HEADSPACE-SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY MASS SPECTROMETRY APPLIED TO DETERMINATION OF VOLATILES IN NATURAL MATRICES

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ABSTRACT

Headspace – solid phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry-ion trap detector (GC-MS-IT) has been used to characterize volatile compounds in several natural matrices. A great number of fibres with different polarities are commercially available, allowing the screening of the highest possible number of components. HS-SPME has several advantages, such as the condensation of extraction, concentration and sample introduction into a single step. In this extraction technique, a medium polarity fibre for flavours (carboxen/polydimethylsiloxane or divinylbenzene/polydimethylsiloxane) is usually used in order to achieve equilibrium between non-polar and polar compounds, thus increasing the screening range. The traditional methodologies using organic solvents, namely dichloromethane (DCM), allow the determination of a considerable number of volatile and semi-volatile compounds in natural matrices. In this review, the main theoretical points involved in GC-MS analysis will be discussed. The application of HS-SPME and other extraction methodologies (hydrodistillation, Soxhlet and solvent extraction), coupled to GC-MS-IT, to several natural matrices (macroalgae, *Brassica oleracea*, *Catharanthus roseus* and mushrooms, with particular emphasis in these last two) will be referred. With this the usefulness of these methodologies in the screening of volatile and semi-volatile compounds will be demonstrated.

KEYWORDS: *Brassica* spp., *Catharanthus roseus*, GC-MS, HS-SPME, macroalgae, mushrooms, natural matrices, *Rumex induratus*, volatile compounds

ABBREVIATIONS: AAT, alcohol acyl transferases; ACH, acyl-CoA thioesterase; ACS, acyl-CoA synthetase; ACX, acyl-CoA oxidase; ACP, acyl carrier protein; ADH, alcohol dehydrogenase; AOC, allene oxide cyclase; AOS, allene oxide synthase; APC, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; CAR/PDMS, carboxen/polydimethylsiloxane; CCD, carotenoid cleavage dioxygenases; CCO, carotenoid cleavage oxygenases; CI, chemical ionization; CW/DVB, carboxax/divinylbenzene; DCM, dichloromethane; DMAP, dimethylallyl diphosphate; DVB/PA/PDMS, divinylbenzene/carboxax/polydimethylsiloxane; ESI, electrospray ionization; FT-ICR, fourier-transform ion cyclotron resonance; GC-IT-MS, gas chromatography-ion trap detector-mass spectrometry; HCA, agglomerative hierarchic cluster analysis; HPL, hydperoxide lyases; HS-SPME, headspace-solid phase microextraction; IPP, isopentenyl diphosphate; KAT, 3-ketothiolase; LOX, lipoxygenase; MALDI, matrix-assisted laser desorption/ionization; MFP, multifunctional protein containing a 2E-enol-CoA hydratase and a 3S-hydroxyacyl-CoA dehydrogenase; OPDA, 12-oxo-phytodienoic acid; PCA, principal component analysis; RF, radio frequency; SBSE, stir bar sorptive extraction; SIM, selected ion monitoring mode; SPE, solid-phase extraction; TOF, time of flight; TSI, thermospray ionisation

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INTRODUCTION

A quick check of the literature will reveal that most of the analytical studies available concern chemical classes with well-established pharmacological properties, such as polyphenols or alkaloids (Chausser-volfson and Gutterman 1997; Ferreres et al. 2008). However, several pharmaceutical properties, namely antioxidant activity (Singhara et al. 2009), antimicrobial, antifungal (Bakkar et al. 2008) among others, have been recently recognized for some volatile compounds. Analysis of the volatile profile in natural matrices is very important for different scientific areas, particularly those concerned with food analysis, cosmetics, ecology, biochemistry, phytochemistry and plant physiology.

Volatile compounds have different chemical characteristics, covering a wide range of polarity, solubility, volatility and pH. These compounds can be found in natural matrices, usually in very low amounts (ppb range), and many of them are highly unstable. Accordingly, the choice of an appropriate extraction method greatly influences the reliability and accuracy of natural products’ analysis. In order to achieve practical and reliable methods for the analysis of complex matrices, several techniques have been developed, including solid-phase microextraction (SPME), extraction-distillation, solid-phase extraction, supercritical fluids, solvent and Soxhlet extraction, among others.

The coupling of different extractive methods to GC-MS-IT allows the separation and identification of distinct compounds, even when they occur in samples at trace amounts.

GC-MS, as one can easily figure, combines two powerful techniques: GC for separation of compounds and MS to their detection and identification. This feature rapidly turn this analytical approach into a most effective and useful technique.

In this review, the main theoretical points involved in GC-MS analysis will be discussed and some examples of application of GC-MS-IT on the analysis of different natural matrices will be presented.

VOLATILE COMPOUNDS

Volatiles cover a wide range of compounds with different characteristics, usually with low molecular weight, which can be metabolites derived from amino acids, fatty acids and carbohydrate compounds. Although volatiles were regarded exclusively as secondary metabolites of plants, nowadays several works have proven that, apart from plants, vegetable materials (leaves, flowers, fruits and roots, among others), some volatiles are emitted from other living organisms, such as fungi (mushrooms), insects, algae or even oysters (Fujimura et al. 1994; Harborne 1999; Josephson et al. 2006; Cho et al. 2007). By interacting with volatiles, the olfactory sensory system is responsible for the flavour preferences that rule human diet (Vichi et al. 2005; Goff and Klee 2006; Edris 2007).

It is believed that volatiles have protective effects in plants, mainly due to their anti-microbial and anti-herbivore activity, acting as repellents for herbivores and pathogens. A number of these compounds also have the ability to attract arthropods that fall upon or parasitize herbivores, thus minimizing further damage to plant tissue. Volatiles can also play a role in attracting pollinators. Some of the molecules have the ability to scavenge reactive oxygen species, protecting against internal oxidative damage (Dudareva et al. 2004; Schwab et al. 2008). Furthermore, recent studies have demonstrated that these compounds play an important role in plant-plant communication. Undamaged plants can adaptively respond to the chemical information emitted by damaged neighbours (Yan and Wang 2006).

From a chemical viewpoint, volatiles can be straight-chain, branched-chain, aromatic or heteroaromatic compounds, constituted by a wide range of chemical groups, such as hydroxyl, carboxyl, carboxyl, ester, lactone, amine and thiol functions. They can be produced in different biosynthetic pathways and can be divided in terpenoids, apocarotenoids and norisoprenoids, lactones, benzenoids and phenylpropanoids, nitrogen and/or sulphur-bearing volatile compounds and fatty acids-derived compounds.

Terpenoids

Terpenoids constitute one of the most diversified families of natural products. So far, over 40000 different structures were identified in several biological systems, including a large number of medically important compounds, such as hormones and cytostatic agents (Schwab et al. 2008).

The universal precursors of terpenoids are the five-carbon compound isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP). The mevalonate pathway was considered to be the unique biosynthetic pathway for the formation of IPP, DMAPP and the subsequent terpenes, until the discoveries by the research groups of Rohmer (Rohmer 1993) and Eisenreich (Eisenreich 1998). These works suggested that pyruvate and triose phosphate served as precursors for the formation of IPP and DMAPP via an alternative pathway. It was also shown that 1-deoxyxylulose, a known precursor of thiamine and pyridoxal, could be diverted very efficiently to terpenoids by E. coli cells. Subsequent studies by several research groups identified 1-deoxy-d-xylulose 5-phosphate as the first intermediate of the alternative terpenoid pathway. Nevertheless, details of the formation of IPP and DMAPP from this intermediate remain unknown (Rohdich et al. 2001) (Fig. 1).

These two terpene biosynthetic pathways have been studied in different taxonomic kingdoms. Animals, fungi and archaeabacteria appear to use exclusively the mevalonate pathway, while eubacteria use either the mevalonate or the deoxyxylulose one (Takagi et al. 2000). Plants use the mevalonate pathway in the cytosol to synthesize sesquiterpenes (C15), phytoesters and ubiquinone, while the deoxyxylulose pathway takes place in the plastids to generate monoterpenes (C10), diiterpenes (C30), gibberellins, abscisic acid and carotenoids (tetramerpenes) (Pichersky et al. 2006; Schwab et al. 2008).

Apocarotenoids and norisoprenoids

Apocarotenoids are compounds involved in a great variety of cellular processes, being found in all life kingdoms. They are commonly produced by oxidative cleavage and later modification of larger carotenoid compounds. Like their precursors, apocarotenoids are non-polar isoprenoids that possess a conjugated double-bound system and, for this reason, exhibit antioxidant properties (Kloer and Schulz 2006). Furthermore, these metabolites have low threshold values and characteristic aroma notes, and have a high commercial value for flavours and perfume industries, particularly ionones, which are found in blackberry, peach and apricot, and in the odour of flowers such as violets. Furthermore, ionones are valuable for its industrial applications as starting materials for other products (Rodriguez-Bustamante and Sánchez 2007).

Depending on the carotenoid precursor and the breakdown position, a great variety of apocarotenoids can be generated. This breakdown can take place via either non-enzymatic or enzymatic routes. The non-enzymatic breakdown comprises photo-oxygenation, auto-oxidation and thermal degradation. On the other hand, the enzymatic breakdown can be accomplished by several enzymes, such as carotenoid cleavage oxygenases (CCO), carotenoid cleavage dioxygenases (CCD), lipoxygenases (LOX), xanthine oxidase, phenoloxidases and peroxidases.

Carotenoids can be cleaved in C9 to C13 positions and C13 products, known as norisoprenoids, represent the most common and widespread group (Rodriguez-Bustamante and Sánchez 2007) (Fig. 2).
Benzenoids/phenylpropanoids

The shikimate pathway links carbohydrate metabolism to the synthesis of aromatic amino acids, which can in turn act as precursors of several primary and secondary metabolites. Phenylpropanoid metabolism provides plants with thousands of compounds that are intermediates in the synthesis of structural cell components (e.g. lignin, suberin, and other cell wall-associated phenolics), while others constitute a diverse array of pigments (e.g. flavonoids and anthocyanins, both of which are usually non-volatile). The aromatic amino acid phenylalanine is also the precursor of volatile benzenoids (C_{6}-C_{1}) and phenylpropanoids (C_{6}-C_{3}), providing the characteristic benzene ring that is further oxidized, acylated or methylated, thus yielding individual scent compounds (van Schie et al. 2006). Phenylpropanoids that are reduced at the C9 position (to aldehyde, alcohol, or alkane/alkene) and/or that contain alkyl additions to the hydroxyl groups of the benzyl ring or to the carboxyl group (i.e. ethers and esters) are volatile. Eugenol and safrol are characteristic compounds of this family, and are frequently the main components of several essential oils.

Benzenoid compounds (C_{6}-C_{1}) are originated from trans-cinnamic acid as a side branch of the general phenylpropanoid pathway. Characteristic compounds of this family known to constitute the floral scent of plants are benzylacetate, methylsalicylate, methylbenzoate, benzylbenzoate, dimethoxytoluene, phenylpropene and methyleugenol (Boatright et al. 2004) (Fig. 3).

Nitrogen- and sulphur-bearing volatile compounds

The pungent and, in some cases, unpleasant odour characteristic of some plants is due to, in a general way, volatile metabolites which incorporate sulphur or nitrogen.

A particular feature of some angiosperm families, namely Brassicaceae, is the presence of glucosinolates. These sulphur and nitrogen-containing secondary metabo-
Glucosinolates are originated from amino acids. Plants that produce and accumulate glucosinolates always possess a thio glucoside glucohydrolase enzyme known as myrosinase, which hydrolyzes the glucose moiety on the glucosinolate main skeleton. The products are glucose and an unstable aglycone that can rearrange to form volatile compounds, such as isothiocyanates, nitriles, and other products, which are responsible for the pungent aroma characteristic of these plants (Halkier and Gershenzon 2006).

Pyrazines constitute a group of heterocyclic nitrogen-containing compounds, being widespread in nature and with a pronounced odour. Several authors suggest a pathway for pyrazine biosynthesis initiated by the formation of a cyclic peptide through the condensation of two amino acids. This pathway only allows the formation of 2,5-dialkylsubstituted metabolites. Another biosynthetic pathway is the condensation of a \( \gamma \beta \)-dicarbonylic compound and an amidated amino acid and allows the formation of both 2,5- and 2,6-alkyl-substituted pyrazine molecules (Beck et al. 2003).

Fatty acids derived compounds

Saturated and unsaturated C\(_6\) and C\(_9\) volatile aldehydes and alcohols are ubiquitous to all kingdoms (Akakabe et al. 2005). These compounds are important contributors to the “green leaf” odour, being produced by the lipoxigenase pathway and involving at least four enzymes: lipoxigenase (LOX), hydroperoxide lyase (HPL), \((Z,2E)\)-enal isomerase and alcohol dehydrogenase (ADH) (Schwab et al. 2008).

LOX catalyzes the oxygenation of fatty acids containing a \((1Z,4Z)\)-pentadiene moiety (e.g. linoleic and linolenic acid) in a region/stereo-selective way. These LOX products are \((Z,E)\)-configured hydroperoxides, which subsequently suffer enzymatic cleavages by HPL, resulting in the formation of short-chain aldehydes with 6 and 9 carbon atoms (Akakabe et al. 2005).

\( \alpha \)-Oxidation leads to the enzymatic degradation of C\(_6\) fatty acids into a C\(_{\alpha-1}\) long chain fatty aldehyde, with concomitant release of CO\(_2\). An \( \alpha \)-dioxigenase-peroxidase has been suggested to operate together with aldehyde dehydrogenase and NAD\(^+\) oxido-reductase to lead to successive fragmentations of fatty acids into shorter chain homologues (Hamberg et al. 1999).

Differently from \( \alpha \)-oxidation, \( \beta \)-oxidation pathway mainly involves a set of several enzymatic reactions: acyl-CoA thioesterase (ACH), acyl-CoA synthetase (ACS), acyl-CoA oxidase (ACX), multifunctional protein containing a \((2E)\)-enoyl-CoA hydratase and a \((3S)\)-hydroxyacyl-CoA dehydrogenase (MFP) and a 3-ketothiolase (KAT), which results in the removal of C\(_2\) units (acyetyl CoA) from the parent fatty acid. This pathway has been reported across different kingdoms (plants, mammals, fungi) (Goepfert and Poirier 2007).

Successive removals of C\(_2\) units followed by the action of an acyl CoA hydrolase leads to volatile acids with buttery and cheese-like odour. Some of these aliphatic acids can also be synthesized de novo by hydrolysis of an acyl carrier protein (acyl ACP). The enzymatic reduction of the parent acyl CoA leads to short- and medium chain aldehydes and alcohols. Esters can be synthesized by acyl CoA, formed during \( \beta \)-oxidation, and alcohols. The enzymes that catalyze the combinations of acyl CoA and alcohols are alcohol acyl transferases (AAT) (Schwab et al. 2008).

Jasmonates are also lipid-derived metabolites that have important functions in plants, such as mediation of the responses to mechanical trauma and pathogenesis, regulation of metabolism and reproduction. Jasmonic acid is not volatile, but some of its metabolites are, with myethyl jasmonate or \( cis \)-jasmonine being good examples. This latter is involved in regulating the behaviour of some insects, repelling herbivorous ones and attracting their predators. There are also evidences suggesting that some volatiles from the jasmonic acid pathway may enable communication among plants (Liechti and Farmer 2002). Fig. 4 represents a schematic pathway of fatty acids derived flavour compounds.
Lactones

\( \gamma \)- and \( \delta \)-lactones are fatty acid derivatives quite widespread in nature, and many of them display pronounced biological activities as attractants for pollination and seed germination stimulants. They also act as pheromones, antiseptics, allergens, or even as cardiotonic compounds. Furthermore, lactones are important flavour and aroma constituents and are extensively used as food additives and in perfumery. For example, 4-butylbutanolide occurs in the flavour of apricot.

Fig. 3 Schematic view of some branches of phenylpropanoid metabolism. Solid arrows indicate enzymic reactions. Abbreviations: PAL, phenylalanine ammonia-lyase; BA2H, benzoic acid 2-hydroxylase; C4H, cinnamate 4-hydroxylase; CA2H, cinnamate 2-hydroxylase; 4CL, 4-coumarate coenzyme A ligase; C3H, \( \rho \)-coumarate 3-hydroxylase; CCOMT, caffeoyl coenzyme A \( O \)-methyl-transferase; CCR, cinnamoyl coenzyme A reductase; CAD, cinnamoyl alcohol dehydrogenase; EOMT, eugenol \( O \)-methyl-transferase.
EXTRACTIVE TECHNIQUES

Several extractive techniques have been developed for the analysis of the volatile profile of natural matrices. The volatile composition is usually quite complex and therefore different approaches and methods of analysis should be used to obtain a complete overview. When several different extractions take place, the sum of the obtained volatiles constitutes a good representation of the matrix’s profile.

Traditional methodologies (extraction-distillation, solvent and Soxhlet extraction and solid-phase extraction) usually imply several steps, including purification and concentration of the extract (with consequent analyte losses), long preparation times and the use of large quantities of organic solvents. As it is performed at low temperatures, this technique can prevent the thermal degradation and hydrolysis of the matrix. Nevertheless, the complex and expensive processes and the need of large amounts of sample (Wang et al. 2008a). Essential oils can undergo chemical alterations and thermal decomposition that can easily destroy some compounds, thus changing the volatile profile.

Solid-phase extraction (SPE) allows obtaining compounds without applying heat. This technique is performed by using an adsorbent surface to extract organic compounds from a liquid phase and, for this purpose, different resins can be applied. Although SPE is limited to semi-volatile and non-volatile compounds (Masqué et al. 1998), it appears to be applicable to both polar and non-polar compounds. Compared to the other techniques referred above, SPE offers a reduction of processing time and quantity of solvent used. Nevertheless, it still requires multiple steps, long extraction times, losses in the evaporation step and risk of contamination (Alpendurada 2000).

The disadvantages of traditional extraction methodologies, previously referred, and especially the use of large quantities of solvents led to the development of new methods, which main advantages are the small volume of solvent used and the relatively fast extraction step.

Super-critical fluid extraction is an interesting technique for the extraction of volatile compounds from vegetable matrices. This technique has several advantages over traditional extraction techniques, such as the possibility to adjust a number of parameters (solvent, extraction temperature and pressure) to obtain better selectivity and extractive efficiency, also allowing the reduction of the use of polluting organic solvents. As it is performed at low temperatures, this technique can prevent the thermal degradation and hydrolysis of the matrix. Nevertheless, the complex and expen-
sive equipment necessary, not available in all laboratories, constitutes its main disadvantage (Pourmortazavi and Hajimirsadeghi 2007; Wang et al. 2008b). Pawlisyn (Pawlisyn 1997) developed an adsorptive technique called solid-phase microextraction (SPME), which can be regarded as replacement and improvement of classical sample preparation methods (Ulrich 2000). SPME was originally developed for environmental analysis (Pawliszyn 1997), although today it is widely used for analysing volatiles in a wide range of areas, such as food, environmental, botanical and clinical analysis, as well as in forensic applications (Alpendurada 2000; Kataoka et al. 2000).

SPME is a well-established sample preparation technique for the analysis of volatile and semi-volatile compounds, presenting many advantages such as high sensitivity and reproducibility, simplicity, absence of solvents, cost and combination of extraction and pre-concentration into a single step (Kataoka et al. 2000). This technique, based on absorption and/or adsorption mechanisms, can be performed in three different sampling modes: direct injection (immersion), HS extraction and extraction with membrane protection (Prosen and Zupancic-Kralj 1999). HS-SPME is the most used for several reasons, being the main one the lack of contact with the sample that reduces the matrix influence and prevents decomposition or contamination of the fibre coating (Alpendurada 2000). Moreover, the time necessary to reach the equilibrium between the analyte and the stationary phase is shorter than for aqueous samples, and so HS-SPME is recommended for compounds with high volatility (Prosen and Zupancic-Kralj 1999). Several coatings are available, the particular choice of a coating being highly influenced by the chemical structure of the target compounds. The careful selection of the polarity and thickness of coating allows the extraction of different compounds, with other variables being equally important, namely temperature, agitation, pH and the addition of a salt to help the release of some compounds (Ulrich 2000).

### Solid Phase Microextraction – Optimization

Achieving good reproducibility, selectivity and sensitivity with SPME requires a careful optimization of the extractive process. The performance of this method is affected by the combination of several parameters, which are all interrelated.

Several fibres are commercially available (Table 1). The selection of the adequate fibre is very important because compounds with different polarity and volatility can be sampled simultaneously by the careful selection of the polarity and thickness of the fibre coating. In general, the “like dissolves like” rule can be applied and by changing the fibre coating we can modulate the compounds extracted (Kataoka et al. 2000). The different fibres are available in various thicknesses. A thin coating has a faster diffusion rate and allows preferentially the retention of semi-volatile compounds. On the other hand, the thick coatings permit the retention of highly volatile compounds with an increased sensitivity; however, they require a longer equilibrium time (Prosen and Zupancic-Kralj 1999). By constructing an extraction-time profile curve, we can observe that the amount of the analyte extracted is a function of time. In order to extract the maximum amount of analyte, the equilibrium time has to be reached, and this is dependent on the partition coefficient of the analyte. Consequently, the shorter exposure time is chosen according to the extraction-time profile curve and the analyte detection limit (Ulrich 2000). Fig. 5 shows a schematic representation of the extraction and desorption procedure using SPME. To achieve the optimal stage of equilibrium several parameters can be changed, such as temperature, agitation, pH, ionic strength, and derivatization.

Agitation is normally used to achieve faster equilibrium, because it enhances the diffusion of analytes to the fibre. The rate of stirring should be constant for all samples and has to be experimentally determined. The increase of extraction temperature also raises the diffusion coefficient of analytes, although it reduces the distribution constant (Ulrich 2000). The pH of the sample is important for weak acid or basic compounds, mainly because the extraction of these compounds is more effective when they are in the undissociated form (Alpendurada 2000). The addition of a soluble salt, usually sodium chloride or sodium sulphate, increases the ionic strength of the solution, thus making organic compounds less soluble and increasing the partition coefficients (Steffen and Pawlisyn 1996; Ulrich 2000). Derivatization can be used when aiming the extraction of very polar compounds. The derivatization agent may be either added into the matrix, or bonded to the SPME fibre, where analytes are adsorbed and derivatized simultaneously (Prosen and Zupancic-Kralj 1999).

### Gas Chromatography and Mass Spectrometry (GC-MS)

Gas chromatography is a powerful tool to analyse volatile compounds. Mixtures to be analysed are injected into an inert gas stream and swept into a tube, which is packed with a solid support coated with a resolving liquid phase. Absorptive interactions between the components in the gas stream and the coating lead to a differential separation of the components of the mixture, which are then swept in

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**Table 1** Characteristics of SPME fibres commercially available.

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Recommended use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polydimethylsiloxane (PDMS)</strong></td>
<td></td>
</tr>
<tr>
<td>100 μm / non-bonded</td>
<td>Low molecular weight or volatile compounds</td>
</tr>
<tr>
<td>30 μm / non-bonded</td>
<td>Non-polar semi-volatiles</td>
</tr>
<tr>
<td>7 μm / bonded</td>
<td>Large molecular weight compounds</td>
</tr>
<tr>
<td><strong>Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)</strong></td>
<td></td>
</tr>
<tr>
<td>65 μm / partially crosslinked</td>
<td>Polar volatile analytes, such as amines and alcohols</td>
</tr>
<tr>
<td>60 μm / partially crosslinked</td>
<td>General purpose SPME fiber for HPLC</td>
</tr>
<tr>
<td><strong>Carboxen/Polydimethylsiloxane (CAR/PDMS)</strong></td>
<td></td>
</tr>
<tr>
<td>75 μm / partially crosslinked</td>
<td>Trace-level volatiles</td>
</tr>
<tr>
<td><strong>Carbowax/Template Resin (CW/TPR)</strong></td>
<td></td>
</tr>
<tr>
<td>50 μm / partially crosslinked</td>
<td>Surfactants (for HPLC only)</td>
</tr>
<tr>
<td><strong>Carbowax/Divinylbenzene (CW/DVB)</strong></td>
<td></td>
</tr>
<tr>
<td>65 μm / partially crosslinked</td>
<td>Polar analytes</td>
</tr>
<tr>
<td><strong>Polyacrylate</strong></td>
<td></td>
</tr>
<tr>
<td>85 μm / partially crosslinked</td>
<td>Polar semi-volatiles</td>
</tr>
<tr>
<td><strong>Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)</strong></td>
<td></td>
</tr>
<tr>
<td>50/30 μm divinylbenzene/Carboxen on PDMS fiber</td>
<td>Trace compounds (MW 40-275)</td>
</tr>
<tr>
<td><strong>Carbowax-Polyethylene Glycol (PEG)</strong></td>
<td></td>
</tr>
<tr>
<td>60 μm / partially crosslinked</td>
<td>Alcohols and polar compounds (MW 40-275)</td>
</tr>
</tbody>
</table>
order through a detector flow cell.

MS is based on the production of ions from the analyte. This is ionized in high vacuum and its ions and fragmentation products are impelled and focused through a magnetic mass analyzer, to be further collected and the amounts of each selected ion measured in a detector.

The combination of GC and MS yields an instrument capable of separating mixtures into their individual components, identifying and providing quantitative and qualitative information on the amounts and chemical structure of each compound.

From an instrumental point of view, three components are essential to perform mass spectrometric experiment: the first is the ion source, the second is the mass analyser and the last one is the detector. These three compartments are submitted to a very high vacuum to prevent the undesirable movement of particles (Karasek and Clement 1991).

**Ion sources**

The choice of the ionization method to be employed is dependent of the physico-chemical properties of the analyte(s) of interest (volatility, molecular weight, thermolability, complexity of the matrix in which the analyte is contained, among others).

The ion sources can be divided in two main classes: those requiring sample in the gas phase prior to ionization, and those able to manage low volatility and high molecular weight samples.

The first class includes electron ionization (EI) and chemical ionization (CI) sources, which are the most diffused nowadays due to their multiple uses in GC-MS. The other ones can be further divided into those operating with solutions, such as Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI) or Atmospheric Pressure Photoionization (APPI), and those based on sample desorption and ionization from a solid substrate: Matrix-Assisted Laser Desorption/Ionization (MALDI) and Thermospray Ionisation (TSI). These last two are mainly applied in liquid chromatography. As our work deals with the identification of volatile compounds, we will focus only on EI and CI ion sources.

EI is based on the interaction of an energetic electron beam, generally 70 eV, with the sample vapour (at a pressure in the range $10^{-7}$-$10^{-5}$ Torr). This interaction leads to the production of a series of ions related to the chemical properties of the compounds under study. CI methods generally apply lower levels of electronic impact (12 eV). This method is employed to select species of interest in complex matrices (Karasek and Clement 1991).

**Analysers**

There are several types of analysers: Magnetic Sector, Time of Flight (TOF), Ion Mobility, Fourier-Transform Ion Cyclotron Resonance (FT-ICR), Quadrupole and Ion Trap. Quadrupole and ion trap are currently the mass analysers most widely employed in GC-MS. Both systems start from the same considerations: ions of different $m/z$ values will interact in a different manner with alternated electrical fields (radio frequency (RF)).

Four hyperbolic and equidistant rods constitute the Quadrupole analyser. Ions “travel” through the rods and are separated along them according to their $m/z$ values.

The Ion Trap analyser is constituted by a central hyperbolic electrode and two other electrodes called the “end caps”. An RF is applied in the central electrode creating a magnetic field in the trap. Molecules coming from the gas chromatographic column go in to the trap. After changing the potential, the electrons also go into the trap. The impact of electrons with the molecule causes its ionization. The formed ions describe then a characteristic orbital in the shape of an eight. The orbital described by the ions increase in its amplitude being sequentially ejected from the trap (first those with lower $m/z$ values) directly to the electron multiplier.

There are some advantages and disadvantages for using the quadrupole and/or the ion trap. Briefly, quadrupole is more tolerant to weak vacuum, though it is adapted to electrospray ionization. In addition, it can reach $m/z$ values of 3000, which is of great interest for electrospray ionization. In addition, it can reach $m/z$ values of 3000, which is of great interest for electrospray ionization. The Ion Trap has the great advantage of being much more sensitive in full scan mode acquisition (monitoring a range of $m/z$). The most important difference between ion trap and quadrupole analysers concerns the separation of the $m/z$ produced into the analyser. In quadrupole, this separation is performed along the rods while in the ion trap the separation is done into the trap. Consequently, ion trap is able to produce full scan spectra with high detection limits; however, it cannot work in SIM.
(Selected Monitoring Ion) mode, which is a characteristic of quadrupole analysers. Ion trap has the possibility to do several fragmentations of each m/z (MS/MS until n levels) in opposition to quadrupole, which works only with one level of MS/MS. Finally, ion trap analyser is able to separate very similar ranges of m/z, which means that its resolution is better. Depending on the objectives of the work, the option for one or another can be made (Watson and Sparkman 2007).

Detectors

The most used detector for GC-MS purposes is the electron multiplier. This component is the part of the mass spectrometer, which is responsible for increasing the signals coming from the analyser. A semi-conductor material (SnO, PbO) in the internal surface plates the electron multiplier and the top of the multiplier is maintained with a negative voltage. Each ion entering in the electron multiplier collides with the surface material and releases two electrons. These two electrons collide with the opposite wall of the electron multiplier releasing two more electrons. This process is repeated successively; for one ion entering in the electron multiplier 100 000 electrons are generated, which corresponds to a gain of 10^5. The electric signal is then processed and the data is treated with the acquisition software (Lavagnini et al. 2006; Watson and Sparkman 2007; MacMaster 2008).

APPLICATIONS

A complete identification and ulterior quantification of volatiles, in complex matrices such as natural products, needs some previous proceedings due to volatiles being present in a wide range of concentrations. Some volatiles can be present in high levels (mg/L or mg/Kg) while others occur in very low ones (ng/L or ng/Kg). As was discussed previously, an ion trap analyser stores all m/z particles for a small period. When a molecule is present in high amounts, the consequence is an “overloading” in the trap, which causes a distortion of mass spectrum of the molecule that may prevent a correct identification. In opposition, when volatile molecules are present in low levels (ng or even pg levels) a very accurate spectrum can be obtained, which is usually coincident with those of most libraries. Consequently, and due to this specificity/limitation, quantification can be analytically difficult. Linearity studies must be performed in certain ranges of concentrations and, when compounds are present in high concentration, dilution of samples is recommended; when SPME is used the time of fibre exposure to the headspace has to be reduced.

Our research group has applied HS-SPME allied to GC-IT-MS to several natural matrices, namely mushrooms, Catharanthus roseus (L.) G. Don, formerly Vinca rosea L. (Apocynaceae), Brassica oleracea L. var. acephala leaves and sprouts, Rumex induratus and algae, among others matrices.

Mushrooms

Wild edible mushrooms are a source of interesting organoleptic properties such as flavour, texture and colour. The taste of edible mushrooms is primarily attributed to several water-soluble substances, including 5’-nucleotides, free amino acids and soluble carbohydrates. Among the diverse volatile compounds, a series of aliphatic eight carbon (C8) components, such as 1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octanol, 1-octen-3-one and 3-octanone, have been reported to be the major contributors to the characteristic mushroom flavour (Cho et al. 2006). Besides that, other compounds, such as esters, terpenes and indoles have been identified and can also be related to mushroom flavour.

The objective of the work of Guedes de Pinho et al. (2008) was to obtain a complete screening of the volatiles and semi volatiles of eleven edible mushrooms (Suillus bellini, Suillus luteus, Suillus granulatus, Tricholomopsis rutihans, Hygrophorus agathosmus, Amanita rubescens, Russula cyanoxantha, Boletus edulis, Tricholoma equestre, Fistulina hepatica, and Cantharellus cibarius). Additionally, the contents of the identified volatiles were correlated with sensorial descriptors. For this propose, HS-SPME and liquid extraction with organic solvents combined to GC-IT-MS was used.

A comparison between three fibres (CAR/PDMS, CW/DVB and DVB/CAR/PDMS) was performed and DVB/CAR/PDMS fibre was selected for the analysis of all mushroom species, as it was the one that could give the most complete profile of the compounds present in the analysed species. In addition, it revealed to be the best and more selective fibre for the identification of aldehydes like methional and phenylacetaldehyde, regarded as important compounds to the mushroom flavour (Barker et al. 1983; Silva Ferreira and Guedes de Pinho 2003).

A sensorial analysis was performed and some descriptors, such as “mushroom-like”, “farm-feed-like”, “floral”, “honey-like”, “hay-herb” and “nutty” were obtained. Applying multivariate analysis (principal component analysis (PCA) and agglomerative hierarchical cluster analysis (HCA)) to the sensorial and chemical data, it was possible to make a correlation between the descriptors and the volatiles. The descriptors selected were “farm-feed” (28%), “mushroom-like” (24%), “floral” (18%), “honey-like” (8%), “nutty” (8%), “hay-herb” (7%) and 7% corresponded to other descriptors, which were discarded. The eleven species were divided in three groups (Fig. 6). The one with “floral” and “honey” descriptors included S. granulatus and S. luteus; the second group, presenting “hay-herb”, “nutty” and “mushroom-like” notes was composed by A. rubescens, C. cibarius, S. bellini and T. equestre; and finally the species characterized by “farm-feed” were H. agathosmus, T. rutihans, R. cyanoxantha, B. edulis and F. hepatica.

HS-SPME and dichloromethane extracts allowed the identification of 64 volatile and non-volatile compounds in the analysed mushroom species. These included 5 volatile acids, 8 non-volatile acids, 7 esters, 9 alcohols, 7 aldehydes, 7 ketones, 11 terpenes, 1 volatile phenol, 2 lactones and 7 ketones, 11 terpenes, 1 volatile phenol, 2 lactones and 7 others compounds. Thirteen compounds were tentatively identified and fifty-one were identified by comparison of the Kovats index and the MS spectrum of the pure standard. Among the volatile acids, we identified five fatty acids (myristoleic, palmitoleic, stearic, linoleic and oleic acids) and three others: benzoic, cinnamic and phenylacetic acids. H. agathosmus presented the highest percentage of myristoleic, palmitoleic, oleic and cinnamic acids. S. bellini was identified as the richest in alcohols, what is considered to be the main odors of the “mushroom-like” aroma (Cho et al. 2006). Among these compounds, C. cibarius presented the highest percentage of 1-octen-3-ol, while A. rubescens was the one with the highest amount of 3-octanol. Statistical results showed that these alcohols have higher correlations with the “nutty” descriptor than with the “mushroom-like” aroma. T. equestre and S. luteus presented the highest levels of aldehydes and benzaldehyde and phenylacetaldehyde were identified in all of the species. Phenylacetaldehyde is considered responsible for “honey” notes (Cho et al. 2006), with S. luteus being the species presenting the highest levels of this compound. B. edulis was the richest specie in methional. This compound has a very low olfactory perception limit and its descriptor is “boiled potato” (Soares da Costa et al. 2004). The panel for these mushroom species did not use this descriptor; however, this species was described with notes of “farm-feed”. A very high correlation between methional and “farm-feed” descriptor has been found, and the presence of this compound could explain its aroma characteristics (Soares da Costa et al. 2004; Cho et al. 2006). Among the identified ketones, two different groups emerged: one was constituted by 3-octanone and 1-octen-3-one, while the other one was composed by volatile norisoprenoids, such as β-ionone, 6-methyl-5-hepten-2-one, trans-geranylelactone and (E,E)-far-
Catharanthus roseus (L.) G. Don is commonly known as the Madagascar periwinkle. This plant is characterized by the presence of several compounds with important pharmacological activities, namely indolic alkaloids (van der Heijden et al. 2004; Sottomayor and Barceló 2005). The water extracts and leaves from C. roseus are traditionally used in folk medicine for preventing some diseases and suppress the sensation of hunger and fatigue (Ross 2003; van der Heijden et al. 2004).

In our work (Guedes de Pinho et al. 2009), 88 compounds were identified in flowers, leaves and stems, by HS-SPME and by dichloromethane extraction, combined with GC-MS. These two methodologies were used concerning the determination of the total amount of volatile and semi-volatile, and other non-volatile compounds, respectively. HS-SPME was applied to fresh plant (to identify the most volatile compounds) and to lyophilized extract (to determine the less volatile compounds). The dichloromethane extraction was performed to characterize the non-volatile compounds.

The most effective fibres for HS-SPME were those characterized by two components: a liquid (PDMS) for the less polar compounds and a solid (DVB, CAR or both) polymeric coating for the more polar constituents. In this work three fibres were evaluated with the following phases: CAR/PDMS, CW/DVB and DVB/PDMS, with the last one having the particularity of being selective to nitrogen-containing compounds.

Using the CAR/PDMS fibre, 25 compounds were identified: n-hexanal, 2-hexen-1-ol, cis-3-hexen-1-ol, hexanol, benzaldehyde, 1,4-cineole, limonene, benzyl alcohol, eucalyptol, 3-methoxy-2,5-dimethylpyrazine, phenylacetaldehyde, limaool, 2-nonen-1-ol, phenylethanol, camphor, iso-butylmethylxypyrirazine, trans-2-decenol, verbenone, β-cyclocitral, bornilacetate, eugenol, valencene, β-ionone, epoxy-β-ionone and (E)-geranylacetone. When the CW/DVB fibre was applied, some diterpenic compounds, such as manool and their oxides and α-bisabolol were detected, in addition to the above-mentioned compounds. Finally, using the DVB/PDMS, 10 aldehydes, 14 alcohols, 8 esters, 10 nitrogen-bearing compounds, 12 terpenes, 8 carotenoid compounds, H. agathosmus and B. edulis; and group 3 included S. luteus, S. granulatus, R. cyanoxantha and F. hepatica (Fig. 7).

**Catharanthus roseus**
derivatives, 4 ketones plus 3 other compounds were identified for the first time in fresh flowers, stems and leaves of *C. roseus*. In general, SPME allowed the determination of 12 aldehydes, 14 alcohols, 10 nitrogen containing compounds, 17 terpenic compounds (including aliphatic mono and diterpenes), 12 carotenoid derivatives, 6 ketones, 1 hydroxycinnamic acid and 3 phenol compounds (Fig. 8).

Dichloromethane extraction allowed the identification of 14 other compounds. Among these compounds, some structures like alkaloid molecules could be identified, as well as 2,2,7,7-tetramethyltricyclo(6.2.1.0(1,6))undec-4-en-3-one, α-farnesene, phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol. In this way, the SPME showed to be a more effective technique.

Moreover, statistical analysis (principal component analysis) allowed the distinction of the different organs of the plant (leaves, stems and flowers) in what concerns their volatile composition. Succinctly, flowers were richer in terpene molecules (including limonene), aldehyde compounds, esters compounds, namely methyljasmonate, and phenols (due to the high amounts of eugenol). Leaves were correlated to carotenoid derivative compounds, sum of ketones and ester compounds. Finally, stems were in good correlation with nitrogen containing compounds, alcohols and miscellaneous compounds (Fig. 9).

A deeper knowledge of *C. roseus* volatile constituents was achieved by the use of HS-SPME fibre. Some of the compounds identified have important bioactivities in the human body.

**Brassica oleracea L.**

Plants from the Brassicaceae family play a major role in worldwide vegetable production and consumption, ranking second after Solanaceae. *Brassica* vegetables, including all cabbage-like ones, are consumed in great quantities all over the world. Concerning the determination of volatiles, several studies have reported the volatile composition of different species of *Brassica*, namely *Brassica rapa* L. var. *perviridis* Bailey (Miyazawa et al. 2005) and *Brassica oleracea* L. var. *botrytis* L. (Valette et al. 2003). These two previous works used hydrodistillation and organic solvents to...
The DVB/PDMS fiber was selected for the analyzes of all leaves of tronchuda cabbage to determine the volatile profile of internal and external compounds. The aim of our work (de Pinho et al. 2009) was to separate the volatile profile of internal and external leaves of tronchuda cabbage grown under different fertilization regimens, using HS-SPME combined with GC-MS-IT. The DVB/PDMS fiber was selected for the analyzes of all samples, as it revealed to be the best and more selective fiber for the identification of sulfur compounds, breakdown products of glucosinolates, considered as important chemical classes for kale characterization. Hence, 34 volatile and non-volatiles components were formally identified and 38 others were tentatively identified. Qualitative and quantitative differences were noticed between internal and external leaves. In general, internal and external leaves exhibited aldehydes, sulphur-bearing volatile compounds, ketones, terpenes and norisoprenoids, and it was possible to denote interesting differences between the leaves.

A parallel study was performed in order to evaluate the variation of volatiles along the seedling development of Brassica oleracea L. var. acephala (kale). In this work, 66 volatile compounds, distributed by several chemical classes were determined: alcohols, carbonyl compounds (ketones, aldehydes and esters), norisoprenoids, terpenes, sulphur-bearing compounds, among others (Fernandes et al. 2009). This is the first work on the volatiles profiling of kale during seeds sprouting, as well as on the leaves of mature plant. According to the results obtained, it may be anticipated that, in agreement with the state of development of the plant, it is possible to find different chemical classes of volatiles.

These results suggest that Brassica spp., namely its seeds and sprouts, can have a large potential for biological activity, which include both negative (outright toxicity) and positive (chemoprotective effect against certain cancers) nutritional attributes.

**Rumex induratus**

*Rumex induratus* is an endemic Iberic herb, growing spontaneously in Northeast Portugal, where leaves are highly appreciated and consumed, specially in salads. Despite the high consumption, its phytochemical characterization is quite scarce. Previous works were performed concerning organic acids and phenolic composition (Ferrer et al. 2001).

**Fig. 9.** Principal component analysis of all volatile compounds analysed by HS-SPME-GC-MS grouped by family classes in flowers, stems and leaves of *C. roseus*. Abbreviations: SCAR, sum of carotenoid molecules; Sket, Sum of ketones; SPHE, sum of phenols; SE, Sum of esters compounds; Salc, Sum of alcohols; Sald, Sum of aldehydes; STER, Sum of terpenes; Ncompounds, nitrogen-containing compounds. Reprinted from Guedes de Pinho P, Gonçalves RF, Valentão P, Pereira DM, Seabra RM, Andrade PB, Sottomayor M (2009) Volatile composition of *Catharanthus roseus* (L.) G. Don using solid-phase microextraction and gas chromatography/mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 49, 674-685, ©2009, with kind permission of Elsevier.

**Fig. 10** Chromatogram in FullScan mode of HS-SPME combined with GC/MS, using Divinylbenzene/PDMS fibre, of the brown algae *F. spiralis*. Identity of compounds: 1 – heptanoic acid, 2 – octanoic acid, 3 – nonanoic acid, 4 – undecanoic acid, 5 – dodecanoic acid, 6 – (Z)-4-hexenol, 7 – (E)-2-hexenal, 8 – (E)-2-heptenal, 9 – phenylacetaldehyde, 10 – (E)-2-octenal, 11 – (E,Z)-2,6-nonadienal, 12 – (E)-2-nonenal, 13 – (E)-cinnamaldehyde, 14 – (E,E)-2,4-decadienal, 15 – methyl dihydrojasmonate, 16 – benzyl benzoate, 17 – methyl palmitate, 18 – benzyl bromide, 19 – 2-bromo-1-cyclohexene, 20 – linalool, 21 – (+)-camphor, 22 – menthone, 23 – menthol, 24 – α-terpineol, 25 – carvone, 26 – geranyl acetone, 27 – β-ionone, 28 – 5,6-epoxy-β-ionone, 29 – dihydroactinidiolide, 30 – dimethyl trisulfide, 31 – benzothiazole, 32 – methylthymol, 33 – eugenol. Chromatographic conditions: Oven temperature - 40°C (for 1 min), 2°C/min to 220°C and held for 30 min. Injector port was heated to 220°C, in splitless mode. Carrier gas - Helium C60, at 1 mL/min, constant flow. Chromatographic column - VF-5 ms 30 m × 0.25 mm × 0.25 μm (FactorFour) from VARIAN.
To establish the volatile composition of *R. induratus* leaves different extractive methods (hydrodistillation, SPME, Soxhlet and solvent extraction) were performed, followed by GC-MS-IT analysis (Taveira et al. 2009). Incidentally, it was possible to identify 81 volatile compounds, distributed by several chemical classes: esters, terpenes, aldehydes, acids, norisoprenoids, ketones, naphthalene derivatives, steroidal derivatives, alcohols, among others. The liquid solvent allowed the extraction of molecules with high molecular weights, while HS-SPME with a DVB/PDMS fibre, extracted the most volatile compounds.

### Algae

Marine algae have been regarded as an attractive source of pharmaceutical agents in several biochemical and pharmaceutical investigations (Kamenarska et al. 2009). *Fucus spiralis* L. is an intertidal marine seaweed that lives in a harsh environment, where it is subjected to repeated immersion and emersion due to tidal fluctuations (Dring 2006). As result, it is exposed to a wide range of environmental stress, having, therefore, a protective antioxidant defense system (Dring 2006). In fact, powerful antioxidant molecules (phlorotannins, ascorbic acid, tocopherols, carotenoids, phenolics, chlorophyll related compounds, bromophenols, catechins, mycosporine-like amino-acids, among others), similar to those of vascular plants, are reported in algae (Dring 2006).

Volatiles play important roles in chemical communication in marine ecosystems, acting as pheromones or allelochemicals, chemical defenses such as deterrence against predators, inhibition of bacterial and fungal fouling, and suppression of competing neighbours (Akakabe and Kajiwara 2008).

The determination of the volatiles in the aqueous extract of *F. spiralis*, was achieved by GC-MS-IT, using a DVB/PDMS fibre.

Thirty-three compounds were identified (Fig. 10, Table 2) and, as far as we know, with the exception of geranyl acetone (26) (Sartin et al. 2001), all of them are described for the first time in *F. spiralis*. These compounds belong to different classes: five acids (1-5), one alcohol (6), eight aldehydes (7-14), three esters (15-17), two halogenated compounds (18-19), six monoterpenes (20-25), four norisoprenoid derivatives (26-29), two sulphated compounds (30-31) and two other volatiles (32-33). Acids were predominant (ca. 47.6%), with monoacetic acid (3) being the main compound (ca. 21.5%) (Table 2) (unpublished data).

In parallel with chemical analysis, studies regarding biological activity of this and other species are required, as oceans remain as one of the most promising sources for bioactive molecules in the nearer future.

### In vivo analysis

Being GC-MS an increasingly powerful and popular analytical approach, its application has been extended to other areas, such as chemical ecology. Volatiles play a very important role in the relations between organisms of different trophic levels, such as insects and plants. As so, GC-MS has been used in the study of the influence of herbivory upon host plants, which responds by synthesizing and releasing a set of compounds with the objective of warning neighbour plants of the presence of a predator or attraction of insect parasitoids. The work of Vercammen et al. (2001) is a good example of a versatile GC-MS system that was used for the *in vivo* monitoring of plant responses when challenged by biotic and abiotic stress. In this work, feeding of the cotton leafworm (*Spodoptera littoralis*) on ivy (*Hedera helix*) and tomato plants (*Lycopersicon esculentum* Mill) was used as a model of insect plant interactions (per. obs.). In the near future, the number of studies applying GC-MS to living organisms is expected to rise.

### Table 2 Average content (in percentage) of volatile compounds identified in lyophilized aqueous extract of *F. spiralis*.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>RT (min)</th>
<th>QI (m/z)</th>
<th>RA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids</td>
<td>1 Heptanoic acid</td>
<td>12.276</td>
<td>60/73/87</td>
<td>6.015</td>
</tr>
<tr>
<td></td>
<td>2 Octanoic acid</td>
<td>15.122</td>
<td>60/73/85</td>
<td>14.765</td>
</tr>
<tr>
<td></td>
<td>3 Nonanoic acid</td>
<td>17.863</td>
<td>60/73</td>
<td>21.508</td>
</tr>
<tr>
<td></td>
<td>4 Undecanoic acid</td>
<td>20.438</td>
<td>60/73/87</td>
<td>4.371</td>
</tr>
<tr>
<td></td>
<td>5 Dodecanoic acid</td>
<td>25.294</td>
<td>60/73/85</td>
<td>0.966</td>
</tr>
<tr>
<td>Alcohols</td>
<td>(Z)-4-Hexenol</td>
<td>4.695</td>
<td>55/67/82</td>
<td>0.538</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>7 (E)-2-Hexenal</td>
<td>5.960</td>
<td>55/69/83</td>
<td>0.220</td>
</tr>
<tr>
<td></td>
<td>8 (E)-2-Heptenal</td>
<td>8.795</td>
<td>57/70/83</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>9 Phenylacetaldehyde</td>
<td>11.411</td>
<td>91</td>
<td>3.990</td>
</tr>
<tr>
<td></td>
<td>10 (E)-2-Octenal</td>
<td>11.807</td>
<td>70/93</td>
<td>0.543</td>
</tr>
<tr>
<td></td>
<td>11 (E,Z)-2,6-Nonadienal</td>
<td>14.516</td>
<td>41/67/70</td>
<td>0.688</td>
</tr>
<tr>
<td></td>
<td>12 (E)-2-Nonenal</td>
<td>14.824</td>
<td>55/70/93</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td>13 (E)-Cinnamaldehyde</td>
<td>18.129</td>
<td>77/103/131</td>
<td>4.488</td>
</tr>
<tr>
<td></td>
<td>14 (E,E)-2,4-Decadienal</td>
<td>19.286</td>
<td>81/152</td>
<td>4.264</td>
</tr>
<tr>
<td>Esters</td>
<td>15 Methyl</td>
<td>27.397</td>
<td>83/93/151</td>
<td>3.254</td>
</tr>
<tr>
<td></td>
<td>16 Benzyl benzoate</td>
<td>30.068</td>
<td>77/91/105</td>
<td>2.847</td>
</tr>
<tr>
<td></td>
<td>17 Methyl palmitate</td>
<td>33.102</td>
<td>87/143/270</td>
<td>0.386</td>
</tr>
<tr>
<td>Monoterpenes</td>
<td>20 Linalool</td>
<td>13.016</td>
<td>93/121</td>
<td>0.869</td>
</tr>
<tr>
<td></td>
<td>21 (+)-Camphor</td>
<td>14.532</td>
<td>81/95/108</td>
<td>0.739</td>
</tr>
<tr>
<td></td>
<td>22 Menthone</td>
<td>14.741</td>
<td>112/139/154</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td>23 Menthol</td>
<td>15.387</td>
<td>81/95/123</td>
<td>8.251</td>
</tr>
<tr>
<td></td>
<td>24 α-Terpineol</td>
<td>15.888</td>
<td>93/121/131</td>
<td>1.220</td>
</tr>
<tr>
<td></td>
<td>25 Carvone</td>
<td>17.285</td>
<td>82/108/150</td>
<td>0.906</td>
</tr>
<tr>
<td>Norisoprenoid derivatives</td>
<td>26 Geranyl acetone</td>
<td>22.581</td>
<td>107</td>
<td>0.274</td>
</tr>
<tr>
<td></td>
<td>27 β-Ionone</td>
<td>23.426</td>
<td>177</td>
<td>0.253</td>
</tr>
<tr>
<td></td>
<td>28 5,6-Epoxy-β-ionone</td>
<td>23.510</td>
<td>123/177</td>
<td>0.446</td>
</tr>
<tr>
<td></td>
<td>29 Dihydroactinidiolide</td>
<td>24.740</td>
<td>111/137/180</td>
<td>1.287</td>
</tr>
<tr>
<td>Sulphated compounds</td>
<td>30 Dimethyl trisulfide</td>
<td>9.170</td>
<td>45/79/126</td>
<td>0.566</td>
</tr>
<tr>
<td></td>
<td>31 Benzothiazole</td>
<td>16.826</td>
<td>69/108/135</td>
<td>2.944</td>
</tr>
<tr>
<td>Others</td>
<td>32 Methylhexaldehyde</td>
<td>16.951</td>
<td>96/124/139</td>
<td>4.180</td>
</tr>
<tr>
<td></td>
<td>33 Eugenol</td>
<td>20.193</td>
<td>164</td>
<td>2.763</td>
</tr>
</tbody>
</table>

**RT** = retention time. **QI** = quantification ions. **RA (%)** = relative area in percentage. **MS** = tentatively identified by NIST05. **Identified by comparison with reference compound.

### CONCLUSION

The study of the volatile composition of natural products is important due to the organoleptic characteristics and biodiversity that these compounds can exhibit. Flavours and scents are directly tied to food preference and palatability and while some volatile metabolites emitted from plants or fungi are pleasant to mammals or insects, other compounds are mainly unpleasant.

The research on these flavour components from the food and beverage, as well as perfumery and cosmetics industries, draws closer our understanding about why and how these compounds are metabolized, leading to several discoveries about the biological activities of some volatiles.
In fact, some of them have protective effect against different kinds of cancer (Singletary 2000), while others play important roles in reproduction and metabolic regulation (Liechti and Farmer 2002). Essential oils, that contain a variety of volatile molecules, are widely used for antiseptic and insecticidal applications (Bakkali et al. 2008). Due to their properties, these metabolites earned increasing attention in pharmacuetics, therapeutics or plague control research.

The recent adoption of simple and sensitive methods using headspace sampling coupled with GC-MS, allowed a substantial increase in the number of identified volatile compounds in the last two decades. The main advantages of HS-SPME, in comparison to other classical extraction techniques, are simplicity, elimination of several steps, non-usage of solvents, high sensitivity, requirement of small amount of sample and low cost. This technique applied to GC-MS has proved to be an important tool to researchers, as a general volatile profile of the matrix in study can be rapidly obtained. For the reasons described above, HS-SPME has become the first choice in quality control laboratories.

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