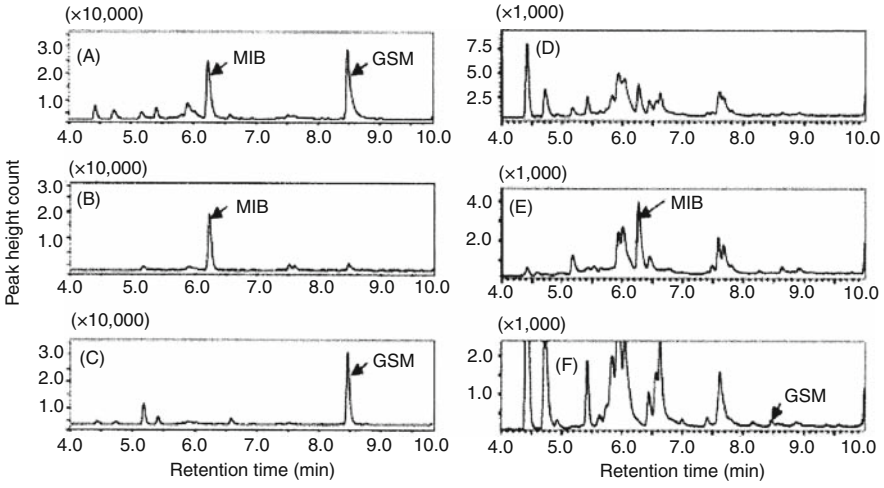

Fig. 5.11 MS total ion (TIC) and SIM chromatograms obtained from standard solution and environmental water sample. (**A**) TIC by HS-SPME (1 ng/mL standard solution), (**B**) SIM ($m/z = 95$) by HS-SPME and (**C**) SIM ($m/z = 112$) by HS- SPME. (**D**) TIC by HS-SPME (pond water, 2.0 mL), (**E**) SIM ($m/z = 95$) by HS-SPME and (F) SIM ($m/z = 112$) by HS-SPME. The ions, $m/z = 95$ and 112, were selected for the detection of MIB=2-methylisoborneol and GSM=geosmin, respectively. Reprinted with permission from Saito et al. (2008)

80–200 *m/z*. It was established that the best extraction efficacy can be achieved by employing PDMS/DVB fibres (55% for MIB and 57% for GSM). Typical chromatograms showing the separation of MIB and GSM are depicted in Fig. 5.11. The LOD values were 0.9 and 0.6 pg/ml, respectively. It was stated that the method can be applied for the analysis of these synthetic fragrances in environmental waters (Saito et al., 2008).

An on-line purge-and trap-gas chromatography-mass spectrometry (PT-GC-MS) technology was developed for the measurement of odorants in various water samples. Besides MIB and GSM 2,4,6-trichloroanisole (2,4,6-TCA), 2-isopropyl-3-methoxypyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP) were included in the experiments. GC analyses were performed in a fused silica capillary column (75 m × 0.53 mm i.d., film thickness, 3.0 μm). Temperature program started at 40°C for 4 min, increased to 240°C at 20°C/min (final hold 15 min). Helium was the carrier gas. Ionising voltage was set to 70 eV, the mass range was 40–300 *m/z*. The details of the GC-MS measurements are listed in Table 5.6. A chromatogram showing the separation of synthetic fragrances is depicted in Fig. 5.12. The validation parameters of the PT-GC-MS method are compiled in Table 5.7. It was established that the technology can be applied for the determination of odorants in ground water samples (Salemi et al., 2006).

Another HS-SPME-GC-MS procedure was employed for the measurement of MIB and GSM in pulp mill effluent treatment ponds. Extraction was performed with a PDMS/DVB fibre at 60°C for 30 min.

The separation of fragrances is illustrated in Fig. 5.13. The concentrations of MIB and GSM in a wastewater treatment plant are listed in Table 5.8 (Watson et al., 2003).

Liquid–liquid extraction using pentane was used for the prepurification of odourous compounds from water.

The LOD values were 0.1 ng/l for IPMP, IBMP, MIB and GSM, 0.5 ng/l for anisole and 1 ng/l for 2,4,6-TCA and *trans, trans*-2,4-heptadienal. This simple, rapid and sensitive method was proposed for the simultaneous determination of odourous compounds in water (Shin and Ahn, 2004).

Table 5.6 Details of the GC–MS program (SIM) applied to the experiments

Compound	t_R (min)	Retention window (min)	Selected ions
IPMP	14.20	12.00[a]–14.50	124, 137[b], 152
IBMP	15.03	14.50–15.30	94, 124[b], 151
MIB	15.87	15.30–16.50	95[b], 108
TCA	17.16	16.50–17.40	195[b], 197, 210
IS	*17.61*	*17.40–18.20*	*94*, *121*, *136* [b]
GSM	18.59	18.20–20.00[a]	112[b], 125

[a] The MS detector was OFF before time 12.00 min and after 20.00 min.
[b] The selected ion (*m/z*) for quantitation.
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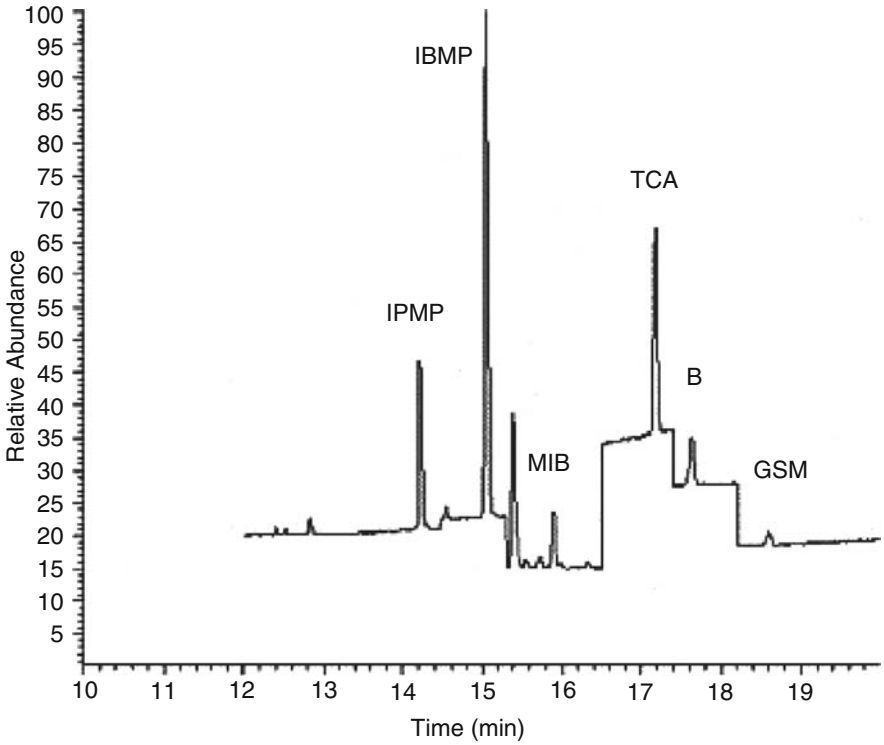

Fig. 5.12 Chromatogram obtained after the extraction of a spiked ground water sample at a concentration of 100 ng/l. Peak identification: IPMP = 2-isopropyl-3-methoxypyrazine, IBMP = 2-isobutyl-3-methoxypyrazine, MIB=2-methylisoborneol, TCA=2,4,6-trichloroanisole, GSM=geosmin. Reprinted with permission from Salemi et al. (2006)

Galaxolide and tonalid among other pollutants such as faecal steroids, caffeine, petroleum and combustion by-products in different WWTPs were separated and identified by GC-MS. Measurements were performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started at 60°C for 2 min, increased to 150°C at 20°C/min, to 290°C at 10°C/min (final hold 20 min). Quantitation and conformation ions *m/z* were 243, 258 and 243, 213 for AHTN and HHCB, respectively. It was established that the measurement of these compounds in surface waters can be used for the assessment of the sources of contaminants (Standley et al., 2000).

The removal of pharmaceuticals, HHCB and AHTN in biological WWTP treatments was studied by GC-MS. Synthetic fragrances were adsorbed on a C18 supports, separated and identified by GC-MS using SIM. The measurements indicated that the biological transformation of fragrances is relatively slow, their removal is mainly due to their adsorption onto sludge (Joss et al., 2005).

Table 5.7 Performance parameters of the PT–GC–MS[a]

Compound	LOD[b] ($S/N = 3$, ng l^{-1})	LOQ[b] ($S/N = 10$, ng l^{-1})	RSD[c] (%)		R^2 value	Recovery[d] (%)	Carry over[e]	
			Low[f]	High[g]			Low[f]	High[g]
IPMP	0.4	1.3	4.9	3.1	0.9962	103	–	<1
IBMP	0.2	0.7	5.1	2.8	0.9987	90	–	<1
MIB	1	3.3	6.4	4.7	0.9931	85	–	2
TCA	0.4	1.3	6.2	3.0	0.9902	83	–	<1
GSM	2	6.7	7.9	4.2	0.9943	94	–	3

[a] Data obtained by extraction of 20 ml of spiked water sample containing 5 g NaCl with 35 ml/min. He as purging gas for 20 min.
[b] Based on the mass chromatogram (base peak, *Table*5.5 and 5.6) after analysis in SIM mode, at the lowest point of the calibration curve.
[c] Relative standard deviation, $n = 5$.
[d] Calculated by comparing the river water and HPLC water samples spiked at the same level.
[e] Obtained by running a blank (non-spiked HPLC water) following a spiked HPLC water sample and stated as percent ratio of peak area in the blank to those in the spiked (standard) water.
[f] At the lowest point of calibration curve (10 ng/l).
[g] At the highest point of calibration curve (200 ng/l).
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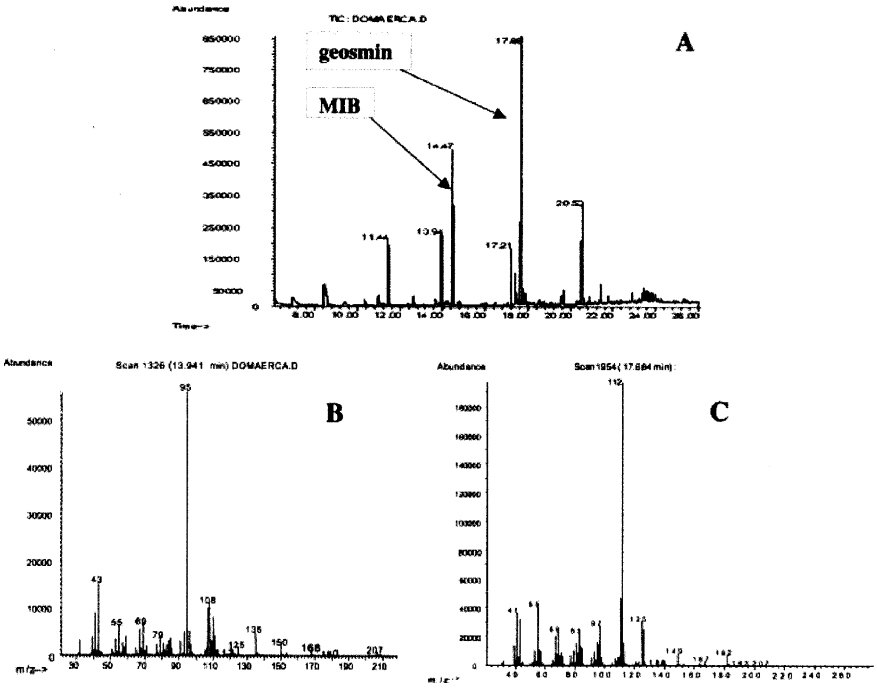

Fig. 5.13 Sample gas chromatogram (**A**) and mass spectra of 2-methylisoborneol (**B**) and geosmin (**C**) measured in aeration cell (September 18, 2001). Compounds were identified from mass spectra and retention times of analytical standards. Reprinted with permission from Watson et al. (2003)

Table 5.8 Geosmin (GSM) and MIB (ng/l) measured at five sites in the Cornwall Pulp Mill wastewater treatment plant, 2001–2002

Site	Sample	MIB	GSM	MIB	GSM	MIB	GSM	MIB	GSM	MIB	GSM	MIB	GSM
1	Canal water	65	45	20	55	–	–	–	–	–	5	–	–
2	Treated canal water	35	40	20	45	–	–	–	–	–	–	–	–
3	Primary outfall	–	12	–	5	–	–	–	–	–	–	–	–
4	Aeration cell	23,960	90,920	25	1,27,510	3,730	26,270	6,440	15,060	6,725	21,913	2,070	1,630
5	Secondary outfall	1740	80	13	75	100	20	No data	No data	110	20	–	15

(–) indicates not detected.
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5.2 Waste Water and Sludge

The chemical decomposition, biodegradation and adsorption of fragrances in WWTPs have also been extensively investigated. The method of preference of their analysis, as in the case of surface and drinking waters, was SPME or HS-SPME coupled with GC-FID or GC-MS using TIC or SIM techniques. Thus, the fate of nitro musks, nitro musk amino metabolites and polycyclic musks in sewage sludge was determined by GC-ion-trap-MS-MS (Herren and Berset, 2000). The removal of synthetic fragrances in WWTPs in the United States and Europe was previously reviewed (Simonich et al., 2002).

The biodegradation of antiepileptics, tranquilizers, analgesics, antibiotics, galaxolide, tonalide and celestolide in a membrane bioreactor (MBR) was assessed by GC-MS as previously reported (Rodriguez et al., 2003). The concentrations of synthetic fragrances and other model compounds in the influent and permeate of MBR are depicted in Fig. 5.14. The results demonstrated that the biodegradation of hydrophobic fragrances is slow, their removal is mainly due to their adsorption into the sludge (Reif et al., 2008).

Pressurised liquid extraction (PLE) followed by SPE and GC-MS was applied for the determination of 61 organic pollutants, among them skatole, AHTN, HHCB, camphor, acetophenone and isoquinone in sediments. SPE was carried out on PS/DVB fibres. GC separation was performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.50 μm). Temperature program started at 40°C for 3 min, increased to 100°C at 4°C/min, to 320°C at 9°C/min. MS conditions were: electron impact ionisation, 70 eV; mass range 45–450 *m/z* for 30 min, 45–550 *m/z* for the last 10 min. Some results are visualised in Fig. 5.15. The recoveries of AHTN in sand, stream sediment and topsoil were 78.05%, 79.05% and 80.4%, respectively. The same recoveries for HHBC were 76.8%, 76.6% and 78.4%, respectively. The

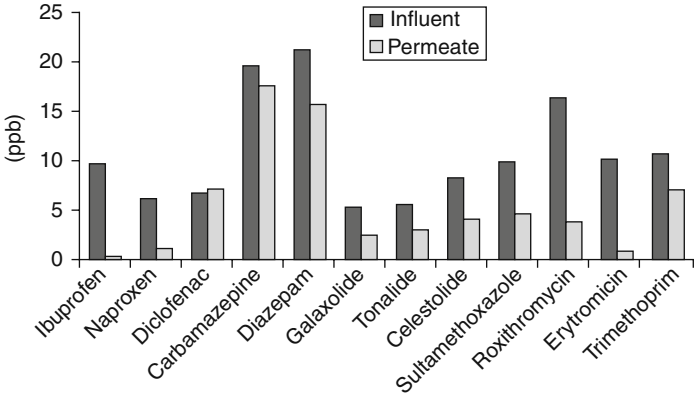

Fig. 5.14 Concentrations of selected PPCPs in the MBR influent and permeate. Reprinted with permission from Reif et al. (2008)

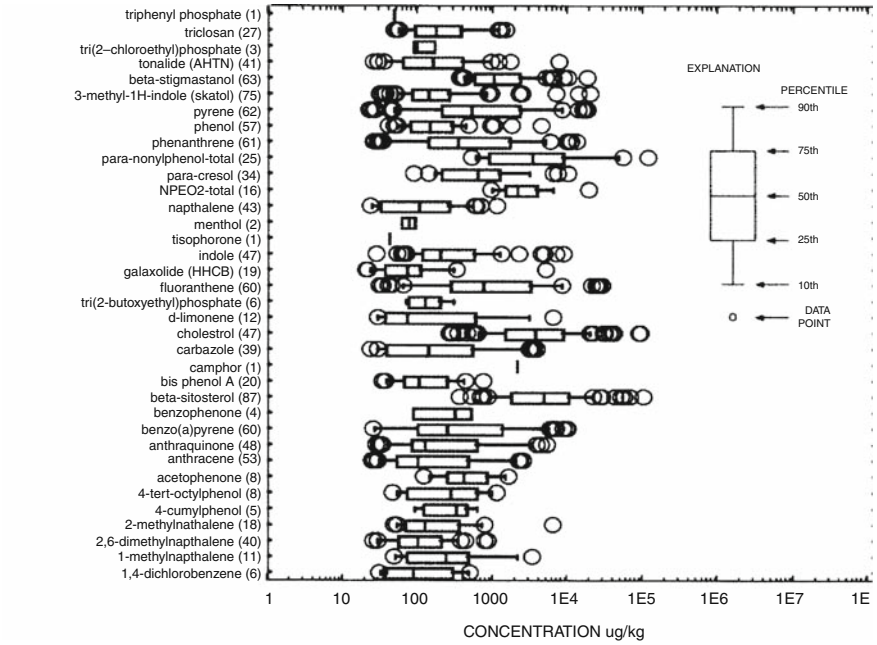

Fig. 5.15 Analysis of 103 environmental soil, sediment, and suspended-sediment samples. The concentration axis is in log scale to accommodate the large concentration ranges for the compounds of interest. The number of compound detections is listed after each compound name. Reprinted with permission from Burkhardt et al. (2005)

method was proposed for the determination of the occurrence, fate, distribution and transport of these pollutants in the environment (Burkhardt et al., 2005).

An on-site SPE method was developed and applied for the measurement of ultra-trace synthetic fragrances in municipal sewage effluent. HHCB, AHTN, ATII, ADBI, DPMI, AHMI and musk ketone were investigated. Samples were purified by SPE, gel permeation chromatography (GPC) and silica sorbent. GC measurements were performed in a capillary column (30 m $\times$ 0.25 mm i.d., film thickness, 0.50 μm). Temperature program started at 90°C, increased to 300°C at 10°C/min, final hold 5 min. MS conditions were: electron impact ionisation, 70 eV; mass range 35–400 *m/z*. The concentrations of synthetic fragrances found in STP effluent are compiled in Table 5.9. It was stated that the method is highly reproducible, the recoveries being 80–97%, and can be employed for the separation and quantitative determination of musk fragrances at very low concentrations (Osemwengie and Steinberg, 2001).

The behaviour of HHCB and AHTN in a STP was investigated by using LLE-PTV-GC-MS. The concentrations of AHTN and HHCB found in STP are compiled in Table 5.10. The measurement demonstrated that the majority of fragrances readily adsorb on sludge (Bester, 2004).

Table 5.9 Concentrations (ng/l) of synthetic musk compounds and nitro musk metabolites in STP effluent stream

Analytes	85 I[a]	65 I[a]	85 I[a]	45 I[a]	60 I[a] (% RSD)
Musk xylene	1.3	<MDL	<MDL	0.3	<MDL
Musk ketone	27.5	21.5	23.4	21.3	<MDL
Musk ambrette	<MDL	<MDL	<MDL	<MDL	<MDL
Musk moskene	<MDL	<MDL	<MDL	<MDL	<MDL
Musk tibetene	<MDL	<MDL	<MDL	<MDL	<MDL
Versalide	<MDL	<MDL	<MDL	<MDL	<MDL
Galaxolide	138	11	152	35.0	40.8(1.8)
Phantolide	4.3	3.1	5.0	2,5	2.4(4.3)
Cashmeran	<MDL	<MDL	<MDL	<MDL	<MDL
Celestolide	2.1	0.3	0.3	0.5	1,4(7.2)
Traseolide	83.8	34.5	126	6.6	<MDL
Tonalide	67.3	47.1	92.2	26.6	36.8(2.5)
4-amino-musk-xylene	1.4	11.6	<MDL	31.5	<MDL
2-amino-musk-xylene	<MDL	<MDL	0.9	<MDL	<MDL
Amino musk ketone	<MDL	<MDL	<MDL	<MDL	<MDL

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The concentrations of galaxolide, tonalide, traseolide, phantolide, celectoide, cashmeran, musk xylene and musk ketone were determined in Canada and Sweden. Canadian samples were preconcentrated by LLE (*n*-pentane and dichloromethane) while Swedish samples were purified by SPE. Canadian samples were analysed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started at 60°C, 3 min hold, increased to 300°C at 3°C/min. MS conditions were: electron impact ionisation, 70 eV; mass range 35–500 *m/z*. Swedish samples were measured in another GC-MS system using a different capillary column (30 or 60 m × 0.25 mm i.d., film thickness, 0.25 μm). Oven temperature program started at 80°C, increased to 150°C at 8°C/min, to 250°C at 2°C, then to 310°C at 8°C. The concentrations of synthetic fragrances found in STP effluent are compiled in 5.11. The comparison of the Canadian and Swedish samples revealed that the concentration of musk fragrances is markedly higher in Canada than in Sweden (Ricking et al., 2003).

Chromatographic methods were developed for the simultaneous determination of antiphlogistics, lipid regulators, cytostatic agents and two polycyclic musk fragrances (AHTN and HHCB) in activated and digested sludge. Fragrances were extracted by PLE (extracting agent, methanol) and USE (extracting agent methanol followed by acetone). Both extracts were further concentrated by SPE using C18 sorbent. Synthetic fragrances were separated in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started at 50°C, ramped to 160°C at 20°C/min, to 280°C at 4°C/min, to 300°C at 20°C/min, final hold 10 min. The retention times of AHTN and HHCB were 15.30 and 15.05 min, respectively. Typical chromatograms showing the good separation capacity of the system are depicted in Fig. 5.16. The recovery values of musks are compiled in Table 5.12.

Table 5.10 Concentrations of AHTN, HHCB and HHCB–lactone in influent and effluent of a German sewage treatment plant (average of 5 days)

	AHTN				HHCB				HHCB–lactone		
	Sludge [ng/g]	Influent [ng/g]	Effluent [ng/g]	Break-through [%]	Sludge [ng/g]	Influent [ng/g]	Effluent [ng/g]	Break-through [%]	Influent [ng/g]	Effluent [ng/g]	Break-through [%]
08.04.02	1480	617	240	39	3038	2182	795	36	270	420	156
09.04.02	1532	713	215	30	3243	2325	691	30	270	370	137
10.04.02	1343	587	206	35	2709	1933	652	34	230	370	161
11.04.02	1746	572	203	35	3342	1857	669	36	215	340	158
12.04.02	1525	427	197	46	3010	1409	669	48	170	335	197
Mean	1525	583	212	37	3068	1941	695	37	231	367	162
SD	145	103	16.7	5.9	244	352	58	7	42	34	22

Additionally, the day-to-day variation is given as standard deviation. Additionally, sludge data for HHCB and AHTN are given. Data derived from duplicate samples each.
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Table 5.11 Results of the analysis of PMF in samples from Canada and Sweden and data for comparison

Location	Cashmeran (DPMI)	Celestolide (ADBI)	Phantolide (AHMI)	Galaxolide (HHCB)	Traseolide (ATTI)	Tonalide (AHTN)	Musk xylene	Musk ketone	Ratio HHCB/AHTN
Enköping (Sweden) Population 21,000	<1	7	4	336	<1	90	<1	<1	3.74
Skene (Sweden) Population 17,280	<1	3	2	218	<1	42	<1	<1	5.14
Gasslösa (Sweden) Population 79.000	<1	6	5	423	<1	104	<1	<1	4.07
Nolhaga (Sweden) Population equiv. 39.500	<1	2	2	157	<1	42	<1	<1	3.77
Ljusne (Sweden) Population 90.000	<1	8	3	407	<1	77	<1	<1	5.32
Strawberry Marsh ATP (Canada) population 90.000	<1	7	2	480	<1	220	<1	<1	2,.8

Table 5.11 (continued)

Location	Cashmeran (DPMI)	Celestolide (ADBI)	Phantolide (AHMI)	Galaxolide (HHCB)	Traseolide (ATTI)	Tonalide (AHTN)	Musk xylene	Musk ketone	Ratio HHCB/AHTN
Lancester (Canada) population 90.000	<1	4	2	205	<1	110	<1	<1	1.86
Mill Cove Plant Bedford (Canada) popul. 350.000	<1	19	6	1300	<1	520	<1	<1	2.50
Stockholm, Göteborg, Malmö (Sweden)	n.m.	n.m.	n.m.	1000–6000	n.m.	n.m.	n.m.	1000–5000	–
Lippe (Germany)	n.m.	<10–20	<10–60	50–1400	n.m.	10–360	n.m.	n.m.	2.00—5.00
Havel-River-Berlin mean moderate to high level (Germany)	n.m.	<20–20	<10–70	230–1590	<10–70	70–530	n.m.	n.m.	3.00—3.29
Elbe Estary and German Bight (North Sea) Germany	n.m.	n.m.	n.m.	0.09–95	n.m.	0.08–67	n.m.	n.m.	1.13–1.85

Table 5.11 (continued)

Location	Cashmeran (DPMI)	Celestolide (ADBI)	Phantolide (AHMI)	Galaxolide (HHCB)	Traseolide (ATTI)	Tonalide (AHTN)	Musk xylene	Musk ketone	Ratio HHCB/AHTN
Berlin area (Germany)	n.m.	20–410	n.m.	30–12,500	n.m.	40–6800	<10	<10–390	0.75–4,58
Hamm STP influent (Germany)	n.m.	20	20	970	n.m.	320	n.m.	n.m.	3.03
Hamm STP effluent (Germany)	n.m.	<10	60	1400	n.m.	360	n.m.	n.m.	3.89
Sewer Schönerlinde (Germany)	n.m.	410	n.m.	10,800	n.m.	5800	<10	320	1.86
Sewage plant effluent (Germany) sample 06/24/97	n.m.	n.m.	n.m.	4200	n.m.	1900	n.m.	n.m.	221
Sewage plant effluent (Germany) sample 07/29/97	n.m.	n.m.	n.m.	3700	n.m.	1700	n.m.	n.m.	2.18
Sewage pond influent (Germany) sample 06/24/97	n.m.	n.m.	n.m.	3600	n.m.	1500	n.m.	n.m.	2.40

Table 5.11 (continued)

Location	Cashmeran (DPMI)	Celestolide (ADBI)	Phantolide (AHMI)	Galaxolide (HHCB)	Traseolide (ATTI)	Tonalide (AHTN)	Musk xylene	Musk ketone	Ratio HHCB/AHTN
Sewage pond effluent (Germany) sample 07/29/97	n.m.	n.m.	n.m.	1700	n.m.	640	n.m.	n.m.	2.66
STP effluent (USA) (n=12)	<MDL	0,3–2,1	2,4–5	40,8–152	<MDL-126	36,8–92,2	<MDL-1,3	<MDL-27,5	1.11—2.05
STP effluent (primary gravitational settling and activated sludge) (USA) (n=4)	n.m.	n.m.	n.m.	16,600± 10,400	n.m.	12,500± 7650	n.m.	n.m.	n.c.
STP effluent (primary gravitational settling and activated sludge) (USA) (n=1)	n.m.	n.m.	n.m.	2053±1314	n.m.	1326±270	n.m.	n.m.	n.c.

Table 5.11 (continued)

Location	Cashmeran (DPMI)	Celestolide (ADBI)	Phantolide (AHMI)	Galaxolide (HHCB)	Traseolide (ATTI)	Tonalide (AHTN)	Musk xylene	Musk ketone	Ratio HHCB/AHTN
STP effluent (primary gravitational settling and carousel) (EU) (n=2)	n.m.	n.m.	n.m.	4620	n.m.	1440	n.m.	n.m.	3.21
STP effluent (primary gravitational settling and oxidation ditch) (USA) (n=2)	n.m.	n.m.	n.m.	1065	n.m.	1235	n.m.	n.m.	0.86
STP effluent (primary gravitational settling and tickling filtering) (USA) (n=3)	n.m.	n.m.	n.m.	1495	n.m.	1010	n.m.	n.m.	1.48
STP effluent (primary gravitational settling and tickling filtering) (EU) (n=2)	n.m.	n.m.	n.m.	2056±655	n.m.	1555±522	n.m.	n.m.	n.c.

Table 5.11 (continued)

Location	Cashmeran (DPMI)	Celestolide (ADBI)	Phantolide (AHMI)	Galaxolide (HHCB)	Traseolide (ATTI)	Tonalide (AHTN)	Musk xylene	Musk ketone	Ratio HHCB/AHTN
STP effluent (primary gravitational settling and rotating biological contractor) (USA) (n=1)	n.m.	n.m.	n.m.	2400	n.m.	1645	n.m.	n.m.	1.46

MDL means methods detection limit. The number means the average and the ± means the SD.
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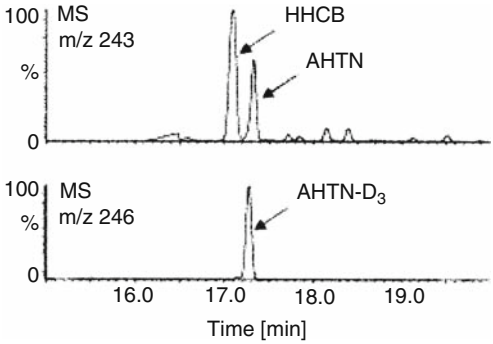

Fig. 5.16 Single-ion monitoring (SIM) mode for HHCB and AHTN. AHTN-D_3 was used as surrogate standard. Reprinted with permission from Ternes et al. (2005)

Table 5.12 Absolute mean recoveries by ultrasonic solvent extraction for activated sludge ($n = 3$), digested sludge ($n = 3$) and groundwater ($n = 3$) after spiking with 1000 ng/g (AHTN) and 2500 ng/g (HHCB) to the sludge and 1 µg/l to the water

	LOQ		Activated sludge	Digested sludge	Water
	Sludge (ng/g)	Water (ng/l)	Absolute recovery (mean ± R.S.D. 1σ (%))	Absolute recovery (mean ± R.S.D. 1σ (%))	Absolute recovery (mean ± R.S.D. 1σ (%))
AHTN	250	20	78 ± 15	74 ± 20	82 ± 9
HHCB	250	20	87 ± 10	64 ± 12	78 ± 8
AHTN-D_3		20	109 ± 7	105 ± 4	88 ± 7

LOQ: limit of quantification.
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It was emphasised that the quality of sludge exerts a considerable impact on the analysis of musk fragrances, therefore, the determination of the individual recoveries for unknown sludge samples is highly advocated. (Ternes et al., 2005).

The behaviour of pharmaceutics, cosmetics and hormones in STP was investigated by using SPE coupled to GC. The LOD and LOQ values and the recovery of galaxolide were 1.2 ng/l, 4 ng/l and 88%, respectively. The same values for tonalide were 1.8 ng/l, 6 ng/l and 90%, respectively. It was found that the overall removal efficacy of STP varied between 70% and 90% for synthetic musk fragrances (Carballa et al., 2004).

5.3 Miscellaneous Environmental Matrices

Besides surface and ground waters, STPs and WWTPs, the concentrations of synthetic musk fragrances and other odorants were measured in a wide variety of more or less complicated accompanying matrices.

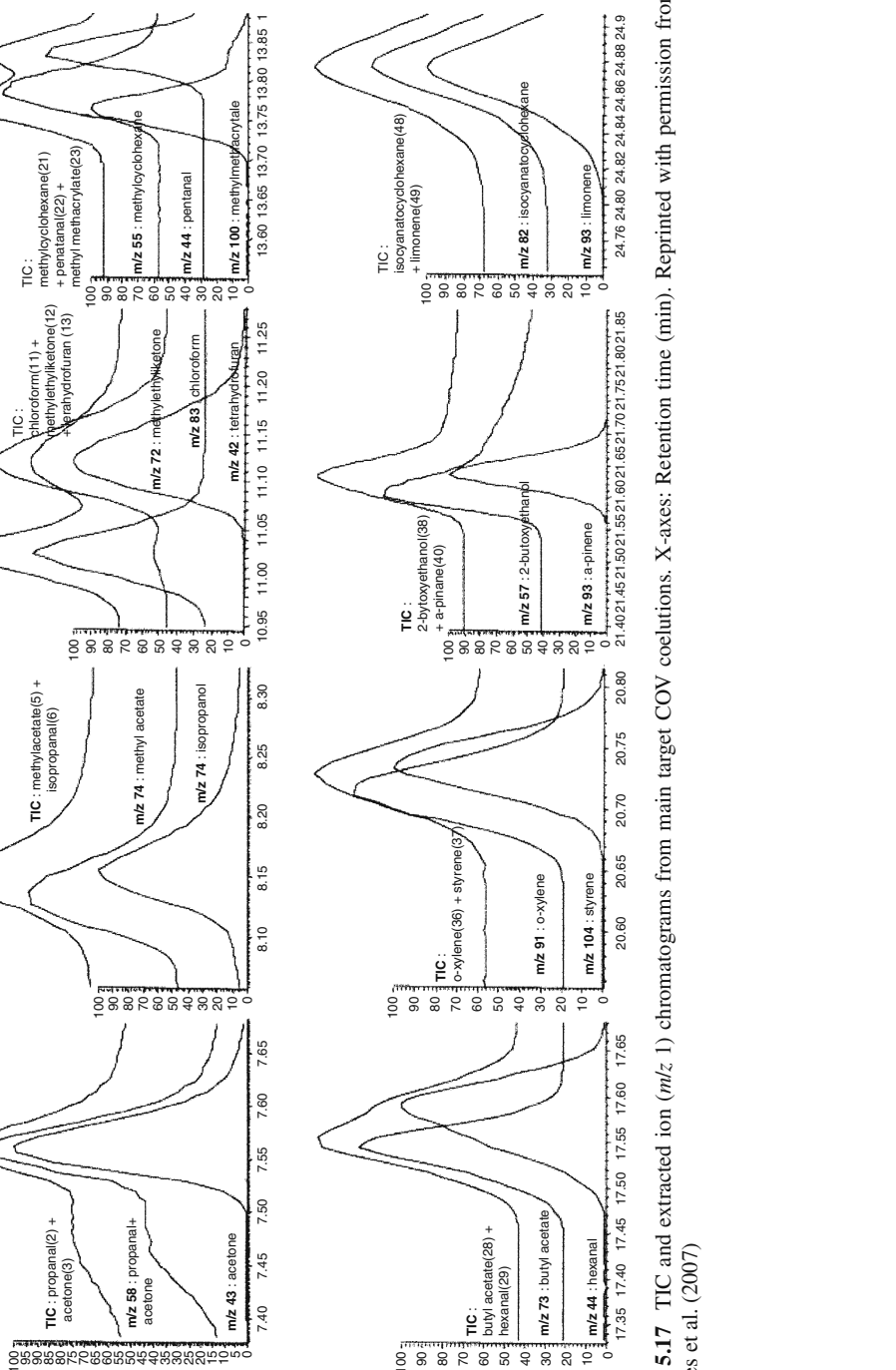

Fig. 5.17 TIC and extracted ion (*m/z* 1) chromatograms from main target COV coelutions. X-axes: Retention time (min). Reprinted with permission from Ribes et al. (2007)

A new method was developed for the analysis of nuisance odours by monitoring of volatile organic compounds. The technology was based on multisorbent adsorption and GC-MS equipped with a thermal desorption (TD) unit. Enrichment of the 57 analytes were performed on different sorbents with weak, medium and high sorption strength. VOCs were separated in a capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started at 40°C, 1 min hold, ramped to 230°C at 6°C/min, final hold 5 min. Helium was employed as carrier gas. The separation of the analytes is shown in Fig. 5.17. The method validation data are compiled in Table 5.13. It was stated that the good reproducibility of the method makes it suitable for the analysis of VOCs (Ribes et al., 2007).

Table 5.13 Summary of method validation data (standards)

Target VOCs	LOD (ng) *m/z* 1	Linearity range (ng) *m/z* 2	RF (response area/ng) *m/z* 1	Target VOCs	LOD (ng) *m/z* 1	Linearity range (ng) *m/z* 2
Ethanol	0.003	0.01	0.01–1300	0.02–1300	13977	4313
Propanal	14	14	89–886	89–886	823	823
Acetone	0.002	4	0.9–470	19–4000	30070	721
Carbon disulphide	0.001	0.001	0.005–537	0.005–540	18428	1426
Methyl acetate	0.01	0.9	0.08–850	0.08–850	3484	723
Isopropanol	0.02	2	0.1–410	3–1430	49397	2278
tert-Butylmethylether	0.2	2	0.8–760	0.8–970	3199	687
n-Hexane	0.004	0.1	0.1–270	0.3–360	27595	6279
Butanal	0.8	4	0.07–782	0.8–782	1442	994
Ethyl acetate	0.02	0.5	1.1–890	0.9–1310	8526	3073
Chloroform	0.01	0.04	0.02–700	2–1740	26657	3040
Methylethylketone	0.002	0.01	0.01–1060	0.05–2360	15309	4565
Tetrahydrofuran	0.03	2	0.08–824	0.08–824	2729	1103
1,1,1-Trichloroethane	0.02	0.1	1.2–790	1.2–790	16003	6292
Cyclohexane	0.01	0.06	0.03–330	0.1–550	20030	20011
Carbon tetrachloride	0.04	0.4	0.4–960	1.5–1930	12732	4393
Isobutanol	1.6	12	0.07–766	7.3–766	1980	217
Benzene	0.001	0.003	0.001–230	0.01–470	65395	9726
1-Butanol	0.08	4	3–420	33–810	20952	9840
Trichloroethylene	0.003	0.1	0.01–350	1.1–700	24206	8091
Methylcyclohexane	0.005	0.01	0.02–230	0.03–230	27184	20293
Pentanal	0.8	4	0.8–500	7–758	3454	1196
Methyl methacrylate	0.5	0.5	0.7–910	0.7–910	11065	11065
Methylisobutylketone	0.02	0.1	0.1–1470	2.4–1960	34752	9265
Toluene	0.005	0.01	001–258	0.2–1800	49150	7185
1,1,2-Trichloroethane	0.03	1	0.06–840	1.9–2400	17473	1600
Tetrachloroethylene	0.003	0.1	0.01–480	0.6–960	22651	10941
Butyl acetate	0.04	0.1	0.4–350	1.5–920	11274	11274
Hexanal	2	30	8–760	59–1010	332	168
N,*N*-Dimethylformamide	14	280	466–1862	466–1862	2922	264
N-Methylformamide	97	194	92–921	242–1938	1537	1180
Ethylbenzene	0.01	0.02	0.03–430	0.7–1260	29083	6181
n-Nonane	0.03	1	0.06–350	1.9–1890	34951	2511

Table 5.13 (continued)

Target VOCs	LOD (ng) *m/z* 1	Linearity range (ng) *m/z* 2	RF (response area/ng) *m/z* 1	Target VOCs	LOD (ng) *m/z* 1	Linearity range (ng) *m/z* 2
m,*p*-Xylene	0.004	0.02	0.02–420	1.0–1250	34514	9266
o-Xylene	0.005	0.02	0.02–197	0.02–197	71251	71251
Styrene	0.02	0.1	0.03–360	0.3–880	48223	48223
Heptanal	0.8	8	7–742	7–742	1256	247
2-Butoxyethanol	6	30	11–1870	100–3200	24561	9210
α-Pinene	0.01	0.1	0.04–300	1.6–1030	52205	4918
Cyclohexanone	0.1	0.5	0.2–870	5–6500	15313	3135
Propylbenzene	0.03	0.1	0.06–270	0.2–580	73964	25920
n-Decane	0.02	0.1	0.1–590	1.2–3090	22715	3347
1,3,5-Trimethylbenzene	0.08	0.09	0.08–1671	0.08–1671	76040	47360
β-Pinene	0.3	3.5	0.08–830	8–830	3955	442
1,2,4-Trimethylbenzene	0.03	0.03	0.06–350	0.1–350	65026	36575
Benzaldehyde	0.03	1.1	0.1–1330	2.0–1330	11585	10305
Isocyanatocyclohexane	10	10	30–1800	30–3710	2108	127
Limonene	0.05	0.1	0.2–530	0.3–2600	25294	6428
p-Dichlorobenzene	0.03	0.5	0.06–370	1.0–770	40625	11036
n-Undecane	0.03	0.3	0.06–290	0.5–880	44634	3041
Phenol	0.6	4.3	1.1–970	8.3–970	54229	14448
1-Octanol	7	14	7.3–1220	29–1220	9486	6326
Naphthalene	0.02	1	0.06–200	2.0–1990	98515	8808
Isothiocyanatocyclohexane	0.2	0.5	0.5–475	1.0–830	36575	27423
2-Methylnaphthalene	0.1	1	0.3–360	2.0–870	79744	23531
1-Methylnaphthalene	0.1	1	0.2–350	2.0–850	79508	24653

Limit of detection (LOD, ng in tube), linearity range (ng in tube) and response factors (RF, response area/ng in tube).
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VOCs and odorant associated with swine barn particulate matter were separated and quantitated by HS-SPME-GC-MS(FID)-olfactometry. Analytes were preconcentrated on PDMS, carboxen/PDMS and CAR/DVB fibres for 3 h and separated by a nonpolar precolumn and a polar column. Injector and detector temperatures were 260°C and 280°C, respectively. Oven temperature started at 40°C, 3 min hold, ramped to 220°C at 7°C/min, final hold 10 min. Helium was employed as carrier gas. The mass detection range of MS was *m/z* 33–280. The following analytes were detected under the experimental conditions, retention time in min in parentheses: H_2S (1.16), pentane (1.38), methyl mercaptan (1.48), 1,1-dichloro-1-fluorethane (1.55), trimethylamine (1.56), 3-pentaneamine (1.83), acetone (1.91), heptane (2.45), butanal (2.53), 3-methyl-butanal (3.21), diacetyl (3.70), pentanal (4.21), hexanal (6.61), ethanone (8.33), 2-heptanone (9.06), heptanal (9.21), styrene (10.15), 6-methyl-2-heptanone (10.56), *N*,*N*-dimethylformamide, 2-pentyl furane, (10.98), 1-hexanol (11.51), octanal (11.78), dodecane (12.01), 2-butoxy-ethanol (12.55), acetic acid (13.1), 3-octen-2-one (13.65), *n*-nonanal (14.16), 2-ethylhexanol (14.53), propanoic acid (14.81), isobutyric acid (15.40), 1-octanol (15.80), butanoic

acid (16.46), 3-methyl-butanoic acid (17.20), γ-hexalactone (18.13), pentanoic acid (18.38), acetamide (18.78), 2,4-nonatienal (18.81), hexanoic acid (20.16), benzenemethanol (20.71), dimethyl sulphone (21.11), heptanoic acid (21.86), phenol (22.66), 2,6-di-*tert*-butyl-4-ethylphenol (23.70), 4-methylphenol (23.78), 2-piperdinone (24.56), 4-ethyl-phenol (25.13), 2′aminoacetophenone (25.95), indole (28.81), 2-methyl-indole (29.50), 5-acetyl-2-methylpyridine (31.00).

It was established that the amount of adsorbed VOCs strongly depended on the size of particulate matter (Cai et al., 2006).

A microwave-assisted solvent extraction method coupled with GC-MS was developed for the measurement of pharmaceuticals and PCPs (musk ketone included) in solid matrices. Soil samples were extracted twice with methylene chloride than with methanol, acetone or hexane. GC-MS separations were performed in a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started at 50°C, 1 min hold, raised to 150°C at 25°C/min, to 204°C at 8°C/min, to 212°C at 4°C/min, to 240°C/min, to 310°C at 20°C/min, final hold 13 min. Helium was employed as carrier gas. The retention time of musk ketone was 13.37 min, the target and qualifier *m/z* 1 and 2 were 279.25, 294.15 and 128.15, respectively. It was found that the recovery values were relatively low but the reproducibility of the method was acceptable (Rice and Mitra, 2007).

Ultrasonic-assisted extraction of various chemicals from soils and sediments has also been performed. After extraction the analytes were further enriched by SPE. Pollutants were separated and quantitatively determined by GC-MS using a capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). Temperature program started at 40°C, 3 min hold, raised to 100°C at 8°C/min (hold 4.50 min), to 290°C at 9°C/min. Helium was employed as carrier gas. The retention time of indole was 15.51 min; the recovery and LOD values were 79.7% and 0.06 μg/g (Bossio et al., 2008).

The occurrence of synthetic musk fragrances in living organisms has also been frequently investigated. Thus, the accumulation of musk fragrance in freshwater fish and mussels (Gaterman et al., 2002a, 2002b) and in marine fish samples was also demonstrated (Kallenborn et al., 2001).

The concentration of various organic pollutants, among them DDT and its degradation products, polychlorinated biphenyls, polybrominated diphenyl esters and synthetic fragrances (crysolide, phantolide, fixolide, traseolide, galaxolide, musk ketone and musk xylene), was measured in fish from remote alpine lakes in Switzerland. Synthetic musk fragrances were determined in a glass capillary column (20 m × 0.30 mm i.d., film thickness, 0.15 μm). Temperature program started at 110°C, 1 min hold, raised to 150°C at 20°C/min, to 220°C at 4°C/min, to 260°C at 20°C, final hold 5 min. Hydrogen was employed as carrier gas.

The concentrations of synthetic musks in the lakes are compiled in Table 5.14. It was concluded from the results that atmospheric distribution processes may contribute to the environmental fate of synthetic musk fragrances (Schmid et al., 2007).

Table 5.14 Compilation of total concentrations of persistent organic pollutants in fish from alpine lakes in the Grisons, Switzerland (ng/g, lipid weight (lw) based)

	L. Tuma	*L. Lunghin*	*L. Moesola*	Surettasee	*L. Diavolezza*	*L. Teo*	*L. Grond*
	L. Tuma	*L. Lunghin*	*L. Moesola*	Surettasee	*L. Diavolezza*	*L. Teo*	*L. Grond*
Synthetic musks							
MX (musk xylene)	1.9	12	1.8	1.9	1.3	2.5	2.2
MK (musk ketone)	2.2	2.6	2.5	2.2	2.0	2.9	2.1
ADBI (Crysolide)	33	28	29	8.7	29	35	27
AHMI (Phantolide)	1.7	2.5	1.2	0.79	6.3	2.0	3.3
ATII (Traseolide)	1.7	2.1	1.9	1.4	1.3	2.1	1.7
AHTN (Fixolide)	30	38	54	20	27	27	20
HHCB (Galaxolide)	50	78	230	42	46	53	44

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The occurrence of fragrances in humans has also been extensively investigated. Thus, the accumulation and degradation of synthetic musks in human subject (Hawkins et al., 2002), in human milk (Lieble et al., 2000) and in human-derived Hep G2 cells were demonstrated (Mersch-Sundermann et al., 2001).

Synthetic musk fragrances were also determined in trout from Danish fish farms and human milk employing GC-MS technology. Homogenated trout samples

Table 5.15 Retention time (R_t) in minutes and the molecular weight and masses (m/z ratios) for GC/HRMS and GC/MS detection of the synthetic musk compounds

Compound	R_t (min)	Molecular weight	SIM masses (m/z)
MS function 1			
DPMI	8.08	206.3	206.167;[a] **191.144**
ADBI	10.41	244.4	244.183; **229.159**
AHMI	11.21	244.4	**244.183**; **229.159**
MS function 2			
Musk ambrette	12.36	268.2	**253.082**; 268.2
Bromocyclene	12.49	393.8	**236.841**; **238.838**
ATII	12.51	258.4	258.198;[b] **215.144**
Musk xylene D_{15}	12.51	312.0	**294.148**
HHCB	12.57	258.4	243.175; **213.164**
AHTN D_3	13.06	261.4	261.217; **246.194**
AHTN	13.09	258.4	258.198; **243.175**
Musk xylene	13.26	297.3	**282.073**
Musk moskene	13.34	278.3	**263.103**; 261; 278
Musk tibetene	14.36	266.3	**251.103**; 176; 266
Musk ketone	15.27	294.3	**279.098**; 247; 191

[a] Ion not used for quantification due to interfering substances.
[b] Not completely separated from an interfering peak from HHCB.
Bold masses were used for quantification.
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Table 5.16 Synthetic musk compounds concentration in μg/kg fresh weight for trout from 50 Danish trout farms in 1999 and 87 farms in 2003 and 2004

Compound	Year	Minimum	Maximum	Median	Average[a]	Standard deviation[a]	Recovery ($n = 10$)
HHCB	1999	n.d. (0.52)	52.6	4.97	8.54	10.1	90
	2003/04	n.d. (0.52)	28.0	1.15	5.87	6.14	81
AHTN	1999	0.44 (0.21)	15.9	1.13	2.24	3.27	94
	2003/04	n.d. (0.61)	7.5	n.d.	2.70	1.86	79
ADBI	1999	n.d. (0.03)	18.3	0.40	1.14	2.87	85
	2003/04	n.d. (0.24)	0.50	n.d.	0.41	0.13	70
AHMI	1999	n.d. (0.07)	21.5	0.11	1.16	3.88	86
	2003/04	n.d. (0.17)	n.d.	–	–	–	71
ATII	1999	n.d. (0.08)	15.4	0.30	0.73	2.22	76
	2003/04	n.d. (0.15)	0.60	n.d.	0.40	0.28	75
Musk tibetene	1999	n.d. (0.03)	13.7	n.d.	1.10	2.71	95
	2003/04	n.d. (0.22)	n.d.	n.d.	–	–	85
Musk moskene	1999	n.d. (0.05)	14.6	0.63	1.03	2.03	83
	2003/04	n.d. (0.33)	n.d.	n.d.	–	–	84
Musk ketone	1999	n.d. (0.13)	5.16	0.65	1.02	0.86	87
	2003/04	n.d. (0.28)	1.0	n.d.	0.65	0.24	83
Musk xylene	1999	n.d. (0.18)	1.59	0.52	0.76	0.34	97
	2003/04	n.d. (0.23)	1.30	n.d.	0.54	0.28	80
Musk ambrette	1999	n.d. (0.10)	1.93	0.09	0.73	0.41	95
	2003/04	n.d. (0.30)	0.32	n.d.	0.32	0.01	79

[a] Calculated on measurements above detection limit. Minimum, maximum, median and average concentrations are listed together with the standard deviation. The detection limit (LOD) for each compound is included in brackets in the column for the minimum values. The recovery for the spiked sample in 10 series is included.
n.d.: not detected below the limit of detection.
(–) No samples above the detection limit.
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were extracted by acetone–pentane (1:3, v/v) while human milk was extracted by ethanol, diethylether, pentane. The extracts were further purified by gel permeation chromatography (GPC) using polystyrene support and by SPE. Synthetic musk fragrances were measured in a capillary column (30 m × 0.25 mm i.d.). Temperature program started at 90°C, 2 min hold, ramped to 180°C at 20°C/min, to 225°C at 3°C/min, to 290°C at 30°C, final hold 15 min. Helium was employed as carrier gas. The results are compiled in Table 5.15. The concentrations of synthetic musk fragrances in trout are compiled in Table 5.16. The results demonstrated that HHCB is the dominating pollutant in trout followed by the other synthetic fragrances. The synthetic musk levels found in human milk samples are listed in Table 5.17. The data illustrate the wide distribution of the individual concentrations of synthetic fragrances in human milk. It was further established that the consumption of farmed trout is not the main source of pollution of humans by synthetic musk fragrances (Duedahl-Olesen et al., 2005).

Table 5.17 Synthetic musk levels (μg/kg fat) in 10 Danish human milk samples collected in 1999

Compound	Minimum	Maximum	Median	Average[a]	Standard deviation[a]	Recovery (run 1 and 2)
HHCB	38.0 (0.59)	422	147	179	111	98–101
AHTN	5.58 (2.0)	37.9	17.5	19.5	9.77	101–108
ADBI	n.d. (0.39)	11.2	5.98	7.78	3.09	90–112
AHMI	n.d. (1.0)	9.94	n.d.	8.03	2.89	101–114
ATII	n.d. (0.22)	2.58	n.d.	–	–	91–110
Musk moskene	n.d. (1.3)	30.6	n.d.	15.1	14.6	101–92
Musk ketone	n.d. (5.0)	26.9	14.9	17.0	6.12	90–94
Musk xylene	n.d. (3.1)	46.4	9.44	23.6	15.6	101–100

[a] Calculated on measurements above detection limit.

Data include the minimum, maximum, median and average concentrations with the recovery calculated for two series ($n = 2$). Detection limits is listed in brackets with the minimum concentration.

n.d.: not detected, below detection limit.

(–) Only one sample above detection limit.

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