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CHEMICAL ALTERATION AND USE OF BEESWAX THROUGH TIME: ACCELERATED AGEING TESTS AND ANALYSIS OF ARCHAEOLOGICAL SAMPLES FROM VARIOUS ENVIRONMENTAL CONTEXTS*

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In order better to interpret the chemical composition of ancient organic residues and artefacts containing beeswax, the degradation of this raw material was accelerated in the laboratory by controlled heating. During the course of degradation, deposits were condensed above the beeswax. Both degraded beeswax and these deposits were analysed. These experiments definitively proved that n-alkane depletion is due to a sublimation process that depends on the molecular weight of these hydrocarbons. The formation of benzoic and cinnamic derivatives due to the degradation of flavonoid precursors initially present in beeswax has also been highlighted for the first time. The analysis of samples from Neolithic and Roman periods led to the identification of beeswax characterized by different degradation patterns linked to their environmental context.

KEYWORDS: GREECE, FRANCE, FAYUM, NEOLITHIC, ROMAN PERIOD, FOURIER TRANSFORM INFRARED SPECTROMETRY, GAS CHROMATOGRAPHY, MASS SPECTROMETRY, DEGRADATION EXPERIMENT, BEESWAX, POTTERY, STRAINER, *N*-ALKANES, ESTERS, PHENOLIC COMPOUNDS

INTRODUCTION

Among the natural resources exploited during ancient times by human societies, the different materials produced in wild beehives, including beeswax, honey and propolis, were probably gathered and used for various purposes as early as the end of the Ice Age. The first evidence for the collection of beehive products dates back to the Neolithic period and consists of rock shelter paintings representing bees and honey-hunting scenes in eastern Spain (Dams 1978; Crane 1983; Guilaine 1994). However, most of our knowledge concerning the use of beeswax dates from Antiquity and is associated with written documents and representations dealing with the economic aspects of bee-products.

Although beeswax is now partly replaced by synthetic or fossil products, it played an important role during Antiquity in technology, in the symbolic and artistic fields, and in the preparation of cosmetics or medicinal commodities (Lucas and Harris 1962; Blanc and Nercessian 1992; Charlton and Newdick 1996; Colinart 1996; Bonet Rosada and Mata Parreño 1997). A sacred and magical character was conferred to honey and beeswax as a result of their natural and enigmatic production from flower nectar within the complex organization of bee

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societies (Charlton and Newdick 1996; Blanc and Nercessian 1997). Beeswax was also used for its hydrophobicity, its plasticity and its healing properties (Dioscoride, translated in Colinart *et al.* 1999). All these characteristics explain the wide range of uses of beeswax since Antiquity (Fig. 1).

Apart from the rock shelter paintings and the archaeological artefacts interpreted as beehives in Pre-Roman Spain (Bonet Rosada and Mata Parreño 1997), little is known about the use of beeswax during prehistoric times. Indeed, when preserved in archaeological settlements, beeswax is not easily recognizable and its identification in ancient vessels or paintings is necessarily based on chemical analyses. The first analytical study of archaeological beeswax was performed by Kühn in 1960, using infrared spectrometry. The application of gas chromatography – mass spectrometry to organic amorphous materials of archaeological origin considerably increased the possibility of identifying beeswax, even when mixed with other materials (Fig. 2). In particular, beeswax was identified in different ceramic vessels dating back to the Neolithic and medieval periods (Evans and Heron 1993; Heron *et al.* 1994; Charters *et al.* 1995; Evershed *et al.* 1997; Regert *et al.* 1999; Evershed 2000; Regert *et al.* in press). The role of beeswax in pottery is still not clearly elucidated, but it may have been used as a sealing agent in order to decrease the permeability of the ceramics (Charters *et al.* 1995). More recently, an interesting investigation on Mochlos lamps from the Minoan culture in Crete gave evidence for the use of beeswax as an illuminant (Evershed *et al.* 1997). This material was not expected in such a context, and this discovery emphasizes the importance of bees and their production in Minoan culture, which is also illustrated by the bee-shaped jewellery manufactured during this period (Delaveau 1987).

Chemical identification of beeswax in archaeological samples is generally based on the detection of characteristic solvent-soluble molecular constituents, including a series of odd-numbered linear hydrocarbons (C_{21}–C_{33}), even-numbered free fatty acids (C_{22}–C_{30}) and long-chain palmitate esters in the carbon range C_{40}–C_{52} (Tulloch and Hoffman 1972; Tulloch 1973; Kolattukudy 1976). Contemporary beeswax presents a very recognizable pattern of *n*-alkanes—of which heptacosane (C_{27}) is the major compound—of esters with a main constituent that contains 46 carbon atoms, and of free fatty acids, of which lignoceric acid (C_{24}) is predominant. The chromatographic profile of ancient beeswax often presents significant differences to that of contemporary beeswax, due to the degradation of this material through

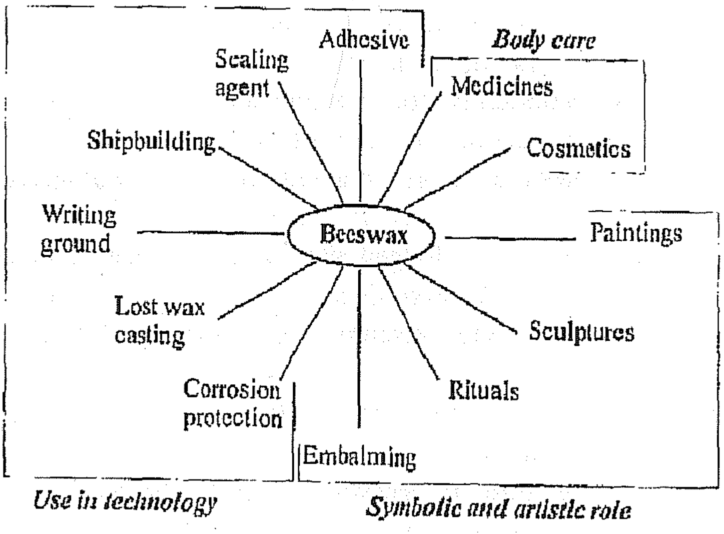


Figure 1 *The different functions of beeswax during Antiquity.*

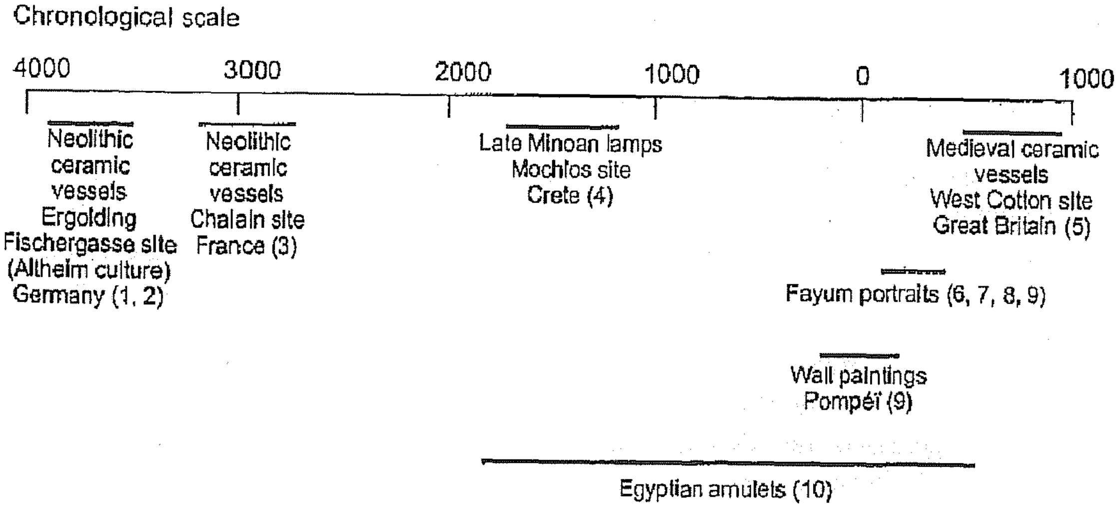


Figure 2 *An overview of the chemical identification of beeswax in archaeological remains in Western Europe and Egypt. 1, Evans and Heron (1993); 2, Heron* et al. *(1994); 3, Regert* et al. *(1999); 4, Evershed* et al. *(1997); 5. Charters* et al. *(1995); 6, Colinart* et al. (1999); 7, Ramer (1979); 8, White (1978); 9, Kühn (1960); 10, Colinart (1987).

time. Contrary to what was attempted, the ester pattern appears to be very stable despite a partial hydrolysis leading to the formation of palmitic acid and long-chain even-numbered alcohols, whereas the alkane pattern is partly modified and depleted (Colinart 1987; Evans and Heron 1993; Heron *et al.* 1994; Regert *et al.* 1999). If a drastic heating of beeswax was proved to be responsible for the total loss of alkanes, no explanation was proposed concerning the physico-chemical process involved in their degradation (Heron *et al.* 1994). Bacterial degradation has been supposed to be responsible for the decrease of hydrocarbons in ancient beeswax, but this assumption has never been seriously assessed (Colinart *et al.* 1999). Lastly, the chemical constituents responsible for the yellowish colour of beeswax have received scant attention and none of them have been studied in archaeological samples.

In order to better understand the decay of beeswax through time, laboratory degradation experiments accelerated by temperature were performed on contemporary beeswax. The chemical transformations were followed both by Fourier transform infrared spectrometry (FTIR) and by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). These experiments yield new insights into the evolution of fatty acid, *n*-alkane and ester patterns, which depend both on chemical and physical mechanisms. Lastly, low molecular weight phenolic compounds, both benzoic and cinnamic acid derivatives, have been shown to be formed during the degradation of beeswax.

These results were compared to those obtained from archaeological samples from very different environmental contexts. Samples of ancient organic remains were collected from Neolithic ceramic vessels from a waterlogged site (Bercy, France: Lanchon 2000) and from a site situated in the Drama plain in northern Greece (Dikili Tash: Treuil 1997), as well as from a Fayum portrait stored in the Louvre Museum, which was initially preserved in an Egyptian tomb characterized by a very dry and unburied environment (Aubert and Cortopassi 1998; Parlaska 1998). Their analysis allowed the identification of beeswax exhibiting various alteration patterns, the interpretation of which is enlightened by the results obtained by degradation experiments. The role of beeswax in these different sites will be examined according to the kind of ceramic vessels in which they were found, and its utilization in works of art in Egypt during the Roman period will be discussed.

MATERIALS AND METHODS

Sample description

Waxes available for degradation experiments were provided by apiarists working in different parts of France. Four different waxes, originating from the regions of Bourgogne, Gironde, Marne and the Doubs, were analysed. Yellow-brownish beeswax from the Doubs was chosen for the degradation experiment.

The archaeological samples from the Neolithic site of Bercy (Paris, France) consisted of 22 ceramic sherds collected during excavations in the 1990s (Lanchon 2000). Most of the samples are characterized by the presence of charred surface organic residues, as is usually observed in waterlogged sites (Regert *et al.* 1999). The first analytical investigation was intended to assess the preservation degree of organic matter; the analyses were performed on different sherds for which vessel morphology and size were not always known.

Different ceramic vessels were sampled in 1993 and 1995 from the Neolithic site of Dikili Tash in northern Greece (Treuil 1997). Although the pottery surface was free of visible residue, the organic matter trapped in the porous fabric was investigated. Among the five sherds studied, one belongs to a spouted vessel and another was attributed to a sieve vessel due to the presence of perforations of 2–3 mm diameter.

A micro-chip from a Louvre Museum portrait of a Fayum woman (Inv. MND 2047; AD 120–130) was also investigated. It was sampled on the pink painting material from the cloth on the right-hand side of the portrait.

The laboratory degradation experiment

Raw beeswax (20 g) was heated at 60 °C and 100 °C for 7 months in a crystallizer dish covered by a Petri dish, in order to collect the volatile products that are liable to be condensed above the beeswax (Fig. 3). Such conditions are efficient for understanding the role of temperature on beeswax degradation, associated either with an intentional human heating during Antiquity or its preservation in a warm and dry context, as is the case in Egyptian tombs. Experimental temperatures close to that of the melting point of beeswax (62–65 °C) were chosen, as well as 40 °C higher in order to accelerate the degradation process.

In order to avoid contamination, the crystallizer dishes containing beeswax were heated in a very pure bath of Fontainebleau quality sand purchased from Prolabo. The samples were analysed by FTIR, GC and GC/MS.

FTIR analysis

The IR spectra were recorded on 13 mm KBr pellets in transmission mode by a Perkin–Elmer Spectrum 2000 between 4000 and 220 cm^{-1}.

Sample preparation for GC and GC/MS analysis

Contemporary beeswaxes were extracted in dichloromethane (1 mg ml^{-1} solutions). Beeswax was rapidly dissolved in the solvent after a few minutes. A portion of this solution was derivatized by trimethylsilylation using 50 μl of BSTFA (*bis*(trimethylsilyl) trifluoroacetamide) with 1% trimethylchlorosilane added, SIGMA T 6381) at 70 °C for 30 min. After evaporation of the derivatization reagent until dryness, the sample was redissolved in dichloromethane before analysis.

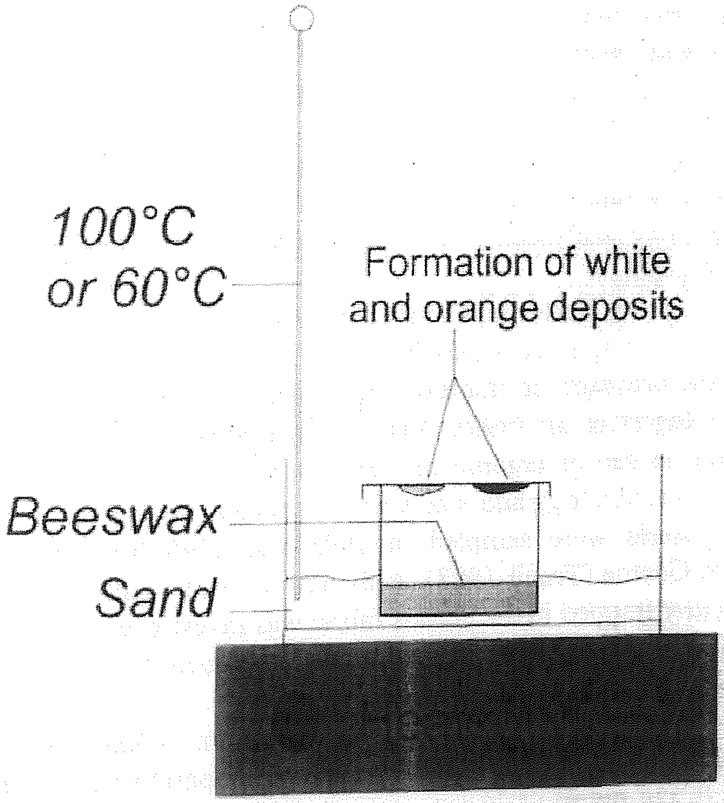


Figure 3 *The scheme of the degradation experiment of beeswax accelerated by temperature.*

Ceramic sherds and charred surface residues were prepared as described by Charters and her collaborators (Charters *et al.* 1995). The carbonized residues from Bercy were collected using a scalpel blade.

The sherd surfaces were first cleaned by scraping with a scalpel blade to remove all surface contaminations. The sherds (around 3 g) and the charred surface residues were then ground into powder in a glass mortar with a pestle. After addition of an internal standard (20 μl of a 1 mg ml^{-1} n-C$_{34}$ solution in n-hexane), the samples were extracted twice for 15 min by sonication in a chloroform/methanol solution (2:1, v/v; HPLC grade). The solution containing the solvent soluble fraction was then transferred in an adapted glass tube and centrifuged at 2000 rpm for 20 min. After evaporation until dryness under a nitrogen stream, the sample was diluted in 500 μl of a chloroform/methanol solution (2:1, v/v; HPLC grade). An aliquot (100 μl) was trimethylsilylated with 50 μl of BSTFA at 70 °C for 30 min. After evaporation, the sample was diluted in 10–50 μl of n-hexane before chromatographic analysis.

Due to its very small size and rare and precious nature, preparation of the Fayum portrait sample was reduced to the main operation: it was simply dissolved in a few drops of dichloromethane and directly injected into the gas chromatograph.

GC analysis

The GC analyses of contemporary beeswax and archaeological samples were performed on a HP 6890 chromatograph equipped with an on-column injector used in the track oven mode and a Varian CP Sil 5 CB column (15 m length, 0.32 mm internal diameter, 0.1 μm phase thickness) preceded by a 1 m precolumn. Helium was used as carrier gas, with a programmed flow as follows: 2 ml min^{-1} for 17 min; 1 ml min^{-2} until 4 ml min^{-1}; 4 ml min^{-1} for 4 min; 4–6 ml min^{-1} at a 1 ml min^{-2} rate; 6 ml min^{-1} for 16 min. The oven temperature was held at 50 °C for 1 min, ramped from 50 to 350 °C min^{-1} at 10 °C min^{-1} and held at 350 °C for 10 min. The detector was used in the FID mode at 350 °C.

The samples recovered on the Petri dish were analysed on the same chromatograph, equipped with a Varian CP Sil 8 CB column (30 m length, 0.25 mm internal diameter, 0.25 μm phase thickness) preceded by a 5 m precolumn. Helium was used as carrier gas, with a constant flow fixed at 2 ml min^{-1}. The oven was programmed at 50 °C for 2 min, from 50 °C to 320 °C at 10 °C min^{-1} and at 320 °C for 10 min. The FID detector was maintained at 350 °C.

GC/MS analysis

The GC/MS analyses were performed with a Finnigan GCQ device, equipped with an ion trap analyser linked to a HP 5890 chromatograph. Injection was performed through a split/splitless injection system used in the splitless mode and maintained at 300 °C.

Contemporary beeswax and archaeological samples were analysed with the same column and temperature programme as for GC analysis. The helium pressure was programmed as follows: 1 min at 5 psi; from 5 psi to 16.8 psi in 30 min; and 16.8 psi for 10 min. The GC/MS interface was held at 320 °C.

The degradation products recovered on the Petri dish were analysed using the same column and temperature as for GC analysis. Helium was held at a constant flow of 1.5 ml min^{-1} and the GC/MS interface was maintained at 300 °C.

Mass spectrometry was performed in the electron impact mode at 70 eV. The ion source temperature was fixed at 180 °C and the mass range was scanned from 50 to 800 in 0.6 s.

RESULTS AND DISCUSSION ON THE STUDY OF CONTEMPORARY RAW AND DEGRADED BEESWAX

Contemporary beeswax was systematically analysed before and after alteration by infrared spectrometry and gas chromatography. Although infrared spectrometry does not allow the specific identification of the different molecular constituents of beeswax, this analytical technique allows the evolution of the functional groups through time to be followed. Interpretations of the spectra obtained relied on comparison with the infrared databank already published (Nakanishi 1962) and discussions given in Wales *et al.* (1989), Mills and White (1994) and Colinart *et al.* (1999).

Most of the molecular constituents of raw and degraded beeswax and archaeological samples were identified by GC analysis, by comparison of the chromatograms obtained to those of reference standards of *n*-alkanes, fatty acids and long-chain alcohols purchased from Sigma Corporation and Carlo Erba. When necessary, these assignments were confirmed by GC/MS analyses. Mass spectra interpretations were based on searching on the NIST database, mainly in the case of phenolic compounds formed by degradation of beeswax, and on the detection of the fragmentation mechanisms for the main homologous components (especially fatty acids, *n*-alkanes, alcohols and long-chain esters). However, due to the use of an ion trap analyser, the mass spectra of the TMS alcohols were characterized by particular rearrangements, which will be discussed below.

Raw contemporary beeswax

All of the raw contemporary beeswaxes analysed were found to be very pure and free of any other waxy material such as paraffin, which may be used in apiary activities. The four samples from different geographical origins presented very similar chromatographic patterns (Fig. 4), in spite of small differences observed in the percentages of the different chemical homologous families (23–25% *n*-alkanes, 2–6% free fatty acids and 49–53% long-chain esters). These results are consistent with the data that have previously been published (Tulloch and Hoffman 1972; Donadieu and Marchiset 1984; Charters *et al.* 1995).

Infrared spectra were also very similar for each raw beeswax studied (Fig. 5). Absorption bands at 720 and 730 cm^{-1} are characteristic of $\delta(CH_2)_n$ plane rotation of linear long carbon chains, which are common to all the long-chain fatty acids, *n*-alkanes and esters present in beeswax. Other bands at 1377, 1464, 1473, 2850, 2918 and 2950 cm^{-1} are also characteristic of hydrocarbon chains. The presence of carboxyl groups from fatty acids and esters are, respectively, responsible for absorption bands at 1711 and 1737 cm^{-1}. The quite intense and large band at 1175 cm^{-1} is relevant to the ν(C–O) vibration of esters.

Physico-chemical degradation processes of alkanes and esters

Heating beeswax for two days at 100 °C resulted in the formation of a white deposit on the Petri dish placed upon the crystallizer dish (Fig. 3). Three days later, another deposit, this time orange-coloured, was also observed. These two deposits were separately sampled and analysed by infrared spectrometry in KBr pellets and by GC and GC/MS after extraction in dichloromethane and trimethylsilylation.

The infrared spectrum obtained on the white residue condensed on the Petri dish was quite similar to that of raw beeswax, especially in the case of C–H bonding (Fig. 5). However, small differences were observed on the ν C=O absorption band of fatty acids which is located at 1703 cm^{-1} for the white residue, instead of 1711 cm^{-1} for raw beeswax. Furthermore, the band at 1737 cm^{-1}, characteristic of ester C=O vibration, present on the beeswax spectrum, was not observed on the white residue spectrum. Although these results do not allow precise

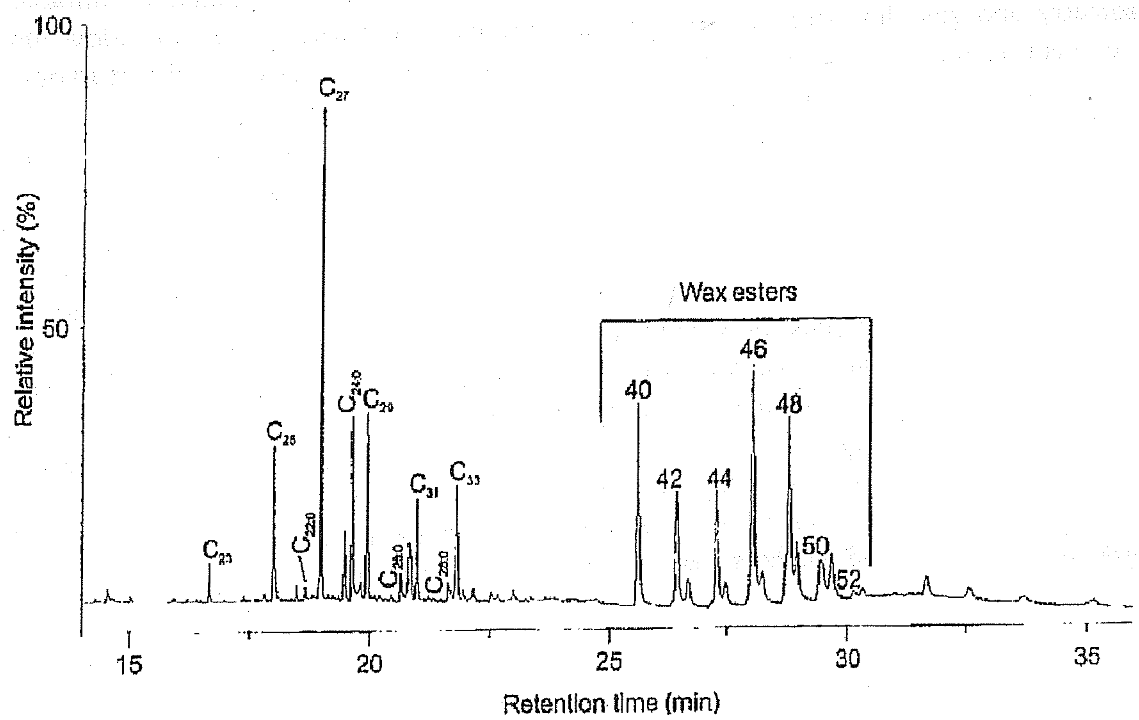


Figure 4 *A characteristic gas chromatogram of contemporary trimethylsilylated contemporary beeswax (from the Doubs, France). C_x corresponds to the n-alkanes containing x carbon atoms; $C_{y:0}$ corresponds to free fatty acids with y carbon atoms.*

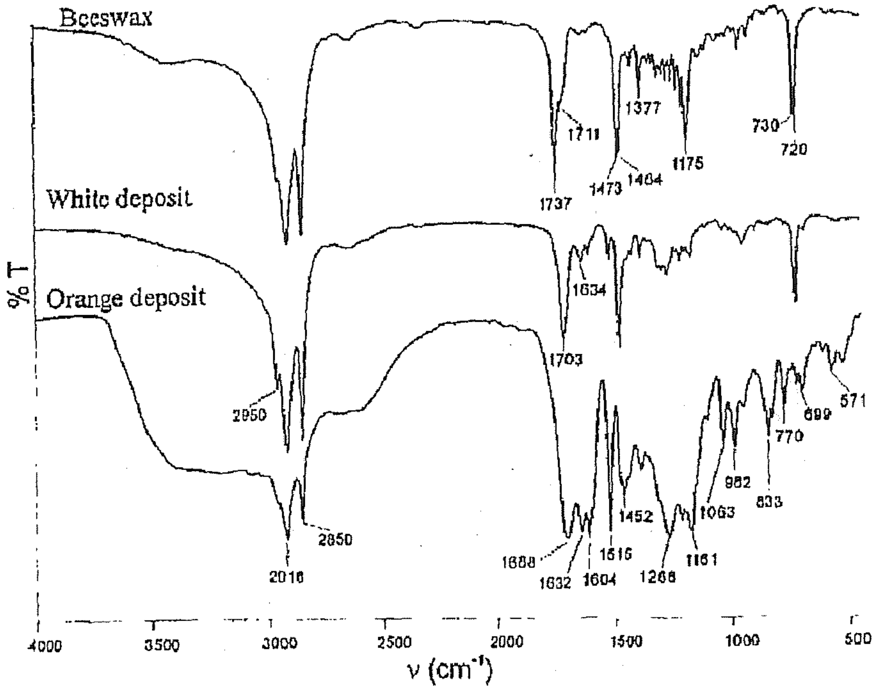


Figure 5 *The infrared spectra of a raw contemporary beeswax from the Doubs (France) and of the white and orange deposits condensed above the beeswax heated at 100 °C.*

identification of the molecular composition of the white residue, they are indicative of the presence of long-chain aliphatic constituents and free fatty acids. In addition, this white residue seems to be characterized by the absence of esters.

Gas chromatography of this residue revealed the presence of six main compounds assigned to palmitic acid and to a series of odd-numbered linear hydrocarbons in the range C_{21}—C_{29}, *n*-tricosane and *n*-pentacosane being predominant (Fig. 6). The difference in fatty acid composition between raw beeswax and the white residue may thus explain the shift of the C=O fatty acid band on the infrared spectrum (Fig. 5). The total absence of esters suspected on the basis of infrared spectrometry was also confirmed.

Although the *n*-alkane distribution in the white residue differs from that known in beeswax, their odd-numbered linear skeleton strongly indicates that they were vaporized from beeswax heated at 100 °C and directly condensed on the cold Petri dish placed over the crystallizer dish. It must be noted that *n*-hentriacontane (C_{31}) and *n*-tritriacontane (C_{33}) identified in beeswax were not vaporized. The distribution of the smallest length carbon-chain compounds, which are over-represented in comparison to those of beeswax, can be explained by the fact that, among homologues, volatility decreases with increasing molecular weight.

The formation of condensed residues was much slower during the experiment performed at 60 °C, since a white deposit was only observed after 6 months. Although it was supposed that an extreme heating was necessarily responsible for the loss of *n*-alkanes (Heron *et al.* 1994; Evershed *et al.* 1997), these experiments show that the phenomenon also occurs by heating at only 100 °C or 60 °C.

The formation of phenolic compounds during beeswax degradation

Contrary to the white residue, the orange residue formed on the Petri dish provided a very complex infrared spectrum, strongly different from that of the raw contemporary beeswax

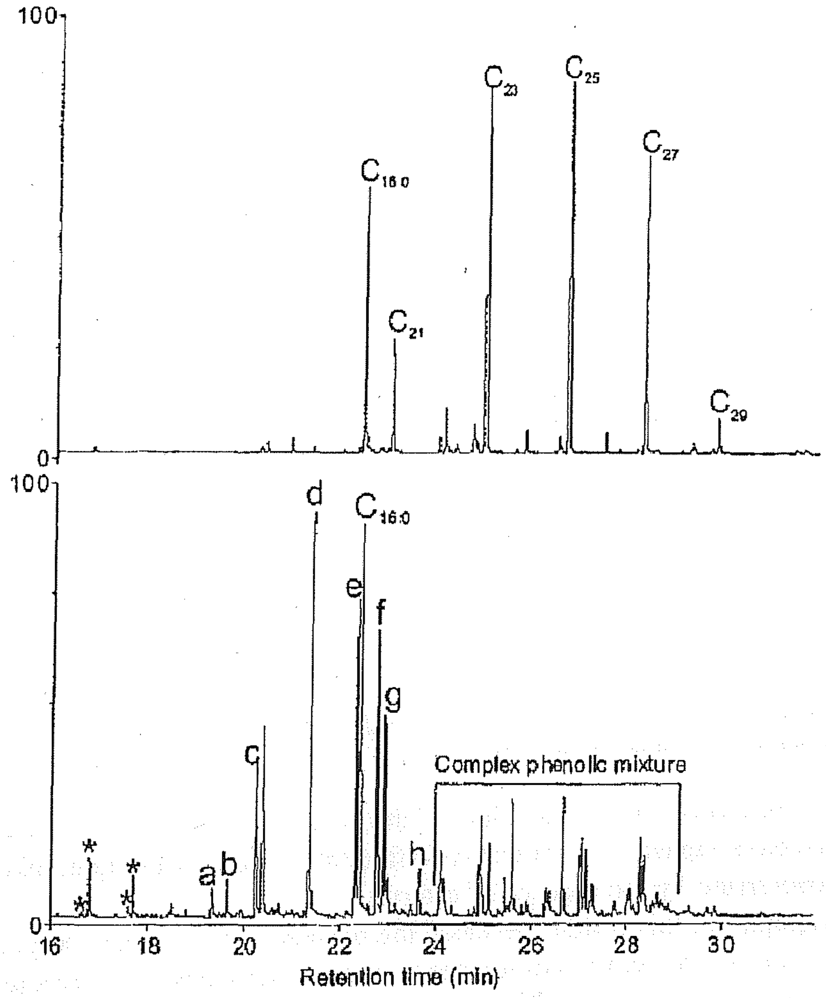


Figure 6 *Gas chromatograms of the white (top) and orange (bottom) trimethylsilylated deposits condensed above the beeswax heated at 100°C. C_x, n-alkane containing x carbon atoms; $C_{16:0}$, palmitic acid; *, benzoic derivatives; letters a–h refer to phenolic compounds detailed in Table 1.*

(Fig. 5). Apart from the C–H vibration bands at 2956, 2918 and 2850 cm^{-1}, the spectrum shows a complex pattern in the range 1700–450 cm^{-1}. Although these bands were not precisely assigned, their pattern is consistent with the presence of aromatic compounds because of (i) absorption bands in the region 1700—1600 cm^{-1} corresponding to δ CH out-of-plane vibration of substituted groups, (ii) weak and sharp in-plane bending bands (1225—950 cm^{-1}) due to hydrogen atoms on the phenyl group and (iii) out-of-plane bending bands of these same chemical bonds below 900 cm^{-1} (Nakanishi 1962).

Palmitic acid was the only molecular constituent clearly identified by its retention time on the chromatogram of the trimethylsilylated orange deposit (Fig. 6). GC/MS analyses were thus performed to elucidate the chemical composition of this deposit: most of the compounds were found to be phenolic derivatives, as revealed by mass spectrometry. The constituents eluting between 16 and 20 min were attributed to benzoic acid derivatives, two of them being clearly identified by a mass spectrometry library search as trimethylsilylated vanillic and protocatechuic acids (Table 1). The following components, characterized by retention times ranging from 20 to 24 min, were identified as trimethylsilylated derivatives of cinnamic acids (Table 1). In particular, trimethylsilylated isoferulic, ferulic and caffeic acids were identifed.

Table 1 *Mass spectra and formulae of benzoic and cinnamic derivatives formed by degradation of raw beeswax*

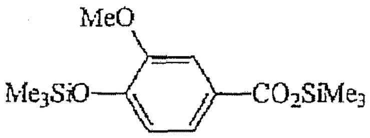
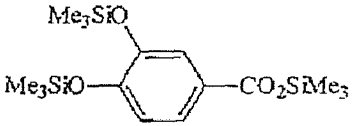
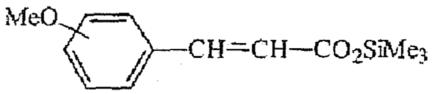
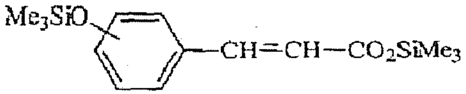
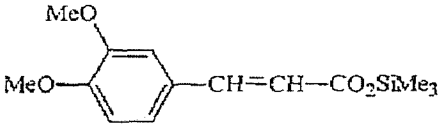
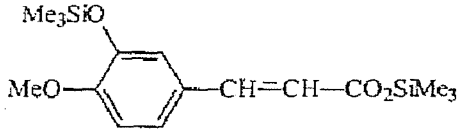
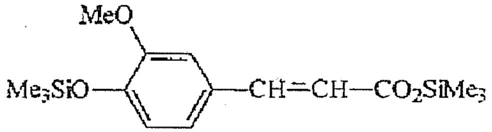
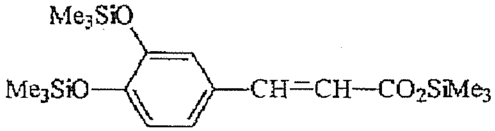
Peak number	*Mass spectrum*	*Chemical name*	*Molecular weight*	*Molecular formula*	*Structures*
a	181(31)-223(13)-267(80)-282(34)-297(100)-312(40)	Vanillic acid, *bis*-TMS derivative	312	$C_{14}H_{24}O_4Si_2$	
b	193(100)-239(8)-281(5)-355(43)-370(46)	Protocatechuic acid, *tris*-TMS derivative	370	$C_{16}H_{30}O_4Si_3$	
c	133(12)-161(43)-191(54)-235(100)-250(38)	m- or p-methoxycinnamic acid, TMS derivative	250	$C_{13}H_{18}O_3Si$	
d	73(19)-219(24)-249(43)-293(100)-308(47)	m- or p-trimethylsilyl ester, coumaric acid	308	$C_{15}H_{24}O_3Si_2$	
e	191(54)221(56)-265(100)-280(70)	3,4-dimethoxycinnamic acid, TMS derivative	280	$C_{14}H_{20}O_4Si$	
f	73(10)-179(7)-191(8)-219(18)-233(11)-249(29)-264(19)-279(6)-293(67)-308(66)-323(82)-338(100)	Isoferulic acid, *bis*-TMS derivative	338	$C_{16}H_{26}O_4Si_2$	

Table 1 *(continued)*

Peak number	*Mass spectrum*	*Chemical name*	*Molecular weight*	*Molecular formula*	*Structures*
g	73(11)-179(6)-191(8)-219(11)-233(3)-249(30)-264(5)-279(19)-293(65)-308(69)-323(100)-338(99)	Ferulic acid, *bis*-TMS derivative	338	$C_{16}H_{26}O_4Si_2$	
h	73(7)-191(7)-219(100)-249(9)-293(4)-381(27)-396(63)	Caffeic acid, *tris*-TMS derivative	396	$C_{18}H_{32}O_4Si_3$	

At longer retention times, a very complex chromatographic pattern is composed of phenolic constituents that still have to be fully identified.

This is the first time that such cinnamic and benzoic derivatives have been shown to be formed by beeswax degradation. The main constituents of beeswax, including *n*-alkanes and esters, cannot be the precursors of such components. Beeswax is known to also contain several flavonoids that are responsible for its colour, as minor constituents (Tomàs-Barberàn *et al.* 1993). These flavonoids, characterized by a phenolic structure, may be considered as the precursors of benzoic and cinnamic derivatives identified on the condensed deposit formed above heated beeswax. Such results should hereafter be taken into account in the interpretation of amorphous organic components from archaeological residues. Indeed, although these components are relatively sensitive to oxidation processes, their presence should be considered as possibly resulting from beeswax alteration.

A comparison of heated beeswax and vaporized organic residues

In order to compare the molecular composition of the deposits condensed on the Petri dish, the beeswax heated at 60 °C and 100 °C was also analysed by infrared spectrometry, GC and GC/MS. The results do not show any significant difference between beeswax before and after heating, despite the vaporization of some of its constituents. This may be explained by the low amount (a few mg) of the matter that has been vaporized compared to the total quantity of beeswax present in the crystallizer dish (20 g). Nevertheless, it must be noted that very low amounts of even-numbered long-chain linear alcohols (C_{26}—C_{34}) appeared in beeswax when heated at 100 °C. These compounds were identified by their mass spectrum, which presents a particular pattern when analysed by an ion trap mass spectrometer (Fig. 7). All these spectra reveal a minor peak at $[M\text{-}15]^+$. The base peak $[M\text{-}15+18]^+$ may be interpreted as resulting from a molecular rearrangement due to a molecule–ion reaction between the residual H_2O and the $[M\text{-}15]^+$ ion. The formation of alcohols in the heated beeswax and the presence of palmitic acid condensed on the Petri dish give rise to the assumption that partial hydrolysis of the wax esters occurred, as mentioned before (Charters *et al.* 1995; Evershed *et al.* 1997). Furthermore,

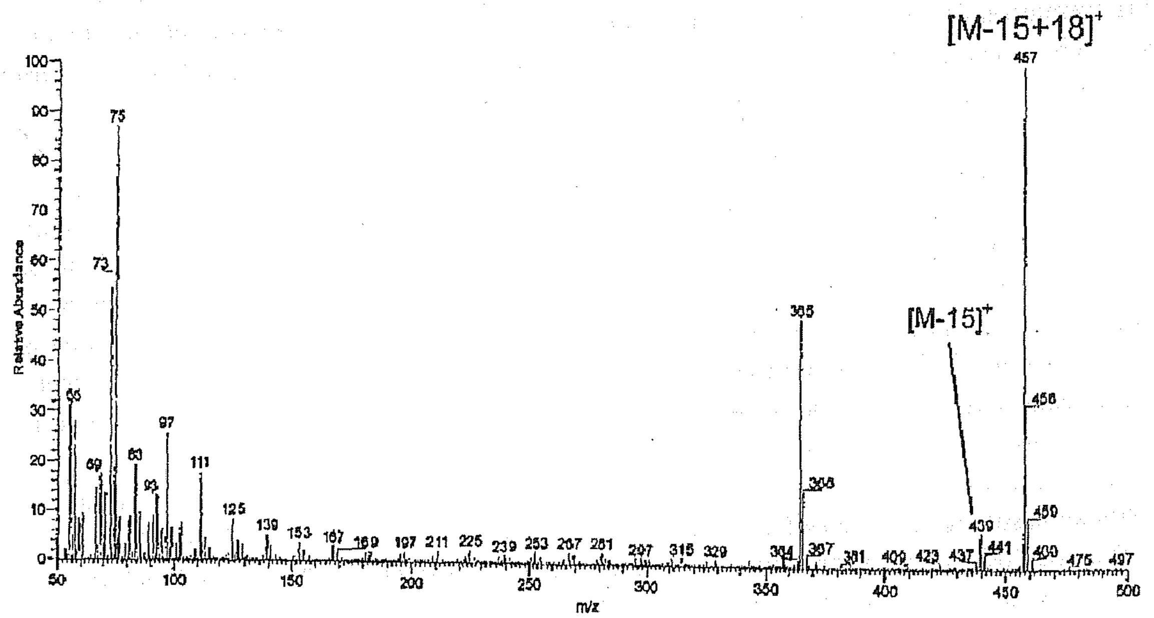


Figure 7 *The mass spectrum of the trimethylsilyl derivative of ceryl alcohol (C_{26}) obtained with an ion trap analyser.*

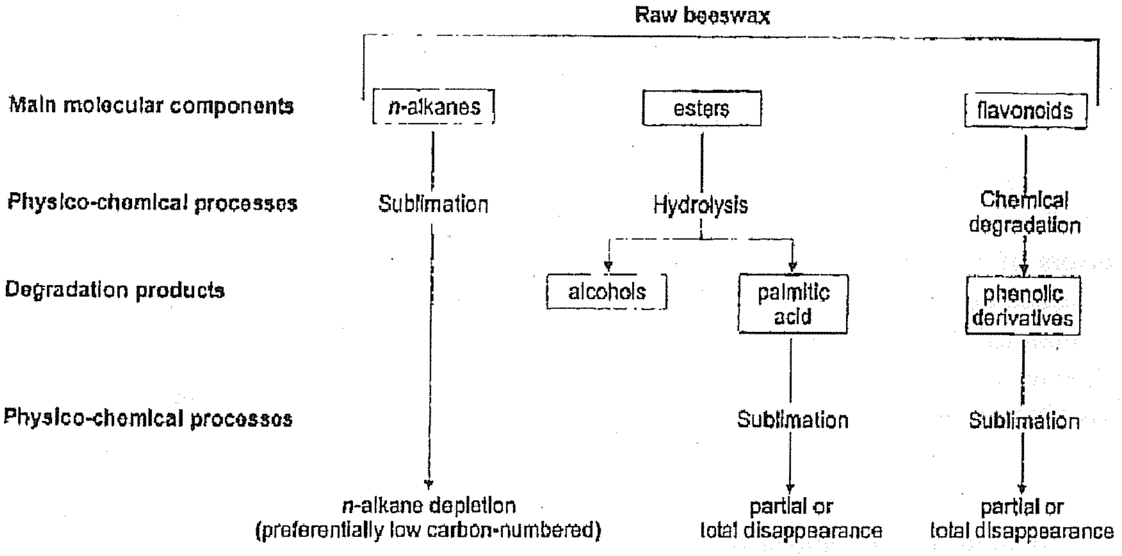


Figure 8 *A summary of the physico-chemical mechanisms involved in beeswax degradation.*

it must be pointed out that palmitic acid formed by wax ester hydrolysis was not detected in heated beeswax but only in the condensed residues, which shows that this fatty acid is vaporized as soon as it is formed because of a melting point (63–64 °C) close to that of *n*-heptacosane and *n*-nonacosane.

The general interpretation of the degradation experiments

Degradation experiments of beeswax accelerated by temperature revealed the various processes involved in its modification through time: both chemical and physical mechanisms may be identified (Fig. 8). First of all, hydrolysis of esters—already discussed elsewhere (Charters *et al.* 1995; Evershed *et al.* 1997)—was observed, and chemical transformation of flavonoids, initially present in beeswax, into smallest phenolic compounds was shown for the first time as far as we know. Although the physico-chemical mechanisms of this transformation are still unclear, the possible formation of benzoic and cinnamic acid derivatives in beeswax should not be ignored. Indeed, such aromatic components are widely distributed groups of substances in the plant kingdom (Bravo 1998) and their potential linkage to beeswax should now be taken into account. On the other hand, sublimation of molecular compounds, either initially present in raw beeswax or formed by hydrolysis of esters and by chemical degradation of flavonoids, may occur. In particular, this phenomenon is responsible for a differential depletion of *n*-alkanes, which occurs after a few days at 100 °C and after a few months at 60 °C. The preferential loss of smallest *n*-alkanes may induce a modification of the *n*-alkane profile through time, as has been observed by several authors (Evershed *et al.* 1997) or the total depletion of these compounds (Heron *et al.* 1994). This phenomenon has been shown to be very rapid at 100 °C (a few days), whereas it began only after 6 months at 60 °C. The decreasing rate of depletion of hydrocarbons with temperature led us to think that at lower temperatures, such as 30 °C or 40 °C—as in Egypt—the phenomenon still exists and may be noticeable after several centuries or millenia. It can thus be assumed that beeswax preserved in a dry and warm environment may be subject to a partial loss of hydrocarbons, even without being intentionally heated. Furthermore, the sublimation of palmitic acid formed by hydrolysis of wax esters may explain the absence of this component in archaeological samples containing beeswax, in which alcohols, resulting from hydrolysis of wax esters, are present.

THE IDENTIFICATION OF BEESWAX IN ARCHAEOLOGICAL SAMPLES

Among the amorphous organic archaeological samples examined, different vessels as well as funerary portraits yielded beeswax in a more or less altered state. Beeswax was indeed undoubtedly identified in four ceramic vessels from the French Neolithic site of Bercy, in one ceramic from the Greek Neolithic site of Dikili Tash and in a Fayum portrait from the Roman period in Egypt.

Beeswax in ceramic vessels from the Neolithic site of Bercy

The site of Bercy, located in the south-west of Paris *intra muros*, was discovered in 1990 during the urban reorganization of the right bank of the River Seine. A rescue excavation carried out in the 1990s revealed a Neolithic village separated from the river by a fence that was at least 53 m long (Lanchon 2000). The site was occupied during the Middle and Late Neolithic periods (4600–3600 BC and 3500–3000 BC, respectively) and at the beginning of the Iron Age (about 500 BC). Pottery material was very abundant on the site: the number of different vessels was estimated at 1500. Some of them are characterized by the presence of charred surface residues. The purpose of our study of this site was to assess the degree of preservation of organic matter in ceramic vessels. Out of the 22 sherds analysed, four can be directly associated with the use of beeswax (Table 2). The chromatographic pattern obtained on the charred surface residues was very complex (Fig. 9), revealing the presence of a large number of molecular constituents. GC analysis allowed the identification of palmitic and stearic acids and of palmitic wax esters in the range C_{40}–C_{52}, whereas the compounds eluting between 16 and 24 min were identified by GC/MS. These analyses revealed the distribution of homologous components of three distinct classes, including odd-numbered *n*-alkanes from 23 to 33 carbon atoms, long-chain even-numbered alcohols and free fatty acids, respectively, in the range C_{24}–C_{34} and C_{20}–C_{34}. The ester and long-chain hydrocarbon distributions were identical to those known in raw contemporary beeswax (Fig. 10). The acid distribution, on the other hand, was broader than that observed in contemporary beeswaxes that we analysed. However, this distribution with the tetracosanoic acid ($C_{24:0}$) predominant, is compatible with the natural variability of free fatty acid distribution in beeswax (Tulloch and Hoffman 1972). The high percentage of palmitic acid, associated with long-chain alcohols ranging from C_{24} to C_{34}, is indicative of the hydrolysis

Table 2 *Quantitative results of the sherds from Bercy containing beeswax*

Archaelogical reference	*Lab. number*	*Sample description*	*Sample weight ground for analysis (g)*	*TLE ($\mu g\ g^{-1}$)*
CAPVAL III, Sect. 4, CVII.56-57 .C11	OD3006C	Charred residue	0.3265	2065
	OD3006D	Ceramic sherd	3.7655	10
CAPVAL III, CVII.56.C2	OD3017C	Charred residue	0.1149	1690
	OD3017D	Ceramic sherd	2.0753	67
CAPVAL III, Sect. 1, CV.22.C2	OD3020C	Charred rsidue	0.2201	460
	OD3020D	Ceramic sherd	2.8712	103
CAPVAL III, CVI.27.C3	OD3042C	Charred residue	0.0874	3203
	OD3042D	Ceramic sherd	1.9454	0

TLE, total lipid extract.

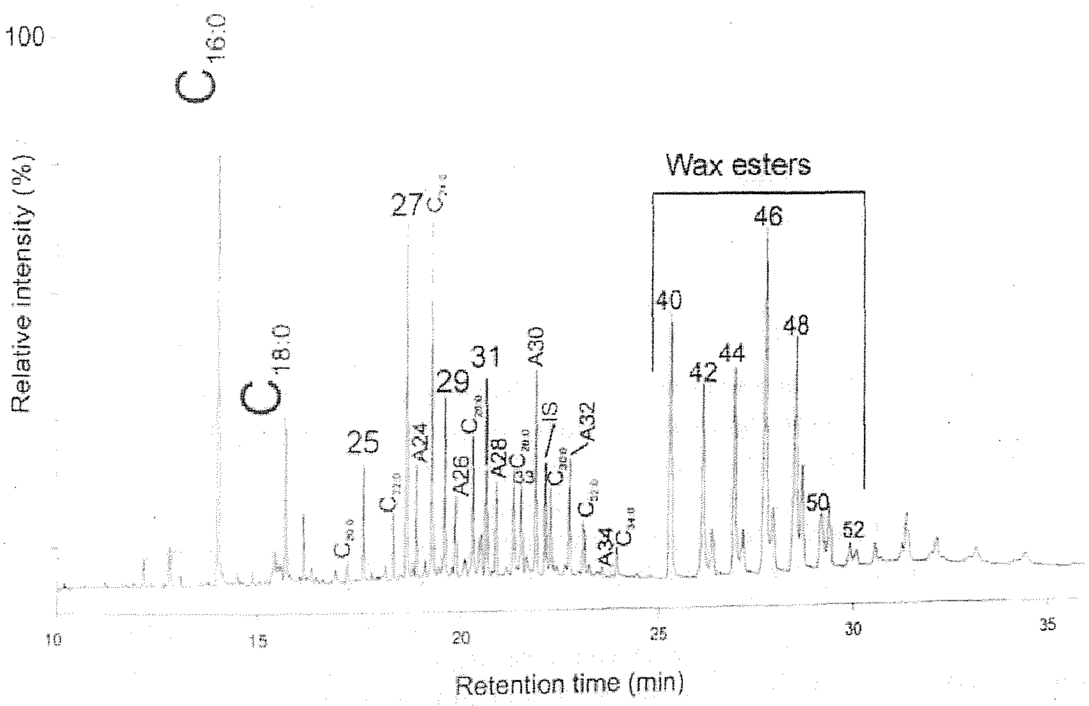


Figure 9 *A gas chromatogram of the trimethylsilylated charred surface residue OD3006C1 from Bercy, showing the presence of altered beeswax. Numbers between 25 and 33 correspond to n-alkanes; Ax corresponds to linear long-chain alcohols containing x carbon atoms and $C_{y:0}$ corresponds to long-chain fatty acids with y carbon atoms. IS, internal standard.*

of esters that occurred through time (Evershed *et al.* 1997). The presence of stearic acid and unsaturated C_{18} fatty acids may be attributed to a fatty matter that has been mixed with beeswax, as noted by other authors (Charters *et al.* 1995). While the concentration of organic matter was quite significant in charred surface residues from Bercy that contained beeswax (Table 2), the associated sherds revealed relatively low amounts of organic matter. Furthermore, even though beeswax was undoubtedly detected and identified in the charred surface residues of four sherds,

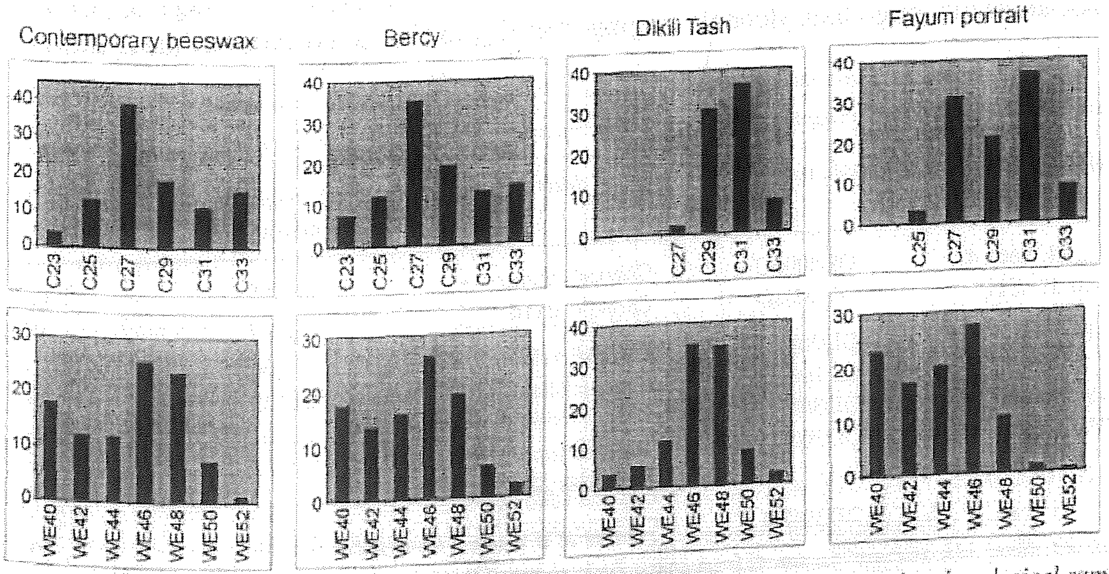


Figure 10 *The relative abundances of n-alkanes and esters from contemporary beeswax and archeological samples from different contexts. C_x corresponds to n-alkanes with x carbon atoms. WEy corresponds to wax esters containing y carbon atoms.*

it penetrated into the porous fabric of only one sherd (Table 2). This difference between the composition of the sherds and their associated charred residues is still difficult to understand, but may be due either to a particular use of the vessel or to a waterproofing treatment.

Beeswax in Neolithic Greek pottery

The tell of Dikili Tash was first inhabited at the beginning of the Late Neolithic period, around 6800–6700 BP (5450–5350 BC). Known since the 19th century in the Drama plain (eastern Macedonia), the site has been partly excavated by a French team since 1986 (Treuil 1997). Most of the ceramics do not present any visible residue on their surface. Preliminary chemical analyses were performed on a few samples to look for any organic matter that was possibly trapped in the porous matrix. Five sherds sampled in the sector excavated by the French team in 1993 and 1995 were examined in the laboratory. One of them (Ref: OD3037, sect. V/E, excavation 93, square 313 102, sac 31, no. 4) revealed a particular chromatographic profile (Fig. 11). GC/MS analyses led to the identification of palmitic acid and four series of homologous compounds, including odd-numbered *n*-alkanes in the range C_{27}–C_{33}, the *n*-hentriacontane (C_{31}) being predominant, long-chain alcohols with an even carbon number ranging from C_{22} to C_{34}, wax esters containing an even carbon number from C_{40} to C_{52}, and even-numbered free fatty acids in very low amount in the range C_{24}–C_{28}.

All of the different homologous compounds identified in the Dikili Tash sample are known in raw or degraded beeswax. However, it must be pointed out that *n*-alkane and wax ester profiles differ sensitively from the chromatographic pattern of raw contemporary beeswax (Fig. 10). The lowest molecular weight hydrocarbons (C_{23} and C_{25}) disappeared and the usual major hydrocarbon in beeswax, *n*-heptacosane, was largely depleted. This is consistent with

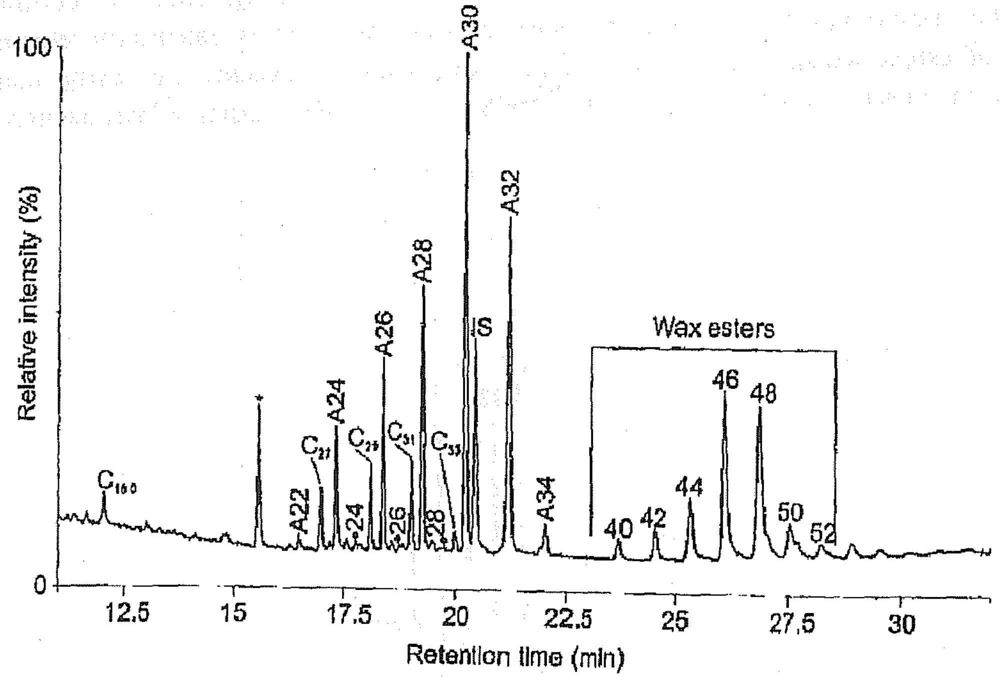


Figure 11 *A gas chromatogram of the organic content of a ground trimethylsilylated sherd OD3037D1 (Sect. V/E, fouille 93, carré 313, 102, sac 31, no. 4) from Dikili Tash, showing the presence of altered beeswax. C_x corresponds to n-alkanes with x carbon atoms; Ay corresponds to linear long-chain alcohols containing y carbon atoms. $C_{16:0}$ is palmitic acid. The black disc with a number is, for long-chain fatty acids, the number corresponding to the carbon atom number. IS, internal standard; *, plasticizer.*

the results obtained from beeswax submitted to accelerated ageing, in that they show a differential vaporization of *n*-alkanes depending on their carbon number.

More surprisingly, alteration of the distribution of wax esters has also occurred. Indeed, the wax ester profile presents a depletion of the lowest molecular weight components. The high amount of alcohols (more than 50% of the total lipid extract) clearly indicates that esters have been hydrolysed. The low amount of palmitic acid, also released by ester hydrolysis, may be explained by a sublimation phenomenon of this compound, similar to that observed on *n*-alkanes. This modification of the wax ester profile seems to be due to the preferential release of shorter-chain alcohol moieties. This phenomenon has also been observed during degradation experiments of milk triacylglycerides, in which the degradation rate of the smallest triglycerides is higher than for that of longer-chain compounds (Dudd and Evershed 1998; Dudd *et al.* 1998).

The identification of beeswax in Egyptian funerary painting from the Roman period

At the end of the 2nd century AD—in other words, at the beginning of Roman domination—an original funerary ritual appeared in the Fayum area in Egypt, where traditional masks associated with mummies were replaced by painted portraits (see, for example, Parlaska 1998). During a restoration campaign of such portraits from two collections stored in the Louvre Museum (Paris) and Dijon Museum of Fine Arts, the analyses performed on micro-samples allowed the identification of beeswax in many of them. In particular, the sample taken from the woman portrait provided a chromatogram (Fig. 12) dominated by wax esters in the range C_{40}–C_{52}, presenting a characteristic profile of beeswax as shown by Figure 10. A series of odd-numbered *n*-alkanes was also identified, ranging from C_{25} to C_{33}, the *n*-hentriacontane (C_{31}) being predominant. As in the case of the sample from the Dikili Tash site, this long-chain hydrocarbon profile may be explained by a loss of alkanes through time by sublimation. Although the sample was not derivatized before analysis, the alcohols potentially released by hydrolysis of esters would have been detected, since such compounds are easily analysed after direct injection (Grappin-Wsevolojsky 1998, unpublished report). Consequently, the

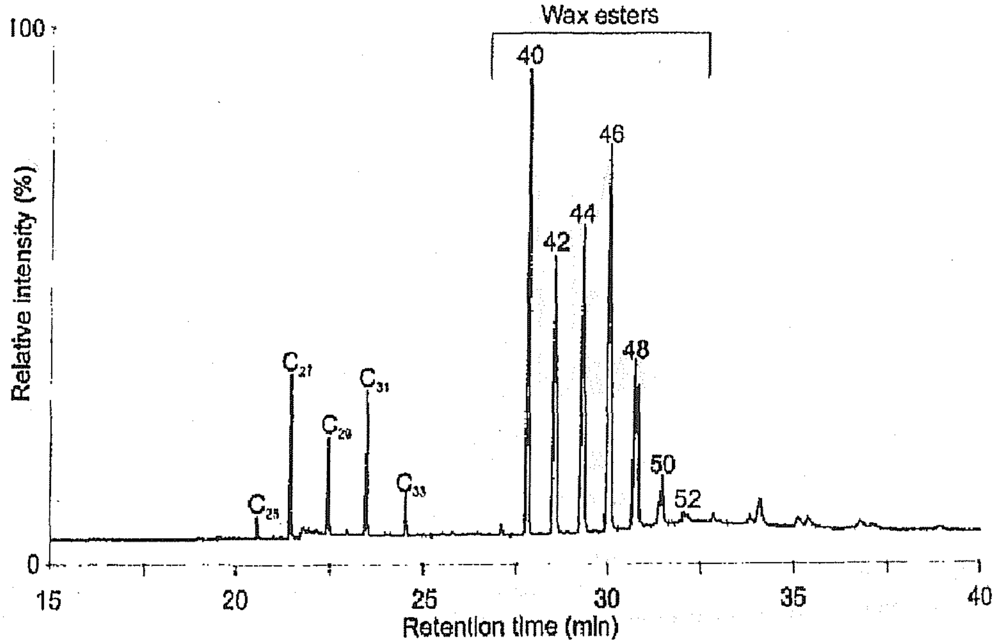


Figure 12 *A gas chromatogram of the organic binder from a Fayum woman portrait (Louvre Museum, Inv. MND 2047, 2nd century AD), showing the presence of altered beeswax. C_x corresponds to n-alkanes with x carbon atoms.*

absence of alcohols and the unmodified ester profile lead to the assumption that beeswax used in Fayum portraits has not undergone hydrolysis, but has been submitted only to the physical process of sublimation.

THE INFLUENCE OF THE NATURAL CONTEXT ON BEESWAX PRESERVATION

In the course of this research, beeswax was encountered in various natural and archaeological contexts: (i) clayey alluvial deposits continually saturated with water (Bercy); (ii) an alluvial cone situated near an ancient swamp (Dikili Tash); and (iii) a dry and warm environment in an Egyptian tomb (the Fayum portrait).

Whatever the natural and archaeological contexts in which beeswax was identified, several common features were noted on the chromatographic profile of the samples. First, the samples from Bercy, Dikili Tash and the Fayum portrait were all found to contain odd-numbered *n*-alkanes and even-numbered wax esters in the range C_{40}–C_{52}. Furthermore, the profile of esters appears to be very stable through time, even when hydrolysis occurs. Generally, long-chain alcohols and palmitic acid are identified as the degradation molecular compounds formed by ester hydrolysis. A modification of the wax ester profile was observed in a single sample, from the site of Dikili Tash, in which the lower molecular weight wax esters (mainly C_{40}, C_{42} and C_{44}) were depleted. However, all the esters present in beeswax appear to be partially preserved. This stable wax ester distribution in beeswax was already reported in various archaeological contexts dating back from the Neolithic period to the Middle Ages (Evans and Heron 1993; Heron *et al.* 1994; Charters *et al.* 1995; Evershed *et al.* 1997; Regert *et al.* 1999). Contrary to the wax ester pattern, the long-chain hydrocarbon profile is more sensitive to an evolution, leading to the loss of the lowest molecular weight compounds. This has been observed in the samples from the Fayum portrait and the Dikili Tash ceramic vessel. These results, in comparison with those of the degradation experiments described above, give rise to the assumption that *n*-alkanes were partly sublimated after having been withdrawn on the site during ancient times. This must be the case regarding the Fayum portrait that had been preserved in a very dry environment, but it must also be pointed out that beeswax could have been heated during Antiquity before use. In particular, it is known that beeswax used in paintings was whitened in sunlight before use (Pliny the Elder, 1969). In the case of beeswax identified in the ceramic strainer from Dikili Tash, it is still difficult to know whether sublimation occurred during the Neolithic period because of heating or under the action of diagenetic processes. On the other hand, the alkane profile was found to be very well preserved in the ceramic vessels from Bercy, because of the waterlogged context of the site. However, this observation is surprising, since the residues in which beeswax was identified were clearly carbonized. Comparatively, beeswax has already been identified in charred surface residues of pottery from a Neolithic lacustrine context at the site of Chalain (Jura, France) and was found to present an altered *n*-alkane profile in which C_{27} and C_{29} linear hydrocabons were identified (Regert *et al.* 1999). These features obtained on beeswax preserved in two comparable contexts seem to indicate that the beeswax from Bercy was not strongly heated, whereas the firing treatment was more extensive at Chalain.

THE USE OF BEESWAX DURING THE NEOLITHIC PERIOD AND ANTIQUITY

Beyond leading to an understanding of physico-chemical mechanisms involved in beeswax alteration through time, the results obtained from the archaeological samples provide new information concerning the use of this material during different periods.

First of all, to our knowledge, the identification of beeswax in the Neolithic ceramic vessel from Dikili Tash corresponds to the oldest use of beeswax directly attested to in Greece. Previous analytical studies of ceramic vessels from the site of Mochlos in Crete have already shown that beeswax was used early on in this part of the world (Evershed *et al.* 1997), but the remains of beeswax dating from the Neolithic period were only known in Germany (Evans and Heron 1993; Heron *et al.* 1994) and France (Regert *et al.* 1999; Regert *et al.* in press).

It must be noted that beeswax identified at the site of Dikili Tash was associated with a particular ceramic vessel pierced with holes of about 2–3 mm in diameter. This type of ceramic sherd, generally considered to come from a strainer, was quite abundant at the site, but complete examples were rarely encountered. It is thus difficult to assess the exact size and shape of the vessel that provided the sherd. More or less complete pierced vessels were also discovered at the neighbouring contemporary settlement of Sitagroi (Renfrew *et al.* 1986, 423 and plate XCVIII) and it may be assumed that the sherd that we studied belonged to such a type of vessel, generally called a strainer or sieve (Renfrew *et al.* 1986, 368, 397). This discovery may be compared to what was reported by Bonet Rosada and Mata Parreño in 1997 concerning the use of a funnel and a grass strainer in hive product processing. Indeed, 'until recently, in the area of Jumilla-Yecla (Murcia), honey was pressed over a funnel using a strainer of esparto grass (*Spita tenacissima)* on top to filter solid particles from the honey when extracting it from the honeycombs' (Bonet Rosada and Mata Parreño, 1997, 44). Although the use of a strainer is described for honey filtering, it may also have been used for beeswax or for the separation of beeswax and honey. Because this is the first report of beeswax associated with a ceramic strainer, this hypothesis has to be considered cautiously. Nevertheless, it establishes new guidelines of investigation concerning the interpretation of the function of pottery.

To our knowledge, Bercy is the third Neolithic site in Western Europe to provide ceramic vessels containing beeswax. The increase of organic analyses performed on ceramic vessels seems to show that beeswax was widely used for a long time. Nevertheless, it is still difficult to clearly understand the function of this material in ceramic vessels. It may have been used to waterproof the porous matrix of ancient pottery, as suggested by several authors (Heron *et al.* 1994; Charters *et al.* 1995), but this proposition has still to be fully demonstrated. Another possibility is that beeswax was part of culinary preparations containing honey. Lastly, the role of beeswax in medicines for its therapeutic properties was perhaps also known by Neolithic people.

The identification of altered beeswax in one of the Fayum portraits confirms the use of this material in Roman paintings. However, further laboratory experiments will be necessary in order to fully distinguish the natural decay of beeswax from a chemical modification due to the anthropogenic transformation of this material performed before its use in painting (Pliny the Elder, 1969).

CONCLUSIONS

This study provides new information concerning the physico-chemical mechanisms involved in beeswax degradation through time and supports the evidence for the wide use of beeswax as early as the Neolithic period. Indeed, the modification of hydrocarbon profiles in beeswax, characterized by a preferential loss of the lower molecular weight *n*-alkanes, may be clearly attributed to a sublimation process induced either by the deliberate heating of beeswax during use or by preservation in a dry and warm context. Hydrolysis of esters, leading to the formation of even-numbered alcohols and palmitic acid, was observed during our experiments. It was also noticed that palmitic acid sublimated until a partial or total depletion. Lastly, phenolic compounds were not identified in any archaeological sample containing beeswax, but their formation was shown as possible during beeswax degradation. These constituents, both benzoic and cinnamic acid derivatives, may be formed by the degradation of flavonoids that are naturally present in beeswax. Such components are widely distributed in the plant kingdom, but we cannot ignore the fact that they may also be formed during beeswax alteration. The interpretation of results obtained from amorphous organic archaeological remains should henceforth take this new fact into account.

Analysis of a series of archaeological samples led to the identification of beeswax in ceramic vessels from Bercy (France) and Dikili Tash (Greece), two Neolithic sites, as well as in a Fayum portrait (Egypt) dated to the Roman period. A useful correlation between the chromatographic pattern of archaeological beeswax and its preservation context could be drawn. Furthermore, the association of beeswax with a ceramic strainer in Greece is indicative of the use of such a vessel in the processing of bee products.

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