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Characterisation of wax works of art by gas chromatographic procedures

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Abstract

To identify the various natural and synthetic substances used by sculptors at the end of the 19th century, several contemporary reference samples were investigated by high temperature gas chromatography (HT GC) and HT GC–MS. Using specific chromatographic conditions and minimising sample preparation, we could separate, detect and identify a wide range of biomolecular markers covering a great variety of molecular weights and volatilities, with a minimum amount of sample, in a single run. Beeswax, spermaceti, carnauba, candellila and Japan waxes as well as pine resin derivatives, animal fats, paraffin, ozokerite and stearin, used as additives in wax works of art, were chemically investigated. In the case of low volatile compounds, transbutylation was performed. The structure of long-chain esters of spermaceti was elucidated for the first time by HT GC–MS analysis. Such a method was then carried out on 10 samples collected on a statuette of Junon by Antoine-Louis Barye (Louvre Museum, Paris, France) and on a sculpture by Aimé-Jules Dalou (*Musée de la Révolution Française*, Vizille, France). The analytical results obtained provide new data on the complex recipes elaborated by sculptors at the end of the 19th century.

Keywords: Beeswax; Japan wax; Spermaceti wax; Carnauba wax; Candellila wax; Pine resin; Paraffin; Ozokerite; Stearin; High temperature gas chromatography–mass spectrometry; Wax sculptures; Cultural heritage

1. Introduction

Waxy substances have been transformed and used by our ancestors as early as the neolithic period, for a large range of activities, as waterproofing substances, illuminant, sealing agent but also for adhesive making and many other technical, medicinal or symbolic purposes [1–16].

Although beeswax was the earliest waxy material exploited by men, many other natural substances have been used thereafter. Chinese insect wax, shellac wax, spermaceti and wool wax, from animal origin; carnauba, candelilla or Japan waxes, secreted by various plants, and fossil materials such as ozokerite or paraffin have also been employed in sculpture manufacture or for the restoration of works of art [10,17,18].

Additives are often mixed with these waxy materials in order to improve their properties. Resins are used to harden and colour the material. Fatty materials increase malleability and softness of waxes. Pigments and dyes colour the material and starch is used as an extender [10,16]. Synthetic materials such as paraffin or ozokerite were used to minimise the amount beeswax, an expensive resource. Stearin, a mixture of palmitic and stearic acids, commercially synthetised by alkaline hydrolysis of animal fats, was also largely employed since 1831, date of its first synthesis by the French chemist Chevreul [17].

Wax works of art may thus contain several substances in various amounts. They are made of very complex molecular mixtures containing different classes of molecules corresponding to a large range of molecular weights, polarities and volatilities. *n*-Alkanes, long-chain alcohols and fatty acids, mono-, poly-, hydroxy-esters and triacylglycerols are encountered in waxes [19] whereas terpenoid components

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are issued from vegetable resins [20]. Both polar (fatty and terpenoid acids) and apolar (*n*-alkanes) compounds are often present in a given sample. Various alteration processes, including hydrolysis of esters and triacylglycerols, migration and crystallisation of *n*-alkanes and fatty acids, sublimation of *n*-alkanes but also oxidation and polymerisation, may occur, mostly depending on the context of preservation [2,5,8].

Naturally occurring organic chemical compounds, such as those used in wax sculptures, are the result of either primary or secondary metabolism of various living beings [21]. Their molecular structure is thus directly related to their natural origin. If the molecules survive through time, they can be preserved without any modification. Nevertheless, in most cases, they have been subjected to various alteration processes that modified their structure but their carbon skeleton may still be preserved. These molecular constituents with a specific carbon skeleton, named biomarkers, supply useful information about the origin and the history of the organic materials studied [22].

The elucidation of the chemical composition of wax works of art can thus provide insight into the natural or synthetic organic substances involved in sculpture making and the origin of the alterations observed. The chronology of the production may also be assessed since some synthetic substances such as paraffin or stearin only began to be used during the 19th century. This knowledge is of considerable importance for determining suitable methods for restoration and preservation of our cultural heritage.

Because such materials are made of complex molecular mixtures, the methods of choice for separating, detecting and identifying the molecular biomarkers are the chromatographic methods.

To our knowledge, the first analytical studies of waxes by gas chromatography were performed in the seventies and eighties with packed columns [23,24] or very short, 1.50 m length, capillary columns [17]. These pioneer approaches provided fingerprints of several commercial substances. Nevertheless, these methods did not always allow a satisfactory resolution and the high molecular weight compounds were often eluted with a notable bleeding of the column. Furthermore, the use of very short columns prevents any coupling with mass spectrometry when structural characterisation is necessary.

More recently, two papers reported the use of supercritical fluid chromatography for the analysis of waxes in art objects [25,26]. If waxes made of paraffin, spermaceti, candelilla, ceresine and beeswax can be easily identified by these methods, some others, especially Japan wax made of triacylglycerols, remain difficult to characterise.

These different analytical approaches minimise the step of sample preparation and avoid any hydrolysis step which is a source of loss of information on the distribution of long-chain compounds. Nevertheless, detection and separation of high molecular weight compounds still remain difficult. To overcome this obstacle, it may be useful to proceed to chemical treatment by methanolysis or alkaline hydrolysis in order to cleave the C–O bonding of esters and triacylglycerols before chromatographic analysis [27,28]. Various distributions of fatty acids and alcohols from the original esters and triacylglycerols are then used for distinguishing the different waxes. Pyrolysis–GC–MS may also be efficient for identifying beeswax in micro-samples [28]. However, in all these cases, the chemical treatment or the pyrolysis step is responsible for the modification of the structure of the initial biomarkers and leads to the obtention of complex chromatograms. Such approaches are thus efficient for characterising a single substance in a sample but the interpretation becomes more difficult when several are present.

One of the challenges of the analysis of materials sampled on works of art is to be able, with a single analysis, on a micro-sample, to identify most of the substances involved in the sample of interest. With this aim, waxy substances and their additives were investigated by HT GC and HT GC–MS, using methods minimising sample preparation and avoiding any structural modification of the molecular biomarkers. Before analysing samples from sculptures from our cultural heritage, reference materials, such as animal and vegetable waxes but also natural and synthetic substances known to have been used as additives in wax sculptures, were investigated. Analyses of micro-samples issued from two sculptures from the 19th century were then undertaken to improve our knowledge on the know-how of the sculptors. In most cases, HT GC and HT GC–MS were performed after a rapid sample preparation including solvent extraction and trimethylsilylation to obtain a characteristic chromatographic fingerprint of the samples without any structural alteration. However, in some cases, a transbutylation step, preceding trimethylsilylation, was also necessary. This method was used to enable the release of monomers involved in polymerised products known to be formed in altered vegetable oils, and to precise carbon length of the fatty acids and alcohols issued from long-chain esters not volatile enough to be studied by HT GC–MS.

The analysis of long-chain esters by HT GC–MS allowed us to provide new data on the structure of the different isomers characteristic of some natural substances, especially in the case of the monoesters from spermaceti, for which no structural information was available until now.

The interest of this methodology was emphasised after the analyses of micro-samples from wax sculptures from the Louvre Museum and the "*Musée de la Révolution Française*" (Vizille, France).

2. Experimental

2.1. Solvents and reagents

All solvents were of HPLC grade. Trimethylsilylating agent, bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane – BSTFA – was purchased from Acros Organics and boron trifluorure/butanol solution (BF_3/BuOH, 14%, w/v) from Regis Chemical. Sodium

carbonate (Na_2CO_3) and sodium sulfate (Na_2SO_4) were supplied by Fischer scientific.

The following commercial standards were used to obtain their retention time in gas chromatography and their mass spectrum: *n*-alkanes (odd carbon atom number from C_{23} to C_{29}); fatty acids (even carbon atom number from C_{14} to C_{28}); *n*-alcohols (even carbon atom number from C_{18} to C_{30}); diacylglycerols; triacylglycerols (trimyristin, tripalmitin and tristearin) and diacids (C_6–C_{10}). All these standards were purchased from Sigma–Aldrich.

2.2. Contemporary reference materials

All the characteristics of the reference materials presented below are based upon information scattered in several papers dealing either with materials from cultural heritage or with the chemistry of natural substances [10,17,20,23,24].

2.2.1. Waxy materials from animal origin

Two animal waxes, namely beeswax and spermaceti wax, were studied. These substances were available from the collection of natural substances of the *Centre de Recherche et de Restauration des Musées de France* (C2RMF). Beeswax, as everybody knows, is a natural product synthetised by bees whereas spermaceti wax is a hard white wax issued from the head cavity of the sperm whale.

2.2.2. Waxy materials from vegetable origin

Carnauba, Candelilla and Japan waxes were investigated in this study. Carnauba wax is produced by the leaves of several palm trees, such as *Copernicia cerifera*, from America, especially in Brazil. Candelilla is produced by the leaves of various species of *Euphorbia*, also growing in the New World, particularly in Mexico and in the South of the United States. Japan wax is a protective coating of kernels from small shrubs (sumac plants, *Rhus* genus) growing in China or Japan.

2.2.3. Additives to waxy products

Ozokerite and paraffin are fossil substances, respectively, obtained from earth waxes found in lignite bed and from distillation of petroleum whereas stearin is a commercial product as already mentioned above. These substances are usually mixed with beeswax but they may also be used as main ingredient of the waxy paste.

Pine resin derivatives are the common organic additives in wax sculptures. Turpentine corresponds to a fresh exsudate from pine trees whereas colophony is a solid residue obtained after distillation of fresh pine resin. Both substances from the collection of natural substances of the C2RMF were investigated here.

Tallow and lard from the collection of natural substances of the C2RMF were also studied since they are known to have been added to waxy materials to make them more supple [16], as well as aged vegetable oils (linseed, walnut and poppyseed oils), which may also be involved in waxy recipes of sculptors.

2.3. Museum samples

Museum samples consisted of 10 micro-chips of waxy materials collected on two different sculptures. One of the sculptures is a statuette of Junon, represented on a peacock, (290 mm × 120 mm) by Antoine-Louis Barye (1796–1875) from the Louvre Museum (Museum reference: RF 4682; laboratory reference: FZ34081). The sculpture was modelled in plaster and then covered with a thin waxy coating. During the restoration of the sculpture, seven samples were collected by one of us (SC) for identifying the nature of the waxy patina (Table 1).

The second sculpture, from the *Musée de la Révolution Française* (Vizille, France), is a preparatory study by Aimé-Jules Dalou (1838–1902) for the preparation of the bronze low relief entitled *Mirabeau répondant au marquis de Dreux-Brézé, séance du 23 juin 1789* located in the French *Assemblée Nationale* in Paris (1883–1891, height 2.36 m; width 6.54 m; museum reference: 1889 MRF 2003-9; laboratory reference: FZ36205). The different materials sampled on this sculpture were collected during the restoration of the work of art. They consist of the waxy substance used by Dalou (sample MR0868), a sample corresponding to an ancient restoration material (sample MR0869) and white crystallisations (sample MR0870) visible at the surface of the sculpture.

Table 1
Description and location of samples from the statuette of Junon by Barye, Louvre Museum, R.F.4682

General aspect	Lab reference	Location on the sculpture	Description under binocular
Red waxy material	MR1022	Behind the right ankle, on the draping	Red, homogeneous and slightly transluscent material
	MR1023	On the back of the statuette, at the end of the left wing of the peacock	Orange sample with small black grains
	MR1025	On the back of the statuette, at the top of the left leg of the peacock	Orange and transluscent material
	MR1030	Behind the right knee of Junon	Dark red material with small black grains
“Anthracite” patina	MR1029	On the middle of the back of Junon	Brown material containing small orange grains
Brown patina	MR1024	Near sample MR1025	Light brown material with small black grains
White crystallisations	MR1026	Under sample MR1023	Not observed

2.4. Iodine test for starch detection

A spot-test reaction using lugol's iodine (a solution of iodine in potassium iodine) was performed in order to reveal the presence of starch [29]. When grains of starch are present, they turn dark blue. This simple, rapid and diagnostic test was thus carried out on museum micro-samples, under binocular, when enough matter was available.

2.5. Extraction and trimethylsilylation of the samples

Reference samples were extracted either with dichloromethane or cyclohexane (in the case of paraffin and ozokerite) for obtaining 1 mg mL^{-1} solutions. An aliquot of 50 μL was sampled, evaporated until dryness under a stream of nitrogen and submitted to trimethylsilylation (50 μL of BSTFA, 30 min, 70 °C). After cooling at room temperature and evaporation of the derivatising agent, the samples were redissolved in 100–200 μL of dichloromethane or cyclohexane. One microlitre of the solution was then injected for HT GC and HT GC–MS analysis.

In the case of museum samples, micro-grains (typically 100–500 μm in diameter) were sampled under binocular with a scalpel blade and deposited in a glass tube. Extraction was performed with 100–200 μL of dichloromethane by ultrasonication for 20 min. An aliquot of 50–100 μL was sampled for trimethylsilylation following the same protocol as indicated for reference samples. If only very tiny amount of sample is available, the solvent extraction step may be avoided and derivatisation is directly achieved on a micro-chip of sample. All the operations of sample preparation are then performed in a single vial, avoiding any loss of matter.

One microlitre of each solution was then injected for HT GC and HT GC–MS analysis using a 15 m column.

2.6. Transbutylation procedure

Transmethylation is routinely used for releasing fatty acids involved in polymers formed through the alteration of siccative vegetable oils [30]. However, the evaporation step following the reaction may provoke the partial loss of some low molecular weight methylated diacids, as we noticed during this procedure. To avoid any modification of the ratio between the molecular constituents released by the transesterification step, we decided to increase the molecular weight of the compounds obtained, and thus to decrease their volatility, by using butanol instead of methanol.

After extraction of the samples as mentioned above, an aliquot of 100 μL was transferred to a 2 mL glass vial and treated with 50 μL of boron trifluorure, butanol (BF_3/BuOH, 14%, w/v, 16 h, 80 °C) after evaporation of the solvent. The solution was then neutralised using a saturated solution of Na_2CO_3; the organic compounds were extracted using CH_2Cl_2 and rinsed with 250 μL of ultrapure water and the organic phase was dried with Na_2SO_4. An aliquot of 100 μL of this organic solution was then transferred into a glass vial. The solvent was evaporated until dryness and the sample was trimethylsilylated in order to proceed to the derivatisation of free hydroxy functions. After evaporation until dryness, the sample was dissolved in CH_2Cl_2 before analysis. One microlitre of each solution was then analysed by GC and GC–MS using a 30 m column.

2.7. HT GC and HT GC–MS analyses of trimethylsilylated samples

Samples obtained after extraction and trimethylsilylation were analysed by HT GC with a Hewlett Packard 6890 Series II gas chromatograph equipped with an on-column injector, a Varian CP Sil 5 CB capillary column (15 m length, 0.32 mm i.d., 0.1 μm film thickness) preceded by a 1 m deactivated precolumn, and a FID detector at 350 °C. The injector was used in the track oven mode. The temperature of the oven was programmed as follows: 1 min at 50 °C, 50–350 °C at 10 °C min^{-1}, 350 °C for 10 min. Helium was used as carrier gas with a programmed flow from 2 mL min^{-1} for 17 min, to 4 mL min^{-1} for 5 min, until 6 mL min^{-1} for 15 min. The increasing rate of helium flow was of 1 mL min^{-2} between each change of helium flow.

HT GC–MS of these samples was carried out with a ThermoFinnigan GCQ device linked with a Hewlett Packard 5890 gas chromatograph equipped with a split/splitless injector used in splitless mode at 320 °C and a CP Sil 5 CB capillary column (15 m length, 0.25 mm i.d., 0.1 μm film thickness). The temperature program of the oven was the same as that used in HT GC. Helium was used as carrier gas with the following pressure program: 5 psi for 1 min, 5–16.8 psi at 0.39 psi min^{-1}, 16.8 psi until the end of the run. The GC–MS interface was held at 340 °C. Mass spectra were recorded in the electron impact mode at 70 eV, and the ion source was held at 180 °C. The mass range was scanned from 50 to 800 in 0.9 s in full scan mode.

2.8. GC and GC–MS analyses of transbutylated samples

The analysis of transbutylated samples was performed by GC using a Hewlett Packard 6890 Series II gas chromatograph equipped with a split/splitless injector used in splitless mode at 310 °C, a Varian CP Sil 5 CB capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) preceded by a 5 m length deactivated column. The molecular constituents were eluted using helium as carrier gas at a constant flow of 1.5 mL min^{-1} with the following temperature program of the oven: 50 °C for 1 min, 50–320 °C at 10 °C min^{-1}, 320 °C for 12 min. The temperature of the FID detector was fixed at 340 °C. GC–MS analyses of the transbutylated samples were carried out with a ThermoFinnigan GCQ device linked with a Hewlett Packard 5890 gas chromatograph. The same column as in GC was used, as well as the same temperature conditions. Helium was used as carrier gas with the following pressure program: 17 psi for 1 min, 17–35 psi at 0.67 psi min^{-1}, 35 psi until the end. The GC–MS interface

temperature was fixed at 310 °C; mass spectra were recorded in the electron impact mode at 70 eV, and the ion source was held at 180 °C. In full scan mode, the mass range was scanned from 50 to 650 in 0.6 s.

3. Results and discussion

Reference and museum samples were first analysed by HT GC and HT GC–MS after extraction and trimethylsilylation in order to detect and identify, in a single run, most of the solvent soluble molecular components present in the samples. If this method is particularly efficient for separating a large range of molecular compounds, some biomarkers may not be clearly identified by HT GC–MS because of their low volatility (case of the long-chain esters from carnauba wax) or their high molecular weight (polymerised materials). When enough matter was available, samples were thus also analysed by GC and GC–MS after a transbutylation step.

3.1. Animal and vegetable waxes

The chromatograms obtained by HT GC on the total lipid extract (TLE) of animal waxes, namely beeswax and spermaceti, are shown in Fig. 1. Several *n*-alkanes, fatty acids and monoesters were identified by comparison of their retention time and their mass spectrum with those of commercial molecular standards. The two reference substances analysed are easily distinguishable by their chromatographic profile. Beeswax contains a homologous series of *n*-alkanes with odd carbon number, from C_{23} to C_{33}, *n*-heptacosane (C_{27}) being the main compound. Free fatty acids (even-numbered compounds C_{22}–C_{34}, C_{24} as main compound) were also identified. Long-chain esters were detected in beeswax and spermaceti. The ester distribution in beeswax is in agreement with the one already published by several authors [2,5,6,8,11] and their mass spectrum is typical of esters derived from palmitic acid ranging from C_{40} to C_{52}. The predominance of palmitic acid and the alcohols released by transbutylation confirm this structure of esters from beeswax (Fig. 2). If the structure of long-chain esters from beeswax was already studied by several authors [6,8,19,31], very few data are available on the esters from spermaceti, particularly due to the fact that the structure of these biomarkers was not investigated by mass spectrometry. Spermaceti mainly contains even-numbered long-chain esters ranging from C_{26} to C_{36}, C_{30} being the main component. Odd-numbered esters (C_{27}–C_{33}) were also detected in low amount. The study of spermaceti after transesterification with a BF_3/BuOH solution showed a distribution of even-numbered fatty acids and long-chain alcohols, respectively, in the range C_{12}–C_{18} and C_{14}–C_{20}, indicative of the fatty acids and alcohols involved in the ester structure (Fig. 2). In order to get more information about the structure of these esters, HT GC–MS analyses were performed on the total lipid extract of the sample. These analyses confirmed that the main biomarkers from spermaceti are

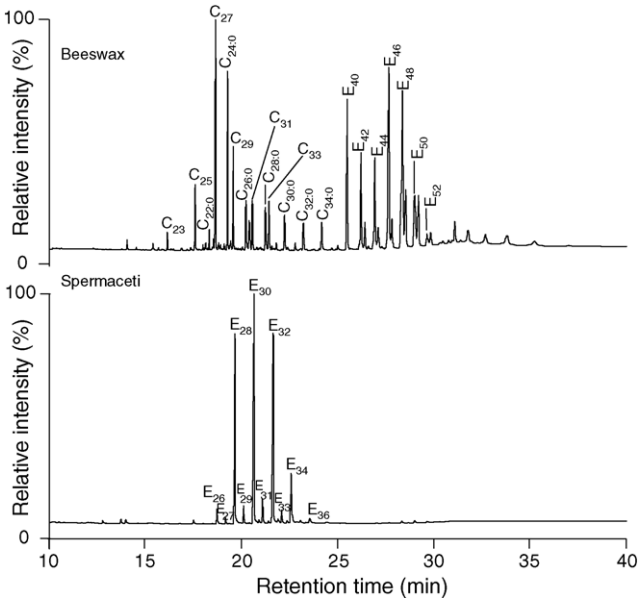

Fig. 1. HT GC-FID chromatograms obtained after solvent extraction and trimethylsilylation of two animal waxes (beeswax and spermaceti) with a 15 m column. The identification of the main molecular compounds was performed by HT GC–MS analysis. $C_{x:0}$: saturated fatty acids with *x* carbon atoms; C_x: *n*-alkane with *x* carbon atoms and E_x: long-chain monoesters with *x* carbon atoms.

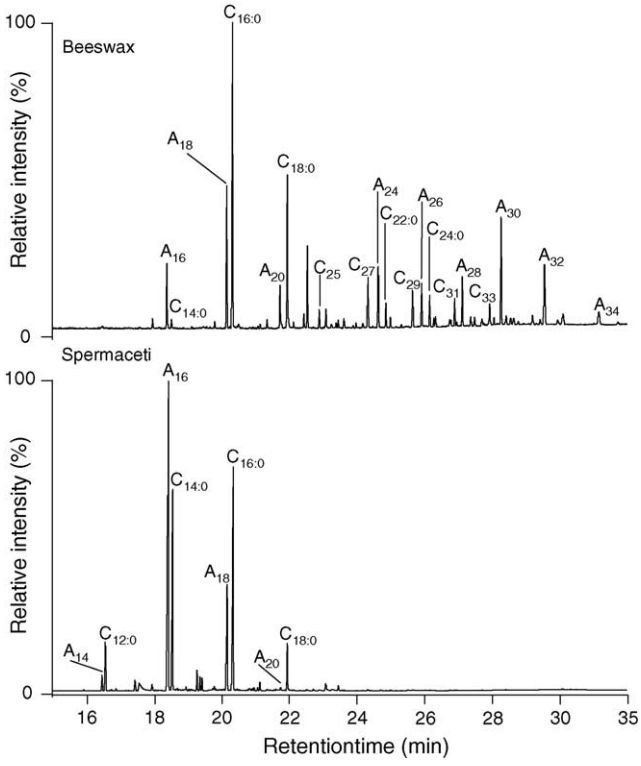

Fig. 2. GC-FID chromatograms obtained after transbutylation of beeswax and spermaceti with a 30 m column. The identification of the main molecular compounds was performed by GC–MS analysis. $C_{x:0}$: saturated fatty acids with *x* carbon atoms; C_x: *n*-alkane with *x* carbon atoms and A_x: linear alcohols with *x* carbon atoms.

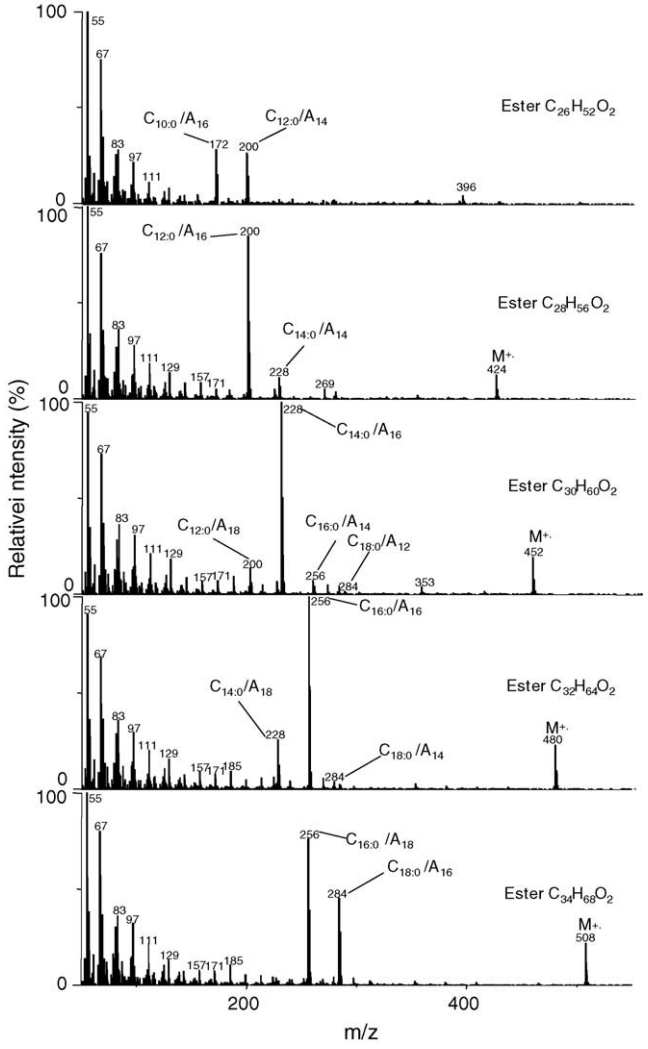

Fig. 3. Mass spectra of the even-numbered esters of spermaceti.

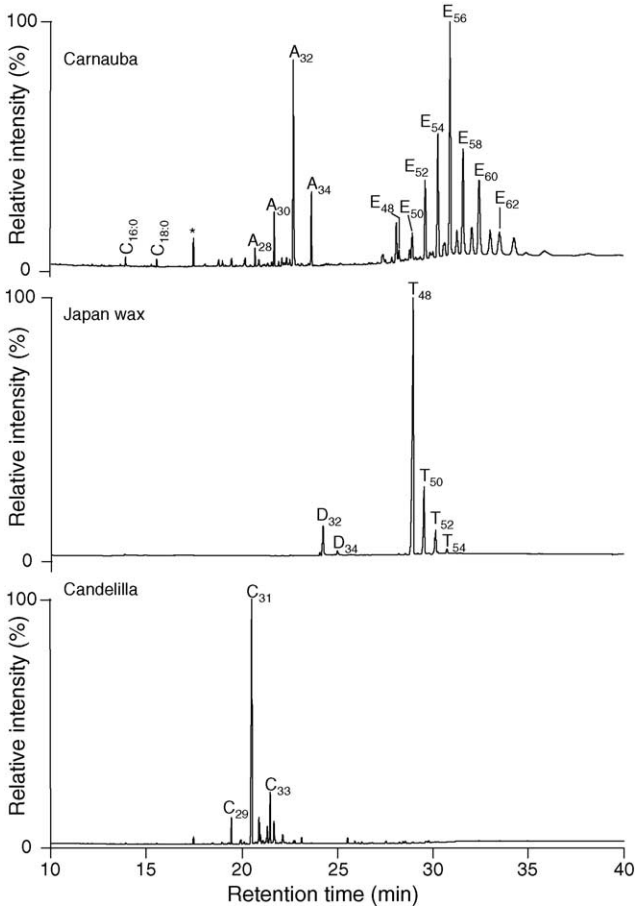

Fig. 4. HT GC-FID chromatograms obtained after solvent extraction and trimethylsilylation of three vegetable waxes with a 15 m column. The identification of the main molecular compounds was performed by HT GC–MS analysis. $C_{x:0}$: saturated fatty acids with *x* carbon atoms; C_x: *n*-alkane with *x* carbon atoms; A_x: linear alcohol with *x* carbon atoms; E_x: long-chain monoester with *x* carbon atoms; D_x: diacylglycerol with *x* carbon atoms and T_x: triacylglycerol with *x* carbon atoms. (*) plasticiser.

even-numbered long-chain esters from C_{26} to C_{36}, together with other esters in very low amount with odd carbon number in the range C_{27}–C_{33}. Long-chain esters are known to provide two main fragments characteristic of the acid moiety of the ester, namely $[C_nH_{2n+1}CO_2H]^{\bullet+}$ and $[C_nH_{2n+1}CO_2H + H^+]$, in a mass spectrometer equipped with an ion trap [12]. Mass spectra obtained for each even-numbered ester of spermaceti are shown in Fig. 3. Most of the spectra present a main peak $[C_nH_{2n+1}CO_2H]^{\bullet+}$ indicating that C_{26} ester is mainly made of hexadecanoyl decanoate, C_{28} ester of hexadecanoyl dodecanoate, C_{30} ester of hexadecanoyl myristate, C_{32} ester of hexadecanoyl palmitate, C_{34} ester of octadecanoyl palmitate and C_{36} ester of octadecanoyl stearate. It thus appears that palmitate esters are not predominating in spermaceti. The esters are indeed mainly made of hexadecanoyl moieties associated with a fatty acid containing 10–18 carbon atoms. All the spectra also show that each chromatographic peak corresponds to the co-elution of several isomers. For example, the peaks at *m/z* 200, 228, 256 and 284 in the mass spectrum of C_{30} ester, respectively, correspond to the radical ions $[C_{11}H_{23}CO_2H]^{\bullet+}$, $[C_{13}H_{27}CO_2H]^{\bullet+}$, $[C_{15}H_{31}CO_2H]^{\bullet+}$ and $[C_{17}H_{35}CO_2H]^{\bullet+}$. These fragments clearly indicate that several esters are co-eluted. They correspond to octadecanoyl laurate, hexadecanoyl myristate, tetradecanoyl palmitate and dodecanoyl stearate.

Minor odd-numbered esters were also detected. As the main esters, they correspond to mixtures of several isomers.

Vegetable waxes provided very different chromatographic patterns for their total lipid extract (Fig. 4). Candelilla presents the simplest composition. It mostly contains three *n*-alkanes C_{29}, C_{31} (main compound) and C_{33}, as already mentioned in literature [17,23,24]. Japan wax also contains few biomarkers, especially triacylglycerols, with tripalmitin as principal component. Diacylglycerols, partly issued from the partial hydrolysis of TAG, were also detected. Consequently, although Japan wax is generally classified in waxy products, probably because of its physical properties similar to those of beeswax, it is a fatty material mainly containing triglycerides [17,20,24]. Carnauba is the more complex

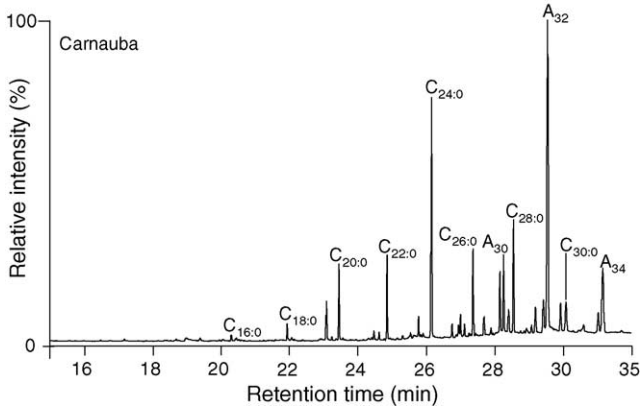

Fig. 5. GC-FID chromatograms obtained after transbutylation of carnauba wax with a 30 m column. The identification of the main molecular compounds was performed by GC–MS analysis. $C_{x:0}$: saturated fatty acids with *x* carbon atoms and A_x: linear alcohols with *x* carbon atoms.

vegetable wax that we analysed. Made of low amount of palmitic and stearic acids, it contains free even-numbered alcohols (C_{28}–C_{34}, C_{32} principal component) and a series of long-chain compounds. If these compounds may be eluted by HT GC, using high temperature programme until 350 °C and a ramp of helium flow during the analysis, with a satisfying resolution and a low column bleeding, it was not possible to proceed to their detection and identification by HT GC–MS using the same chromatographic conditions as for the other substances. Indeed, the esters of carnauba are very long-chain compounds containing even-numbered constituents from C_{48} to C_{62}, C_{56} being the major compound. It is thus possible to elute them by HT GC using only very specific conditions, especially an helium flow of 6 mL min^{-1} at the end of the run. Such an important value of helium flow cannot be used when coupling HT GC with mass spectrometry due to the necessity of maintaining a sufficient vacuum in the mass spectrometer. The results obtained on the transbutylated sample were thus of great importance to obtain information on the alcohols and fatty acids involved in the formation of esters from carnauba (Fig. 5). A series of even-numbered fatty acids from C_{16} to C_{30}, of which tetracosanoic acid (C_{24}) is predominant, and three main linear long-chain alcohols containing 30, 32 and 34 carbon atoms (C_{32} as major compound) were identified. Contrary to beeswax characterised by esters that all contain the same acid moieties, it appears that the esters from carnauba wax contain a large range of fatty acids mostly associated with a single alcohol (C_{32}).

3.2. Paraffin, ozokerite and stearin

Total lipid extracts of paraffin and ozokerite, analysed by HT GC after extraction with hexane, are characterised by a series of homologous linear alkanes, containing both even and odd carbon number (Fig. 6) [17,18,24]. Paraffin contains *n*-alkanes from C_{21} to C_{35}, of which *n*-heptacosane (C_{27}) is the main compound [17]. However, one must note that the distribution of these *n*-alkanes may slightly change depending

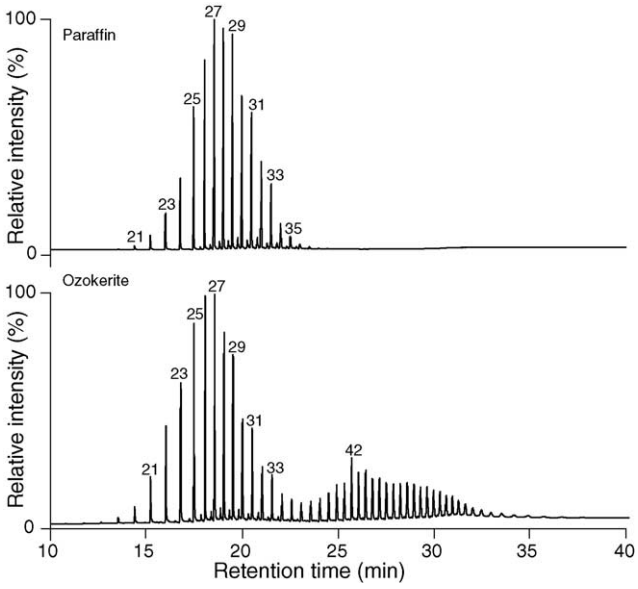

Fig. 6. HT GC-FID chromatograms obtained after solvent extraction of two fossil waxes (paraffin and ozokerite) with a 15 m column. Each number *x* on the chromatograms corresponds to *n*-alkane with *x* carbon atoms.

on the raw petroleum matter distilled. The chromatographic pattern of ozokerite is more complex. Ozokerite contains a wide range of *n*-alkanes with carbon atoms from 18 to 60 in two broad bands peaking at 27 and 42. Because this matter is a fossil substance from earth deposits of wax, the distribution of the alkanes may also differ depending on the natural processes of formation [18].

The chromatogram of stearin, obtained by HT GC after extraction and trimethylsilylation, is very simple: it only contains two peaks attributed by their retention time to palmitic and stearic acids, fatty acids released by animal fats during the industrial process of stearin making.

3.3. Pine resin derivatives

Two kinds of conifer derivatives were analysed by HT GC after extraction and trimethylsilylation: turpentine and colophony. Both substances provide a complex chromatographic pattern from which it was possible to identify diterpenoid components (Fig. 7). Depending on their way of formation, naturally by biosynthesis in pine resin, or by alteration either during the heating operation of distillation or due to natural alteration, these constituents may be classified in two categories, namely biomarkers and alteration markers [32]. The molecular compounds separated and detected in these conifer derivatives were identified by the study of their mass spectrum [33–36]. Table 2 summarises the main peaks of each diterpenoid marker.

Turpentine mainly contains biomarkers, especially pimaric, isopimaric, palustric and abietic acids. A degradation marker, dehydroabietic acid, was also identified. Colophany contains dehydro-7-dehydroabietic and dehydroabietic acids (main compound), and a low amount of

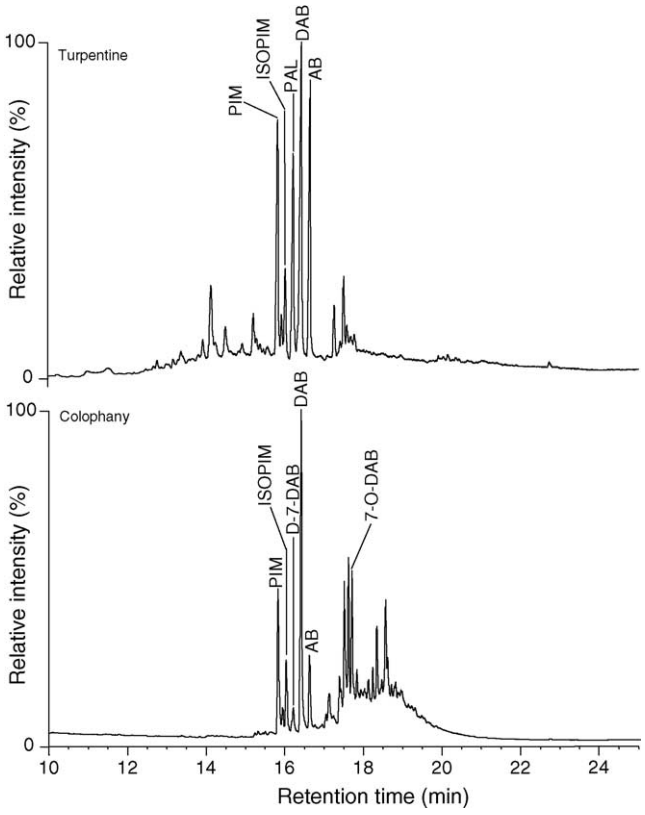

Fig. 7. HT GC-FID chromatograms obtained after solvent extraction and trimethylsilylation of pine resin derivatives, e.g. turpentine and colophony (15 m column). PIM: pimaric acid; ISOPIM: isopimaric acid; D-7-DAB: dehydro-7-dehydroabietic acid; PAL: palustric acid; DAB: dehydroabietic acid; AB: abietic acid and 7-O-DAB: 7-oxo-dehydroabietic acid.

pimaric, isopimaric and abietic acids. 7-Oxo-dehydroabietic, an oxidation marker of abietic acid was also identified.

Thus, turpentine is characterised by a low yield of alteration compared to colophany, which may be explained by the fact that turpentine is directly obtained from the resin whereas colophany is the solid residue resulted from the distillation of pine resin.

3.4. Fatty materials

The most common fatty materials involved in wax sculptures are animal fats but vegetable oils are also mentioned by some authors [37]. The main vegetable oils used in the field of art are siccative oils that polymerise through time. They

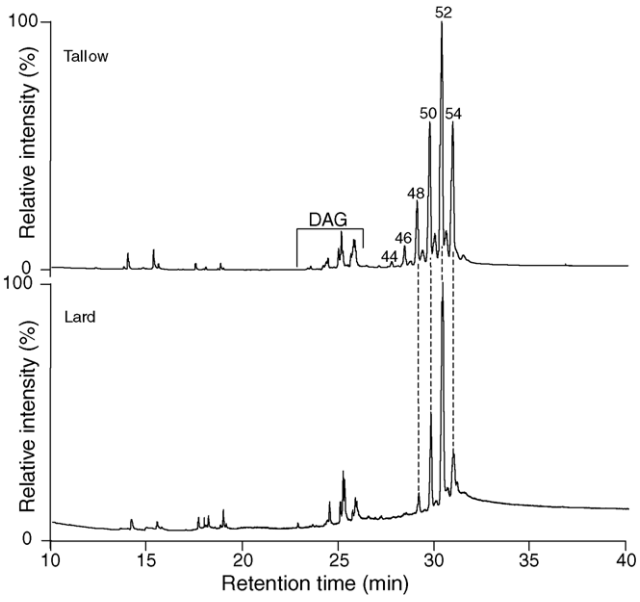

Fig. 8. HT GC-FID chromatograms obtained after solvent extraction and trimethylsilylation of two animal fats (15 m column). DAG: diacylglycerols. Each number *x* on the chromatograms corresponds to triacylglycerols with *x* carbon atoms.

were thus analysed after transbutylation by GC whereas animal fats were investigated by HT GC after extraction and trimethylsilylation.

Gas chromatograms of tallow and lard are dominated by triacylglycerols (Fig. 8). Fatty acids and diacylglycerols, issued from the beginning of hydrolysis of their precursors (triglycerides), were also detected. The distribution of triacylglycerols is slightly different for the two substances: the compound containing 52 carbon atoms for the acid moieties, is predominant in each case but the distribution of triacylglycerols is narrower in the case of lard (C_{48}–C_{54} instead of C_{44}–C_{54} for tallow), as already mentioned by other authors [38].

Transbutylation of aged vegetable oils provided very similar chromatogram for each sample, except from a quantitative standpoint (Fig. 9). Palmitic (P), stearic (S) and unsaturated acids ($C_{18:n}$) were identified, associated with oxidation markers, namely α,ω-diacids from 6 to 10 carbon atoms. Azelaic acid, formed by oxidation of unsaturated acids in 9 position, quite common in vegetable oils, is the main diacid formed. P/S ratio are in agreement with those published in the

Table 2
Mass spectra characteristics of the diterpenoid compounds identified in pine resin derivatives

Biomolecular constituents	Mass spectra
Pimaric acid	73 (100), 91 (77), 121 (57), 241 (16), 256 (12), 359 (8), 374 (7)
Isopimaric acid	73 (89), 91 (69), 241 (100), 256 (65), 359 (5), 374 (2)
Dehydro-7-dehydroabietic acid	195 (32), 237 (100), 252 (22), 355 (3), 370 (3)
Palustric acid	73 (77), 91 (49), 241 (100), 256 (13), 359 (21), 374 (23)
Dehydroabietic acid	73 (34), 239 (100), 250 (3), 357 (6), 372 (5)
Abietic acid	73 (59),185 (44), 213 (23), 241 (57), 256 (100), 374 (14)
7-Oxo-dehydroabietic acid	73 (48), 187 (17), 253 (100), 268 (73), 327 (12), 386 (7)

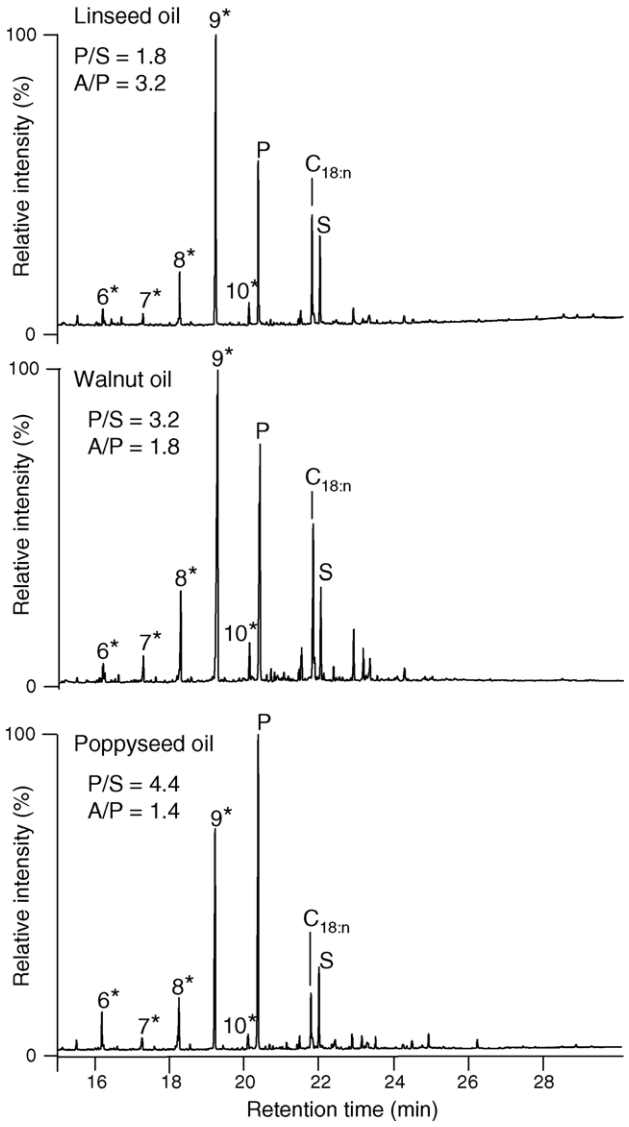

Fig. 9. GC-FID chromatograms of transbutylated aged vegetable oils analysed with a 30 m column. P: palmitic acid; S: stearic acid; A: azelaic acid. The numbers 6* to 10* correspond to linear α,ω-diacids containing 6–10 carbon atoms.

literature since P/S is less than 2 in linseed oil, between 2 and 3 in walnut oil and more than 3 for poppyseed oil [18,39,40]. Transbutylation of the samples would thus be an interesting method for detecting the presence of a siccative oil in a wax sculpture. However, because such an oil would always be mixed with a wax, the ratio of saturated fatty acids could not be used to precise the nature of the oil since all waxy products also release fatty acids of that kind.

Although all the natural and synthetic substances potentially involved in wax sculptures are made of complex molecular mixtures, they all present very different and characteristic chromatographic patterns, either because they contain specific biomarkers (case of diterpenoid components from pine resin derivatives) or because they differ by their biomarker distribution (case of triacylglycerols in Japan wax and animal fats and of long-chain monoesters in waxes such as beeswax, spermaceti or carnauba wax). These specific compositions are thus of great interest for elucidating the nature of the materials involved in wax sculptures, as it is discussed below.

3.5. Museum samples

All the samples were observed under a binocular in order to assess their colour and their homogeneity. When enough matter was available, a spot-test reaction using lugol's iodine was performed in order to reveal the presence of starch and the samples were then prepared for analysis by HT GC and HT GC–MS and by GC and GC–MS when enough matter was available for analysis.

3.5.1. Statuette of Junon by Barye

At least three kinds of different materials were suspected after a fine and detailed observation of the surface of the sculpture: a red wax, a brown one and a third material presenting an anthracite aspect. Moreover, white efflorescences, probably corresponding to a surface alteration [16], were observed and sampled to be studied (Table 1). Seven micro-samples, the size of the head of a pin for the largest, were collected at the surface of the sculpture, using a scalpel blade, to identify these different materials. The samples were stored in a glass vial until analysis.

The lugol test was only carried out on samples for which enough matter was available, namely on samples MR1023 and MR1025. The test was positive for both samples.

All the samples were then extracted and trimethylsilylated for HT GC and HT GC–MS analysis. However, because of the tiny amount of matter in each sample, no transbutylation was performed, preventing the study of any polymerised oil which could have been used by the sculptor. Nevertheless, the high temperature gas chromatograms obtained were very instructive and allowed the characterisation of three different recipes used by Barye (Fig. 10).

Even-numbered palmitate long-chain esters, ranging from C_{40} to C_{50}, were identified in six samples. The distribution of these esters was very close to that known in beeswax for three samples (MR1022, MR1023 and MR1025), triacontanoyl palmitate (C_{46}) being the main compound, followed, from a quantitative standpoint by tetracosanoyl palmitate (C_{40}). In these samples, a series of homologous *n*-alkanes, of which *n*-heptacosane is the main compound, as well as several saturated fatty acids (palmitic $C_{16:0}$, stearic $C_{18:0}$ and $C_{22:0}$–$C_{26:0}$ even-numbered acids) were also identified. Tetracosanoic acid is known to be the main free fatty acid present in beeswax and the distribution of odd-numbered *n*-alkanes is also consistent with the presence of beeswax. However, even-numbered *n*-alkanes, which are not issued from beeswax were also detected. It thus appears that paraffin was added to beeswax. Even-numbered *n*-alkanes are only issued from paraffin whereas odd-numbered *n*-alkanes come both from beeswax and paraffin. The origin of palmitic and stearic acids, ubiquitous saturated acids, is difficult to assess. They can

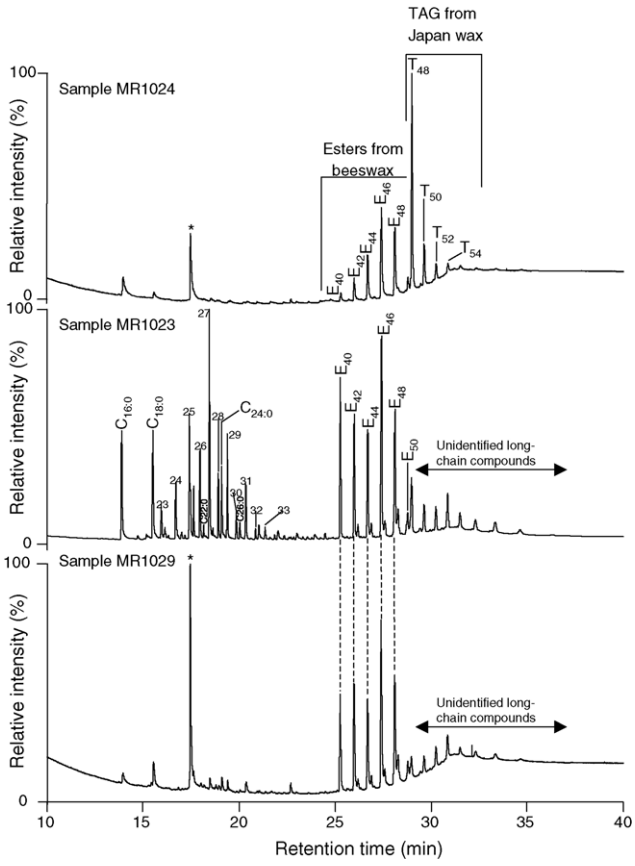

Fig. 10. HT GC-FID chromatograms obtained after solvent extraction and trimethylsilylation of three samples from the sculpture of Junon analysed with a 15 m capillary column. $C_{x:0}$: saturated fatty acids with *x* carbon atoms; C_x: *n*-alkane with *x* carbon atoms and E_x: long-chain monoester with *x* carbon atoms.

correspond to the use of stearin but they can also result from a partial hydrolysis of the beeswax esters. However, in that case, long-chain alcohols should also have been detected and palmitic acid would have been present in higher amount.

In another sample (sample MR1029), the profile of esters is also very close to that of beeswax, even though tetracosanoyl palmitate (C_{40}) is a bit depleted. However, none of the *n*-alkanes neither free fatty acids were identified in this sample. That may be explained by a heating treatment. Indeed, the artist may have heated beeswax before use, either to mix it with additives or to increase its plasticity. Such a process is known to induce the volatilisation of the low molecular compounds, especially *n*-alkanes [2,5,8]. Furthermore, a migration of *n*-alkanes towards the surface of the sculpture may have occurred. This hypothesis is confirmed by the results obtained on white crystallisations (sample MR1026) that were shown to be mostly composed of odd-numbered *n*-alkanes ranging from C_{25} to C_{31}, with C_{27} as main compound.

In samples MR1023 and MR1029, the high temperature chromatograms obtained are also characterised by the presence of high molecular compounds eluting after 30 min. Whether they correspond to long-chain esters or triacylglycerols is difficult to assess but they may be interpreted as long chain compounds from beeswax, as already discussed by other authors [6,17].

The last sample (sample MR1024) was also characterised by an altered profile of esters from beeswax. High molecular weight compounds eluting after the long-chain esters from beeswax were also detected. These components were identified as triacylglycerols with a distribution characteristic of Japan wax. Barye used a mixture of beeswax and Japan wax for performing a brown patina located on a wing of the peacock. Adding this exotic wax to beeswax had probably the purpose to obtain a more malleable and brilliant substance [37]. The use of Japan wax in sculpture is not frequently described in the literature. However, its use by Barye was already mentioned by Colinart in 1987 [17] in a sketch entitled "*Lionne dévorant une gazelle*" (lioness devouring a gazelle, Musée du Louvre, sculpture department, R.F.R. 11). David d'Angers (1788–1856) has also used Japan wax in a portrait of Caroline Murat, the youngest of Napoleon's sisters (Musée du Louvre, sculpture department, R.F. 1712) [17]. This new discovery of Japan wax in a sculpture of Barye tends to indicate that its use by this sculptor may not have been occasional.

3.5.2. Preparatory study by Dalou

The micro-chemical test with lugol's iodine was negative for samples MR0868 (waxy matter from the sculpture) and MR0869 (sample issued from an ancient step of restoration), indicating the absence of starch. This test was not performed on white crystallisations (sample MR0870) because of the low amount of matter available for analysis.

The first sample (MR0868), analysed by HT GC after extraction and trimethylsilylation, provided quite a complex chromatographic profile on which several molecular components were identified (Fig. 11). The main compound (b) was identified by mass spectrometry as TMS 7-oxo-dehydroabietic acid. This diterpene was accompanied by dehydroabietic acid (compound a), another degradation marker of pine resin. Two other compounds, named (c) and (d) on Fig. 11, were also detected, but they could not be precisely identified.

The series of compounds eluting at retention times higher than 28 min are characterised by a retention time and a distribution similar to that of Japan wax. They have been identified as triacylglycerols, tripalmitin being the major compound. Diacylglycerols, formed by partial hydrolysis of these former biomarkers were also identified, at retention times comprised between 24 and 26 min.

A series of homologous *n*-alkanes containing odd and even-numbered carbon atoms, ranging from C_{23} to C_{35} clearly indicates the addition of paraffin. However, because long-chain palmitate esters (C_{44}, C_{46} and C_{48}) characteristic of beeswax were also detected in low amount, the odd-numbered *n*-alkanes are also partly issued from beeswax.

At least, palmitic and stearic acids were identified but it is difficult to know if they indicate the presence of stearin or if

they were formed by natural hydrolysis of the triacylglycerols from Japan wax.

These results tend to show that Dalou used quite a complex mixture made of oxidised pine resin and Japan wax as the main materials, but also paraffin, beeswax in very low amount and perhaps stearin.

The restoration sample appeared to be mainly constituted of beeswax (long-chain esters and *n*-alkanes) to which paraffin and a very few amount of pine resin (presence of dehydroabietic acid) were added. However, three particular compounds, which were never identified before in any wax sculpture according to our knowledge, were also detected (compounds E_{32}, E_{34} and E_{36} on Fig. 11). Their mass spectrum allowed us to identify them as long-chain esters of raw formula $C_{32}H_{64}O_2$ ($M = 480$), $C_{34}H_{68}O_2$ ($M = 508$) and $C_{36}H_{72}O_2$ ($M = 536$). The mass spectra of the esters $C_{32}H_{64}O_2$ and $C_{36}H_{72}O_2$ present a significant peak, respectively, at *m/z* 256 and 284 characteristic of hexadecanoyl palmitate for the first one and octadecanoyl stearate for the second one. The chromatographic peak identified as $C_{34}H_{68}O_2$ appears to correspond to the co-elution of octadecanoyl palmitate (*m/z* 256 for $[C_{15}H_{31}CO_2H]^{\bullet+}$) and hexadecanoyl stearate (*m/z* 284 for $[C_{17}H_{35}CO_2H]^{\bullet+}$). The origin of these constituents is difficult to assess. Although they are present in spermaceti, their distribution does not present any analogy with the one characteristic of this material. They may either come from a natural waxy material still to be identified or from the alteration of the biomarkers present in the sample.

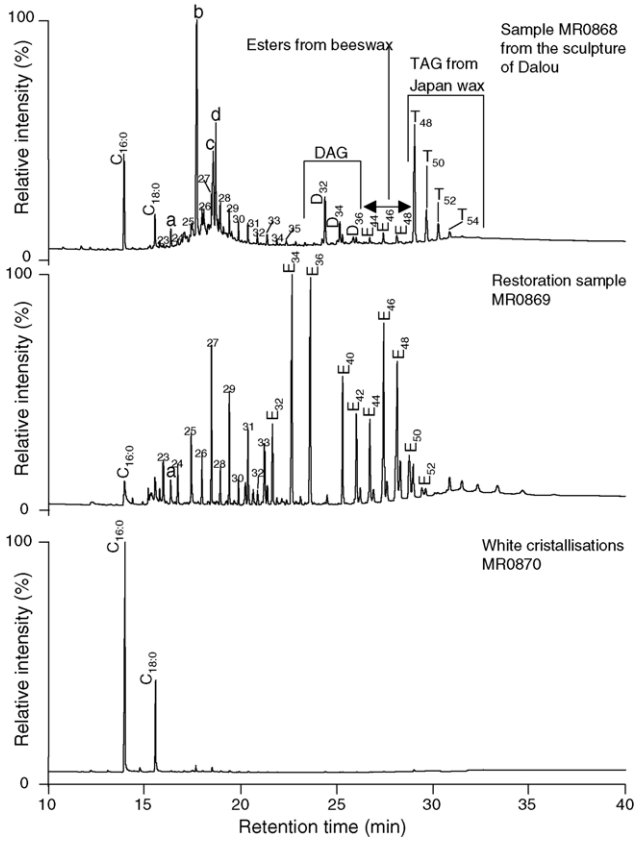

Fig. 11. HT GC-FID chromatograms obtained after solvent extraction and trimethylsilylation of three samples from Dalou's sculpture, by analysis with a 15 m chromatographic column. $C_{x:0}$: saturated fatty acids with *x* carbon atoms; C_x: *n*-alkane with *x* carbon atoms and E_x: long-chain monoester with *x* carbon atoms. (a) TMS dehydroabietic acid; (b) TMS 7-oxo-dehydroabietic acid; (c) and (d) unknown compounds. (*) plasticiser.

White crystallisations only contain palmitic and stearic acids which have migrated to the surface of the sculpture, revealing another way of alteration than for the statuette of Junon.

Because enough matter was available for analysis, these samples were also submitted to transbutylation in order to reveal markers from polymerised siccative vegetable oils but no diacid was detected, indicating the absence of such materials.

4. Conclusions

This study provides a rapid, micro-destructive and very informative method for identifying a wide range of natural and synthetic substances which may have been exploited for wax sculptures creation. By minimising sample treatment and using a gas chromatographic method with specific analytical conditions, it is possible to separate, detect and identify molecular compounds ranging on a large scale of molecular weights and volatilities. The analysis may be performed on very tiny samples, the size of the head of a pin.

This method avoids any hydrolysis step, always preventing a detailed structure identification of the main biomarkers, namely long-chain esters, present in most of the natural waxes. Beeswax, spermaceti, carnauba, candelilla and Japan waxes, as well as pine resin derivatives, paraffin, ozokerite and animal fats were analysed by this way. In the case of spermaceti, HT GC–MS analyses allowed to precise the structure of the different isomers of even-numbered esters ranging from C_{26} to C_{34}, as well as odd-numbered esters detected in low amount. By a fine study of the mass spectra obtained, it was shown that esters were mainly composed of hexadecanol or octadecanol moieties associated with a range of fatty acids containing 10–20 carbon atoms, whereas the idea that esters from spermaceti were mostly constituted of palmitic acid moieties prevailed until now.

The results obtained on contemporary reference samples were then used to interpret the data obtained on 10 samples taken from two sculptures from the 19th century. The data obtained are summarised on Table 3. First of all, one must note that the methodology developed on model samples was shown to be perfectly suitable for micro-samples from our cultural heritage, even in the case of waxy pastes containing very complex molecular mixtures resulting from the use of several substances. Combining macro- and micro-observations, chemical test with lugol's iodine for starch detection and GC and GC–MS analyses, it was possible to elucidate the different recipes elaborated by the sculptors.

Table 3
Summary of the results obtained on samples taken on two sculptures from the 19th century

Description	Sample numbers	Results
Waxy materials from the statuette of Junon by Barye	MR1022, MR1023, MR1025	Beeswax (main material), paraffin, starch (only searched in samples MR1023 and MR1025), and perhaps stearin
	MR1029	Altered beeswax
	MR1024	Japan wax and altered beeswax
Write crystallisations	MR1026	Palmitic and stearic acids
Waxy material from the sculpture by Dalou	MR0868	Pine resin (main material), Japan wax, paraffin, beeswax (in very few amount), and perhaps stearin
Waxy material sampled on an ancient restoration on the sculpture by Dalou	MR0869	Beeswax (main material), paraffin and traces of pine resin
Write crystallisations	MR0870	*n*-Alkanes from beeswax

In most cases, beeswax was identified, generally in notable amount, except in the sample from Dalou's sculpture, in which only traces of this material were detected. Paraffin was usually added to beeswax, probably to minimise the amount of beeswax, this substance being quite expensive. A large amount of pine resin, now largely oxidised, was also added in the case of Dalou's sculpture. The role of this material was probably to harden the wax.

The use of Japan wax is attested in the sample from Dalou's sculpture and in one sample from the statuette of Junon by Barye. In the first case, it was used as the main waxy material whereas in the second, it was mixed with beeswax. Such results strengthen the role of Japan wax, also mentioned in works of art by Barye and David d'Angers [17] at the end of the 19th century.

Stearin, composed of palmitic and stearic acids, may also have been used, but this is difficult to certify since these acids may also have been formed by hydrolysis of the main biomarkers of beeswax and Japan wax. Starch was identified in two samples from the statuette of Junon but it was not detected in any sample from Dalou's sculpture. It is also possible that black carbon was added to the waxy materials to colour the sculptures since few black grains were observed under binocular in most samples.

Finally, the white crystallisations observed on both sculptures were attributed to a mixture of *n*-alkanes in one case and of free fatty acids in the other. Their concentration at the surface of both sculptures is probably due to their migration towards the surface under temperature variation.

These new results on the composition of waxy materials used for sculpting during the 19th century shed new light on the innovation abilities of sculptors at this period. Indeed, the diversity of substances identified for each sculpture clearly shows that the sculptors used a wide range of materials during this period, in order to obtain pastes with specific colours and properties, depending on their artistic purpose.

This study, based upon the analysis of a large range of reference materials and tiny samples from 19th century sculptures, emphasizes the heuristic power of researches devoted to the characterisation of complex organic materials from our cultural heritage, which not only allow to provide a better understanding of sculptors behaviours, but also ensure a better chemical elucidation of the structure of biomarkers present in natural substances which were used for several purposes through time.

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