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Optimization of headspace solid phase microextraction for gas chromatography/mass spectrometry analysis of widely different volatility and polarity terpenoids in olibanum

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Abstract

The aim of this study was the optimization of headspace SPME conditions for trapping diterpenes present in frankincense (olibanum). Diterpenes like cembrenes or incensole and its derivatives are characteristic of olibanum. So in order to detect by SPME the occurrence of olibanum in archeological objects, it appears essential to have the best extraction conditions for these diterpenes that will be in very small quantities. Both sampling time and extraction temperature were studied and five fiber coatings were tested: polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and carbowax/divinylbenzene (CW/DVB). The PDMS/DVB fiber was found to be the most efficient for trapping olibanum characteristic diterpenes, with a sampling time of 1 h and a sampling temperature of 80 °C.
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1. Introduction

One of the laboratory research axes is conception and development of analysis methods for the characterization of natural organic substances present in archaeological objects and especially those of the Ancient Egypt. Mummification balms in particular have retained our attention and we are trying to determine their chemical composition [1]. Resins like pine resin, mastic and gum resins, namely frankincense or myrrh, were possibly used in mummification balms [2,3]. Identification of specific biomarkers in recent samples of these resins or gum resins is essential to determine afterwards their presence in ancient balms or unguents. These are very complex mixtures including fats, resins, waxes, gums, fossil hydrocarbons, ... [2,4]. In this paper, we focus our study on one gum resin: frankincense.

Among the methods of sample treatment for GC/MS analysis, headspace SPME seems to be one of the most efficient. In fact it presents a lot of advantages. First, it is a non-destructive and non-invasive method. This point is of importance when dealing

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with archaeological samples. Secondly, it concentrates volatile compounds and so allows their detection even at trace level; it requires only small amounts of sample. Third, non-volatile compounds such as fat bodies coming from vegetable oils or animal fats used in balm making, or from human tissues of the mummy itself, are often present in samples together with polysaccharides from various origins. Fat bodies and polysaccharides are not extracted by such a method. Sample pretreatment is absolutely necessary before their GC/MS analysis. It consists in a degradation followed by a derivatization. However this hard sample pretreatment often involves isomerizations of terpenes-like compounds. Thus, characterization of resins or gum resins in archaeological samples is difficult as it can be ambiguous. So, an alternative method to analyze them is needed. SPME avoids fastidious sample pretreatment and is an efficient screening method for the detection of resin or gum resin in archaeological samples. Moreover, it allows to choose further sample treatment in order to have more information. If presence of terpenes is detected by SPME, a milder sample pretreatment to avoid their isomerization will be chosen.

In a previous paper [5], we have successfully used headspace SPME with a PDMS fiber for the analysis of pine resin, galbanum, labdanum, mastic, myrrh and olibanum. In this paper, we focused our attention only on the characterization of frankincense.

Frankincense, also called olibanum, is a natural oleo-gum resin that exudes from incisions in the bark of *Boswellia* trees. The genus *Boswellia* of the Burseraceae family includes nearly 23 species of small trees that grow mainly in Arabia, on the eastern coast of Africa and in India. Frankincense is composed of about 5–9% highly odorous essential oil (mono- and sesquiterpenes), 65–85% alcohol-soluble resins (diterpenes, triterpenes), and the remaining water-soluble gums (polysaccharides) [6,7]. The sole terpenoid part of frankincense will be studied here. It contains high volatile mono- and sesquiterpenes, low volatile diterpenes and very low volatile triterpenes. Obviously, headspace SPME cannot be applied to the last class of components. In attempt to determine which resin was used during the embalming mummies process, the presence of the high volatile mono- or sesquiterpenes is not discriminative. In fact these compounds can be found in other resins like mastic or pine resin. However, diterpenes like incensole or isoincensole and their oxide or acetate derivatives (see Fig. 1) are characteristic biomarkers of olibanum. Although diterpenoid hydrocarbons possessing the cembrane

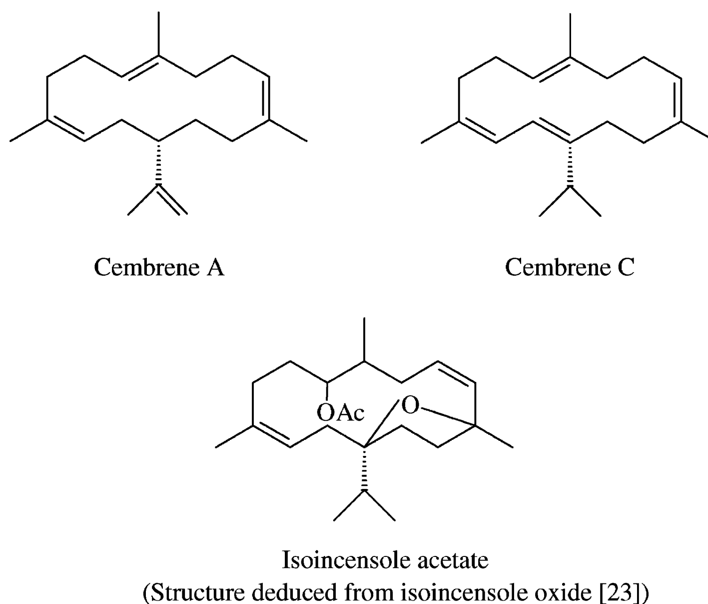


Fig. 1. Structure of cembrene A, cembrene C and isoincensole acetate.

skeleton have been isolated from a variety of terrestrial and marine organisms, their occurrence and particularly that of cembrenes A and C (see Fig. 1) is a supplementary proof of the presence of olibanum in a sample. Our study has been focused on the finding of the best experimental conditions to trap low volatile diterpenes on the SPME fiber.

SPME was widely used for analysis of volatile organic compounds and especially terpenes in essential oils or in living flowers to elaborate perfume [8–20]. In this paper, we reported the best experimental conditions for trapping diterpenes from a gum resin. We have tested five fiber coatings polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and carbowax/divinylbenzene (CW/DVB), four sampling temperatures (20, 40, 60 or 80 °C) and four sampling times (20, 40, 60 or 80 min).

2. Experimental

2.1. Chemicals and samples

The olibanum sample come from Somalia and was supplied by Wolf (type 8767). It was graciously given by Dr. J. Connan. CH_2Cl_2 used for solvent extraction of the olibanum was of analytical grade and supplied by Merck (Darmstadt, Germany).

2.2. Fibers

Five different SPME fibers: PDMS with coating thickness 100 μm, PDMS/DVB 65 μm (Stable Flex), CAR/PDMS 85 μm (Stable Flex), CW/DVB 70 μm (Stable Flex), DVB/CAR/PDMS 50/30 μm (Stable Flex) were purchased from Supelco (Bellefonte, PA, USA). All fibers were new and conditioned according to the suppliers' instructions.

2.3. GC/MS conditions

GC/MS analyses were performed on a 3400 gas chromatograph (Varian, Walnut Creek, CA, USA) coupled to an INCOS 50 quadrupole mass spectrometer (Finnigan, San Jose, CA, USA). Analytes were separated on a DB5 (J&W Scientific, Folsom, USA) capillary column of 30 m × 0.25 mm with a phase thickness of 0.25 μm. The injector temperature was set at 250 °C and the temperature program was: 40 °C during 1 min, 9 °C/min increase rate up to 130 °C, followed by a 2 °C/min increase rate to 230 °C. The carrier gas was helium with a column head pressure of 10 psi. Splitless injection (1 min) was used. The temperatures of the transfer line and the source were 250 and 150 °C, respectively.

The mass spectrometer was operated in electron-impact (EI) mode at 70 eV, in the scan range *m/z* 29–400. Compounds were identified by use of the NIST98 spectral library, as well as literature MS data [21–24] and by comparison of their retention indices, relative to C_9–C_{22} alkanes.

2.4. SPME procedure

Before every extraction, the fibers were conditioned at 250 °C for 10 min.

All extractions were performed in 2 ml glass vials equipped with screw cap and PTFE/silicone septa, using 2 mg of powdered frankincense. The vial was immersed of 5 mm in a thermostated bath at 20, 40, 60 or 80 °C. The SPME fiber was maintained 1 cm above the solid sample. Because of the solid nature of the samples, no internal standard was used. To calculate the Kovats indices, 0.2 μl of a mixture of 14 alkanes (C_9–C_{22}) was added to 2 mg of powdered frankincense just before SPME sampling.

In order to have the most homogeneous matrix and reach the best reproducibility for fiber comparison, 100 mg of frankincense were freshly powdered in a 5 ml glass vial from which 2 mg are taken for each experiment.

After extraction, the analytes were thermally desorbed during 5 min at 250 °C in the injector of the gas chromatograph.

2.5. Solvent extraction procedure

Two milligrams of powdered olibanum were dissolved in 0.5 ml dichloromethane. The mixture was kept in an ultrasonic waves bath for 10 min. After centrifugation, the insoluble part (polysaccharides) was eliminated and 1 μl of the solvent soluble material was injected in the gas chromatograph. In parallel to

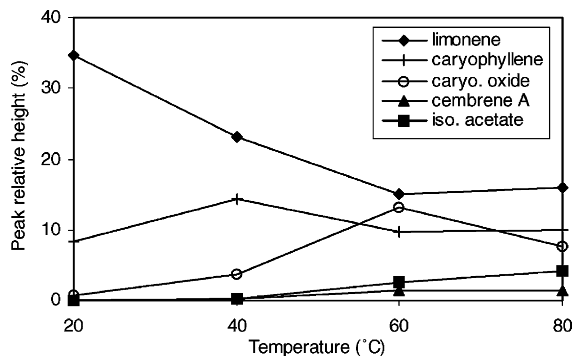


Fig. 2. Temperature profiles for three classes of analytes: monoterpenes: limonene, sesquiterpenes: β-caryophyllene, caryophyllene oxide (caryo. oxide), and diterpenes: cembrene A, isoincensole acetate (isoacetate). PDMS fiber, sampling time 40 min.

this study, polysaccharides could be analyzed as previously described [25].

3. Results and discussion

3.1. Choice of the best sampling temperature

Diterpenes are low volatile compounds, so sampling times will be long and extraction temperature high. The sampling time of these preliminary experiments was arbitrarily fixed at 40 min. This time was sufficiently long to permit the diterpenes to be trapped. Heating solid samples helps to release analytes from their matrix into the headspace, increasing the vapor pressure of analytes that favors the extraction process [26,27]. Four extraction temperatures were tested: 20, 40, 60 and 80 °C. In order to avoid sample deterioration and loss of thermolabile analytes, no temperature higher than 80 °C was tested. Indeed, it was reported in literature that some terpenes like β-caryophyllene undergo oxidation at elevated temperature [28]. The SPME fiber was the commonly used PDMS 100 μm. Results obtained for the three classes of terpenes are presented in Fig. 2.

The three classes of terpenes have different temperature profiles. These differences can be related to their boiling points, which are related to their molecular weight and their number of carbons. Watanabe et al. obtained similar profiles by plotting peak area of some other chemical compounds class versus heating temperature. Optimal sampling temperatures were found to be dependent on the number of carbons of the anesthetic. Dibucaine, which has 20 carbons, did not reach equilibrium even at a temperature of 140 °C [29].

Monoterpenes are high volatile compounds (limonene: bp_{763} 175.5–176.5 °C) and leave the solid matrix very quickly. An increase in temperature does not favor their vaporization from the matrix to the gaseous phase, but causes their desorption from the fiber to the gaseous phase, which then becomes the major process. We observe therefore a decrease in peak area. The same temperature profile was obtained by Yeung et al. for SPME sampling of menthone and menthol in taste-masked tablets. They found that 45 °C was the best sampling temperature and noted that a further increase in temperature resulted in a loss of recovery of these two terpenes [30]. Similarly, Schäfer et al. have established that, the distribution constants between the fiber coating and the gaseous phase for terpinolene, limonene and camphene were exponentially decreasing with increasing temperature. They chose 40 °C as the optimal sampling temperature for these monoterpenes [31].

The comportment of sesquiterpenes which are less volatile (β-caryophyllene: bp_{760} 262 °C) is quite different. In a first time their migration from the matrix to the gaseous phase is predominant, hence we observe an increase in peak area. But beyond a certain temperature, which depends on the boiling point, the migration from the fiber to the gaseous phase predominates. Therefore peak area decreases. We can note that the optimal trapping temperature is lower (40 °C) for β-caryophyllene than for his oxide derivative (60 °C), which is less volatile.

For the diterpenes, which are the less volatile compounds, the migration from the matrix to the gaseous phase predominates even at 80 °C. They do not reach their optimal trapping temperature. So in order to better observe diterpenes on the chromatograms, we choose a sampling temperature of 80 °C.

3.2. Effect of the sampling time and comparison of different fibers

Five SPME fiber coatings (PDMS, PDMS/DVB, CAR/PDMS, DVB/CAR/PDMS, CW/DVB) and four sampling times (20, 40, 60 and 80 min) were tested. The sampling temperature was 80 °C.

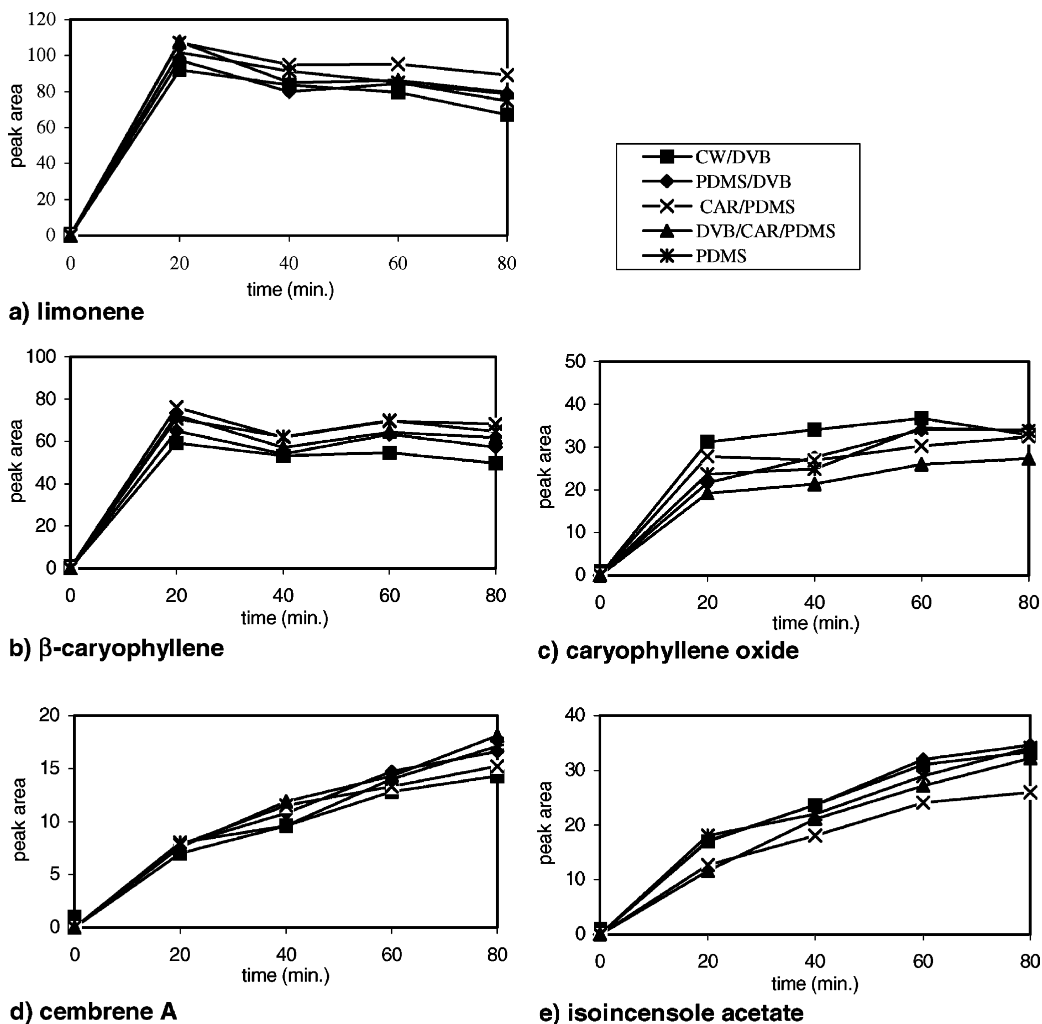


Fig. 3. Sampling time profiles for five terpenes with five different fibers. Sampling temperature 80 °C.

The influence of the sampling time on the amount of extracted terpenes by the different fiber coatings can be observed in Fig. 3. Sampling profile seems to be only dependent on the volatility of the studied compound. For mono- and sesquiterpenes, independently of the nature of the fiber coating, the extraction optimum is reached in about 20 min. However, for the less volatile diterpenes, 80 min are not enough to extract them completely from the matrix, the optimal extraction time being longer.

Such a behavior is well known and well documented. For the monoterpenes α-terpinene and α-pinene, Zabaras et al. found at 25 °C an optimal exposure time of 10 min but 60 min were not enough for the two less volatile sesquiterpenes viridiflorene and α-gurjunene to reach equilibrium [32]. Here, no difference of sampling time occurs between monoterpenes and sesquiterpenes because of the higher extraction temperature. Other studies have shown that sesquiterpenes require a very long time to reach equilibrium [33]. In the same way, Vereen et al. have observed during headspace SPME of the foliage of the Fraser fir, that the monoterpenes β-pinene and limonene reached equilibrium in 30 min at room temperature, while the monoterpene acetate derivatives (fenchyl acetate, bornyl acetate and isobornyl acetate)

needed 3 h and the two sesquiterpenes α-humulene and β-caryophyllene did not equilibrate over a period of 4 h [34]. For these two compounds, Field et al. reported that the optimal sampling time was 4 h at 50 °C during headspace SPME on hops [28]. In this study, we can observe for monoterpenes a slight decrease in peak area when increasing the sampling time. It was already reported that in case of multicomponent systems, a competition occurs for the "active places" of the coating of the SPME fiber. By increasing extraction time the higher-boiling compounds might displace the previously sorbed lower-boiling ones [35,36]. Moreover, Coleman has proved the marked tendency of SPME fibers to selectively adsorb the most alkyl-substituted components. Then, for SPME in aqueous solutions containing more than one organic compound, it appeared that the adsorption of the most non-polar alkyl substituents could ultimately retard, displace, exclude, or compete more favorably for adsorption than the most polar compounds do [37].

Diterpenes require more than 80 min to reach equilibrium. This is expected for compounds that exhibit low vapor pressure in combination with high partition coefficient between the fiber coating and the gaseous phase. Zhang and Pawliszyn pointed out that, while sensitivity of the fiber to the less volatile compounds is high, low coefficients between the sample and the headspace would result in long extraction times. For example, the equilibration time was about 8 min for naphthalene and 30 min for acenaphthalene, while neither phenanthrene nor chrysene did reach equilibrium within 70 min at room temperature. During headspace SPME the amount of such compounds present in the gaseous phase is absorbed by the fiber coating at a much faster rate than their release from the matrix, thus the amount of mass in the headspace at any time is small and a long time is required to reach equilibrium [38]. In our case, we can say that, equilibration between the frankincense powder and the headspace is the rate-determining step for sampling low volatile diterpenes.

In order to avoid too long experimental time for one sample, we choose 60 min as the sampling time. Obviously, with a sampling time of 60 min and a sampling temperature of 80 °C, we are not at the partition equilibrium for diterpenes extraction; SPME has therefore not the maximum sensitivity. However, peak intensities are sufficiently high to detect the presence of diterpenes in a sample. Moreover, a proportional relationship exists between the amount of analyte adsorbed by the SPME fiber and its initial concentration in the sample matrix before reaching partition equilibrium [39,40]. SPME quantifications are then feasible if necessary, by a rigorous reproducibility of the experimental conditions.

Table 1
Values of the criterion function F_{ij} calculated for five different fiber coatings

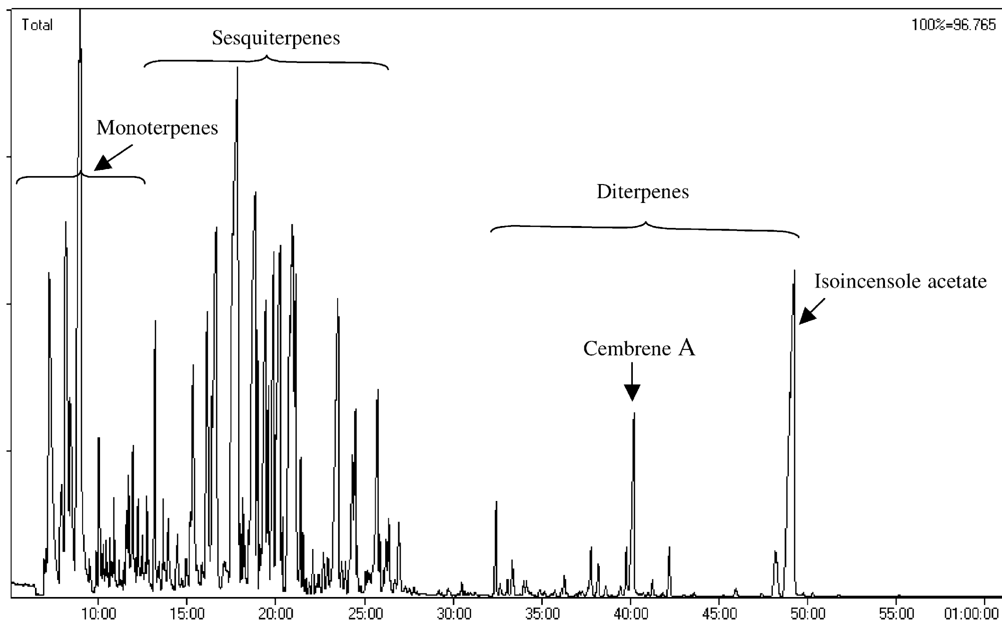
Fiber coating	F_{ij} for monoterpenes	F_{ij} for sesquiterpenes	F_{ij} for diterpenes
PDMS	1.04	1.06	1.01
CAR/PDMS	1.13	1.07	0.88
CAR/PDMS/DVB	1.04	1.04	0.98
PDMS/DVB	0.95	1.03	1.10
CW/DVB	0.84	0.80	1.03

Sampling time 60 min, extraction temperature 80 °C. Monoterpenes are: α-pinene, β-myrcene, α-phellandrene and limonene; sesquiterpenes are: α-cubebene, α-copaene, β-elemene, β-caryophyllene, α-humulene, γ-muurolene, β-eudesmene and caryophyllene oxide; diterpenes are cembrene A and isoincensole acetate.

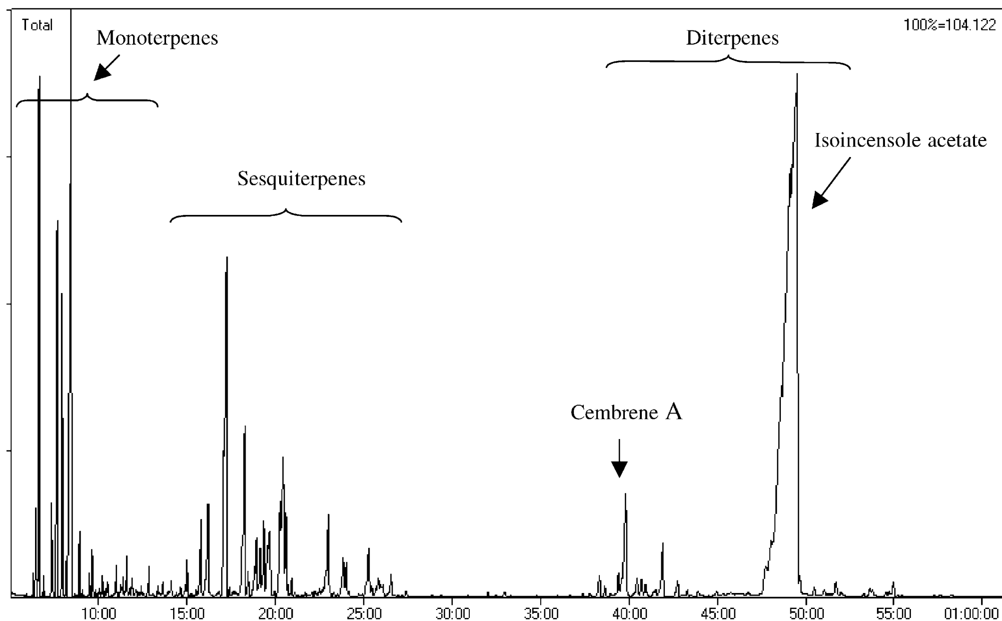
On the other hand, we observe that for each compound the sampling profile is the same for the five fiber coatings. We can deduce that the quantity of terpene trapped is independent of the nature of the fiber coating. Yeung et al. have also observed that optimal sampling conditions of menthone and menthol are similar for three different fibers: PDMS, PDMS/DVB, polyacrylate [30]. These behaviors can be explained easily, as the interactions between compounds and fibers are not specific. It is not very surprising because of the nature of studied terpenes, which are principally composed of carbon and hydrogen.

A mathematical selection based on the function F_{ij} can be used in order to better discriminate the various fiber coatings. This criterion function was introduced by Zuba et al. in order to compare six commercially available fiber coatings for the headspace extraction of polar volatile compounds (Eq. (1)) [41]. F_{ij} is the ratio of the mean of H_{ij} for n analytes for one fiber, and the mean of H_{ij} for n analytes for the five fibers; where H_{ij} is the peak height of i analyte with use of j fiber coating. Results are summarized in Table 1.

$$F_{ij} = \frac{1/n\sum_i H_{ij}}{1/5(1/n\sum_{ij} H_{ij})} \quad (1)$$



a) Chromatogram of frankincense obtained with SPME



b) Chromatogram of frankincense obtained with dichloromethane extraction

Fig. 4. Comparison between SPME and dichloromethane extraction.

Table 2
Compounds detected in frankincense by SPME and by solvent extraction

Peak no.	Compound	Kovats index	SPME (relative peak area (%))	CH_2Cl_2 extraction (relative peak area (%))
1	α-Thujene	916	0.1	0.4
2	α-Pinene	942	**4.0**	**3.6**
3	Camphene	949	0.9	0.1
4	Sabinene	963	0.3	0.4
5	β-Pinene	966	0.4	0.2
6	β-Myrcene	994	**4.1**	**3.2**
7	α-Phellandrene	1003	0.5	1.7
8	*o*-Methyl-anisole	1005	1.2	0.1
9	*p*-Cymene	1014	1.3	2.6
10	Limonene	1020	**7.4**	**5.1**
11	Eucalyptol (1,8-cineole)	1022	0.9	0.8
12	γ-Terpinene	1054	0.1	0.3
13	Linalool	1077	1.0	0.3
14	(−)-*trans*-Pinocarveol	1129	0.4	0.1
15	*cis*-Verbenol	1131	0.1	0.1
16	(−)-4-Terpineol	1182	0.1	tr[a]
17	(+)-α-Terpineol	1193	0.4	0.2
18	*S*-*cis*-Sabinol	1202	0.6	0.2
19	Verbenone	1221	0.1	tr
20	*trans*-Carveol	1225	0.5	0.1
21	*cis*-Carveol	1230	0.1	tr
22	Cumaldehyde	1235	0.1	tr
23	Carvone	1237	0.3	tr
24	3,5-Dimethoxytoluene	1264	1.6	0.2
25	Bornyl acetate	1275	0.3	0.1
26	Cuminol	1276	0.1	tr
27	δ-Elemene	1339	0.2	0.1
28	Terpinyl acetate	1344	0.4	tr
29	α-Cubebene	1347	1.9	0.2
30	α-Copaene	1383	2.8	0.6
31	β-Bourbonene	1388	0.9	0.1
32	β-Elemene	1393	5.6	1.0
33	β-Caryophyllene	1426	**12.3**	**5.7**
34	β-Gurjunene (calarene)	1427	1.0	0.1
35	α-Guaiene	1441	0.2	tr
36	Aromadendrene	1444	0.3	tr
37	α-Humulene (α-caryophyllene)	1454	5.3	2.3
38	Allo-aromadendrene	1456	1.3	0.2
39	γ-Muurolene	1475	3.0	0.6
40	Germacrene D	1479	1.4	0.4
41	β-Eudesmene (β-selinene)	1483	3.3	0.7
42	Valencene	1485	0.8	0.4
43	α-Muurolene	1501	2.7	1.5
44	δ-Guaïene (α-bulnesene)	1503	0.3	0.1
45	γ-Cadinene	1509	2.9	1.3
46	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	1511	0.9	3.3
47	δ-Cadinene	1524	1.9	0.7
48	1,2,3,4,6,8a-Hexahydro-1-isopropyl-4,7-dimethyl-naphthalene	1528	0.6	0.2
49	[1s-(1a,4ab,8aa)]-1,2,4a,5,6,8a-Hexahydro-4,7-dimethyl-1-(1methylethyl)-naphthalene	1531	0.3	0.1
50	Caryophyllene oxide	1582	4.7	1.1

Table 2 (*Continued*)

Peak no.	Compound	Kovats index	SPME (relative peak area (%))	CH_2Cl_2 extraction (relative peak area (%))
51	Oxygenated sesquiterpene ($C_{15}H_{26}O$)	1604	0.8	0.6
52	1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1607	0.6	0.3
53	Cubenol	1609	0.2	0.1
54	τ-Cadinol	1634	1.6	0.8
55	δ-Cadinol	1636	0.3	0.1
56	β-Eudesmol	1649	0.1	0.1
57	α-Cadinol	1651	0.3	0.7
58	Oxygenated sesquiterpene ($C_{15}H_{24}O$)	1653	0.6	0.2
59	Oxygenated sesquiterpene ($C_{15}H_{24}O$)	1661	0.6	0.3
60	Dimer of α-phellandrene 1[b]	1795	0.8	0.1
61	Dimer of α-phellandrene 2[b]	1904	0.4	0.1
62	Unidentified diterpene 3[b]	1945	0.3	0.2
63	Cembrene A	1959	2.1	1.9
64	Cembrene C	2002	0.4	0.8
65	Unidentified diterpene 4[b]	2141	0.7	12.3
66	Isoincensole acetate	2152	**8.2**	**40.4**

[a] tr: compound at trace level (<0.1%).

[b] Spectral data of the unidentified diterpenes, *m/z* (relative intensity): dimer of α-phellandrene 1: 93 (100), 92 (58.4), 136 (26.9), 77 (21.6), 43 (12.5), 41 (11.8), 65 (2.9), 55 (2.3), 105 (2.0), no M^+; dimer of α-phellandrene 2: 93 (100), 92 (55.0), 41 (39.4), 136 (20.0), 77 (15.0), 69 (14.7), 43 (12.8), 105 (9.7), 55 (6.5), 229 (5.0), 272 (2.2, M^+), 257 (1.4); unidentified diterpene 3: 41 (100), 69 (91.3), 91 (25.1), 105 (15.7), 119 (15.1), 79 (14.7), 55 (13.6), 133 (7.8), 147 (7.5), 161 (6.9), 229 (6.8), 203 (5.2), 187 (4.2), 272 (3.5, M^+), 257 (1.7); unidentified diterpene 4: 59 (100), 41 (99.4), 43 (85.2), 67 (78.9), 81 (77.9), 93 (77.8), 55 (59.7), 107 (58.1), 121 (48.4), 135 (24.3), 136 (23.9), 147 (17.7), 161 (16.5), 189 (12.4), 272 (10.7), 202 (7.6), 175 (7.2), 229 (6.8), 257 (5.8), 290 (0.1, M^+).

can be simplified to:

$$F_{ij} = \frac{\sum_i H_{ij}}{1/5(\sum_{ij} H_{ij})}$$

We can first observe that F_{ij} values are very close for the five fiber coatings. Zuba found very different F_{ij} values (from 0.40 to 1.79) for the six fibers he tested. Nevertheless, we can note that the CW/DVB fiber gave the worst results for mono- and sesquiterpenes and the CAR/PDMS fiber the worst results for diterpenes. This can be explained by the nature of the two fiber coatings. The CW/DVB coating is polar and therefore is more suitable for polar compounds and less effective in recovering the non-polar ones [37,42]. In fact, if we compare, for example, the trapped amount of the two sesquiterpenes caryophyllene and its oxide derivative and the two diterpenes cembrene A and the more polar isoincensole acetate, we can notice that the CW/DVB fiber is more effective for caryophyllene oxide and isoincensole acetate, than for caryophyllene and cembrene A. CAR/PDMS is composed of porous carbon that is better for adsorption of small molecules. Another disadvantage of the CAR/PDMS fiber is that it has been shown to rearrange some monoterpenes to *p*-cymene during headspace SPME, giving erroneous results [43]. In order to have the maximum information on an archaeological sample, these two fibers must be eliminated. Among the three fibers remaining, PDMS/DVB gave the best result for diterpenes ($F_{ij} = 1.10$) and good results for mono and sesquiterpenes ($F_{ij} = 0.95$ and 1.03, respectively). It is not surprising because of the non-polar nature of the PDMS/DVB coating. Indeed PDMS favors the absorption of non-polar analytes and can have hydrophobic interactions with the lipophilic terpenes. DVB possesses insaturations due to the aromatic rings allowing π–π interactions with double bonds of terpenes. PDMS/DVB was also found to be the best fiber coating for headspace SPME sampling of volatile flavor components, mainly terpenes, of various juice samples [42]. Bicchi et al. have also observed that PDMS/DVB, CAR/PDMS and CAR/DVB/PDMS were the most effective fibers among eight commercially fibers, for the recovery of mono- and sesquiterpenes from various medicinal plants [20]. Therefore, PDMS/DVB will be henceforth chosen for later analysis of frankincense or archaeological samples.

3.3. Comparison between headspace SPME and solvent extraction

In order to ensure that headspace SPME allows extraction of almost all the volatile or semi-volatile compounds of frankincense, a classical extraction with CH_2Cl_2 was performed. In presence of dichloromethane, the hydrophilic polysaccharides present in the gum part of the olibanum precipitated. Then only the resinic part, i.e. terpenoids remained in solution. The two chromatograms can be seen in Fig. 4. The main components and their relative amounts are summarized in Table 2.

Although the two modes of extraction do not give exactly the same chromatogram, headspace SPME gives a good image of the terpenoid composition of olibanum.

As can be seen in Table 2, this frankincense sample is particularly rich in isoincensole acetate, which is the major component. It also contains large amounts of limonene and β-caryophyllene and in smaller proportions α-pinene and β-myrcene. His chemical composition is similar to those found for olibanum essential oil from *Boswellia thurifera* (*B. sacra*) [44].

4. Conclusion

Headspace SPME coupled with GC–MS is a rapid and simple method enabling extraction and identification of the mono-, sesqui-, and diterpenes of frankincense. Sampling time and sampling temperature, as well as the fiber coating were optimized in order to detect the low volatile diterpenes, which are biochemical markers of olibanum. The optimized extraction conditions were 60 min at 80 °C with the PDMS/DVB fiber. This method can be applied to the study of other resins or gum resins.

Because SPME concentrates compounds, we can apply it to archaeological samples susceptible of containing frankincense or other resin or gum resin. But they must be sufficiently big and hard to have confined volatile and semi-volatile terpenes in their heart. Because headspace SPME is a good screening method for the presence of resin in archaeological samples, it allows further sample treatment orientation in order to find other resin biomarkers as, for example, triterpenes.

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References

[1] P. Méjanelle, J. Bleton, S. Goursaud, A. Tchapla, J. Chromatogr. A 767 (1997) 177.
[2] A. Lucas, J.R. Harris, in: Ancient Egyptian Materials and Industries, Histories and Mysteries of Man LTD, London, 1962, p. 90.
[3] N. Baum, in: S.H. Aufrère (Ed.), Encyclopédie religieuse de l'Univers végétal. Croyances phytoreligieuses de l'Egypte ancienne, vol. 1, Orientalia Monspeliensa X, Montpellier, 1999, p. 421.
[4] A. Tchapla, J. Bleton, S. Goursaud, P. Méjanelle, in: S.H. Aufrère (Ed.), Encyclopédie religieuse de l'Univers végétal. Croyances phytoreligieuses de l'Egypte ancienne, vol. 1, Orientalia Monspeliensa X, Montpellier, 1999, p. 445.
[5] S. Hamm, J. Bleton, A. Tchapla, J. Sep. Sci., submitted for publication.
[6] A.O. Tucker, Econ. Bot. 40 (4) (1986) 425.
[7] G. Khan, M.I.H. Farooqi, Int. J. Pharmacognosy 29 (1991) 302.
[8] J. Rohloff, Phytochemistry 59 (2002) 655.
[9] W.M. Coleman, T.A. Perfetti, B.M. Lawrence, J. Chromatogr. Sci. 36 (1998) 575.
[10] M. An, T. Haig, P. Hatfield, J. Chromatogr. A 917 (2001) 245.
[11] M.C. Diaz-Maroto, M.S. Perez-Coello, M.D. Cabezudo, Chromatographia 55 (2002) 723.
[12] W.M. Coleman, B.M. Lawrence, Flavour Fragr. J. 12 (1997) 1.
[13] W.M. Coleman, B.M. Lawrence, J. Chromatogr. Sci. 38 (2000) 95.
[14] L.N. Fernando, I.U. Grün, Flavour Fragr. J. 16 (2001) 289.
[15] G. Flamini, P.L. Cioni, I. Morelli, Flavour Fragr. J. 17 (2002) 147.
[16] G. Knupp, P. Kusch, M. Neugebauer, J. Chem. Educ. 79 (2002) 98.
[17] Z. Liu, M.J. Wene, J. Chromatogr. Sci. 38 (2000) 377.
[18] C. Shang, Y. Hu, C. Deng, K. Hu, J. Chromatogr. A 942 (2002) 283.
[19] L. Jirovetz, G. Buchbauer, M.B. Ngassoum, M. Geissler, J. Chromatogr. A 976 (2002) 265.
[20] C. Bicchi, S. Drigo, P. Rubiolo, J. Chromatogr. A 892 (2000) 469.
[21] R.P. Adams, in: Identification of Essential Oils by Ion Trap Mass Spectroscopy, Academic Press, New York, 1989.
[22] G. Vernin, C. Boniface, J. Metzger, Y. Maire, A. Rakotorijaona, D. Fraisse, C. Parkanyi, Dev. Food Sci. 24 (1990) 511.
[23] S. Basar, A. Koch, W.A. König, Flavour Frag. J. 16 (2001) 315.
[24] A.O. Barakat, J. Rullkötter, Org. Mass Spectrom. 28 (1993) 157.

[25] P. Méjanelle, J. Bleton, A. Tchapla, S. Goursaud, in: Z. El Rassi (Ed.), Carbohydrate Analysis by Modern Chromatography and Electrophoresis, Journal of Chromatography Library, vol. 66, Elsevier, Amsterdam, 2002, p. 845.
[26] Z. Zhang, J. Pawliszyn, J. High Resolut. Chromatogr. 16 (1993) 689.
[27] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[28] J.A. Field, G. Nickerson, D.D. James, C. Heider, J. Agric. Food Chem. 44 (1996) 1768.
[29] T. Watanabe, A. Namera, M. Yashiki, Y. Iwasaki, T. Kojima, J. Chromatogr. B 709 (1998) 225.
[30] D.Y.-H. Yeung, T. Lee, G. Grant, M. Ma, E. Kwong, J. Pharm. Biomed. Anal. 30 (2003) 1469.
[31] B. Schäfer, P. Hennig, W. Engewald, J. High Resolut. Chromatogr. 18 (1995) 587.
[32] D. Zabaras, S.G. Wyllie, Flavour Fragr. J. 16 (2001) 411.
[33] A.J. Matich, D.D. Rowan, N.H. Banks, Anal. Chem. 68 (1996) 4114.
[34] D.A. Vereen, J.P. McCall, D.J. Butcher, Microchem. J. 65 (2000) 269.
[35] A. Keszler, K. Heberger, J. Chromatogr. A 845 (1999) 337.
[36] K.G. Furton, J. Bruna, J.R. Almirall, J. High Resolut. Chromatogr. 18 (1995) 625.
[37] W.M. Coleman, J. Chromatogr. Sci. 35 (1997) 245.
[38] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[39] J. Ai, Anal. Chem. 69 (1997) 3260.
[40] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[41] D. Zuba, A. Parczewski, M. Reichenbächer, J. Chromatogr. B 773 (2002) 75.
[42] M.E. Miller, J.D. Stuart, Anal. Chem. 71 (1999) 23.
[43] D. Zabaras, S.G. Wyllie, J. Sep. Sci. 25 (2002) 685.
[44] M.T. Baratta, H.J.D. Dorman, S.G. Deans, A.C. Figueiredo, J.G. Barroso, G. Ruberto, Flavour Fragr. J. 13 (1998) 235.