4

Gas Chromatographic Analysis of Plant and Insect Surface Compounds: Cuticular Waxes and Terpenoids

Beata Szafranek, Monika Paszkiewicz,
Marek Gołębiowski and Piotr Stepnowski
University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland

1. Introduction

The surfaces of all higher plants are covered by a layer of cuticular waxes. These are composed mainly of long-chain aliphatic components but also of cyclic compounds. The primary role of the waxes is to prevent uncontrolled water loss. The chemical composition of plant cuticular waxes can affect the resistance of plants to herbivores and herbivore behaviour. Cuticular waxes and their separate components enhance or deter insect oviposition, movement or feeding.

Most plants have trichomes on their aerial surfaces. The trichomes may be simple hairs or more specialized glandular trichomes, whose main function may be the production and accumulation of chemicals such as essential oils. The vast majority of these consists of monoterpenoids, sesquiterpenoids and diterpenoids with a high vapour pressure. They may be absorbed on the cuticular wax layer. The trichome secretions are closely related to plant-insect or plant-microbe interactions. Terpenoids can attract, repel or initiate defence reactions in insects. Apart from their ecological roles, plant terpenoids are widely used in the pharmaceutical and fragrance industries. The properties of essential oils are correlated with their qualitative and quantitative compositions.

The surfaces of insects are also covered by a layer of wax. Insect cuticular waxes are also involved in various types of chemical communication between individuals of a species and reduce the penetration of chemicals and toxins as well as infectious microorganisms. Analyses and identification of insect waxes is the first step towards developing methods of insect control.

Improvements in analytical techniques have led to the characterization of plant and insect surface compounds and provided new insights in chemical ecology. Moreover, an enormous number of plant terpenoid analyses are associated with their pharmaceutical and fragrance applications. Qualitative and quantitative analyses of cuticular waxes and terpenoids are usually achieved by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Peak identification is based primarily on retention times, retention indices and comparison of recorded spectra with an MS library. This review will describe gas

chromatographic applications in the analysis of various classes of cuticular waxes and terpenoids.

2. Cuticular waxes

Cuticular waxes are commonly present on the surfaces of higher plant and insect species, but they often differ from one another in composition. Generally speaking, cuticular waxes consist of complex mixtures of long-chain nonpolar compounds, with the molecular weights of individual components ranging from about 200 to 600 Da, but from time to time exceeding 1000 Da.

2.1 Chemical composition of plant cuticular waxes

Plant surface waxes are complex mixtures of relatively non-polar aliphatic and cyclic compounds (Baker, 1982; Bianchi, 1995; Jetter et al., 2006). The surface waxes of plants consist of various groups of long-chain lipids such as hydrocarbons, wax esters (esters of long-chain alcohols and fatty acids, alkyl esters), fatty acids, long-chain alcohols, aldehydes, ketones, β-diketones and hydroxy-β-diketones. Different classes of compounds may contain a series of homologues with different numbers of carbon atoms in the chain, but those most often encountered have from 20 to 36 carbon atoms, although wax esters with more than 60 carbons are also known. Some less common constituents include branched-chain hydrocarbons, alkenes, terpene hydrocarbons, diols, ω-hydroxyacids, branched-chain fatty acids, branched-chain esters, methyl esters, benzoic acid esters, acetates, polyesters of ω-hydroxyacids (estolides), and many others. Cyclic compounds are also common in surface waxes: terpenoids (triterpenols, triterpenoid acids, ketones and aldehydes) and sterols, and their esters; aromatic compounds have been found in only a very few plant species, however. The relative percentages of homologous compounds often form typical patterns that can be helpful in the interpretation of gas chromatograms. For example, the distribution patterns of n-alkanes are typically bell-shaped. The chemical composition of plant surface waxes depends mainly on the plant species and may be different on its various organs. Surface waxes cover not only the leaves but also the stem, fruits, flowers and seeds. Their composition may also depend on plant age and environmental conditions such as illumination, temperature or the presence of chemicals. The composition of surface waxes developing during the evolution of plants became increasingly complex and varied. The chemical compositions and biochemical aspects of plant surface waxes are described in more detail in several monographs (Hamilton, 1995a; Kolattukudy, 1976; Riederer & Müller, 2006).

2.2 Chemical composition of insect cuticular waxes

The cuticular waxes of insect species may contain the following chemical classes: hydrocarbons, fatty acids, alcohols, triacylglycerols and wax esters (Gołębiowski et al., 2011; Nelson & Blomquist, 1995). The waxes of some species also contain aldehydes, ketones, esters and sterols. The wax compositions of insects can vary depending on stage, sex, age, and their position in the colony hierarchy. Cuticular waxes can also vary within species as a response to living conditions such as temperature, dryness and available food. The major function of insect waxes is protection against desiccation, but they also prevent microbial infections, affect the adsorption of chemicals and play a role in chemical communication

between species. Hydrocarbons, e.g. n-alkanes, n-alkenes and methyl-branched hydrocarbons, often constitute a major fraction of insect cuticular waxes: they were found to be the major compounds in the cuticular waxes of adults, nymphs and eggs of *Zygogramma exclamationis* (Nelson and Charlet, 2003). 79.4% of cuticular waxes isolated from *Calliphora vicina* larvae were fatty acids (Gołębiowski et al., 2008a). In contrast, the fatty acids in *D. pini* exuviae extracts made up only 2.0% of all waxes (Gołębiowski et al., 2010). Alcohols ranging from $C_{24:0}$ to $C_{34:0}$ constituted the major lipid fraction of the cuticular waxes from *Heliothis virescens* pupae (Buckner et al., 1996). Wax esters consisting of even-numbered-carbon compounds from C_{38} to C_{64} were the major compounds (86%) present in the cuticle of *Bemisia argentifolii* nymphs and exuviae (Buckner et al., 1999). Cuticular waxes of insects also contain aldehydes as minor constituents. This group of waxes were identified in *Heliothis virescens* pupae, *Helicoverpa zea* pupae (Buckner et al., 1996), *Aleurodes singularis* adults and exuviae (Nelson et al., 1998), *Bemisia argentifolii* nymphs and exuviae (Buckner et al., 1999), *Bemisia tabaci* and *Trialeurodes vaporariorum* adults (Buckner et al., 1994). Triacylglycerols are compounds of cuticular waxes but they could also be contaminants derived from body lipids. For example, triacylglycerols were identified in the waxes of *Melanoplus sanguinipes* and *M. packardii* adults (Soliday et al., 1974). Fatty acid methyl esters are not common constituents of insect cuticular waxes. Both methyl and ethyl esters were identified in the cuticular waxes of *Acanthoscelides obtectus* Say (Gołębiowski et al., 2008b). Like fatty acid methyl esters, ketones are minor constituents of cuticular waxes. The cuticular lipid of *Ceutorrhynchus assimilis* contains 98% of C_{29}, smaller amounts of C_{26} (<1%) and traces of C_{28}, C_{30} and C_{31} (Richter & Krain, 1980). The ketones of *C. assimilis* are very similar to those in the surface waxes of *Brassica napus*, which suggests that they could be of host plant origin. Sterols are also minor constituents of cuticular lipids. Cholesterol is a cell membrane component in insects and a precursor of steroid hormones such as ecdysone. Insects lack the capacity to synthesize sterols, so they convert phytosterols to cholesterol *via* dealkylation. Cholesterol is the most abundant sterol in the cuticular waxes of insects, occurring, for example, in the waxes of *M. sanguinipes* and *M. packardii* (Soliday et al., 1974).

2.3 Isolation and class separation of plant cuticular waxes

Plant cuticular waxes are usually extracted by dipping the plant material in an organic solvent of intermediate polarity for 10 - 60 sec (Bakker et al., 1998; Hamilton, 1995b; Jetter et al., 2006; Stammitti et al., 1996). The solvents used include chloroform, dichloromethane, hexane, petroleum ether and toluene. A two-step extraction with the use of two portions of solvent may improve recovery. The classical extraction method is usually carried out at room temperature but occasionally at the boiling point of the solvent for the complete extraction of insoluble polymeric forms of aldehydes (Haas et al., 2001; Szafranek et al., 2008a). The different sides of the same plant organ (the adaxial and abaxial sides of leaves) may be characterized by cuticular waxes with different compositions, so their extraction is more complicated (Jetter et al., 2000; Buschhaus et al., 2007a). Epicuticular waxes can also be isolated by mechanical methods (Ensikat et al., 2000; Jetter et al., 2000; Riedel et al., 2007), which distinguish the epicuticular waxes outside the plant cuticle from the cuticular waxes embedded in the cuticle matrix. The extraction of 1 to 10 cm^2 of the plant surface area should yield a sufficient amount of wax for a single GC-FID or GC-MS analysis (Jetter et al., 2006).

GC is the most commonly used method to selectively identify and quantify the components of cuticular waxes. A schematic diagram of the procedures used in the analyses of plant cuticular waxes is given in Figure 1. Because of the complex nature of plant and insect cuticular waxes, fractionation according to chemical class is usually required before identification of individual components can be achieved. Special care has to be taken in the case of quantitative analyses. Internal standards representing all compound classes should be added early during the wax extraction before fractionation. Traditionally, separation schemes have been developed by using preparative column chromatography (LC) or thin layer chromatography (TLC) (Hamilton, 1995b). A typical example might involve LC separation on silica gel or alumina and gradient elution with increasing concentrations of ethyl acetate in petroleum ether. The separation can then be monitored by TLC. A rapid form of LC is flash chromatography where solvent is forced down the column by air pressure (Still et al., 1978), a procedure that has recently been used for the fractionation of eggplant cuticular waxes (Haliński et al., 2009).

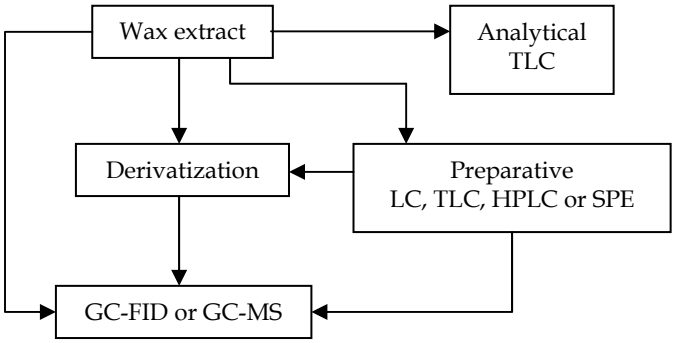

Fig. 1. Summary of typical procedures used in the analysis of plant cuticular waxes.

High-performance liquid chromatography (HPLC) has not been widely used in the analysis of cuticular waxes since these have no useful UV chromophores and because of the difficulties of separating components with different polarities (Hamilton, 1995b). Hwang et al. (2002) developed an HPLC method using a silica gel column and an evaporative light scattering detector (ELSD) to analyse sorghum wax. Szafranek & Synak (2006) and Szafranek et al. (2008b) used HPLC-ELSD for the preparative fractionation of potato cuticular waxes. HPLC separation of cuticular waxes has a better resolution and is more efficient than column chromatography.

Solid-phase extraction (SPE) is today the most popular sample preparation method but still hardly used in the fractionation of plant cuticular waxes. Nass et al. (1998) has developed a method involving the separation of the wax sample into two fractions using simple SPE in order to avoid artefacts in trimethylsilyl derivatization; the fraction containing aldehydes could be GC analysed without silylation. Perez-Camino et al. (2003) have described a procedure for the simultaneous determination of long-chain aliphatic aldehydes, and aliphatic and triterpenic waxes in olive oils. A fraction containing these compounds was isolated from the oil using SPE on silica-gel cartridges. Commercial SPE cartridges are convenient for the isolation of lipid classes, so they may be used more extensively in the

future. Solid phase microextraction (SPME) is a promising new technique for sample extraction/isolation before GC analysis, but as far as we know, it has been applied to the cuticular waxes of insects, but not yet to those of plants.

2.4 Isolation and separation of insect cuticular waxes

Extraction procedures aim to remove all of an insect's cuticular waxes. For this, a non-polar solvent is usually used, for example, pentane, hexane and petroleum ether for 10 seconds or chloroform for 1 min (Gołębiowski et al., 2011; Nelson & Blomquist, 1995). The use of a non-polar solvent minimizes the possible extraction of internal lipids, which are mostly free fatty acids and glycerides. Non-polar solvents are used for extracting waxes rich in hydrocarbons, whereas chloroform extracts the oxygenated compounds in cuticular waxes. Chloroform-methanol mixtures are used for short extractions to avoid the possible extraction of internal waxes. The choice of extraction solvent and extraction time depend on the quantity and quality of the waxes. For example, the cuticular waxes of the larvae and adults of khapra beetles *Trogoderma granarium* were obtained by immersing the insects in dichloromethane for 20 seconds (Maliński et al., 1986). A longer extraction was used to separate the cuticular waxes of four *Periplaneta* species (Said et al., 2005); in this case, insects anaesthetized in CO_2 were immersed in dichloromethane for 2 min. A 30-second immersion in chloroform was sufficient to obtain the cuticular waxes from *Tribolium destructor* larvae and beetles (Hebanowska et al., 1990). One immersion does not extract all of the cuticular waxes, however: two short extractions are more effective for the complete removal of the cuticular waxes without extraction of internal waxes. Sometimes, internal waxes are extracted because the cuticle is damaged or the extraction time is too long. Triacylglycerols are major components of internal waxes, but they are present in the cuticular waxes of insects, too. The presence of triacylglycerols in cuticular wax extracts can be a useful indicator that internal waxes have been extracted. A two-step extraction was used for the cuticular waxes of adults of *Frankliniella occidentalis* (Gołębiowski et al., 2007). The insects were first extracted in petroleum ether for 30 s, after which they were immersed in dichloromethane for 2 min.

Wax extracts are separated into classes of waxes by TLC, LC or HPLC. Table 1 gives examples of the methods for separating insect cuticular waxes.

Insects	Separation	References
T. granarium	Hydrocarbons eluted with hexane on a column filled with silica gel activated at 150 °C for 24 hr	Maliński et al., 1986
B. argentifolii	Lipid classes separated by high performance TLC using plates coated with silica gel. Developing solvent: hexane/diethyl ether/formic acid (80:20:1 v/v/v). Visualization: 5% concentrated sulphuric acid in 95% ethanol	Buckner et al., 1999
A. obtectus	Lipid classes separated by HPLC-ELSD. The mobile phase consisted of petroleum ether (solvent A) and dichloromethane with the addition of 15% acetone and 1.5% isopropanol (solvent B). The gradient was programmed to change linearly from 100% A to 100% B within 20 min.	Gołębiowski et al., 2008b

Table 1. Examples of the isolation and separation of insect cuticular waxes

Recently, SPME and solid injection (SI) have been used for extracting cuticular waxes of insects. SPME and SI can be used in combination with GC or GC–MS. SPME is a sample preparation technique using a fused-silica fibre coated on the outside with an appropriate stationary phase. The compound in the sample is directly extracted and concentrated on the fibre coating. The method saves preparation time and solvents, and it can improve detection limits. SPME can be used routinely in combination with GC, GC–MS, HPLC and liquid chromatography-mass spectrometry. SPME-GC and SPME-GC-MS are applicable to the extraction of volatile and semi-volatile organic compounds from biological samples. The following types of stationary phase can be used: non-polar polydimethylsiloxane (PDMS), polar polyacrylate (PA), Carboxen (Car)/divinylbenzene (DVB), Carbowax/templated resin (polar), mixed-polarity PDMS/DVB, Car/PDMS and DVB/Car/PDMS (Gołębiowski et al., 2011). Compounds of different volatility and polarity can be sampled simultaneously by carefully selection of the polarity and thickness of the fibre coating. The application of SPME to the analysis of insect cuticular hydrocarbons is based mainly on direct contact (DC-SPME) between the fibre and the cuticular surface. SPME adsorption from heated samples (headspace SPME, HS-SPME) is also used; in this technique the fibre is exposed to the vapour phase above a gaseous, liquid or solid sample. The cuticular fatty acid methyl esters from *Calliphora vicina* were analysed by HS-SPME (Gołębiowski, *data unpublished*). The photo of the sampling procedure of insect volatiles is given in Figure 2.

Fig. 2. Static headspace sampling of insect volatiles with a SPME device.

The cuticular hydrocarbons from *Bagrada hilaris* were analysed by SPME-GC-MS (Pasquale et al., 2007) having been sampled using fibres coated with polydimethylsiloxane (PDMS, 100 µm), polyacrylate (PA, 80 µm) and Carbowax–divinylbenzene (CW-DVB, 65 µm). The fibres were conditioned in the gas chromatograph injector port: PDMS at 250 °C for 30 min, PA at 300 °C for 2 h, and CW-DVB at 220 °C for 30 min. Sealed vials with the insects were thermostatted at temperatures of 130 and 150 °C for 10 min. The headspace volatiles were absorbed on the exposed fibre for 2 min. The loaded fibre was then desorbed in the gas chromatograph inlet port for 2 min. 12 hydrocarbons were identified in the cuticular waxes of *B. hilaris*: they consisted of a homologous series of n-alkanes ranging from C_{17} to C_{29}. Although the hydrocarbon profiles of males and females were qualitatively similar, quantitative differences were observed. Two sampling techniques for the GC-MS analysis of free fatty acids from exocrine glands were used (Maile et al., 1998). Solvent-free sampling, either by SPME or SI, are the ideal conditions for the analysis of insect samples. The insect waxes are analysed without any danger of contamination. Small insects or their parts are first sealed in a soft soda glass capillary tube. This is inserted in the gas chromatograph injector port, then heated for 3 min, after which GC analysis commences. The SPME sampling of fatty acids from solutions has been examined with different fibres; the stationary phases used as coatings were polydimethylsiloxane (PDMS, 7 µm), polyacrylate (PA, 85 µm), and Carbowax-divinylbenzene (CW-DVB, 65 µm). The best results for saturated fatty acids were obtained with PDMS-coated fibres, whereas PA fibres are suitable for palmitoleic, oleic and linoleic acids. Solid injection was used to analyse the hydrocarbons of the small ant *Cardiocondyla wroughtonii* (Turillazzi et al., 2002) and can be used to analyse solid samples, including body parts, glands or even small size insects. The solid injector technique involves inserting sealed glass capillaries into the GC-MS injector. Samples are inserted into the groove, which is then moved to align with two holes that allow passage of the carrier gas over the sample in the GC once injected. 44 hydrocarbons were identified on the mass spectra obtained with solid injection. This method proved to be a superior means of extracting hydrocarbons from insects.

2.5 GC separation and detection of cuticular waxes

Gas chromatography was first applied to analyse cuticular waxes in the early 1960s, and its use has progressed with the introduction of capillary columns. Many of the more recently published papers deal with gas chromatography analysis (GC-FID or GC-MS) rather than with liquid chromatography (LC, TLC or HPLC). The aim of GC is to resolve the individual constituents of the complex wax mixture in order to obtain information on their qualitative and quantitative composition. This can be done in the following ways:

a. GC analyses of intact cuticular wax extracts or fractions;
b. chemical derivatization of functional groups containing active hydrogen and then GC analyses;
c. chemical hydrolysis of high-molecular-weight esters and then GC analyses of the liberated moieties.

The detectors most frequently used for GC analysis of cuticular waxes are the flame ionization detector (FID) and mass spectrometer (MS) (Evershed, 1992a; Hamilton, 1995b; Riederer & Müller, 2006). Occasionally, other detectors can be applied. For example, Grossi & Raphel (2003) used GC coupled to an atomic emission detector (AED) for the identification of a series of 1-chloro-n-alkanes in the leaf waxes of three halophytes.

GC analysis of cuticular waxes is carried out mainly on fused-silica capillary columns of different diameter (0.1-0.32 mm) and length (10-50 m). In our opinion, the most common stationary phases used for the analysis of cuticular wax are:

a. 100% dimethylpolysiloxane [for example, DB-1 equivalent to OV-1, RTX-1, HP-1, SE-30, CP-Sil 5CB, EC-1 (Grace, 2011)]
b. 5% phenyl/95% methylpolysiloxane [for example, DB-5 equivalent to OV-5, RTX-5, HP-5, SE-54, EC-5 (Grace, 2011)].

The direct analysis of wax extracts or, alternatively after the conversion of all hydroxyl-containing compounds into the corresponding trimethylsilyl (TMSi) derivatives, is often used to obtain GC profiles of plant cuticular waxes. For example, Buschhaus et al. (2007b) reported alkanes, primary alcohols, alkyl esters, triterpenoids and minor components in the waxes of rose leaves. GC analysis was carried out with the temperature programmed to rise from 50 °C to 320 °C and held for 30 min at 320 °C. Generally speaking, gas chromatographic elution with the oven temperature programmed to rise from 40 or 50 °C to 320 or 340 °C is the usual procedure in the analysis of plant cuticular waxes containing typical constituents such as hydrocarbons, fatty acids, alcohols, aldehydes, ketones, diols, esters and triterpenoids (Buschhaus et al., 2007a; Jetter, 2000; Jetter et al., 1996; Jetter & Riederer, 2000; Szafranek & Synak, 2006). Figure 3 shows the typical GC pattern of intact cuticular wax extract.

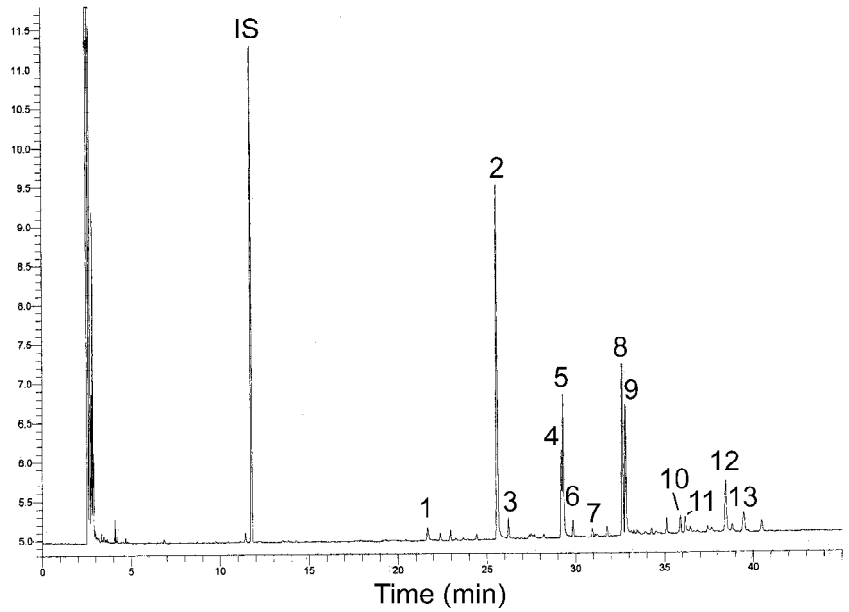

Fig. 3. GC-FID chromatogram of marigold (*Tagetes patula* L.) leaf cuticular waxes obtained on an RTX-5 capillary column with a temperature programme from 180 °C to 320 °C at 4 °C/min. (1) 1-Docosanol; (2) 1-tetracosanol coeluted with n-heptacosane; (3) tetracosanoic acid methyl ester; (4) n-nonacosane; (5) 1-hexacosanol; (6) hexacosanoic acid methyl ester; (7) n-triacontane; (8) n-hentriacontane; (9) 1-octacosanol; (10) n-tritriacontane; (11) 1-triacontanol; (12) β-amyrin; (13) α-amyrin; (IS) internal standard.

Insect cuticular waxes, consisting mainly of hydrocarbons, fatty acids, alcohols, aldehydes, and wax esters, have been analysed with the oven temperature programmed to rise from about 150 °C to 300 or 320 °C (Buckner et al., 1996; Nelson et al., 1994; Pasquale et al., 2007; Turillazzi et al., 2002) or from about 100 °C to 300 or 320 °C (Gołębiowski et al., 2007; Said et al., 2005). The initial temperature was significantly lower for the analysis of cuticular hydrocarbons, aldehydes, alcohols and wax esters in *Bemisia argentifolii* (50 °C to 320 °C) (Buckner et al., 1999), the fatty acids in *Dendrolimus pini* (50 °C to 310 °C) (Gołębiowski et al., 2010) and the surface waxes in *Acanthoscelides obtectus* (30 °C to 320 °C) (Gołębiowski et al., 2008b). The initial temperature depends on the molecular weights of the compounds to be analysed. For example, the fatty acids of *D. pini* ($C_{8:0}$ – $C_{34:0}$) were analysed by GC as methyl esters (Gołębiowski et al., 2010). Owing to their volatility even at relatively low temperatures, short-chain fatty acids (C_4 to C_{10}) should be GC analysed with special care (Evershed, 1992a): temperature-programming from the lowest possible temperature (ca. 30 °C) is necessary to prevent the shorter chain compounds co-eluting with the solvent.

Wax esters, steryl esters, triterpenoid esters or triacylglycerols are analysed at slightly higher temperatures. For example, wax esters from *Salix* leaves were GC–FID analysed and the oven temperature was programmed to increase from 200 °C to 380 °C, then held for 20 min at 380 °C (Szafranek et. al., 2008a). It should be noted that the high temperatures (generally above 300 °C) required for the elution of intact high-molecular-weight compounds can cause the loss of some thermally labile compounds (e.g. unsaturated compounds) (Evershed, 1992a). Wax esters can be GC analysed on non-polar capillary columns. Recently, Stránský et al. (2006) analysed a series of more than 200 wax ester standards using a temperature gradient programme from 240 to 340 °C. The GC analysis of n-alkanes up to C_{54} and esters up to C_{52}–C_{53} could be performed under these chromatographic conditions. The problem with wax ester analysis, however, is that separation largely occurs according to carbon number; hence, the chromatographic peaks represent mixtures of non-separated wax esters. Some ester isomers elute at nearly the same time: for example 18:0-20:0 (an 18:0 acid moiety with a 20:0 alcohol moiety) and 20:0-18:0. The study shows the influence of the number of double bonds in the ester molecule on chromatographic behaviour in comparison with the corresponding saturated ester. Saturated esters have the longest retention times. Esters with three double bonds are eluted before esters with one or two double bonds.

High-temperature gas chromatography (HTGC) and HTGC coupled to mass spectrometry has great potential for the determination of a great number of high-molecular-weight compounds, e.g. sucrose fatty acid esters, ethoxylated fatty alcohols, triglycerides and wax esters (Pereira & Neto, 1999; Pereira et al., 2004). HTGC non-polar and medium polar capillary columns can be used up to 400°C and in some cases 480 °C. On the other hand, high-resolution GC commercial capillary columns can be used up to 370 °C in the routine analysis of compounds of 1200 Da and more. This enables the direct analysis of extracts and fractions of natural products, in some cases without prior derivatization or clean-up. The injection method in HTGC is very important. Cold on-column or programmable temperature vaporizing (PTV) injection are the preferred techniques, as they eliminate discrimination against high boiling analytes. For example, high molecular weight alkanoates (three series of α-tocopherol, β-tocopherol and phytol esterified to fatty acids) in Amazonian plants were analysed by HTGC (Pereira et al., 2002). The cuticular waxes from ivy leaves

containing ester waxes (alkyl alkanoates and alkyl coumarates) were analysed directly by GC-MS, HTGC-MS and ESI-MS/MS by Santos et al. (2007). However, with ESI-MS/MS analysis the detection of a wider range of ester waxes was possible than with HTGC-MS.

Fatty acids are GC analysed after their conversion to non-polar derivatives such as methyl or silyl esters (Evershed, 1992a). Often, esters are prepared as derivatives of the fatty acids liberated from wax esters or sterol esters by hydrolysis (Sümmchen et al., 1995; Szafranek & Synak, 2006). The details and other important considerations of fatty acid analysis have been well summarized by Christie (1994). Generally speaking, fatty acid methyl esters (FAME) can be GC analysed employing non-polar stationary phases like other cuticular wax components. Unsaturated compounds should be analysed on polar stationary phases (e.g. Carbowax). The determination of FAME often requires high resolution for the separation of positional and configurational isomers. The elution order is as follows (Evershed, 1992a):

a. branched-chain FAME: increasing alkyl-chain branching reduces the retention time compared to a linear saturated counterpart and the elution order is independent of the polarity of the stationary phase;
b. positional isomers of unsaturated FAME: the ECL values increase with increasing distance of the double bond from the carboxyl group on both polar and non-polar phases;
c. geometric isomers of unsaturated FAME: the *E*-isomer elutes before the *Z*-isomer on a polar column. The order is reversed on a non-polar column.

Hydrocarbons are easily analysed by GC because of their chemical and thermal stability (Evershed, 1992a). The elution order of n-alkanes reflects the differences in their molecular weights. The effects of chain-branching and isomerism follow the same trends as for fatty acid methyl esters. Insects contain a variety of methyl-branched alkanes that have been widely investigated since the 1970s (Nelson & Blomquist, 1995). With certain combinations of the number and position of methyl branches, methyl-branched alkanes may have GC similar retention times to those of n-alkanes with one or two carbon atoms fewer in the chain. Elution times may overlap for methyl-branched alkanes that differ by one or even two carbons in the chain (Carlson et al., 1998). Since separation of branched-chain alkanes is difficult, it is usually necessary to perform GC-MS.

Fatty alcohols can be GC analysed as native compounds, but their separation is improved by preparing silyl or acetate derivatives (Szafranek & Synak, 2006; Shepherd et al., 1999; Stammitti et al., 1996). Ketones and aldehydes are analysed without derivatization (Szafranek & Synak, 2006; Nass et al. 1998), although aldehydes can be converted to more stable dimethyl acetal derivatives (Drozd, 1981) or other derivatives. See Table 2 for examples of derivatives used in the GC analysis of less common cuticular wax components.

2.6 Derivatives of cuticular wax components for GC analyses

The most common derivatives used in the GG analysis of cuticular waxes containing hydroxy-compounds are trimethylsilyl derivatives (TMSi ethers and esters). They are volatile but not stable, particularly in acidic or wet conditions. Analytes are derivatized with common silylation reagents such as BSA [N,O-bis(trimethyl-silyl)acetamide], BSTFA [N,O-bis(trimethyl-silyl)trifluoroacetamide] and others (Blau & Halket, 1995; Drozd, 1981). TMCS (trimethylchlorosilane) can be used as catalyst. TMSi derivatives should be prepared

immediately prior to the analysis because of their possible sensitivity to moisture. In the case of GC-FID analyses, silicone dioxide, produced by the decomposition of silyl derivatives in a flame, is deposited on the electrodes and slowly decreases the sensitivity of the detector. The reaction is simply carried out in a septum-closed vial by dissolving the sample of cuticular waxes in the reagent mixture and leaving it in the heating block for some time. For example, the reagent mixture of BSTFA-pyridine at 70 °C for 30 min (Jetter et al. 2000; Jetter & Riederer, 2000; Vermeer et al., 2003); BSTFA–TMCS (99:1, v/v) at 90 °C for 15 min (Haliński et al., 2009); BSA-TMCS (85:15, v/v) at 70 °C for 30 min (Szafranek & Synak, 2006); BSA-TMCS (85:15, v/v) at 100 °C for 60 min (Gołębiowski et al., 2008b; 2010). Little (1999) described the artefacts in TMSi derivatization reactions and the formation of several artefacts in the silylation of aldehydes and ketones. Aldehydes have been SPE separated and GC analysed without silyl derivatization (Nass et al., 1998).

Dimethyl acetals can be prepared by heating the aldehydes under reflux with 2% anhydrous methanolic HCl for 2 h, after which the methanolic solution is cooled and neutralized with a small excess of sodium carbonate. Finally, the acetals are extracted from methanol with light petroleum (Drozd, 1981); alternatively, an aldehyde mixture with 1% sulphuric acid in methanol is left overnight in a vial at 50 °C (Christie, 1994).

High-molecular-weight esters, such as wax esters, steryl esters, triterpenoid esters and triacylglycerols, are often hydrolysed, after which the liberated moieties are GC analysed; however, this procedure provides only limited compositional information. The very convenient one-pot small-scale hydrolysis–silylation procedure, applicable to ester-emulsifiers, oils, fats, wax esters and other hydrolysable lipids, has been proposed by the IUPAC (Brüschweiler and Hautfeune, 1990). The procedure involves the saponification of esters (ca. 10 mg) in 0.5 N ethanolic KOH solution (0.25 mL) at 80 °C for 3 h, evaporation to dryness in a stream of nitrogen and then silylation of the hydrolysis products. This enables the components of very small samples to be determined (< 0.1 mg). This procedure was used for the hydrolysis of wax esters and triterpenoid esters in potato and eggplant cuticular waxes (Szafranek & Synak, 2006; Haliński et al., 2009). A solution of 1-2% (v/v) concentrated sulphuric acid in methanol can be easily used for the transesterification of acyl lipids and the esterification of free fatty acids (Christie, 1994). More procedures for ester hydrolysis and transesterification and for fatty acid esterification are described in detail by Christie (1994).

The free hydroxyl groups of long-chain alcohols and other compounds are frequently acetylated prior to GC analysis. Acetic anhydride in pyridine (5:1, v/v) is a mild reagent for acetylation: the sample (up to 50 mg) is dissolved in acetic anhydride in pyridine (2 mL) and left at room temperature overnight, following which the excess reagents are removed in a stream of nitrogen (Christie, 1994). Another procedure was applied for alcohols in potato waxes (Szafranek & Synak, (2006) after Blau & Halket (1995)): the sample (ca. 0.1 mg) was dissolved in chloroform (0.5 mL), and acetic anhydride (0.1 mL) in acetic acid (0.2 mL) was added. The sample was left overnight in a glass vial at 50 °C, and the excess reagents were removed in a stream of nitrogen.

Dimethyl disulphide adducts are easily prepared derivatives for the GC-MS location of double bonds in alkenes and fatty acid methyl esters (Evershed, 1992b). The procedure is as follows: a small (ca. 0.5 mg) sample is dissolved in 50 μL dimethyl disulphide, and 50 μL

carbon disulphide is added with 300 μg iodine; the mixture is kept at 60 °C for 40 h in a sealed vial, after which the reaction is quenched with aqueous $Na_2S_2O_3$; the organic phase is extracted and evaporated in a stream of nitrogen (Vincenti et al., 1987).

See the monographs by Blau & Halket (1995), Christie (1994) and Drozd (1981) for more derivatization procedures prior to GC analysis.

2.7 GC identification of cuticular waxes

Gas chromatography (GC-FID and GC-MS) can be used for both qualitative and quantitative analyses. Complete identification can be effected if GC retention data and mass spectral data are together taken into consideration. GC-FID identification is based on the comparison of retention times of analytes and authentic standards determined under identical GC conditions or by their co-chromatography. Unfortunately, standards of cuticular wax constituents are rarely commercially available. Some standards may be produced in the laboratory from pure components or natural extracts. For example, the carbon number in the analyses of wax esters can be assigned by comparison of their retention times with that of a synthetic wax ester of known structure (Evershed, 1992a). Beeswax is well-characterized so it can be used as a standard mixture. In beeswax wax esters, the predominant fatty acid moiety is hexadecanoic acid and the chain lengths of the alcohol moieties range from C_{26} to C_{36}.

For other compounds, the literature should be consulted for retention data. These are presented in a number of ways, usually as relative retention times, as Kovats retention indices (KI) or programmed-temperature retention indices (RI), or as equivalent chain length values (ECL). Kovats retention indices are based on a linear relationship between the logarithms of the adjusted retention times ($\log t'_R$) of a homologous series of compounds (saturated straight-chain alkanes) and their carbon chain lengths (Castello, 1999). The drawback of the Kovats retention index is related to the column temperature: KI values change with changing temperature. The Kovats retention index is determined under isothermal GC oven conditions and defined as

$$KI = 100z + 100\frac{\log t'_{R,X} - \log t'_{R,Z}}{\log t'_{R,Z+1} - \log t'_{R,Z}}, \quad (1)$$

where $t'_{R.X}$ is the adjusted retention time of unknown compound *X*; $t'_{R.Z}$ is the adjusted retention time of an n-alkane with *z* carbon atoms; $t'_{R.Z+1}$ is the adjusted retention time of an n-alkane with (*z+1*) carbon atoms; compound X is eluted between both n-alkanes; $t'_{R.Z} < t'_{R.X} < t'_{R.Z+1}$.

The programmed-temperature retention index is used when linear temperature programmes are carried out and defined as:

$$RI = 100z + 100\frac{t'_{R,X} - t'_{R,Z}}{t'_{R,Z+1} - t'_{R,Z}} \quad (2)$$

The retention times of the n-alkanes should behave linearly as a function of the carbon number, i.e. the difference between the retention time of two adjacent homologues is the same in the whole programmed analysis (Castello, 1999).

Similarly, ECL are used for the identification of an unknown FAME under isothermal GC oven conditions (Evershed, 1992a):

$$ECL = n + \frac{\log t'_{R,X} - \log t'_{R,n}}{\log t'_{R,n+1} - \log t'_{R,n}} \quad (3)$$

where $t'_{R.X}$ is the adjusted retention time of the unknown FAME *X*; $t'_{R.n}$ is the adjusted retention time of a saturated straight-chain FAME with *n* carbon atoms; $t'_{R.n+1}$ is the adjusted retention time of a saturated straight-chain FAME with (*n+1*) carbon atoms; compound X is eluted between both saturated straight-chain FAMEs; $t'_{R.n} < t'_{R.X} < t'_{R.n+1}$.

Recently, Stránský et al. (2006) determined programmed-temperature retention indices for a series of wax ester standards on a DB-1 (100% dimethylpolysiloxane) fused silica column.

Itoh et al. (1982) determined the relative retention times of 168 acetate derivatives of sterols and triterpene alcohols on non-polar OV-1 (dimethyl silicone) and slightly polar OV-17 (50% phenyl-50% methyl silicone) glass capillary columns. Values are relative to cholesterol acetate.

The equivalent chain-lengths (ECL) of the methyl ester derivatives of 79 unsaturated fatty acids have been determined by GC with fused silica columns coated with Carbowax 20M (polyethylene glycol), Silar 5CP, CP-Sil 84 (Chrompak/Varian) and a 5% phenyl-methyl silicone (Christie, 1988). CP-Sil 84 is equivalent to AT-SILAR-90, DB-23, Rtx-2330, SP-2330 [poly(80% biscyanopropyl)-20% cyanoproylphenyl siloxane] (Grace, 2011).

Insect cuticular hydrocarbons are commonly identified on the basis of retention indices (Nelson & Blomquist, 1995). Pomonis et al. (1989) determined the Kovats retention indices of monomethyl-pentacosanes, some internally branched dimethylalkanes and 2,x-dimethylheptacosanes on a cross-linked methyl silicone fused silica capillary column (Hewlett-Packard). Carlson et al. (1998) described a protocol for the identification of methyl-branched hydrocarbons in insect cuticular waxes. In this protocol, programmed-temperature retention indices are assigned to peaks, then the patterns in GC peaks that probably contain homologues are marked to assist subsequent GC-MS interpretation. The authors also included data from the literature covering most of the insect methylalkanes.

2.8 GC-MS identification of cuticular waxes

Combined GC-MS is the standard technique used for identifying cuticular waxes, and electron ionization (EI) is the most widely used MS ionization technique in their analysis. The most frequent and simple method of identification involves comparison of the recorded mass spectra with those in standard mass spectral libraries or with those of authentic standards. Unfortunately, the spectra of wax constituents are rarely published in MS libraries (e.g. NIST Chemistry WebBook, Wiley Registry of Mass Spectral Data, MSDC). For compounds that remain unidentified in the above MS libraries, the mass spectra found in the literature can be consulted. Table 2 gives the references of published mass spectra or fragmentation patterns for some less common cuticular wax components.

Wax class	Examples of compounds (derivatives)	References
Secondary alcohols	2-Heptacosanol (native, acetate derivative, TMSi ether)	Szafranek & Synak, 2006
	Nonacosan-13-ol (TMSi ether)	Wen et al., 2006
Ketones	2-Heptacosanone	Szafranek & Synak, 2006
	Nonacosan-10-one (methyl oxime)	Jetter & Riederer, 2000
Diketones	Nonacosane-10,11-dione (TMSi ether, quinoxaline derivative)	Jetter & Riederer, 2000
Hydroxyaldehydes	3-Hydroxyhexacosanal (native, TMSi ether)	Vermeer et al., 2003
	5-Hydroxyoctacosanal (native, TMSi ether, acetate derivative)	Wen & Jetter, 2007
Esters	Tetradecanoic acid isopropyl ester	Szafranek & Synak, 2006
	Hexacosyl hexadecanoate Tetracosyl coumarate (TMSi ether)	Santos et al., 2007
	Benzoic acid esters	Gülz et al., 1987
Phenylalkyl esters	3-(4′-Hydroxyphenyl)-propyl esters of fatty acids (TMSi ethers, acetate esters)	Jetter et al., 2002
Ketols	15-Hydroxynonacosan-14-one, 16-Hydroxynonacosan-14-one (TMSi ether)	Wen & Jetter, 2009
	10-Hydroxynonacosan-11-one/11-hydroxynonacosan-10-one (TMSi ether, methyloxime TMSi ether)	Jetter & Riederer, 2000
Diols	Nonacosane-14,15-diol Nonacosane-13,15-diol (TMSi ethers)	Wen & Jetter, 2009
	Hexacosane-1,7-diol (TMSi ethers)	Jetter et al., 1996
	β-Alkanediols (TMSi ethers, isopropylidene ethers), γ-alkanediols (TMSi ethers)	Jetter, 2000
	1,5-Alkanediols (TMSi ethers, acetates)	Wen & Jetter, 2007
	Hentriacontanediol and octacosanediol isomers (TMSi ethers, acetates)	Wen et al., 2006
δ-Lactones (1,5-Alkanolides)	Hexacosanolide, octacosanolide	Jetter & Riederer, 1999
Triterpenoids	Amyrin, lupeol (TMSi derivatives)	Gülz et al., 1987
Sterols	Campesterol, stigmasterol (TMSi derivatives)	Gülz et al., 1987

Table 2. Literature data for mass spectra or fragmentation diagrams of less common cuticular wax components

The interpretation of mass spectra of common wax components are described elsewhere (Hamilton, 1995b; Evershed, 1992b; Christie, 1994). The mass spectra of fatty acids, alcohols, wax esters and other lipids can be found in an open access website (The AOCS Lipid Library, 2011). Briefly, the identification is performed on the basis of the characteristic fragment and molecular ions. For example, the mass spectra of saturated fatty acid methyl

esters generally exhibit characteristic fragment ions at *m/z* 74 and 87 (Evershed, 1992b). The relative abundance of *m/z* 74 decreases with increasing unsaturation, thereby complicating the interpretation of mass spectrum even more. The molecular ion $M^{+\cdot}$ confirms the number of carbons and the degree of fatty acid unsaturation. Christie (1998) summarized the use of picolinyl ester and dimethyloxazoline derivatives for the analysis of unsaturated fatty acids and the interpretations of their mass spectra.

The ions at *m/z* 73 and 75 are very common in the mass spectra of trimethylsilyl derivatives (TMSi) (Evershed, 1992b). TMSi esters of fatty acids possess characteristic ions at *m/z* 73, 75, 117, 129, 132, 145 and $[M\text{-}CH_3]^+$. TMSi ethers of long-chain alcohols are characterized by the ions at *m/z* 73, 75, 103 and $[M\text{-}CH_3]^+$. The mass spectra of long-chain alcohol acetates show the following ions – *m/z* 43, *m/z* 61 $[CH_3COOH_2]^+$ – together with the corresponding fragment at $[M\text{-}60]^{+\cdot}$ and $[M\text{-}60\text{-}28]^{+\cdot}$, but molecular ions are not always detected (Christie, 1994). In our opinion, acetate derivatives are less informative than TMSi derivatives, so it is more convenient to prepare the same TMSi derivatives for every component of the wax extract. Mass spectra of underivatized long-chain alcohols contain little structural information and are easily confused with those of monoalkenes (Christie, 1994). The characteristic ions are 55, 57, 69, 83, 97, 111 and weak $[M\text{-}18]^{+\cdot}$, $[M\text{-}46]^{+\cdot}$.

The mass of spectrum of an underivatized long-chain aldehyde is characterized by the ions *m/z* 82, 96, $[M\text{-}18]^{+\cdot}$ and a weak $M^{+\cdot}$ (Hamilton, 1995b; Christiansen et al., 1969).

The mass spectrum of the wax ester of the type RCOOR' show the important diagnostic ions in the alcohol or acid moieties: $[RCOOH_2]^+$, $[R'\text{-}1]^+$ and $[COOR']^+$ (Hamilton, 1995b).

Recently, the mass spectral characteristics of three classes of wax esters were presented by Zhang et al. (2010). The authors developed a system from GC/MS data for the identification of wax esters in complex samples.

Mass spectra of branched-chain alkanes: 2-methylalkanes are identified by peaks at $[M\text{-}43]^+$ and the less intense peak at $[M\text{-}15]^+$, whereas 3-methylalkanes have peaks at $[M\text{-}29]^+$ and a less intense peak at $[M\text{-}57]^+$(Hamilton, 1995b and the references therein).

3. Terpenoids

Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. These compounds belong to the isoprenoid group. Even though isoprene itself has not been found in nature, its polymers, terpenic hydrocarbons and their oxygen derivatives are very often present in large quantities in different species. The single isoprene unit, therefore, represents the most basic class of terpenes, the hemiterpenes. An isoprene unit bound to a second isoprene is the defining characteristic of terpene, which is also a monoterpene (C_{10}). Sesquiterpenes contain three isoprene units (C_{15}), while diterpenes (C_{20}) and triterpenes (C_{30}) contain two and three terpene units respectively. Tetraterpenes consist of four terpene units, polyterpenes more than four such units. In nature, terpenes occur predominantly in the form of hydrocarbons, alcohols and their glycosides, ethers, aldehydes, ketones, carboxylic acids and esters. The chemical diversity of plant terpenoids originates from often complex terpenoid biosynthetic pathways. McGarvey & Croteau (1995) reviewed terpene biosynthesis. Mapping strategies were used to determine the variance and composition of amino acids at terpene synthase active sites. In a more recent

review of terpene synthase genes, Zwenger & Basu (2007) performed an *in silico* analysis of publicly available microarray data using Genevesitgator software (Zimmerman et al., 2004). Apart from broad structural diversity, terpenes also exhibit a wide array of biological actions. Essential oils have antimicrobial, antiparasitic, insecticidal and antioxidant/prooxidant activities that often represent the combined bioactivity of multiple components. Many plant terpenoids are cytotoxic towards tumour cells and are applied as chemotherapeutic or chemopreventive compounds (Bhalla, 2003; Bifulco, 2005). Terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants. Many insects metabolize the terpenes they have obtained with their plant food to growth hormones and pheromones.

3.1 Terpene isolation from plants

Organic solvent extraction has long been used for isolating essential oils from natural products (Taylor, 1993). To isolate terpenes from plant material dichloromethane extraction (Bowman et al., 1997) and methanol extraction can be applied (Verma et al., 1990). A two-day extraction with a mixture containing pentane and dichloromethane was used to isolate guajava essential oil (Vernin et al., 1991). Water extraction has proved to be an effective technique for isolating essential oil from citrus fruits (Lancas & Cavicchioli, 1990). Also, sequential extraction with toluene, citric acid and benzene has been proposed as a step prior to the determination of vinblastine in rose essential oil (Volkov & Grodnitskaya, 1994). The most common technique used to obtain essential oils from plant materials is steam or hydro-distillation. Analysis of the terpene hydrocarbon fraction of the essential oil obtained by the steam distillation of the fruit of *Schinus molle* L. was described by Bernhard & Wrolstad (1963). This technique has been applied extensively as a step prior to GC-MS for compositional studies of essential oils, as in the case of valerian (Bos et al., 1997), curcuma (Zwaving & Bos, 1992), cinnamon (Jayaprakasha et al., 1997) and rosemary, sage and lavender (Guilleèn et al., 1996). Steam distillation was also applied to obtain potato alcohols (Szafranek et al., 2006) and sesquiterpenes from potato varieties (Szafranek et al., 2005). Kawamura et al. (1999) reported using pressurized-liquid extraction (PLE) methods for extracting taxol from the bark of *Taxus cuspidate*. An interesting result from this study was that although in conventional extraction methods the taxol content of the water extract was very low, this was improved by use of the elevated temperature and pressure conditions of PLE. The influence of some experimental parameters on the PLE of *P. gaudichaudianum* Kunth leaves was elucidated (Peres et al., 2006). The optimization of the main variables involved in the PLE process (extraction temperature and time) was done using the extraction yield and the GC-MS profile of the extracts. The best results were obtained for the following parameters: 10 min and one extraction cycle at 85 °C.

Currently, supercritical fluid extraction (SFE) represents a new separation technique that is very suitable for the isolation of volatile compounds from plant matrices (Sedlakova et al., 2011). α-Cellulose has been used as a model plant matrix for investigating the conditions required to optimize the SFE of typical plant constituents – limonene, caryophyllene, carvone, eugenol and santonin – using CO_2 as the extraction medium (Smith & Burford, 1992); 250 bar and 40 °C were chosen as the optimum conditions. The effects of adding modifiers to the supercritical fluid were also examined. The chemical composition and antimicrobial activity of essential oil-rich fractions obtained by supercritical CO_2 extraction from *Rosmarinus officinalis* L. were investigated (Santoyo et al., 2005). The most active

fraction was obtained using 4% ethanol as modifier (extraction pressure – 25 MPa; extraction temperature – 60 °C). SFE with CO_2 was used for natural essential oil extraction from a Portuguese-grown rose geranium (*Pelargonium* sp.) (Gomes et al., 2007); the best extraction conditions were: extraction time 15–30 min, temperature 40 °C, pressure 90–100 bar. The CO_2 geranium extract had a superior organoleptic quality, with a very fresh natural floral–fruity character and a pale yellow colour, most suitable for use in perfumery.

New approaches such as stir bar sorptive extraction (SBSE) have recently been used with interesting applications. SBSE is one of the most prominent techniques recently employed by some authors to determine volatiles from grapes (Caven-Quantrill & Buglass, 2006; Moreno et al., 2008; Zalacain et al., 2007; Pedroza et al., 2010).

3.2 Headspace methods

Headspace (HS) methods are used for the separation of volatile compounds from complex solid matrices such as plant materials. Reproducible and rapid identification of volatile compounds in aromatic plants can be achieved when static HS sampling is coupled to GC-MS (Esteban et al., 1996). More recently, the capabilities of automated HS sampling in the analysis of volatile compounds from *Origanum vulgare* was developed (Garciá & Sanz, 2001). The application of automatic HS–GC to the determination of the safrole content in different *Asarum* species from China and Europe was also described (Stuppner & Ganzera, 1998).

Solid-phase microextraction is a more recent solvent-free extraction method eliminating most of the drawbacks of other extractive methods, such as solvent use, thermal decomposition of compounds, excessive time preparation and high cost. To detect terpenes using SPME fibres, one of three sampling techniques is generally applied: (i) exposing fibres to the headspace of vials containing liquid or solid samples (Isidorov et al., 2003; Adam et al., 2005; Kos et al., 2006; Santos et al., 2006; Vichi et al., 2006), (ii) exposing fibres to air circulating over the sample (Zini et al., 2001; Isidorov et al., 2005), or (iii) inserting fibres into a vial containing the sample (Lopez et al., 2006). Because of the small dimensions of the sampling device and the simplicity and speed of the extraction procedure, HS-SPME is able to collect fragrances from live plants with minimum disturbance of the specimen, under both laboratory and field conditions. So far, there have been many qualitative experiments reporting the relative composition of individual compounds based on chromatogram peak areas (Isidorov et al., 2003; Santos et al., 2006; Vichi et al., 2006). The usefulness of eight available SPME fibres was elucidated to evaluate the recoveries of some terpene components with different polarities and structures present in the headspace of four aromatic and medicinal plants: rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and valerian (*Valeriana officinalis* L.) (Bicchi et al., 2000). The results showed that the most effective fibres were those consisting of two components, i.e. a liquid phase (polydimethylsiloxane) and a porous solid (carboxen or divinylbenzene, or both). PDMS-SPME was used to study volatile compounds released by intact and mechanically damaged leaves of *Abies fraseri* (Vereen et al., 2000). After 5 min of extraction, monoterpenes such as 3-carene predominate; after 3 h, the major component in the chromatograms is bornyl acetate, with minor amounts of heavier compounds, e.g. camphor and borneol. PDMS/DVB fibres were used to quantify emissions of methyl chavicol, an oxygenated terpene from live branches (*Pinus ponderosa*) (Bouvier-Brown et al., 2007). In order to check the intra- and interpopulational variability of the terpene pattern, a total of 74

Juniperus communis samples were investigated by HS-SPME, GC-MS and GC-FID (Filipowicz et al., 2009). SPME−GC-MS was also used to determine monoterpene compounds in *Mentha piperita* (Rohloff, 1999). Author also compared the results obtained using SPME and steam distillation. Compared to solvent-based samples from essential oil distillation, relatively higher amounts of high-volatile monoterpenes and smaller quantities of less volatile compounds such as menthol and menthone were detected in SPME sampling.

3.3 Separation and detection of terpenes

Gas chromatography possesses inherent advantages that make it particularly attractive for the characterization and quantitative analysis of terpene mixtures. These include high separation efficiencies, short residence times in the chromatographic column, and the use of an inert atmosphere during analysis, the lack of azeotropes, and applicability to very small samples. In most cases capillary columns with dimethyl polysiloxane (methyl silicone) non-polar and Carbowax 20M polar phases are used. Carbowax 20M phases include DB Wax, BP-20, PEG 20M and HP 20, while methyl silicone phases include SE-30, SF-96, OV-1, OV 101, BP 1, CPSIL 5CB, SP 2100, DB 1, DB 5 and HP 1 (Davies, 1990). Among these fused-silica capillary GC columns, DB 1 or DB 5 and CPSil 5 are usually preferred.

One of the most important developments in gas chromatography was the introduction of enantioselective capillary columns with high separation efficiency in the mid-1960s by Gil-Av et al. (1966). At first, phases based exclusively on chiral diamide structures were used. The first optimum performance was achieved with the use of Chirasil-Val, a methyl–polysiloxane phase containing about 6% branched aliphatic side chains with L-valine in a diamide linkage, and similar polymeric chiral diamide stationary phases (König, 1987) possessing excellent thermal stability. Finally, capillary columns using different hydrophobic cyclodextrin derivatives were introduced. The first GC separations of enantiomers using CDs were obtained by Koscielski & Sibilska in 1983; they separated β- and α-pinene, and the corresponding pinanes and δ-3-carene with a column packed with underivatized CDs. CDs are generally carried in non-polar to moderately polar polysiloxanes, as first proposed by Schurig & Novotny (1990). The main reasons for this are the wider range of operating temperatures, the inertness and efficiency of columns prepared by high temperature silylation, the possibilities of tuning column polarity by using different diluting phases, the small amounts of CD necessary to prepare columns, the shorter analysis times, and the possibility of measuring the thermodynamic parameters involved in enantiomer discrimination. Papers published over the period 1989-94 concerning applications of cyclodextrin derivatives (CDDs) to the GC separation of volatile racemates in the essential oil, extract, flavour and aroma fields were reviewed by Bicchi et al. (1995).

Enantioselective GC has found a wide variety of applications, for instance, studies of citronellol (Ravid et al., 1992) and α-terpineol (Ravid et al., 1995) in a variety of species and verbenone in rosemary oils (Ravid et al., 1997). Chiral phases were applied to the separation of linalool and linalyl acetate in a variety of plant species (König et al., 1992; Cassabianca et al., 1998), extracts of *Angelica* seeds and roots (Holm et al., 1997) and other monoterpenoids in geranium oils (Kreis & Mosandl, 1993). Separation of monoterpenes in Scots pine and juniper oils (Hiltunen & Laakso, 1995), *Abies* (Holm et al., 1994) and *Picea* (Persson et al., 1996) oils and limonene, linalool, citronellal and β-citronellol (*Cymbopogon winterianus*)

(Lorenzo et al., 2000) also relied on chiral analysis. Studies of tea tree oil and other members of the *Myrtaceae* (Leach et al., 1993), sesquiterpenes (König et al., 1999; Cornwell et al., 2000; Bülow and König, 2000) and diterpenes (Pietsch and König, 1997; Pietsch and König, 2000) were also carried out.

The most common detection method used in gas chromatography is FID. The nitrogen–phosphorus detector (NPD) can be used to identify nitrogen-containing compounds (Stashenko et al., 1996). Another possibility is the use of oxygen flame ionization detection (O-FID) for the selective determination of oxygenates (Betts, 1994; Schneider et al., 1982). Mass spectrometry is a very useful tool for detecting complex terpene mixtures.

3.4 GC identification of terpenes

Retention indices are fundamental to making retention a reliable identification tool for GC. Identification is based on the direct comparison of retention times with standards or a precise knowledge of retention indices (Davies, 1990; Stashenko et al., 1993; Stashenko et al., 1996). Some 900 Kovats indices of 400 individual compounds were summarized from the general literature (Davies, 1990). A compilation such as that of Adams (1995) reveals an enormous number of compounds that are present in essential oils. The programmed-temperature retention indices listed in Adams' monograph were obtained on a DB-5 column. Identification is hampered, because the retention indices of many related components are similar. The presence of unsaturated bonds, various branched and cyclic compounds, and oxygenated analogues (e.g. alcohols and ketones) further complicate the issue. Problems still surround the variation of stationary-phase polarity and mobile-phase characteristics as a function of temperature in programmed analysis. An effective approach is to combine the retention data from stationary phases of different polarity. Apart from comparing obtained retention indices with values published in the literature, terpenes can also be identified using co-injection with standards.

3.5 GC-MS identification of terpenes

Nowadays the combination of GC-MS in electron impact mode is a well-established technique for the routine analysis of essential oils. With this technique, additional information can be obtained from mass spectra. However, it has to be emphasized that identification of terpenes based only on mass spectra is also virtually impossible. Molecular rearrangement and isomerization processes in unsaturated hydrocarbons result in very similar mass spectra lacking characteristic fragmentation patterns. There are several ways of solving this problem. One is to use GC-MS-MS (tandem mass spectrometry), which analyses each component of such complex peaks separately. Another is single ion monitoring (SIM), a very selective method that has turned out to be the most reliable procedure for quantitative analysis. Apart from these techniques, combined data of retention times, Kovats indices (Richmond and Pombo-Villar, 1997) and mass spectral data enable the unambiguous identification of sesquiterpenoid constituents. Numerous papers have recently been published on GC-MS techniques used to analyse essential oils and volatile compounds (MacLeod & Ames, 1991; Elias et al., 1997; Szafranek et al., 1998; Szafranek et al., 2005; Szafranek & Szafranek, 2008; Gołębiowski et al., 2008c; Gołębiowski et al., 2009).

The mass fragmentation of terpenes was widely discussed by Budzikiewicz et al. (1963), and the features of the mass spectra of mono-, bi- and tri-cyclic terpenoids were investigated

(Yermakov et al., 2010). It was established that the mass spectra of these compounds are absolutely identical in the mass values of fragment ion peaks, although there are minor differences in their relative intensities. The characteristic ions in the spectra of all compounds were $[M\text{-}CH(CH_3)_2]^+$ and $[M\text{-}CH(CH_3)_2\text{–}H_2]^+$. Eleven terpenoid derivatives from the extract including three steroids and eight pentacyclic triterpenes were identified by GC-MS in an extract of the stem bark of *Ficus mucuso* (Djemgou et al., 2009). HS volatiles from flowers, as well as green and ripe mango fruit of cv. Ataulfo from Soconusco, Chiapas, were identified by GC-MS (Sandoval et al., 2007). Chemical identification was confirmed by comparison of the mass spectra pattern with the NIST 2002 computer library and the retention times of synthetics. A new *cis*-sabinene hydrate chemotype was detected in large thyme (*Thymus pulegioides* L.) by Groendahl & Ehlers (2008). Analyses were done using an GC coupled to an inert mass selective detector with an ion source of 70.0 eV at 230 °C. Two columns of different polarities were used: one was an EC-Wax capillary column with oven conditions that included an isothermal hold at 60 °C for 5 min, followed by a ramp of 10 °C/min to 250 °C. The other was a HP-5MS capillary column with an initial oven temperature of 60 °C, followed by a ramp of 3 °C/min to 246 °C. Terpenes were identified using retention times of standards, the NIST 2005 mass spectral library and/or programmed-temperature retention indices. Five chemotypes were detected: carvacrol, linalool, geraniol and thymol, all of which are known to occur in large thyme. In addition, essential oils containing the monoterpene *cis*-sabinene hydrate as the dominant component were found. No sabinene hydrate chemotype has previously been detected in large thyme, although it does occur in other thyme species.

Nevertheless, sometimes mass spectra measured in EI mode are problematic, because they miss the molecular ions, especially esters. In this case, the application of GC-MS in chemical ionization (CI) mode using various reagent gases often yields valuable additional information. Negative ion chemical ionization (NCI) with OH as the reactant ion (Hendriks et al., 1985; Cazaussus et al., 1988) is an additional method. However, there is no characteristic fragmentation pattern in the mass spectra of terpenes obtained by NCI. For sesquiterpenes with one or two non-conjugated double bonds this problem can be solved by using trioxo(*tert*-butylimido)osmium(VIII) (Rücker et al., 1990). This reagent forms cyclic osmate ester amides as intermediates which, after reduction, yield vicinal mono-amino alcohols, bis-amino alcohols and aminotriols. The derivatives formed in such a reaction have characteristic mass spectra that can be used as a fingerprint for the identification of the respective parent compound.

3.6 Two-dimensional GC

While GC-FID is the traditional method for essential oil quantification, GC-MS is the most common analytical method for component identification. However, the wide concentration range of the analytes (from ppb to percentage levels), as well as the presence of numerous isomers (terpenes and oxygenated terpene structures), make qualitative analysis difficult. In addition, the mass spectra of these compounds are usually very similar, so peak identification often becomes very difficult and sometimes impossible.

Recently, comprehensive, two-dimensional gas chromatography (GC×GC) has been extensively used in studies of essential oils. Comprehensive GCxGC enhances the peak capacity for a chromatographic run, allowing better separation in complex sample analysis. GCxGC technology allows the use of two different separation mechanisms in order to increase the

separation power of the chromatographic system. The two directly coupled columns in GCxGC provide orthogonal separation of compounds, and importantly, enable the simultaneous two-column separation of the whole sample. Thus a combination of a non-polar column with a polar column may be a good first choice for a suitable dual column set for essential oils. The principles, practical and theoretical aspects, and the most significant developments of GC×GC were reviewed by Mondello et al. (2008). There are several reports of GCxGC used for determining essential oils (Dimandja et al., 2000; Shellie et al., 2003; Shellie et al., 2001).

The high resolution GC×GC separation of an essential oil and the identification of selected separated components by time-of-flight mass spectrometry (TOF-MS) was reported by Shellie et al., (2001). These authors showed that GC×GC allows orthogonal separation mechanisms on the two columns to achieve separation of components (e.g. borneol and terpinen-4-ol, and *cis*-caryophyllene and β-farnesene) that would otherwise be unresolved on a single column. The authors concluded that peak compression led to the generation of fast second-dimension GC peaks and a ca 25 times better detection sensitivity than with conventional GC elution. This allows many more compounds to be detected when using the GC×GC approach. GC-MS and GC×GC with FID were used in the analysis of peppermint (*Mentha piperita*) and spearmint (*Mentha spicata*) essential oils. Components including acetates, alcohols, furans, ketones, sesquiterpenes and terpenes were detected. The GC×GC chromatogram of peppermint essential oil displays 89 peaks compared to the 30 peaks in the GC-MS chromatogram; likewise, 68 peaks were detected in the GC×GC chromatogram of spearmint compared to 28 in GC-MS. This technique has been successfully used in the industrial analysis of plant materials to improve component separation and identification. In addition, analysis of *Artemisia annua* L. volatile oils using multi-dimensional gas chromatography has indicated that this technique can achieve the complete separation of a wide range of terpenes (Ma et al., 2007). These authors found nearly 700 components, the majority of which were terpenes. The investigation of the volatile compounds of dried rhizomes of *Coptis chinensis* was carried out by Gao et al. (2011). Volatile profiles were established and compared after headspace solid-phase microextraction using a PDMS/DVB fibre coupled to comprehensive 2D GC×GC-TOFMS. Analyses were performed and compared on two column-phase combinations (non-polar/polar and polar/non-polar). Terpenoids represented the most numerous group of compounds identified.

4. Conclusion

Modern analytical techniques can provide accurate and precise profiles of plant and insect cuticular waxes as well as terpenoids. For structural studies the most effective procedures are those based on a combination of mass spectra and GC retention index data.

5. Acknowledgment

The work was financed by Grant DS/8200-4-0085-1 from the University of Gdańsk and by Grant N N303 504238 from the Polish Ministry of Science and Higher Education (2010-2013).

6. References

Adam, M.; Juklova, M.; Bajer, T.; Eisner, A. & Ventura, K. (2005). Comparison of three different solid-phase microextraction fibres for analysis of essential oils in yacon

(*Smallanthus sonchifolius*) leaves. *Journal of Chromatography A*, Vol.1084, No.1-2, (August 2005), pp. 2-6, ISSN 0021-9673

Adams, R.P. (1995). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Allured Publishing, ISBN 0-931710-42-1, Carol Stream, USA

Baker, E.A. (1982). Chemistry and morphology of plant epicuticular waxes, In: *The Plant Cuticle*, D.J. Cutler, K.L. Alvin, & C.E. Price (Eds.), 139-165, Linnean Society of London [by] Academic Press, ISBN 0121999203, London

Bakker, M.I.; Baas, W.J.; Sijm, D.T.H.M. & Kollöffel, C. (1998). Leaf wax of *Lactuca sativa* and *Plantago major*. *Phytochemistry*, Vol.47, No.8, (April 1998), pp. 1489-1493, ISSN 0031-9422

Bernhard, R.A. & Wrolstad R. (1963) The essential oil of *Schinus molle*: the terpene hydrocarbon fraction. *Journal of Food Science*, Vol.28, No.1, (January 1963), pp. 59–63, ISSN 1750-3841

Betts, T.J. (1994). Evaluation of a "Chirasil-Val" capillary for the gas chromatography of volatile oil constituents, including sesquiterpenes in patchouli oil. *Journal of Chromatography A*, Vol.664, No.2, (June 1994), pp. 295-300, ISSN 0021-9673

Bhalla, K.N. (2003). Microtubule-targeted anticancer agents and apoptosis. *Oncogene*, Vol.22, No.57, (December 2003), pp. 9075-9086, ISSN 0950-9232

Bianchi, G. (1995). Plant waxes, In: *Waxes: Chemistry, Molecular Biology and Functions*, R.J. Hamilton (Ed.), 175-222, The Oily Press, ISBN 0-9514171-5-0, Dundee, Scotland

Bicchi, C.; Drigo S. & Rubiolo, P. (2000). Influence of fibre coating in headspace solid-phase microextraction–gas chromatographic analysis of aromatic and medicinal plants. *Journal of Chromatography A*, Vol.892, No.1-2, (September 2000), pp. 469-485, ISSN 0021-9673

Bicchi, C.; Manzin, V.; D'Amato, A. & Rubiolo, P. (1995). Cyclodextrin derivatives in GC separation of enantiomers of essential oil, aroma and flavour compounds. *Flavour and Fragrance Journal*, Vol.10, No. 3, (May/June 1995), pp. 127–137, ISSN 1099-1026

Bifulco, M. (2005). Role of the isoprenoid pathway in ras transforming activity, cytoskeleton organization, cell proliferation and apoptosis. *Life Science*, Vol.77, No.14 (August 2005), pp. 1740-1749, ISSN 0024-3205

Blau, K. & Halket, J.M. (Eds.). (1995). *Handbook of Derivatives for Chromatography*, John Wiley & Sons, ISBN 0-471-92699-X, Chichester

Bos, R.; Woerdenbag, H. J.; Hendriks, H. & Scheffer J.J.C. (1997). Composition of the essential oils from underground parts of *Valeriana officinalis* L. *s.l.* and several closely related taxa. *Flavour Fragrance Journal*, Vol.12, No.5, (September/October 1997), pp. 359-370, ISSN 1099-1026

Bouvier-Browna, N.C.; Holzinger, R.; Palitzsch, K. & Goldstein, A.H. (2007). Quantifying sesquiterpene and oxygenated terpene emissions from live vegetation using solid-phase microextraction fibers. *Journal of Chromatography A*, Vol.1161, No.1-2, (August 2007), pp. 113–120, ISSN 0021-9673

Bowman, J.M.; Braxton, M.S.; Churchill, M.A.; Hellie, J.D.; Starrett, S.J.; Causby, G.Y.; Ellis, D.J.; Ensley, S.D.; Maness, S.J.; Meyer, C.D.; Sellers, J.R.; Hua, Y.; Woosley, R.S. & Butcher D.J. (1997). Extraction method for the isolation of terpenes from plant tissue and subsequent determination by gas chromatography. *Microchemical Journal*, Vol.56, No.1, (May 1997), pp. 10-18, ISSN 0026-265X

Brüschweiler, H. & Hautfeune, A. (1990). Determination of the ester emulsifiers components content after hydrolysis and silylation by gas chromatography: results of a collaborative study and the standardised method. *Pure and Applied Chemistry*, Vol.62, No.4, pp. 781–783, ISSN 1365-3075

Buckner, J.S.; Hagen M.M. & Nelson D.R. (1999). The composition of the cuticular lipids from nymphs and exuviae of the silverleaf whitefly, *Bemisia argentifolii*. *Comparative Biochemistry and Physiology*, Vol.124B, No.2, (October 1999), pp. 201-207, ISSN 1096-4959

Buckner, J.S.; Mardaus M.C. & Nelson D.R. (1996). Cuticular lipid composition of *Heliothis virescens* and *Helicoverpa zea* pupae. *Comparative Biochemistry and Physiology,* Vol.114B, No.2, (June 1996), pp. 207-216, ISSN 1096-4959

Buckner, J.S.; Nelson D.R. & Mardaus M.C. (1994). The lipid composition of the wax particles from adult whiteflies, *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Insect Biochemistry and Molecular Biology,* Vol.24, No.10, (December 1994), pp. 977-987, ISSN 0965-1748

Budzikiewicz, H.; Wilson, J.M. & Djerassi C. (1963). Mass spectrum in structural and stereochemical problems. XXXII, pentacyclic triterpenes. *Journal of the American Chemical Society*, Vol.85, No.22, (November 1963), pp. 3688-3699, ISSN 0002-7863

Bülow, N. & König W.A. (2000). The role of germacrene D as a precursor in sesquiterpene biosynthesis: investigations of acid catalyzed, photochemically and thermally induced rearrangements. *Phytochemistry*, Vol.55, No.2, (September 2000), pp. 141-168, ISSN 0031-9422

Buschhaus, C.; Herz, H. & Jetter, R. (2007a). Chemical composition of the epicuticular and intracuticular wax layers on the adaxial side of *Ligustrum vulgare* leaves. *New Phytologist*, Vol.176, No.2, (October 2007), pp. 311–316, ISSN 1469-8137

Buschhaus, C.; Herz, H. & Jetter, R. (2007b). Chemical composition of the epicuticular and intracuticular wax layers on adaxial sides of *Rosa canina* leaves. *Annals of Botany*, Vol. 100, No. 7, (December 2007), pp. 1557–1564, ISSN 1095-8290

Carlson, D.A.; Bernier, U.R. & Sutton, B.D. (1998). Elution patterns from capillary GC for methyl-branched alkanes. *Journal of Chemical Ecology*, Vol.24, No.11, (November 1998), pp 1845-1865, ISSN 1573-1561

Cassabianca, H.; Graff, J.B.; Faugier, V.; Fleig, F. & Grenier, C. (1998). Enantiomeric distribution studies of linalool and linalyl acetate. a powerful tool for authenticity control of essential oils. *Journal of High Resolution Chromatography*, Vol.21, No.2, (February 1998), 107-112, ISSN 0935-6304

Castello, G. (1999). Retention index systems: alternatives to the n-alkanes as calibration standards. *Journal of Chromatography A*, Vol.842, No.1-2, (May 1999), pp. 51-64, ISSN 0021-9673

Caven-Quantrill, D.J. & Buglass A.J. (2006). Comparison of micro-scale simultaneous distillation–extraction and stir bar sorptive extraction for the determination of volatile organic constituents of grape juice. *Journal of Chromatography A*, Vol.1117, No.2, (June 2006), pp. 121–131, ISSN 0021-9673

Cazaussus, A.; Pes, R.; Sellier, N. & Tabet, J.C. (1988). GC-MS and GC-MS-MS analysis of a complex essential oil. *Chromatographia*, Vol.25, No.10, (October 1988), pp. 865-869, ISSN 0009-5893

Christiansen, K.; Mahadevan, V.; Viswanathan C.V. & Holman, R.T. (1969). Mass spectrometry of long-chain aliphatic aldehydes, dimethyl acetals and alk-1-enyl ethers. *Lipids*, Vol.4, No.6, (November 1969), pp. 421–427, ISSN 1558-9307

Christie, W.W. (1988). Equivalent chain-lengths of methyl ester derivatives of fatty acids on gas chromatography A reappraisal. *Journal of Chromatography A*, Vol.447, pp. 305-314, ISSN 0021-9673

Christie, W.W. (1994) *Gas Chromatography and Lipids,* The Oily Press, ISBN 0-9514171-0-X, Dundee, Scotland, Available from: < http://www.pjbarnes.co.uk/free/gcpdf.htm>

Christie, W.W. (1998). Gas chromatography–mass spectrometry methods for structural analysis of fatty acids. *Lipids*, Vol.33, No.4, (April 1998), ISSN 1558-9307

Cornwell, C.P.; Reddy, N.; Leach, D.N. & Wyllie S.G. (2000). Origin of (+)-δ-cadinene and the cubenols in the essential oils of the *Myrtaceae*. *Flavour and Fragrance Journal*, Vol.15, No.5, (September/October 2000), pp. 352-361, ISSN 1099-1026

Davies N.W. (1990). Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. *Journal of Chromatography A*, Vol.503, 1-24, ISSN 0021-9673

Dimandja, J.M.D.; Stanfill, S.B.; Grainger, J. & Patterson Jr., D.G. (2000). application of comprehensive two-dimensional gas chromatography (GC×GC) to the qualitative analysis of essential oils. *Journal of High Resolution Chromatography*, Vol.23, No.3, (March 2000), pp. 208–214, ISSN 0935-6304

Djemgou, P.C.; Ngandeu, F.; Hegazy, M.E.F.; Nkanwen, E.R.; Neguim, G.; Chosson, E.; Verite P. & Tane, P. **(**2009). GC-MS analysis of terpenes from *Ficus mucuso*. *Pharmacognosy Research*, Vol.1, No.4, (July-August 2010), pp. 197-201, ISSN 0974-8490

Drozd, J. (1981). *Chemical Derivatization in Gas Chromatography*, Elsevier, ISBN 0-44441917-9, Amsterdam

Elias, V.O.; Simoneit, B.R.T. & Cardoso, J.N. (1997). Analysis of volatile sesquiterpenoids in environmental and geological samples. *Journal of High Resolution Chromatography*, Vol.20, No.6, (June 1997), pp. 305-309, ISSN 0935-6304

Ensikat, H.J.; Neinhuis, C. & Barthlott, W. (2000). Direct access to plant epicuticular wax crystals by a new mechanical isolation method. *International Journal of Plant Sciences*, Vol.161, No.1, (January 2000), pp. 143–148, ISSN 10585893

Esteban, J. L.; Martínez-Castro, I.; Morales, R.; Fabrellas, B. & Sanz J. (1996). Rapid identification of volatile compounds in aromatic plants by ATD/GC/MS. *Chromatographia*, Vol.43, No.1-2, (July 1996), 63-72, ISSN 0009-5893

Evershed, R.P. (1992a). Gas chromatography of lipids. In: *Lipid analysis. A practical approach*. R.J. Hamilton & S. Hamilton (Eds.), 113-151, Oxford University Press, ISBN 0199630984, Oxford

Evershed, R.P. (1992b). Mass spectrometry of lipids. In: *Lipid analysis. A practical approach*. R.J. Hamilton & S. Hamilton (Eds.), 263-308, Oxford University Press, ISBN 0199630984, Oxford

Filipowicz, N., Madanecki, P., Gołębiowski, M., Stepnowski, P. and J. R. Ochocka (2009). HS-SPME/GC analysis reveals the population variability of terpene contents in *Juniperus communis* needles. *Chemistry & Biodiversity*, Vol.6, No.12, (December 2009), pp. 2290-2301, ISSN 1612-1880

Gao, X.; Yang, X.; Mitrevski, B.S. & Marriott P.J. (2011). Headspace solid-phase microextraction combined with GC×GC-TOFMS for the analysis of volatile compounds of *Coptis* species rhizomes. *Journal of Separation Science*, Vol.34, No.10, (May 2011), pp. 1157-1166, ISSN 1615-9314

Garciá, M.A. & Sanz J. (2001). Analysis of *Origanum vulgare* volatiles by direct thermal desorption coupled to gas chromatography–mass spectrometry. *Journal of Chromatography A*, Vol.918, No.1, (June 2001), pp. 189-194, ISSN 0021-9673

Gil-Av, E.; Feibush, B. & Charles-Siegler, R. (1966). Separation of enantiomers by gas liquid chromatography with an optically active stationary phase. *Tetrahedron Letters*, Vol.7, No.10, 1009-1015, ISSN 0040-4039

Gołębiowski, M.; Boguś, M.I.; Paszkiewicz, M. & Stepnowski, P. (2010). The composition of the free fatty acids from *Dendrolimus pini* exuviae. *Journal of Insect Physiology*, Vol.56, No.4, (April 2010), pp. 391–397, ISSN 0022-1910

Gołębiowski, M.; Boguś, M.I.; Paszkiewicz, M. & Stepnowski, P. (2011). Cuticular lipids of insects as a potential biofungicides: methods of lipids composition analysis. *Analytical and Bioanalytical Chemistry*, Vol.399, No.9, (March 2011), pp. 3177-3191, ISSN 1618-2650

Gołębiowski, M.; Maliński, E.; Boguś, M.I.; Kumirska, J. & Stepnowski, P. (2008a). The cuticular fatty acids of *Calliphora vicina*, *Dendrolimus pini* and *Galleria mellonella* larvae and their role in resistance to fungal infection. *Insect Biochemistry and Molecular Biology*, Vol.38, No.6, (June 2008), pp. 619-627, ISSN 0965-1748

Gołębiowski, M.; Maliński, E.; Nawrot, J. & Stepnowski, P. (2008b). Identification and characterization of surface lipid components of the dried-bean beetle *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *Journal of Stored Products Research*, Vol.44, No.4, pp. 386–388, ISSN 0022-474X

Gołębiowski, M.; Maliński, E.; Nawrot, J.; Szafranek, J. & Stepnowski, P. (2007). Identification of the cuticular lipid composition of the Western Flower Thrips *Frankliniella occidentalis*. *Comparative Biochemistry and Physiology*, Vol.147B, No.2, (June 2007), pp. 288–292, ISSN 1096-4959

Gołębiowski, M.; Ostrowski, B.; Paszkiewicz, M.; Czerwicka, M.; Kumirska, J.; Halinski, Ł.; Malinski, E. & Stepnowski, P. (2008c). Chemical composition of commercially available essential oils from blackcurrant, ginger, and peppermint. *Chemistry of Natural Compounds*, Vol.44, No.6, (November 2008), pp. 794-796, ISSN 0009-3130

Gołębiowski, M.; Paszkiewicz, M.; Halinski, Ł.; Malinski, E. & Stepnowski, P. (2009). Chemical composition of commercially available essential oils from eucalyptus, pine, ylang, and juniper. *Chemistry of Natural Compounds*, Vol.45, No.2, (March 2009), pp. 278-279, ISSN 0009-3130

Gomes, P.B.; Mata, V.G. & Rodrigues, A.E. (2007). Production of rose geranium oil using supercritical fluid extraction. *The Journal of Supercritical Fluids*, Vol.40, No.1, (February 2007), pp. 50-60, ISSN 0896-8446

Grace. (2011). Capillary column phase cross reference, In: *Gas chromatography*, 2011-08-26, Available from: <http://pdf.directindustry.com/pdf/grace-davison-discovery-sciences/gc/9119-66111.html>

Groendahl, E. & Ehlers, B.K. (2008). A new cis-sabinene hydrate chemotype detected in large *Thyme* (*Thymus pulegioides* L.) growing wild in Denmark. *Journal of Essential Oil Research*, Vol.20, No.1, (January/February 2008), pp. 40–41, ISSN 1041-2905

Grossi, V. & D. Raphel (2003). Long-chain (C_{19}–C_{29}) 1-chloro-n-alkanes in leaf waxes of halophytes of the Chenopodiaceae. *Phytochemistry*, Vol.63, No.6, (July 2003), pp. 693–698, ISSN 0031-9422

Gülz, P.G.; Scora, R.W.; Müller, E. & Marner, F.J. (1987). Epicuticular leaf waxes of *Citrus halimii* Stone. *Journal of Agricultural and Food Chemistry*, Vol.35, No.5, (September 1987), pp. 716–720, ISSN 1520-5118

Guilleèn, M.D.; Cabo N. & Murillo J. (1996). Characterisation of the essential oils of some cultivated aromatic plants of industrial interest. *Journal of the Science of Food and Agriculture*, Vol.70, No.3, (March 1996), pp. 359-363, ISSN 1097-0010

Haas, K.; Brune, T. & Rücker, E. (2001). Epicuticular wax crystalloids in rice and sugar cane leaves are reinforced by polymeric aldehydes. *Journal of Applied Botany (Angewandte Botanik)*, Vol.75, pp. 178–187, ISSN 0949-5460

Haliński, Ł.; Szafranek, J.; Szafranek, B.M.; Gołębiowski, M. & Stepnowski, P. (2009). Chromatographic fractionation and analysis of the main components of eggplant (*Solanum melongena* L.) leaf cuticular waxes. *Acta Chromatographica*, Vol.21, No.1, (March 2009), pp. 127–137, ISSN 1233-2356

Hamilton, R.J. (1995b). Analysis of waxes, In: *Waxes: Chemistry, Molecular Biology and Functions*, R.J. Hamilton (Ed.), 311-349, The Oily Press, ISBN 0-9514171-5-0, Dundee, Scotland

Hamilton, R.J. (Ed.). (1995a). *Waxes: Chemistry, Molecular Biology and Functions*, The Oily Press, ISBN 0-9514171-5-0, Dundee, Scotland

Hebanowska E.; Maliński E.; Latowska A.; Dubis E.; Pihlaja K.; Oksman P.; Nawrot J. & Szafranek J. (1990). A comparison of cuticular hydrocarbons of larvae and beetles of the *Tribolium destructor*. *Comparative Biochemistry and Physiology*, Vol.96B, No.4, pp. 815-819, ISSN 1096-4959

Hendriks, H.; Bos, R. & Bruins, A.P. (1985). Analysis of the essential oil of *Eupatorium cannabinum* by combined gas chromatography-mass spectrometry using electron impact and negative ion chemical ionization. *Planta Medica*, Vol.51, No.6, (December 1985), pp. 541-542, ISSN 0032-0943

Hiltunen, R. & Laakso, I. (1995). Gas chromatographic analysis and biogenetic relationships of monoterpene enantiomers in Scots pine and juniper needle oils. *Flavour and Fragrance Journal*, Vol.10, No.3, (May/June 1995), pp. 203-210, ISSN 1099-1026

Holm, Y.; Laakso, I. & Hiltunen, R. (1994). The enantiomeric composition of monoterpene hydrocarbons as a chemotaxonomic marker in *Abies sachalinensis* (fr. schm.) mast. and *Mayriana miy*. et kudo needle essential oils. *Flavour and Fragrance Journal*, Vol. 9, No.5, (September/October 1994), pp. 223-227, ISSN 1099-1026

Holm, Y.; Vuorela, P. & Hiltunen, R. (1997). Enantiomeric composition of monoterpene hydrocarbons in n-hexane extracts of *Angelica archangelica* L. roots and seeds. *Flavour and Fragrance Journal*, Vol.12, No.6, (November/December 1997), pp. 397-400, ISSN 1099-1026

Hwang, K.T.; Cuppett, S.L.; Weller, C.L. & Hanna, M.A. (2002). HPLC of grain sorghum wax classes highlighting separation of aldehydes from wax esters and steryl esters. *Journal of Separation Science*, Vol.25, No.9, (June 2002), pp. 619–623, ISSN 1615-9314

Isidorov, V. & Vinogorova V.T. (2005). Experimental determination and calculation of distribution coefficients between air and fiber with polydimethylsiloxane coating

for some groups of organic compounds. *Journal of Chromatography A*, Vol.1077, No.2, (June 2005), pp. 195-201, ISSN 0021-9673

Isidorov, V.; Vinogorova, V. & Rafalowski, K. (2005). Gas chromatographic determination of extractable compounds composition and emission rate of volatile terpenes from Larch needle litter. *Journal of Atmospheric Chemistry*, Vol.50, No.3, (November 2005), pp. 263-278, ISSN 0167-7764

Isidorov, V.A.; Vinogorova, V.T. & Rafalowski, K. (2003). HS-SPME analysis of volatile organic compounds of coniferous needle litter. *Atmospheric Environment*, Vol.37, No.33, (October 2003), pp. 4645-4650, ISSN 1352-2310

Itoh, T.; Tani, H.; Fukushima, K.; Tamura, T. & Matsumoto, T. (1982). Structure-retention relationship of sterols and triterpene alcohols in gas chromatography on a glass capillary column. *Journal of Chromatography A*, Vol.234., No.1, (January 1982), pp. 65-76, ISSN 0021-9673

Jayaprakasha, G.K.; Jaganmohan, R. & Sakariah, K.K. (1997). Chemical composition of the volatile oil from the fruits of *Cinnamomum zeylanicum* Blume. *Flavour Fragrance Journal*, Vol.12, No.1, (January 1997), pp. 331-333, ISSN 1099-1026

Jetter, R. & Riederer, M. (1999). Homologous long-chain δ-lactones in leaf cuticular waxes of *Cerinthe minor*. *Phytochemistry*, Vol.50, No.8, (April 1999), pp. 1359-1364, ISSN 0031-9422

Jetter, R. & Riederer, M. (2000). Composition of cuticular waxes on *Osmunda regalis* fronds. *Journal of Chemical Ecology*, Vol.26, No.2, (February 2000), pp 399-412, ISSN 1573-1561

Jetter, R. (2000). Long-chain alkanediols from *Myricaria germanica* leaf cuticular waxes. *Phytochemistry*, Vol.55, No.2, (September 2000), pp. 169-176, ISSN 0031-9422

Jetter, R.; Klinger, A. & Schäffer, S. (2002). Very long-chain phenylpropyl and phenylbutyl esters from *Taxus baccata* needle cuticular waxes. *Phytochemistry*, Vol.61, No.5, (November 2002), pp. 579-587, ISSN 0031-9422

Jetter, R.; Kunst, L. & Samuels, A.L. (2006). Composition of plant cuticular waxes, In: *Biology of the Plant Cuticle*, M. Riederer, & C. Müller (Eds.), 145-181, Blackwell Publishing Ltd, ISBN 978-1-4051-3268-8, Oxford, UK

Jetter, R.; Riederer, M.; Seyer, A. & Mioskowski, C. (1996). Homologous long-chain alkanediols from *Papaver* leaf cuticular waxes. *Phytochemistry*, Vol.42, No.6, (August 1996), pp. 1617-1620, ISSN 0031-9422

Jetter, R.; Schäffer, S. & Riederer, M. (2000). Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. *Plant, Cell and Environment*, Vol.23, No.6, (June 2000), pp. 619–628, ISSN 1365-3040

Johnson, R. H.; Hull-Sanders, H.M. & Meyer, G.A. (2007). Comparison of foliar terpenes between native and invasive *Solidago gigantea*. *Biochemical Systematics and Ecology*, Vol.35, No.12, (December 2007), pp. 821-830, ISSN 0305-1978

Kawamura, F.; Kikuchi, Y.; Ohira, T. & Yatagai, M. (1999). Accelerated solvent extraction of paclitaxel and related compounds from the bark of *Taxus cuspidate*. *Journal of Natural Product*, Vol.62, No.2, (February 1999), pp.244-247, ISSN 0163-3864

Kolattukudy, P.E. (Ed.). (1976). *Chemistry and Biochemistry of Natural Waxes*, Elsevier, ISBN 0444414703, Amsterdam

König, W.A. (1987). *The Practice of Enantiomer Separation By Capillary Gas Chromatography*, Hüthig-Verlag, ISBN 978-3778513248, Heidelberg

König, W.A.; Bülow, N. & Saritas, Y. (1999). Identification of sesquiterpene hydrocarbons by gas phase analytical methods. *Flavour and Fragrance Journal*, Vol.14, No.6, (November/December 1999), pp. 367-378, ISSN 1099-1026

König, W.A.; Krüger, A.; Icheln, D. & Runge, T. (1992). Enantiomeric composition of the chiral constituents in essential oils. Part 1: Monoterpene hydrocarbons. *Journal of High Resolution Chromatography*, Vol.15, No.3, (March 1992), 184-189, ISSN 0935-6304

Kos, G. & Ariya P.A. (2006). Determination of a wide range of volatile and semivolatile organic compounds in snow by use of solid-phase micro-extraction (SPME). *Analytical and Bioanalytical Chemistry*, Vol.385, No.1, (May 2006), pp. 57-66, ISSN 1618-2642

Kreis P. & Mosandl, A. (1993). Chiral compounds of essential oils. Part XIII. Simultaneous chirality evaluation of geranium oil constituents. *Flavour and Fragrance Journal*, Vol.8, No.3, (May/June 1993), pp. 161-168, ISSN 1099-1026

Lancas, F. M. & Cavicchioli M. (1990). Analysis of the essential oils of Brazilian citrus fruits by capillary gas chromatography. *Journal of High Resolution Chromatography*, Vol.13, No.3, (March 1990), 207-209, ISSN 0935-6304

Leach, D.N.; Wyllie, S.G.; Hall, J.G. & Kyratzis, I. (1993). Enantiomeric composition of the principal components of the oil of *Melaleuca alternifolia*. *Journal of Agricultural and Food Chemistry*, Vol. 41, No.10, (October 1993), pp. 1627-1632, ISSN 0021-8561

Little, J.L. (1999). Artifacts in trimethylsilyl derivatization reactions and ways to avoid them. *Journal of Chromatography A*, Vol.844, No.1-2, (June 1999), pp. 1–22, ISSN 0021-9673

Lopez, P.; Huerga, M.A.; Batlle, R. & Nerin, C. (2006). Use of solid phase microextraction in diffusive sampling of the atmosphere generated by different essential oils. *Analytica Chimica Acta*, Vol.559, No.1, (February 2006), pp. 97-104, ISSN 0003-2670

Lorenzo, D.; Dellacassa, E.; Atti-Serafini, L.; Santos, A.C.; Frizzo, C.; Paroul, N.; Moyna, P.; Mondello, L. & Dugo, G. (2000). Composition and stereoanalysis of *Cymbopogon winterianus* Jowitt oil from Southern Brazil. *Flavour and Fragrance Journal*, Vol.15, No.3, (May/June 2000). pp. 177-181, ISSN 1099-1026

Ma, C.; Want, H.; Lu, X.; Li, H.; Liu, B. & Xu, G. (2007). Analysis of *Artemisia annua* L. volatile oil by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. *Journal of Chromatography A*, Vol. 1150, No.1-2, (May 2007), pp. 50-53, ISSN 0021-9673

MacLeod, G. & Ames, J.M. (1991). Gas chromatography-mass spectrometry of the volatile components of cooked scorzonera. *Phytochemistry*, Vol.30, No.3, (February 1991), pp. 883-888, ISSN 0031-9422

Maile, R.; Dani, F.R.; Jones, G.R.; Morgan, E.D. & Ortius, D. (1998). Sampling techniques for gas chromatographic-mass spectrometric analysis of long-chain free fatty acids from insects exocrine glands. *Journal of Chromat*ography, Vol.816A, No.2, (August 1989), pp. 169-175, ISSN 0021-9673

Maliński E.; Hebanowska E.; Szafranek J. & Nawrot J. (1986). The composition of the hydrocarbons of the larvae of the Khapra beetles, *Trogoderma granarium*. *Comparative Biochemistry and Physiology*, Vol.84B, No.2, pp. 211-215, ISSN 1096-4959

Marriott, P.; Shellie, R.; Fergeus, J.; Ong, R. & Morrison, P. (2000). High resolution essential oil analysis by using comprehensive gas chromatographic methodology. *Flavour*

and Fragrance Journal, Vol.15, No.4, (July/August 2000), pp. 225–239, ISSN 1099-1026

McGarvey, D. J. & Croteau R. (1995). Terpenoid metabolism. *Plant Cell*, Vol.7, No.6, (July 1995), pp. 1015-1026, ISSN 1040-4651

Mondello, L.; Tranchida, Q.P.; Dugo, P. & Dugo, G. (2008). Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. *Mass Spectrometry Reviews*, Vol.27, No.2, (March/April 2008), pp. 101–124, ISSN 1098-2787

Moreno, J.J.; Cerpa-Calderón, F.; Cohen, S.D.; Fang, Y.; Qian, M. & Kennedy J.A. (2008). Effect of postharvest dehydration on the composition of pinot noir grapes (*Vitis vinifera* L.) and wine. *Food Chemistry*, Vol.109, No.4, (August 2008), pp. 755–762, ISSN 0308-8146

MSDC. (1991). *Eight Peak Index of Mass Spectra*, The Mass Spectrometry Data Centre (MSDC), The Royal Society of Chemistry, ISBN 0-85186-417-1, Cambridge, UK

Nass, R.; Markstädter, C.; Hauke, V. & Riederer, M. (1998). Quantitative gas chromatographic analysis of plant cuticular waxes containing long-chain aldehydes. *Phytochemical Analysis*, Vol.9, No.3, (May/June 1998), pp. 112–118 ISSN 1099-1565

Nelson, D.R. & Blomquist, G.J. (1995). Insect waxes, In: *Waxes: Chemistry, Molecular Biology and Functions*, R.J. Hamilton (Ed.), 1-90, The Oily Press, ISBN 0-9514171-5-0, Dundee, Scotland

Nelson, D.R. & Charlet, L.D. (2003). Cuticular hydrocarbons of the sunflower beetle, *Zygogramma exclamationis. Comparative Biochemistry and Physiology,* Vol.135B, No.2, (June 2003), pp. 273–284, ISSN 1096-4959

Nelson, D.R.; Buckner, J.S. & Fatland, C.L. (1994). The composition of external lipids of adult whiteflies, *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Comparative Biochemistry and Physiology,* Vol. 109B, No.2-3, (October-November), pp. 293–303, ISSN 1096-4959

Nelson, D.R.; Guershon, M. & Gerling, D. (1998). The surface wax composition of the exuviae and adults of *Aleyrodes singularis. Comparative Biochemistry and Physiology,* Vol.119B, No.4, (June 1998), pp. 655–665, ISSN 1096-4959

NIST Chemistry WebBook. (2011). *NIST Standard Reference Database*, 2011-08-26, Available from: < http://webbook.nist.gov/chemistry/>

Pasquale, C.; Guarino, S.; Peri, E.; Alonzo, G. & Colazza, S. (2007). Investigation of cuticular hydrocarbons from *Bagrada hilaris* genders by SPME/GC-MS. *Analytical and Bioanalytical Chemistry,* Vol.389, No.4, (October 2007), pp. 1259–1265, ISSN 1618-2650

Pedroza, M.A.; Zalacain, A.; Lara, J.F. & Salinas, M.R. (2010). Global grape aroma potential and its individual analysis by SBSE–GC–MS. *Food Research International*, Vol.43, No.4, (May 2010), pp. 1003–1008, ISSN 0963-9969

Pereira, A.S. & Neto, F.R.A. (1999). High-temperature high-resolution gas chromatography: breaching the barrier to the analysis of polar and high molecular weight compounds. *Trends in Analytical Chemistry*, Vol.18, No.2, (February 1999), pp. 126-136, ISSN 0165-9936

Pereira, A.S.; Padilha, M.C. & Neto, F.R.A. (2004). Two decades of high temperature gas chromatography (1983–2003): what's next? *Microchemical Journal*, Vol.77, No.2, (August 2004), pp. 141– 149, ISSN: 0026-265X

Pereira, A.S.; Siqueira, D.S.; Elias, V.O.; Simoneit, B.R.T.; Cabral, J.A. & Neto, F.R.A. (2002). Three series of high molecular weight alkanoates found in Amazonian plants. *Phytochemistry*, Vo.61, No.6, (November 2002), pp. 711–719, ISSN 0031-9422

Peres, V.F.; Saffi, J.; Melecchi, M.I.; Abad, F.C.; Martinez, M.M.; Oliveira, E.C.; Jacques, R.A. & Caramão E.B. (2006). Optimization of pressurized liquid extraction of *Piper gaudichaudianum* Kunth leaves. *Journal of Chromatography A*, Vol.1105, No.1-2, (February 2006), pp. 115-118, ISSN 0021-9673

Perez-Camino, M.C.; Moreda, W.; Mateos, R. & Cert, A. (2003). Simultaneous determination of long-chain aliphatic aldehydes and waxes in olive oils. *Journal of Chromatography A*, Vol.983, No.1-2, (January 2003), pp. 283–288, ISSN 0021-9673

Persson, M.; Sjödin, K.; Borg-Karlson, A.K.; Norin, T. & Ekberg I. (1996). Relative amounts and enantiomeric compositions of monoterpene hydrocarbons in xylem and needles of *Picea abies*. *Phytochemistry*, Vol.42, No.5, (July 1996), pp. 1289-1297, ISSN 0031-9422

Pietsch, M. & König, W.A. (1997). Enantiomeric composition of the chiral constituents of essential oils - part 3: Diterpene hydrocarbons. *Journal of High Resolution Chromatography*, Vol.20, No.5, (May 1997), pp.257-260, ISSN 0935-6304

Pietsch, M. & König, W.A. (2000). Enantiomers of sesquiterpene and diterpene hydrocarbons in *Araucaria* species. *Phytochemical Analysis*, Vol.11, No.2, (March/April 2000), pp. 99-105, ISSN 1099-1565

Pomonis, J.G.; Hakk, H. & Fatland, C.L. (1989). Synthetic methyl- and dimethylalkanes Kovats indices, [^{13}C]NMR and mass spectra of some methylpentacosanes and 2,X-dimethylheptacosanes. *Journal of Chemical Ecology*, Vol.15, No.9, (September 1989), pp. 2319-2333, ISSN 1573-1561

Ravid, U.; Putievsky, E. & Katzir, I. (1995). Determination of the enantiomeric composition of α-terpineol in essential oils. *Flavour and Fragrance Journal*, Vol.10, No.4, (July/August 1995), pp. 281-284, ISSN 1099-1026

Ravid, U.; Putievsky, E.; Katzir, I. & Lewinsohn, E. (1997). Enantiomeric composition of linalol in the essential oils of *Ocimum* species and in commercial basil oils. *Flavour and Fragrance Journal*, Vol.12, No.4, (July 1997), pp. 293-296, ISSN 1099-1026

Ravid, U.; Putievsky, E.; Katzir, I.; Ikan, R. & Weinstein, V. (1992). Determination of the enantiomeric composition of citronellol in essential oils by chiral GC analysis on β-cyclodextrin phase. *Flavour and Fragrance Journal*, Vol.7, No.5, (October 1992), pp. 235-238, ISSN 1099-1026

Richmond, R. & Pombo-Villar E. (1997). Gas chromatography-mass spectrometry coupled with pseudo-Sadtler retention indices, for the identification of components in the essential oil of *Curcuma longa* L. *Journal of Chromatography A*, Vol.760, No.2, (January 1997), pp. 303-308, ISSN 0021-9673

Richter I. & Krain H. (1980). Cuticular lipid constituents of cabbage seedpod weevils and host plant oviposition sites as potential pheromones. *Lipids*, Vol.15, No.8, (August 1980), pp. 580-586, ISSN 1558-9307

Riedel, M.; Eichner, A.; Meimberg, H. & Jetter, R. (2007). Chemical composition of epicuticular wax crystals on the slippery zone in pitchers of five *Nepenthes* species and hybrids. *Planta*, Vol.225, No.6, (May 2007), pp. 1517–1534, ISSN 0032-0935

Riederer, M. & Müller, C. (Eds.). (2006). *Biology of the Plant Cuticle*, Blackwell Publishing Ltd, ISBN 978-1-4051-3268-8, Oxford, UK

Rohloff, J. (1999). Monoterpene composition of essential oil from peppermint (*Mentha* × *piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrometry analysis. *Journal of Agricultural and Food Chemistry*, Vol.47, No.9, (September 1999), pp. 3782–3786, ISSN 0021-8561

Rücker, G.; Neugebauer, M. & Daldrup B. (1990). Mass spectrometric identification of unsaturated terpene hydrocarbons by GC–MS after derivatization with trioxo(tert-butylimido)osmium(VIII). *Flavour and Fragrance Journal*, Vol.5, No.1, (March 1990), pp. 1-18, ISSN 1099-1026

Said, I.; Costagliola, G.; Leoncini, I. & Rivault, C. (2005). Cuticular hydrocarbon profiles and aggregation in four *Periplaneta* species (Insecta: Dictyoptera). *Journal of Insect Physiology*, Vol.51, No.9, (September 2005), pp. 995–1003, ISSN 0022-1910

Sanchez-Palomo, E.; Diaz-Maroto, M.C. & Perez-Coello, M.S. (2005). Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC–MS. *Talanta*, Vol.66, No.5, (June 2005), pp. 1152-1157, ISSN 0039-9140

Sandoval, I.S.; Hernández, A.S.; Valdez, G.V. & Cruz-López, L. (2007). Volatiles of mango var. Ataulfo characterized by SPME and capillary GC/MS spectroscopy. *Journal of Mexican Chemical Society*, Vol.51, No.3, (June 2007), pp. 145-147, ISSN 1870-249X

Santos, A.M.; Vasconcelos, T.; Mateus, E.; Farrall, M.H.; Gomes da Silva, M.D.R.; Paiva M.R. & Branco M. (2006). Characterization of the volatile fraction emitted by phloems of four pinus species by solid-phase microextraction and gas chromatography–mass spectrometry. *Journal of Chromatography A*, Vol.1105, No.1-2, (February 2006), pp. 191-198, ISSN 0021-9673

Santos, S.; Schreiber, L. & Graça, J. (2007). Cuticular waxes from Ivy leaves (*Hedera helix* L.): analysis of high-molecular-weight esters, *Phytochemical Analysis*, Vol.18, No.1, (January 2007), pp. 60–69, ISSN 1099-1565

Santoyo, S.; Cavero, S.; Jaime, L.; Ibañez, E.; Señoráns, F.J. & Reglero, G. (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *Journal of Food Protection*, Vol.68, No.4, (April 2005), pp. 790-795, ISSN 0362-028X

Schneider, W.; Frohne, J.C. & Bruderreck, H. (1982). Selektive gaschromatographische messung sauerstoffhaltiger verbindungen mittels flammenionisationsdetektor. *Journal of Chromatography A*, Vol.245, No.1, (August 1982), pp. 71-83, ISSN 0021-9673

Schurig, V. & Novotny, H.P. (1990). Gas chromatographic separation of enantiomers on cyclodextrin derivatives. *Angewandte Chemie*, International Edition English, Vol.29, No.9, (September 1990), pp. 939-957, ISSN 1521-3773

Sedlakova, J., Kocourkova, B., & Kubaň, V. (2001). Determination of essential oil content and composition in caraway (*Carum carvi* L.). *Czech Journal of Food Sciences*, Vol.19, No.1, 31-36, ISSN 1212-1800

Shellie, R. A.; Marriott, P.J. & Huie, C.W. (2003). Comprehensive two-dimensional gas chromatography (GC × GC) and GC × GC quadrupole MS analysis of Asian and American ginseng. *Journal of Separation Science*, Vol.26, No.12-13, (August 2003), pp. 1185–1192, ISSN 1615-9314

Shellie, R.; Marriott, P. J. & Morrison, P. (2001). Concepts and preliminary observations on the triple-dimensional analysis of complex volatile samples by using GC×GC−TOFMS. *Analytical Chemistry*, Vol.73, No.6, (February 2001), pp. 1336-1344, ISSN 0003-2700

Shepherd, T.; Robertson, G.W.; Griffiths, D.W.; Nick, A. & Birch, E. (1999). Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L.). *Phytochemistry*, Vol.52, No.7, (December 1999), pp. 1239-1254, ISSN 0031-9422

Smith, R. M. & Burford, M.D. (1992) Optimization of supercritical fluid extraction of volatile constituents from a model plant matrix. *Journal of Chromatography A*, Vol.600, No.2, (May 1992), pp. 175-181, ISSN 0021-9673

Soliday, C.L.; Blomquist, G.J. & Jackson, L.L. (1974). Cuticular lipids of insects. VI. Cuticular lipids of the grasshoppers *Melanoplus sanguinipes* and *Melanoplus packardii*. *Journal of Lipid Research,* Vol.15, No.4, (July 1974), pp. 399-405, ISSN 1539-7262

Stammitti, L.; Derridj, S. & Garrec, J.P. (1996). Leaf epicuticular lipids of *Prunus laurocerasus*: importance of extraction methods. *Phytochemistry*, Vol.43, No.1, (September 1996), pp. 45-48, ISSN 0031-9422

Stashenko, E.E.; Martinez, J.R.; Macku, C. & Shibamoto, T. (1993). HRGC and GC–MS analysis of essential oil from colombian ylang-ylang (*Cananga odorata* Hook fil. et Thomson, forma genuina). *Journal of High Resolution Chromatography*, Vol.16, No.7, (July 1993), 441-444, ISSN 0935-6304

Stashenko, E.E.; Puertas, M. A. & Combariza, M.Y. (1996). Volatile secondary metabolites from *Spilanthes americana* obtained by simultaneous steam distillation-solvent extraction and supercritical fluid extraction. *Journal of Chromatography A*, Vol.752, No.1-2, (November 1996), pp. 223-232, ISSN 0021-9673

Still, W.C.; Kahn, M. & Mitra, A. (1978). Rapid chromatographic technique for preparative separations with moderate resolution. *The Journal of Organic Chemistry*, Vol.43, No.14, (July 1978), pp 2923–2925, ISSN 0022-3263

Stránský, K.; Zarevúcka, M.; Valterová, I. & Wimmer, Z. (2006). Gas chromatographic retention data of wax esters. *Journal of Chromatography A*, Vol.1128, No.1-2, (September 2006), pp. 208–219, ISSN 0021-9673

Stuppner, H. & Ganzera, M. (1998). Determination of safrole in different *Asarum* species by headspace gas chromatography. *Chromatographia*, Vol.47, No.11-12, (June 1998), pp. 685-688, ISSN 0009-5893

Sümmchen, P.; Markstädter, C. & Wienhaus, O. (1995). Esters of *Picea abies* needle cuticular wax. *Phytochemistry*, Vol.40, No.2, (September 1995), pp 599-600, ISSN 0031-9422

Szafranek, B.; Chrapkowska, K.; Waligóra, D.; Palavinskas, R.; Banach, A. & Szafranek, J. (2006). Leaf surface sesquiterpene alcohols of the potato (*Solanum tuberosum*) and their influence on Colorado beetle (*Leptinotarsa decemlineata* Say) feeding. *Journal of Agricultural and Food Chemistry*, Vol.54, No.20, (October 2006), pp. 7729-7734, ISSN 0021-8561

Szafranek, B. & Szafranek, J. (2008). Volatiles of *Solanum* spp.: analysis, composition and ecological significance. *Fruit, Vegetable and Cereal Science and Biotechnology*, Vol.2, No.1, pp. 145-155, ISSN 1752-3419

Szafranek, B.; Chrapkowska, K.; Pawińska, M. & Szafranek J. (2005). Analysis of leaf surface sesquiterpenes in potato varieties. *Journal of Agricultural and Food Chemistry*, Vol.53, No.8, (April 2005), pp. 2817-2822, ISSN 0021-8561

Szafranek, B.; Maliński E. & Szafranek, J. (1998). The sesquiterpene composition of leaf cuticular neutral lipids of ten polish varieties of *Solanum tuberosum*. *Journal of the*

Science of Food and Agriculture, Vol.76, No.4, (April 1998), pp. 588–592, ISSN 1097-0010

Szafranek, B.; Synak, E.; Waligóra, D.; Szafranek, J. & Nawrot, J. (2008b). Leaf surface compounds of the potato (*Solanum tuberosum*) and their influence on Colorado potato beetle (*Leptinotarsa decemlineata*) feeding. *Chemoecology*, Vol.18, No.4, (December 2008), pp. 205-216, ISSN 1423-0445

Szafranek, B.; Tomaszewski, D.; Pokrzywińska, K. & Gołębiowski, M. (2008a). Microstructure and chemical composition of the leaf cuticular waxes in two *Salix* species and their hybrid. *Acta Biologica Cracoviensia Series Botanica*, Vol.50, No.2, pp. 49–54, ISSN 0001-5296

Szafranek, B.M. & Synak, E.E. (2006). Cuticular waxes from potato (*Solanum tuberosum*) leaves. *Phytochemistry*, Vol.67, No.1, (January 2006), pp. 80–90, ISSN 0031-9422

Taylor, S. (1993). Improved determination of vanillin and related phenolic components in vanilla (*Vanilla fragrans* (Salisb.) Ames) by high performance liquid chromatography. *Flavour and Fragrance Journal*, Vol.8, No.5, (September/October 1993), pp. 281-287, ISSN 1099-1026

The AOCS Lipid Library. (2011). Mass spectrometry of fatty acid derivatives, In: *The Lipid Library*, 2011-08-24, Available from: <http://lipidlibrary.aocs.org/ms/arch_xyz/index.htm#misc>

Turillazzi, S.; Sledge, M.F.; Cremer, S. & Heinze, J. (2002). A method for analysing small-size specimens in GC-MS. *Journal of Insect Social Life*, Vol.4, pp. 169–175

Vereen, D. A.; McCall, J. P.; Butcher, D. J. (2000). Solid phase microextraction for the determination of volatile organics in the foliage of Fraser fir (*Abies fraseri*). *Microchemical Journal*, Vol.65, No.3, (October 2000), pp.269-276, ISSN 0026-265X

Verma, R. K.; Uniyal, G.C. & Gupta, M.M. (1990). High performance liquid chromatography of poppy straw. *Indian Journal of Pharmaceutical Science*, Vol.52, No.6, (November/December 1990), pp. 276-278, ISSN 0250-474X

Vermeer, C.P.; Nastold, P. & Jetter, R. (2003). Homologous very-long-chain 1,3-alkanediols and 3-hydroxyaldehydes in leaf cuticular waxes of *Ricinus communis* L. *Phytochemistry*, Vol.62, No.3, (February 2003), pp 433-438, ISSN 0031-9422

Vernin, G.; Vernin, E.; Vernin, C. & Metzger, J. (1991). Extraction and GC-MS-SPECMA data bank analysis of the aroma of *Psidium guajava* L. fruit from Egypt. *Flavour and Fragrance Journal*, Vol.6, No.2, (June 1991), pp. 143-148, ISSN 1099-1026

Vichi, S.; Guadayol, J.M.; Caixach, J.; Lopez-Tamames, E. & Buxaderas, S. (2006). Monoterpene and sesquiterpene hydrocarbons of virgin olive oil by headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry. *Journal of Chromatography A*, Vol.1125, No.1, (August 2006), pp. 117-123, ISSN 0021-9673

Vincenti, M.; Guglielmetti, G. ; Cassani, G. & Tonini, C. (1987). Determination of double bond position in diunsaturated compounds by mass spectrometry of dimethyl disulfide derivatives. *Analytical Chemistry*, Vol.59, No.5, (March 1987), pp. 694–699, ISSN 1520-6882

Volkov, S.K. & Grodnitskaya, E.I. (1994). Application of high-performance liquid chromatography to the determination of vinblastine in *Catharanthus roseus*. *Journal of Chromatography B*, Vol.660, No.2, (October 1994), pp. 405-408, ISSN 1570-0232

Wen, M. & Jetter, R. (2007). Very-long-chain hydroxyaldehydes from the cuticular wax of *Taxus baccata* needles. *Phytochemistry*, Vol.68, No.20, (October 2007), pp. 2563-2569, ISSN 0031-9422

Wen, M. & Jetter, R. (2009). Composition of secondary alcohols, ketones, alkanediols, and ketols in *Arabidopsis thaliana* cuticular waxes. *Journal of Experimental Botany*, Vol.60, No.6, (April 2009), pp. 1811–1821, ISSN 1460-2431

Wen, M.; Au, J.; Gniwotta, F. & Jetter, R. (2006). Very-long-chain secondary alcohols and alkanediols in cuticular waxes of *Pisum sativum* leaves. *Phytochemistry*, Vol.67, No.22, (November 2006), pp 2494-2502, ISSN 0031-9422

Yermakov, A.I.; Khlaifat A.L., Qutob, H.; Abramovich, R.A. & Khomyakov, Y.Y. (2010). Characteristics of the GC-MS mass spectra of terpenoids ($C_{10}H_{16}$). *Chemical Sciences Journal*, pp. 1-7, ISSN 21503494

Zalacain, A.; Marin, J.; Alonso, G.L. & Salinas, M.R. (2007). Analysis of wine primary aroma compounds by stir bar sorptive extraction. *Talanta*, Vol.71, No.4, (March 2007), pp. 1610–1615, ISSN 0039-9140

Zhang, L-x.; Yun, Y-f.; Liang, Y-z. & Cao, D-s. (2010). Discovery of mass spectral characteristics and automatic identification of wax esters from gas chromatography mass spectrometry data. *Journal of Chromatography A*, Vol.1217, No.23, (June 2010), pp. 3695-3701, ISSN 0021-9673

Zimmermann, P.; Hirsch-Hoffmann, M.; Hennig, L. & Gruissem, W. (2004). GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. *Plant Physiology*, Vol.136, No.1, (September 2004), pp. 2621-2632, ISSN 0032-0889

Zini, C.A.; Augusto, F.; Christensen, E.; Smith, B.P.; Caramao, E.B. & Pawliszyn J. (2001). Monitoring biogenic volatile compounds emitted by *Eucalyptus citriodora* using SPME. *Analytical Chemistry*, Vol.73, No.19, (October 2001), pp. 4729-4735, ISSN 0003-2700

Zwaving, J.H. & Bos, R. (1992). Analysis of the essential oils of five *Curcuma* species. Flavour *Fragrance Journal*, Vol.7, No.1, (January 1992), pp. 19-22, ISSN 1099-1026

Zwenger, S. & Basu, C. (2007). *In silico* analysis of terpene synthase genes in *Arabidopsis thaliana*. *EXCLI Journal*, Vol.6, pp. 203-211, ISSN 1611-2156

Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications

Edited by Dr. Bekir Salih

ISBN 978-953-51-0127-7
Hard cover, 346 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

The aim of this book is to describe the fundamental aspects and details of certain gas chromatography applications in Plant Science, Wine technology, Toxicology and the other specific disciplines that are currently being researched. The very best gas chromatography experts have been chosen as authors in each area. The individual chapter has been written to be self-contained so that readers may peruse particular topics but can pursue the other chapters in the each section to gain more insight about different gas chromatography applications in the same research field. This book will surely be useful to gas chromatography users who are desirous of perfecting themselves in one of the important branch of analytical chemistry.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Beata Szafranek, Monika Paszkiewicz, Marek Gołębiowski and Piotr Stepnowski (2012). Gas Chromatographic Analysis of Plant and Insect Surface Compounds: Cuticular Waxes and Terpenoids, Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications, Dr. Bekir Salih (Ed.), ISBN: 978-953-51-0127-7, InTech, Available from: http://www.intechopen.com/books/gas-chromatography-in-plant-science-wine-technology-toxicology-and-some-specific-applications/gas-chromatographic-analysis-of-plant-and-insect-surface-compounds-cuticular-waxes-and-terpenoids

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821