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Characterisation of beeswax in works of art by gas chromatography–mass spectrometry and pyrolysis–gas chromatography–mass spectrometry procedures

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Abstract

Pyrolysis (Py) with in situ derivatisation with hexamethyldisilazane–gas chroma-break tography–mass spectrometry (GC–MS) and a gas chromatography–mass spectrometry procedure based on microwave-assisted saponification were used to identify the organic components in small sized beeswax samples. With the latter procedure quantitative recoveries can be made and hydrocarbons, alcohols and ω-1-diols in the neutral fraction, and fatty acids and ω-1-hydroxy acids in the acidic fraction can be efficiently separated and detected. Both procedures were used to characterise a wax anatomic sculpture "The Plague" (1691–1694) by Gaetano Zumbo, resulting in the identification of beeswax and a Pinaceae resin. The GC–MS analysis brought to light some essential differences in beeswax composition between the raw material and the old modelled wax thus giving some clear indications about the recipe used by the sculptor.

Keywords: Art analysis; Waxes; Pyrolysis; Derivatization, GC; Hydrocarbons; Alcohols; Fatty acids; Hydroxy acids

1. Introduction

In both the fields of the cultural heritage and food, beeswax represents the most commonly used natural wax of animal origin. Since the prehistory, it has been used as lighting or as waterproofing and sealing agent in pottery [1–6]. The Egyptians used it in balms for mummies [7] and in shipbuilding [8]. It was used by the Greeks and Romans to waterproof stone surfaces, and both as a protective and coloured varnish to soften the whiteness of marble [9]. Until the Middle Ages, beeswax was used as a binder in a painting technique named encaustic [9–11]. Beeswax has been commonly used for the realisation of wax sculptures over the centuries [12], and between the 17th and 20th centuries the ceroplastic technique was developed for the realisation of anatomic sculptures and botanical models [13].

The characterisation of this material and of the degradation compounds induced by ageing and pollution is of paramount importance for a better understanding of artistic techniques, for gaining insights into the habits of ancient cultures, and for assessing the state of conservation. Such a diagnosis, together with historical information, means that suitable restoration work can be planned and the right environmental conditions for conservation can be established.

Beeswax is a natural product with various origins (Asiatic bees *Apis dorsata*, *Apis florea* e *Apis indica*, African bees *Apis mellifera adansonii*, and European bees *Apis mellifera*) whose qualitative average composition is quite constant and is made up of hydrocarbons (14%), monoesters (35%), diesters (14%), triesters (3%), hydroxymonoesters (4%), hydroxypolyesters (8%), monoacid esters (1%), acid polyesters (2%), free acids (12%), free alcohols (1%), and non identified compounds (6%) [14,15]. Due to the low volatility of these compounds, the characterisation of beeswax is generally based on a preliminary step of hydrolysis to breakdown esters, followed by derivatisation for acidic and alcoholic moieties and gas chromatography analysis. Analytical methods based on gas chromatography–mass spectrometry (GC–MS) [1,6,7,16,17] or high-temperature GC–MS operating in the electron impact ionisation (EI) mode [18–20] and chemical ionisation mode have been successfully used to determine beeswax in works of art and in archaeological finds.

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One of the main problems, as testified in the literature, is the inefficient preliminary step of saponification to which beeswax is very resistant. This may lead to quite lengthy processes, and poor recoveries from the sample. Moreover, low volatile mono, hydroxyesters and polyesters tend to clog up the gas chromatographic system. For instance, the ASTM method for the determination of the saponification number of natural and synthetic waxes entails refluxing the sample with an excess of potassium hydroxide in ethanol for over 2 h [21]. Another procedure is based on a saponification in batches with 10% KOH in methanol:water (1:1) for 3 h at 60 °C [22]. Other techniques require the dissolution of the sample in a chloroform–methanol solution, followed by the addition of 10% NaOH in methanol at 70 °C for 30 min [18] or by BF_3 in methanol at 20% at 90 °C for 1 h [23]. Significantly, in both the refluxing and batch techniques the yields of the procedures are rarely reported. The use of microwave has proved to give faster and more efficient heating than conventional procedures, and has been used to determine the saponification number in synthetic waxes [24].

A successful approach to solve the problem of beeswax characterisation avoiding the sample pre-treatment step is pyrolysis (Py), followed by gas chromatography coupled with mass spectrometry (Py–GC–MS). This technique gives satisfactory results for the characterisation of raw material [25], and also of samples from works of art [26,27]. However, the identification of degradation compounds due to oxidation processes, hydrolysis or microbial alteration is very difficult and has to be carried out with great caution. Better results in the analysis of raw material have been obtained using pyrolysis with thermally-assisted hydrolysis and methylation [28–30]. Although the tetramethylammonium hydroxide thermochemolysis method has been extensively applied to polymeric organic materials [31], the interpretation of product mixtures must be made with care, above all due to decarboxylation reactions undergone by carboxylic acids [32], isomerization of polyunsaturated fatty acids [33], formation of dehydration products [34] and α-methylation of acidic functionalities [35].

Recently, pyrolysis with in situ hexamethyldisilazane silylation has been employed for the characterisation of several organic materials of interest in the field of art [36–41] showing good properties for the identification of old materials. Anyway no application for beeswax has been reported.

This paper presents and compares two analytical procedures developed for the characterisation of beeswax in micro-samples (<1 mg) from works of art. One is based on saponification assisted by microwave and followed by multiple solvent extractions, silylation and GC–MS analysis. The other is based on the Py–GC–MS investigation of the products of the thermally-assisted reaction in the presence of hexamethyldisilazane. In order to evaluate the performance of these procedures and to obtain information on the unknown recipe used and the state of conservation, the two methods were employed in the characterisation of a sample taken from the wax anatomic sculpture "The Plague" (1691–1694) by Gaetano Zumbo which is conserved at the museum "La Specola" in Florence (Italy). This paper reports the most significant results, which have brought to light the previously unknown technique that Gaetano Zumbo used in order to create a material that was easy to handle, resistant in time and surprisingly realistic.

2. Experimental

2.1. Reagents

All solvents were HPLC grade (Baker, Deventer, The Netherlands). Hexamethyldisilazane (HMDS) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Sigma, Milan, Italy) were used without any further purification. Tridecanoic acid and hexadecane, used as an internal standard, and palmitic acid, were supplied by Sigma with a >99% purity.

2.2. Samples

Pure beeswax from *A. mellifera* was purchased from a local apiarist. Beeswax solution was prepared dissolving the wax in chloroform (3.2 μg/g) which was stored at 4 °C.

A sample (sample name: Z; mass: 6.3 mg) of the inner part of a woman's leg from "The Plague" by Gaetano Zumbo was collected and divided into two aliquots. The wax representations ("The Plague", "The Triumph of Time", "The Corruption of Bodies") known as "Waxes of Plague" were made in Florence (1691–1694) for Cosimo III de' Medici. They are surprisingly lifelike, and are of great artistic and anatomical value.

2.3. Analytical procedure based on Py with in situ sylilation GC–MS

A few μg of samples together with 5 μl of hexamethyldisilazane were inserted into quartz tubes (4 cm × 0.53 mm) and placed into a continuous mode microfurnace pyrolyzing injection system Pirojector (SGE, Austin, TX, USA) operating at 600 °C. The pressure in the furnace was kept at 14 p.s.i. and the purge flow was 0.5 ml/min (1 p.s.i. = 6894.76 Pa). The pyrolysis chamber was connected through a programmed-temperature vaporiser (PTV) injector to a 6890N GC System gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled with a 5973 Network Mass Selective Detector (Agilent Technologies) single quadrupole mass spectrometer. The MS transfer line temperature was 280 °C; the MS ion source temperature was kept at 230 °C and the MS quadrupole temperature at 150 °C. The mass spectrometer was operating in the EI positive mode (70 eV) and the mass range was from 50 to 750 *m*/*z*. For the gas chromatographic separation an HP-5MS fused silica capillary column (5% diphenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm i.d.,

0.25 μm film thickness, J&W Scientific/Agilent Technologies, Palo Alto, CA, USA) with a deactivated silica pre-column (2 m × 0.32 mm i.d., J&W Scientific/Agilent Technologies) was used. The PTV injector was used in split mode at 300 °C, the split ratio being dependent on the sample size. The chromatographic conditions were: 31 °C isothermal for 8 min, 10 °C/min up to 240 °C and isothermal for 3 min, 20 °C/min up to 300 °C and isothermal for 30 min. The carrier gas was used in constant flow mode (He, purity 99.995%) at 1.0 ml/min.

2.4. Analytical procedure based on saponification assisted by microwaves and GC–MS analysis

Samples (50–1000 μg) were hydrolysed with 10% KOH in ethanol in a microwave oven model MLS-1200 MEGA Milestone [FKV, Sorisole (BG,) Italy], using the following conditions: 60 min at 80 °C, 200 W and 15 min of N_2 venting. The selected power allowed the chosen temperature to be reached in a negligible time with respect to the duration of the saponification. The hydrolysate was extracted twice with a mixture of *n*-octane and *n*-hexane, 1:2 (neutral fraction). It was then acidified with hydrochloric acid (6 M) and extracted twice with diethyl ether (acidic fraction). The ether extract was dried and re-dissolved in a mixture of acetone and *n*-octane, 2:1. An aliquot of each fraction, admixed with a standard solution of tridecanoic acid (first internal standard), was dried under a gentle flow of nitrogen and added with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, isooctane and a standard solution of hexadecane (second internal standard). The reaction was performed in closed conic vials at 60 °C for 30 min.

A 2 μl volume of the derivatized solutions was analysed with a Trace GC gas chromatograph Thermoquest (Thermo Finnigan, San Jose, CA, USA) equipped with a PTV injector, and coupled with a Polaris Q mass selective detector (Thermo Finnigan) ion trap mass spectrometer. The MS transfer line temperature was kept at 280 °C and the MS ion source temperature at 250 °C. The mass spectrometer was operating in the EI positive mode (70 eV) and mass spectra were acquired in the scan range 50–1000 *m*/*z*. For the gas chromatographic separation an HP-5MS fused silica capillary column (5% diphenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific/Agilent Technologies) was used. The injector was in the "CT splitless with surge" mode at 280 °C with a surge pressure of 100 kPa. The chromatographic oven was programmed as follows: 80 °C for 2 min, 10 °C/min to 200 °C, 30 °C/min to 330 °C, 330 °C for 60 min. The carrier gas was used in the constant flow mode (He, purity 99.995%) at 1.2 ml/min.

The SIM mode was used for the quantitative determinations of hexadecane, tridecanoic acid and hexadecanoic acid trimethylsilyl (TMS) esters, acquiring the following ion fragments (*m*/*z*): 43 and 57 for hexadecane, 117 and 271 for tridecanoic acid, 117 and 313 for hexadecanoic acid.

Linear calibration curves for hexadecanoic acid were obtained in the range of 6 and 12 ng/mg with $R^2 = 0.9999$. Analytical quality control was checked by running daily the standard solution between samples, and the data were reported in Shewerd control charts. Compounds identification was achieved by a library search (Wiley 275), by a comparison with mass spectra of standard compounds (lauric, myristic, palmitic, oleic, stearic and tetracosanoic acids; suberic, azelaic and sebacic acids) or by the study of the mass spectra for the identification of the hydroxy acids and diols. Very often chemical ionisation with methane was performed in order to support the peak assignment.

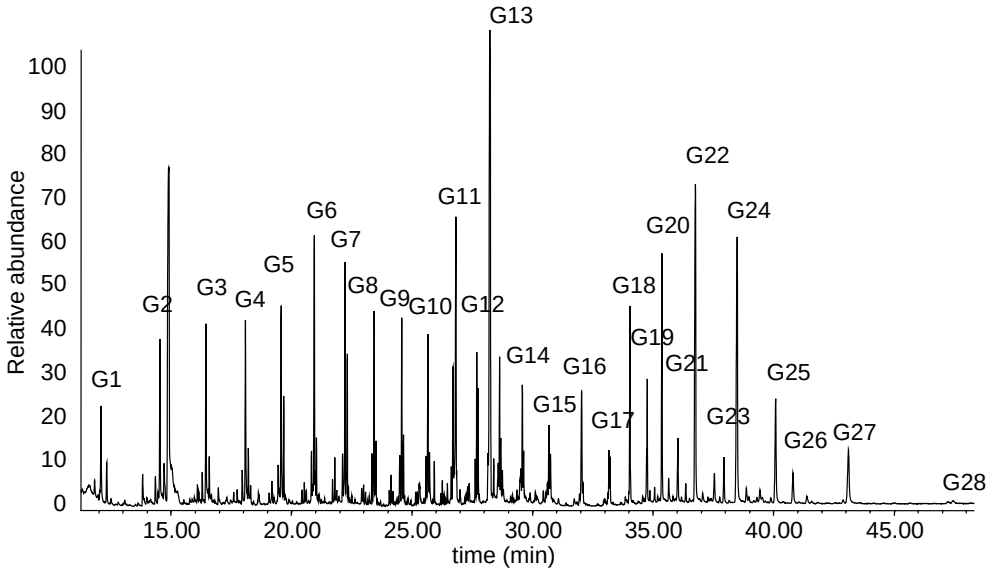

Fig. 1. Total ion chromatogram of the thermally-assisted reaction of a beeswax sample in the presence of hexamethyldisilazane. The identified compounds, grouped in clusters (Gi) are reported in Table 1.

3. Results and discussion

3.1. Characterization of beeswax by Py with in situ silylation GC–MS

Fig. 1 shows the total ion chromatogram of a beeswax sample pyrolysed in the presence of hexamethyldisilazane, showing a quite complex pattern and a very characteristic fingerprint which may help in the identification of this material in old samples. The procedure allows most of monoesters, diesters, hydroxymonoesters and hydroxypolyesters to be broken down. Since most of them are palmitic acid esters, the palmitic acid TMS derivative in the chromatogram has the major abundance. This procedure avoids problems connected with the low volatility of polar compounds produced during pyrolysis. In fact, the transfer from the pyrolysis system to the gas chromatograph injection port is facilitated by the formation of the volatile TMS derivatives. Table 1 reports the identified compounds, divided into groups, with their relative retention times and highlights the presence of linear chain alkanes, alkenes,

Table 1
Identified compounds in the total ion chromatogram of the thermally-assisted reaction of a beeswax sample in the presence of hexamethyldisilazane

Group	Retention time (min)	Compound
G1	11.82	1,8-Nonanediene
	13.08	1-Nonene
	13.33	Nonane
G2	14.34	Decadiene
	14.53	1-Decene
	14.78	Decane
G3	16.14	Hexanoic acid (TMS derivative)
	16.28	Undecadiene
	16.44	1-Undecene
	16.57	Undecane
	16.95	3,5,5-Trimethyl-3-cyclohexen-1-one
G4	17.59	Heptenoic acid (TMS derivative)
	17.73	Heptanoic acid (TMS derivative)
	17.95	Dodecadiene
	18.03	Dodecene
	18.21	Dodecane
	18.29	Decanal
	18.62	3,5-Dimethylphenol (TMS derivative)
G5	19.06	Octenoic acid (TMS derivative)
	19.27	Nonenol (TMS derivative)
	19.37	Nonanol (TMS derivative)
	19.87	Octanoic acid (TMS derivative)
	19.44	Tridecadiene
	19.56	Tridecene
	19.67	Tridecane
G6	20.42	Nonenoic acid (TMS derivative)
	20.52	Nonanoic acid (TMS derivative)
	20.60	Decenol (TMS derivative)
	20.71	Decanol (TMS derivative)
	20.82	Tetradecadiene
	20.87	Dodecenone
	20.93	Tetradecene
	20.98	Dodecanone
	21.03	Tetradecane
G7	21.69	Decenoic acid (TMS derivative)
	21.79	Decanoic acid (TMS derivative)
	21.86	Undecenol (TMS derivative)
	21.96	Undecanol (TMS derivative)
	22.11	Pentadecadiene
	22.17	Tridecenone
	22.21	Pentadecene
	22.72	Tridecanone
	22.31	Pentadecane
G8	22.9	Undecenoic acid (TMS derivative)
	22.98	Undecanoic acid (TMS derivative)
	23.07	Dodecenol (TMS derivative)
	23.14	Dodecanol (TMS derivative)
	23.32	Hexadecadiene
	23.42	Hexadecene
	23.49	Hexadecane
G9	24.05	Dodecenoic acid (TMS derivative)
	24.12	Dodecanoic acid (TMS derivative) (lauric acid)
	24.2	Tridecenol (TMS derivative)
	24.28	Tridecanol (TMS derivative)
	24.48	Heptadecadiene
	24.56	Heptadecene
	24.64	Heptadecane
G10	25.14	Tridecenoic acid (TMS derivative)
	25.22	Tridecanoic acid (TMS derivative)
	25.28	Tetradecenol (TMS derivative)
	25.35	Tetradecanol (TMS derivative)
	25.57	Octadecadiene
	25.65	Octadecene
	25.72	Octadecane
	25.9	Hexadecanal
G11	26.19	Tetradecenoic acid (TMS derivative)
	26.25	Tetradecanoic acid (TMS derivative)
	26.22	Nonadecadiene
	26.31	Pentedecenol (TMS derivative)
	26.38	Pentadecanol (TMS derivative)
	26.69	Nonadecene
	26.74	Nonadecane
	26.81	Heptadecanone
G12	27.17	Pentadecenoic acid (TMS derivative)
	27.24	Pentadecanoic acid (TMS derivative)
	27.30	Hexadecenol (TMS derivative)
	27.36	Hexadecanol (TMS derivative)
	27.61	Eicosadiene
	27.68	Eicosene
	27.73	Eicosane
G13	28.14	Hexadecenoic acid (TMS derivative)
	28.22	Hexadecanoic acid (TMS derivative) (palmitic acid)
	28.26	Heptadecenol (TMS derivative)
	28.31	Heptadecanol (TMS derivative)
	28.56	Heneicosadiene
	28.62	Heneicosene
	28.67	Heneicosane
	28.74	Nonadecanone

Table 1 (*Continued*)

Group	Retention time (min)	Compound
G14	29.08	Heptadecanoic acid (TMS derivative)
	29.16	Heptadecanoic acid (TMS derivative)
	29.15	Octadecenol (TMS derivative)
	29.21	Octadecanol (TMS derivative)
	29.51	Docosadiene
	29.57	Docosene
	29.62	Docosane
G15	30.04	Octadecenoic acid (TMS derivative)
	30.10	Octadecanoic acid (TMS derivative) (stearic acid)
	30.17	Nonadecenol (TMS derivative)
	30.23	Nonadecanol (TMS derivative)
	30.60	Tricosadiene
	30.67	Tricosene
	30.64	Tricosane
G16	31.06	15-Hydroxyhexadecanoic acid (TMS derivative)
	31.38	Eicosenol (TMS derivative)
	31.45	Eicosanol (TMS derivative)
	31.94	Tetracosadiene
	32.06	Tetracosene
	32.09	Tetracosane
G17	32.68	Heneicosenol (TMS derivative)
	32.74	Heneicosanol (TMS derivative)
	33.12	Pentacosadiene
	33.16	Pentacosene
	33.21	Pentacosane
G18	33.64	Docosenol (TMS derivative)
	33.68	Docosanol (TMS derivative)
	34.04	Esacosene
G19	34.36	Triacosenol (TMS derivative)
	34.43	Triacosanol (TMS derivative)
	34.72	Heptacosene
	34.75	Heptacosane
	34.85	Pentacosanone
	35.05	Tetracosanol (TMS derivative)
G20	35.35	Octacosene
	35.62	Tetracosanoic acid (TMS derivative)
G21	36.02	Nonacosane
	36.17	Eptacosanone
	36.35	Hexacosanol (TMS derivative)
G22	36.74	Triacontene
	37.04	Hexacosanoic acid (TMS derivative)
G23	37.40	Untriacontene
G24	37.53	Untriacontane
	37.74	Nonacosanone
	37.93	Octacosanol (TMS derivative)
	38.43	Dotriacontene
	38.86	Octacosanoic acid (TMS derivative)
G25	38.89	Untriacontanone
	40.08	Tricosanol (TMS derivative)
G26	40.78	Tetratriacontene
	41.37	Tricosanoic acid (TMS derivative)
	42.86	Tritriacontanone
G27	43.10	Dotriacosanol (TMS derivative)
	47.21	Pentatriacontanone
G28	47.65	Tetracosanol (TMS derivative)

Table 2
Identified compounds in chromatograms reported in Figs. 2–4

Number	Compound
1	Triacosane
2	Tetracosane
3	Pentacosane
4	Heptacosane
5	Tetracosanol (TMS derivative)
6	Nonacosane
7	Hexacosanol (TMS derivative)
8	1,23-Tetracosanediol (TMS derivative)
9	Untriacontane
10	Untriacontene
11	Octacosanol (TMS derivative)
12	1,25-Hexacosandiol (TMS derivative)
13	Tritriacontane
14	Tritriacontene
15	Triacontanol (TMS derivative)
16	1,27-Octacosandiol (TMS derivative)
17	Dotriacontanol (TMS derivative)
18	Tetratriacontanol (TMS derivative)
19	Hexadecanoic acid (TMS derivative)
20	9(*E*)-Octadecenoic acid (TMS derivative)
21	Octadecanoic acid (TMS derivative)
22	14-Hydroxyhexadecanoic acid (TMS derivative)
23	15-Hydroxyhexadecanoic acid (TMS derivative)
24	Eicosanoic acid (TMS derivative)
25	16-Hydroxyoctadecanoic acid (TMS derivative)
26	17-Hydroxyoctadecanoic acid (TMS derivative)
27	Docosanoic acid (TMS derivative)
28	19-Hydroxyeicosanoic acid (TMS derivative)
29	Tetracosanoicacid (TMS derivative)
30	21-Hydroxydocosanoic acid (TMS derivative)
31	Hexacosanoicacid (TMS derivative)
32	23-Hydroxytetracosanoic acid (TMS derivative)
33	Octacosanoicacid (TMS derivative)
34	25-Hydroxyhexacosanoic acid (TMS derivative)
35	Triacontanoicacid (TMS derivative)
36	Dotriacontanoicacid (TMS derivative)
37	Tetratriacontanoicacid (TMS derivative)
38	Tetracosyl hexadecanoate
39	Hexacosyl hexadecanoate
40	Tetracosyl-15-hydroxyhexadecanoate (TMS derivative)
41	Octacosyl hexadecanoate
42	Hexacosyl-15-hydroxyhexadecanoate (TMS derivative)
43	Triacontyl hexadecanoate
44	Octacosyl-15-hydroxyhexadecanoate (TMS derivative)
45	Dotriacontyl hexadecanoate
46	Triacontyl-15-hydroxyhexadecanoate (TMS derivative)
47	Tetratriacontyl hexadecanoate
48	Dotriacontyl-15-hydroxyhexadecanoate (TMS derivative)

Table 3
Recoveries of palmitic acid as a function of the saponification time obtained on five replicates of beeswax solution (50 μg)

Saponification time (min)	Recoveries of palmitic acid (%, w/w)	Relative standard deviation (R.S.D., %)
20	14.5	4.9
40	14.4	7.2
60	20.1	6.0
120	15.0	11.5

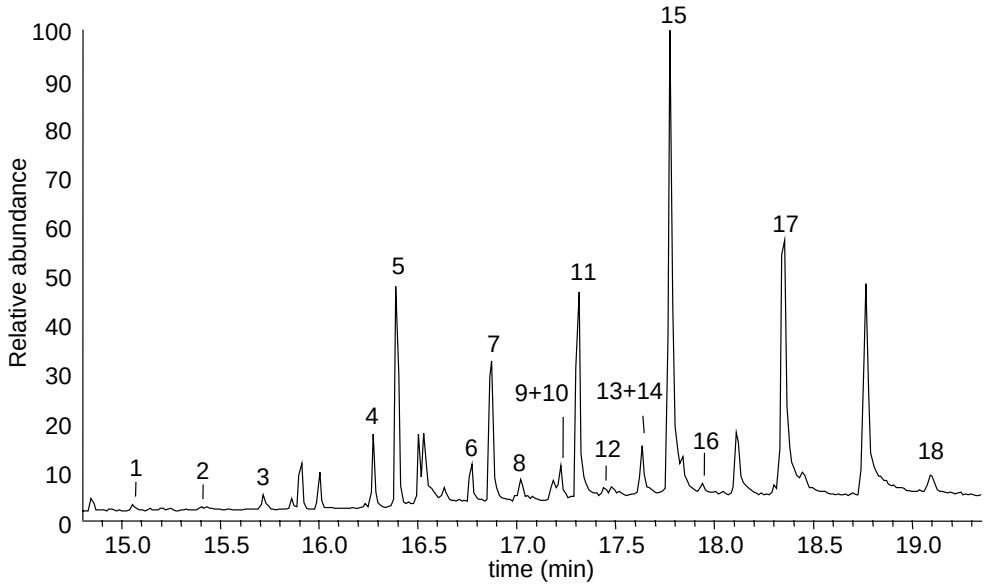

Fig. 2. Total ion chromatogram of the neutral fraction of a beeswax sample, obtained by the GC–MS procedure. The identified compounds are reported in Table 2.

dienes, ketones, aldehydes, acids and alcohols, from low to high carbon atom numbers, some of them being those that naturally occur in wax and others produced by pyrolysis. Their formation may be ascribed to a radical fragmentation of hydrocarbons, mono- and polyesters, free alcohols, and acids.

3.2. Characterization of beeswax by microwave-assisted saponification and GC–MS analysis

The analytical procedure applied to a beeswax sample enabled the detection and efficient separation of hydrocarbons, alcohols and ω-1-diols in the neutral fraction (Fig. 2) and of fatty acids and ω-1-hydroxy acids in the acidic fraction (Fig. 3), respectively. The chromatographic pattern of that makes up the compounds is similar to those reported in the literature [23] and the identified compounds are reported in Table 2. All the compounds are detectable up to a beeswax sample size of few micrograms: thus, its identification in the heterogeneous micro-samples from works of art may be accurately performed.

3.2.1. Saponification assisted by microwave

Several hydrolysis agents (HCl_{ETOH} 5%, KOH_{MEOH}10%: KOH_{aq}10% (2:1), KOH_{ETOH} 2.5%, KOH_{ETOH} 10%) were tested and KOH 10% in ethanol was chosen. Since palmitic acid is the most abundant compound in the hydrolysed wax, representing about 15% (w/w) [42], the estimation of the saponification yield was related to its recovery. This meant that the beeswax yield was considered quantitative when the palmitic acid reached its highest value and the presence of esters was not detected. Table 3 reports the recoveries of palmitic acid as a function of the saponification time obtained on five replicates of beeswax solution (50 μg): the best yield was achieved using 80 °C and 60 min of reaction time. A shorter reaction time implies low recoveries, whereas a longer reaction time or an increase in temperature leads to the formation of a brown precipitate thus highlighting a partial sample degradation. A comparison with the chromatogram (Fig. 4 and the peak assignment reported in Table 2) of the neutral fraction of a non saponified sample highlights the absence of cerides between 20 and 50 min. The non-detectability of beeswax esters, as mentioned before, underlines the efficacy of the proposed analytical procedure.

The palmitic acid yield was better and was achieved in less time if compared with that (3.2 ± 13.8% on five replicates) obtained by applying a common literature method [22] which employs 10% KOH in methanol:water (1:1) for 3 h at 60 °C in a water bath. The final recovery of palmitic acid 20.1% with a relative standard deviation of 6% may be assumed as the limiting value for which the hydrolysis of a beeswax sample may be considered complete in the absence of degradation products.

3.3. Characterization of organic matter in old samples

Fig. 5 shows the chromatogram obtained by the Py–GC–MS analysis of an aliquot of sample Z, compared to the pyrogram obtained from a beeswax sample. Most of the chromatographic peaks correspond to those previously identified in pure beeswax: they show different relative abundances. Furthermore, dehydroabietic acid was detected, thus indicating that a Pinaceae resin is mixed with beeswax.

In the neutral and acidic fractions of sample Z according to the GC–MS procedure all the compounds of a beeswax and

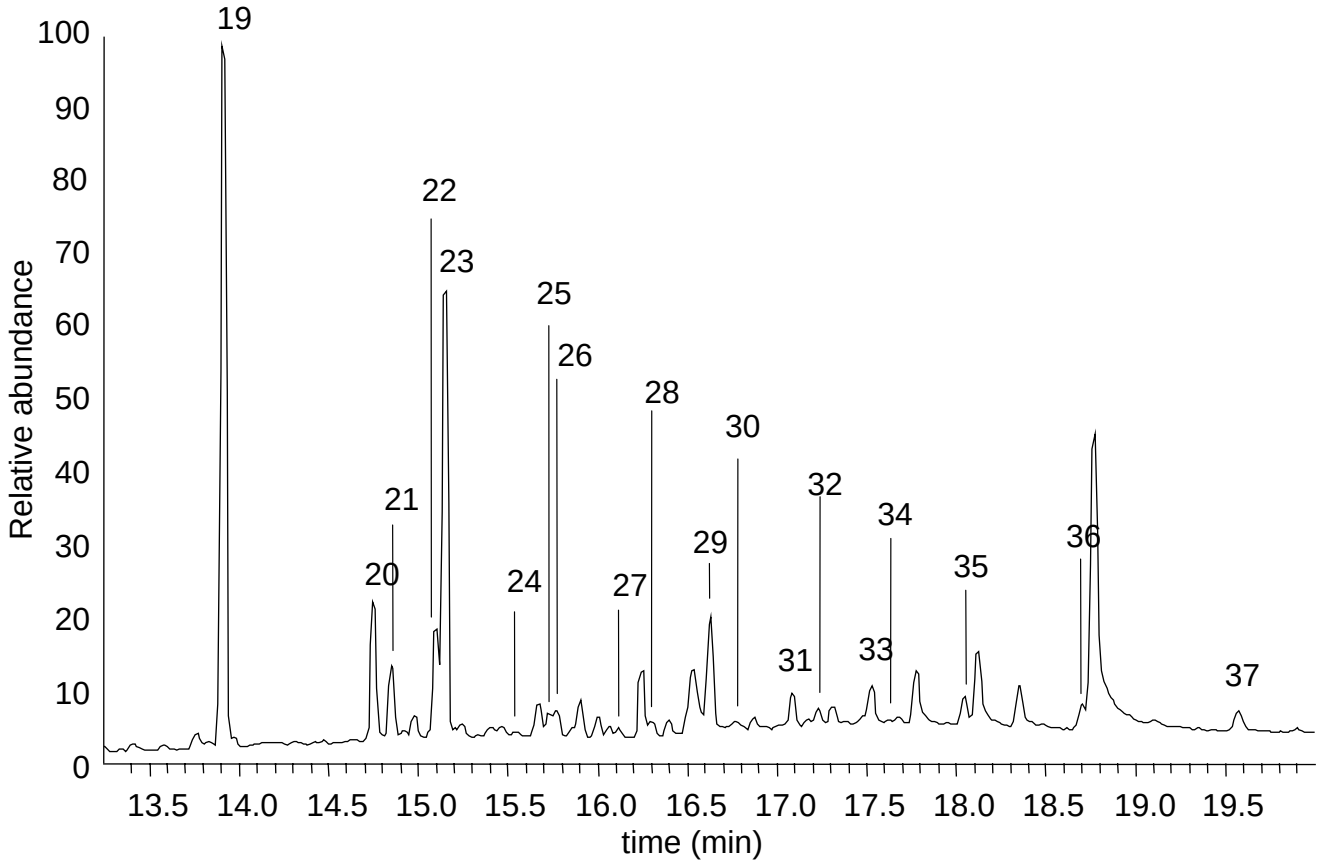

Fig. 3. Total ion chromatogram of the acidic fraction of a beeswax sample, obtained by the GC–MS procedure. The identified compounds are reported in Table 2.

the dehydroabietic and 7-oxodehydroabietic acids, markers of a Pinaceae resins [43], are present (see Table 4). In particular, the main constituent of the sample is beeswax whereas the Pinaceae resin only represents a small amount. These results also highlight that the fatty acid profile is quite different from that expected for beeswax, as shown in Fig. 6. Furthermore, the presence of high quantities of the *trans* isomer of 9-octadecenoic acid in addition to the *cis* isomer

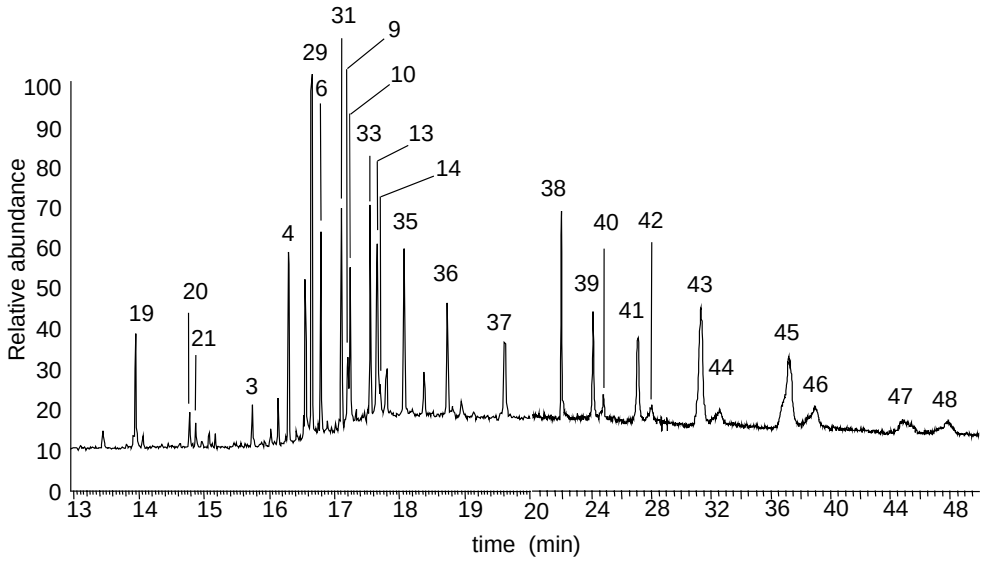

Fig. 4. Total ion chromatogram of the neutral fraction of a non saponified beeswax sample. The identified compounds are reported in Table 2.

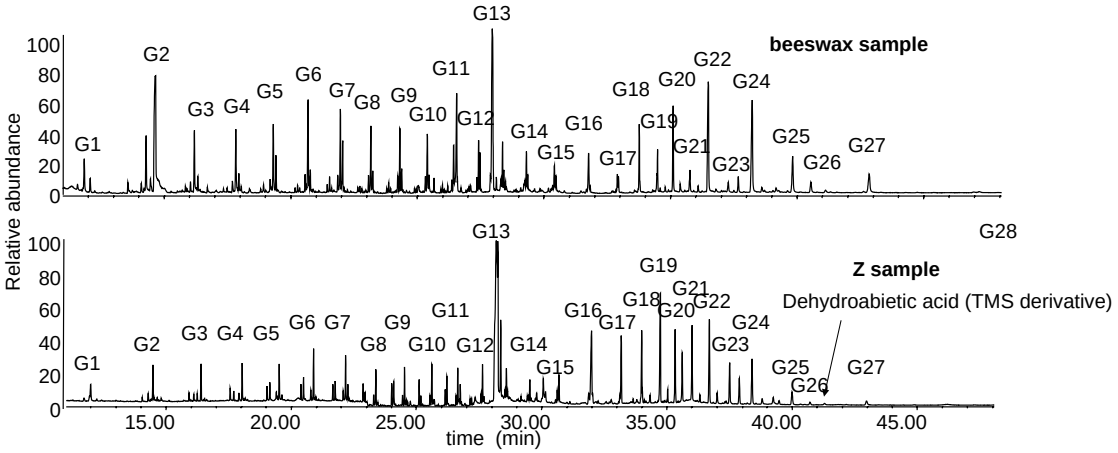

Fig. 5. Total ion chromatogram of the thermally-assisted reaction of Z sample from "The Plague" and of a beeswax sample in the presence of hexamethyldisilazane.

Table 4
Identified compounds in the neutral and acidic fraction obtained by the GC–MS procedure of Z sample from "The plague"

Identified compounds	Chromatogram	Related material
Monocarboxylic acids (ranging from C_{12} to C_{34})[a]	Acidic fraction	Beeswax
ω-1-Hydroxy acids (ranging from C_{12} to C_{32})	Acidic fraction	Beeswax
Linear alkanes (ranging from C_{19} to C_{33})	Neutral fraction	Beeswax
Linear alcohols (ranging from C_{24} to C_{36})	Neutral fraction	Beeswax
α-(ω-1)-Diols (ranging from C_{24} to C_{32})	Neutral fraction	Beeswax
Dehydroabietic acid	Acidic fraction	Pinaceae resin
7-Oxodehydroabietic acid	Acidic fraction	Pinaceae resin

[a] *cis* and *trans* isomers of 9-octadecenoic acid are present.

naturally occurring in beeswax [44], indicates that the wax was thermally treated [45]. Moreover, in the neutral fraction, the pattern of the alcohols exhibits an unexpected behaviour (Fig. 7). In particular, tetracosanol rather than triacontanol is the main component and the relative amounts of alcohols with a higher carbon number are very small. All this would seem to suggest the employment of a treated wax. In particular, the absence of alcohols with a high number of carbon atoms suggests that the heavier esters, i.e. the polyesters and the ones formed with the heavier alcohols, have somehow

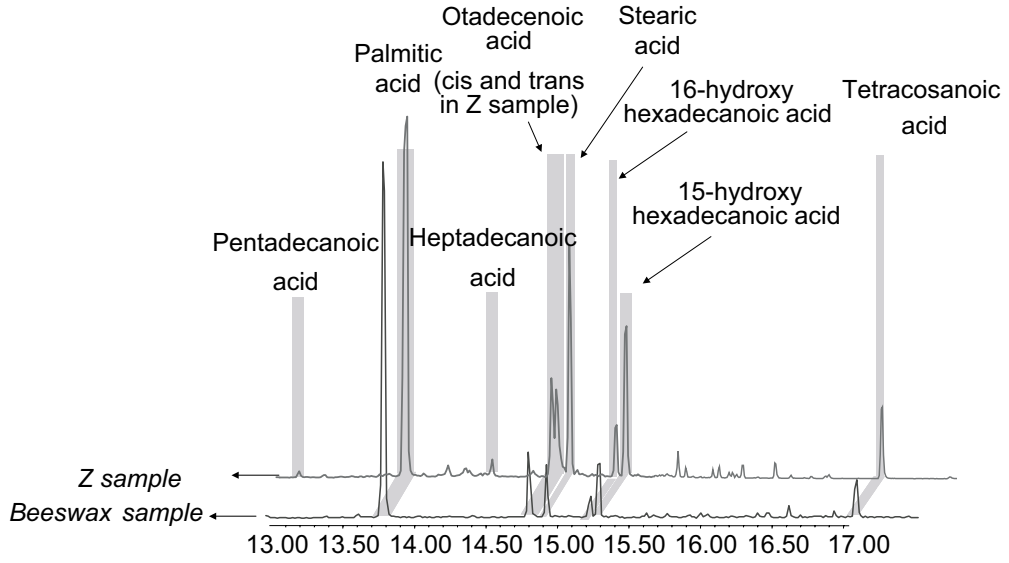

Fig. 6. Total ion chromatogram of the acidic fraction of the Z sample from "The Plague" between 13 and 17 min.

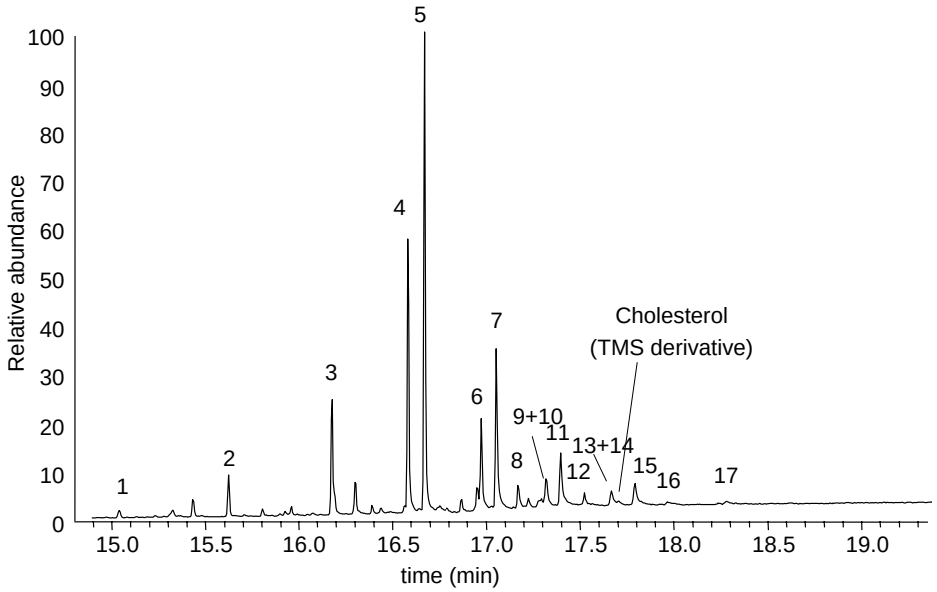

Fig. 7. Total ion chromatogram of the neutral fraction of the sample from "The Plague" obtained by the GC–MS procedure. The identified compounds are reported in Table 2.

been removed. A possible explanation is that the sculptor melted the wax, and after a partial cooling, he collected the fluid discharging the supernatant layer containing solidified compounds. This aspect seems in contrast with the increased quantities of oleic and stearic acids observed. Indeed, the above physical treatment produces a wax softer and probably more transparent than the original one. Thus, in order to increase its hardness, the wax modeller added a Pinaceae resin and a fatty material, probably of animal origin. The hypothesis on the presence of a fatty material which justifies the presence of high amounts of oleic and stearic acids, is confirmed by the identification of cholesterol in the neutral fraction and of odd numbered fatty acids (pentadecanoic acid and heptadecanoic acid) in the acidic one [46].

4. Conclusions

The Py–GC–MS procedure is a very fast technique that can be very successfully used to characterise the organic material of a sample. It has the advantage that no sample pre-treatment is necessary thus reducing possible losses and contaminations. The use of hexamethyldisilazane as an on-line derivatizing agent avoids the problems due to the formation of non-volatile products during pyrolysis, allowing their displacement transfer to the gas chromatographic system. Unfortunately, the non-trivial relationship between products of pyrolysis and the original material, makes the results obtained rather difficult to interpret.

The microwave-assisted saponification procedure followed by GC–MS analysis is certainly more laborious than the pyrolysis method, but gives detailed results of simpler interpretation. Furthermore, the quite good yield of the saponification reaction means that micro-samples can be analysed.

The procedures applied to a sample taken from "The Plague" by Gaetano Zumbo confirm the above remarks: the pyrolysis procedure allowed the identification of beeswax and a Pinaceae resin and the GC–MS analysis led to a clearer understanding of the treatments used by the artist in the realisation of the wax sculpture. The GC–MS results highlight that beeswax employed in the creation of "The Plague" was added to a Pinaceae resin, probably Venice Turpentine, and to a fatty material of animal origin. A harder material, with better protective properties against dirty and powder deposition, and brighter than the original one, with characteristics extremely similar to the colour of human skin [13] was thus obtained.

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