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Abstract: Thermo analytical techniques and gas chromatography/mass spectrometry (GC/MS) were used to evaluate the presence of chemical-physical interactions between pine pitch and beeswax used as additive The mixtures found in several archaeological objects demonstrate that by modifying the physical and chemical properties of pitch and tar, our ancestors were able to add a variety of organic materials, such as waxes or animal fats.We studied pine pitch replicas from Pinus sylvestris prepared following a test from the field of experimental archaeology. Varying proportions of beeswax were added and then the resulting pitches were studied by a multi-analytical approach comprising the use of thermo analytical techniques (DSC, TG and TG-FTIR) and GC/MS, which provides molecular information. The same approach was also used to study a mixture of pitch from Pinus sylvestris L. and beeswax ("Zopissa"), whose relative proportions were unknown, and two archaeological adhesives collected from glass opus sectile fragments found in the northern necropolis of Antinoopolis (Egypt, 4th-5th century AD). Our thermo-analytical techniques managed to determine the relative proportion of pine pitch and additives, such as beeswax, in unknown archeological mixtures, and to evaluate the presence of interactions between pitch and additives.

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Dear Prof. Fabbri,

we would like to thank the reviewers and you for the positive comments and the constructive suggestions in accordance to which the manuscript *"Thermal degradation chemistry of archaeological pine pitch containing beeswax as additive"* by Celia Duce, Sibilla Orsini, Alessio Spepi, Maria Perla Colombini, Maria Rosaria Tiné, Erika Ribechini, has been improved.

You find enclosed the answer to the reviewer comments, the revised text and figure 4.

Best regards,

Prof.ssa Maria Rosaria Tinè

Response to Reviewer #2:

This is an interesting paper for scientists in the field of conservation of cultural heritage, which is devoted to the elucidation of processes taking place in pitch+wax based adhesives of archaeological origin. The research performed is rigorous and provides abundant numerical and graphical data for sustaining the experiments carried out in the laboratory, as it is usual in the team leaded by Prof. Dr. Colombini. For these reasons, I recommend this paper for publication in JAAP. Nevertheless, I kindly ask the authors to take into account the following remarks and comments:

Major remarks:

1- The manuscript is not well organized and it is highly confuse in the essential aspect concerning the aim of the research carried out and the conclusions achieved:

In the "Introduction" section is written (page 4 line 4): "the aim of this work is to evaluate the effect of the addition of beeswax on the physical and chemical properties of pine pitch" Evaluation of physical properties of any material used as an adhesive in heritage field should include a complete determination of optical and mechanical properties (color, elastic modulus, etc.) as well as hydric and reological properties, among others. Chemical properties should include not only identification of chemical composition but also microstructure. No data relative to these parameters has been provided in the paper.

The sentence "the aim of this work is to evaluate the effect of the addition of beeswax on the physical and chemical properties of pine pitch" was changed into "the aim of this work is to evaluate the presence of chemical-physical interactions between pine pitch and beeswax used as additive", both in the Abstract (line 17-19) and in the Introduction (line 68-69).

On the other hand the title of the paper is "thermal degradation chemistry of archaeological pine pitch containing beeswax as an additive". This title adjusts better to the contents of the paper. Nevertheless, the authors should justify the interest for studying the thermal behavior of pitch-beeswax adhesive. Were these adhesives subjected to heating treatments for using in Antiquity and Modern Art and, for this reason, is interesting the study of their possible thermal degradation? What type of heating was used in the traditional application treatments, oxygen-rich atmosphere or in oxygen-poor atmosphere? Quotations should be provided. What temperatures were reached in the traditional application treatments? Quotations should be provided.

On the contrary, if the research has been performed for studying the thermal degradation of pitch additived with beeswax due to natural ageing, it is not probable that the adhesive reaches temperatures up to 60-70 ºC. Therefore, most processes described in the experiments performed on laboratory specimens, which occur between 100-500ºC, have interest scarcely.

TGA is a thermo-analytical technique widely used to investigate the physical-chemical interactions among different components in a mixture by comparing the different thermal degradation behavior of the mixture with respect to the pure components. As reported in the introduction (lines 82-86), in this work we used this methodology to investigate how the physical structure of pine pitch is modified by the addition of beeswax. It was not our purpose to simulate (by heating in oxygen or nitrogen atmosphere) a natural thermal degradation process or to reproduce traditional treatments of art materials.

This important question of the aims of the paper still remains more unclear in the "Conclusions" section where description of the modifications of the thermal behavior of the pitch due to the addition of wax is almost limited to the mention of formation of unknown adducts while abundant results of quantitative content of beeswax of archaeological samples are provided. Thus, the "Conclusions" section suggests that the aim of the study rather was to implement a multi-analytical

method for identifying binary mixtures (pitch-wax) in archaeological samples by using thermoanalytical techniques. In summary, it seems more appropriate to entitle the paper: "Characterization of archaeological samples of pine pitch containing beeswax as an additive by thermoanalytical techniques and GC-MS"

In the introduction, lines 76-77, a new sentence relative to the possibility to reveal the quantitative composition of ancient adhesives was added:

"and to give an idea about the relative proportion of pine pitch and beeswax in unknown archaeological adhesives"

Moreover, in the Conclusions, lines 457-464, more emphasis is now on the formation of new adducts between pine pitch and beeswax. In this view, new sentences were added:

"In particular, the comparison of the DSC curves of the pine pitch/beeswax mixtures with those of the pure components, reveals that the DSC profile of the mixture is not just a simple combination of the DSC curves of the components. In addition, the thermal degradation of pine pitch/beeswax mixtures under nitrogen shows a small mass loss in the range 365-375°C which was not present in the degradation curve of the single components and can be related to the formation of a new adduct. Moreover, the thermal degradation of pine pitch/beeswax mixtures in air shows that the increase in the mass losses of the mixtures in the range 269-291°C is higher than that it would have been if only the mass loss of pure beeswax at 270°C had contributed to it."

2- The manuscript is a profuse description of the results obtained by a multi-technique strategy and thus, abundant data are provided. In my opinion, the length of the different parts of the "results and discussion" section is not well balanced in detriment of the description of the more interesting laboratory specimen: the mixture of pitch and beeswax (i.e. only a sentence is devoted to the adduct (composition is not provided) formed between pitch and beeswax despite it is one of the most important findings that justify the study carried out on thermal degradation.

Chapter 3.3 of the Results and Discussion section: "Characterization of pine pitch samples with added beeswax", is entirely dedicated to the characterization of the mixture of pitch and beeswax. Our paper reports for the first time information on the presence of new compounds/adducts between pine pitch and beeswax. Lines from 326-329 and 352-368 are devoted to the description of the experimental evidence of the presence of pitch/beeswax adducts. The determination of the composition of these compounds, however, would require a fully dedicated research that is beyond the scope of this work.

3- Quantification of the content of beeswax in archaeological samples is made by comparing values of mass loss of lab specimens in TG analyses. Nevertheless, the authors do not mention the performance of a calibration of this parameter. Is there a linear dependence of mass loss upon wax concentration? A graph representing mass loss vs concentration of wax should be provided, at least, as supplementary material.

The procedure followed for the evaluation of the beeswax content, based on the combined analysis of both DSC and TG results, is reported in detail in the text, lines 408-416. The whole of this information cannot be graphically represented.

Minor remarks:

1- page 4 lines 20-48: The introduction section could be shortened by re-writing these paragraphs summarizing the experiments carried out. (They are repeated almost exactly in section 2).

The introduction has been modified and shortened according to the suggestion of the reviewer.

2- page 5 line 23: Pinus sylvestris L. is the botanical species of the tree from which the pitch is obtained, other term such as "terpenoid resin" or "pitch" or "product extracted from Pinus.." should be used here.
Pinus sylvestris L. was substituted by "pitch from *Pinus sylvestris L*"

3- Page 9 line9: According to the context "isomerization" should replace "aromatization" used in this sentence. In other case, authors should better explain how aromatic compounds are formed from the mixture of abietic acid and its isomers apparently formed after pyrolysis.
Done

4.- Page 14 line 16-33: the authors indicate that archaeological samples were analysed by TG-FTIR using different instrument conditions that those for laboratory specimens used as reference materials due to the low amount of sample. A series of new experiments with laboratory specimens should be performed in identical conditions to those of the archaeological samples in order to avoid uncertainties in the conclusions achieved.

Due to the small amount of the archaeological samples, we did on these samples only TG-FTIR analysis (which requires a heating rate of 20°C/min) but not TG analysis. TG experiments on the reference mixtures were performed at both 10°C/min and 20°C/min, but only the experiments at 10°C/min were discussed because they provide the most precise results. It was not possible to perform TG experiments at 10°C/min on the archaeological samples due the fact that samples were finished. As explained in the text (lines 387-392), this does not affect the interpretation of the results.

Answer to Editor's comments:

Line numbering is mandatory. Please add line numbers and number the lines of your manuscript continuously throughout.
Done

Where appropriate please mark the changes made into the document with red colored font. Do not use the revision tool in the final pdf version and/or the Track Changes feature.
Done

Please replace [1,2,3,4,5,6,7,8,9] with [1-9]; and wherever it may apply.
Done

Figure 4: check structures; may be "abietic acid (7)" double bond shifted or name replaced with "palustric acid".
Done. In Figure 4, the structure of abietic acid is now correct

Section 2.5: "iso" should not be italicized.
Done

Page 7 . The production of lactones at 81 °C is quite interesting, if possible please add more information from literature; [26] which chapter?

We added the chapter of the book in ref. [26] and a new reference [27].

Our sentence about the formation of lactones was not clear enough. In fact, lactones are not formed at 81°C during TG analysis but they were formed during the manufacture of pitch at higher temperature. For this reason, we modified the text adding some clarifications (lines 171-174)

"These compounds could be formed from the lipid extractives (free fatty acids, triglycerides, waxes) or from the polysaccharides present in the pine wood during the thermal treatment used to obtain the pitch and then entrapped in the pine pitch matrices [25-27]."

THERMAL DEGRADATION CHEMISTRY OF ARCHAEOLOGICAL PINE PITCH CONTAINING BEESWAX AS AN ADDITIVE

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Abstract

Thermo analytical techniques and gas chromatography/mass spectrometry (GC/MS) were used to evaluate the presence of chemical-physical interactions between pine pitch and beeswax used as additive The mixtures found in several archaeological objects demonstrate that by modifying the physical and chemical properties of pitch and tar, our ancestors were able to add a variety of organic materials, such as waxes or animal fats.

We studied pine pitch replicas from *Pinus sylvestris* prepared following a test from the field of experimental archaeology. Varying proportions of beeswax were added and then the resulting pitches were studied by a multi-analytical approach comprising the use of thermo analytical techniques (DSC, TG and TG-FTIR) and GC/MS, which provides molecular information. The same approach was also used to study a mixture of pitch from *Pinus sylvestris* L. and beeswax ("Zopissa"), whose relative proportions were unknown, and two archaeological adhesives collected from glass *opus sectile* fragments found in the northern necropolis of Antinoopolis (Egypt, 4th-5th century AD). Our thermo-analytical techniques managed to determine the relative proportion of pine pitch and additives, such as beeswax, in unknown archeological mixtures, and to evaluate the presence of interactions between pitch and additives.

1. Introduction

Since early times, the resinous substances secreted by trees have been widely used either in their natural form or as tar and pitch to waterproof the planking of ships and vessels, and as adhesives [1-9]. Tar and pitch have been of great importance in terms of their properties, such as insolubility in water, adhesion and glasslike characteristics. Tar and pitch were obtained by subjecting resinous materials to hard-heating(pyrolysis)-type processes. The exact methods used for the production of pitch and tar before the use of kilns have not yet been completely clarified. Archaeological and documentary evidence as well as experimental archaeology tests suggest that various methods could have been used [10-12. The most ancient documentary evidence is *De Historia Plantarum* (4th - 2nd century BC) by Teophrastus, who wrote that the Macedonians made huge piles of cloven trunks, covered them with peat and soil, and then burnt the wood, letting the tar and pitch run out in a canal.

During the pyrolysis process to obtain pitch and tar, terpenoids, the main compounds of resins, undergo chemical modifications, such as aromatization, demethylation and decarboxylation. This gives rise to the formation of new compounds with a lower molecular weight and a high degree of aromatization [13]. In addition, during the hard-heating (pyrolysis)-type processes of wood from pine and firs, the formation of methylated diterpenoid acids was established: the high amount of methanol in the gas phase reacts easily with diterpenoid acids to produce, above all, methyl-dehydroabietate [4,13,14].

The mixtures found in several archaeological objects demonstrate that in order to modify the physical and chemical properties of pitch and tar, a variety of organic materials, such as waxes or animal fats, were added [1,8,15-17]. For example, mixtures of pine pitch, beeswax and *brassicaceae* seed oil were used as adhesives in the manufacture of *opus sectile* and mosaic decorations [15]. In the ancient world, beeswax and *brassicaceae* seed oil were used to change the physical and chemical characteristics of the pine pitch thus allowing more malleable and mouldable adhesives to be obtained. In addition, the fact that brassica oil was used would seem to demonstrate that its semi-siccative properties were already well known in ancient Egypt. All this testifies to the high technological skills of the craftsmen who created the *opus sectile* and mosaic decorations.

In another case, the presence of triterpenoid fatty acyl esters, which are naturally present in birch bark or in tar and pitch derived from birch, suggest that an animal fat was intentionally added to birch bark tar to produce a modified adhesive/waterproofing material [16]. The formation of stable bonds between triterpenoids and fatty acids to give the corresponding fatty acyl esters was

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attributed by our forefathers to mixing and heating processes, suggesting not only an intentional
67 addition but also an advanced technological level [16].

68 The aim of this work is to evaluate the presence of chemical-physical interactions between pine
69 pitch and beeswax used as additive. Pine pitch replicas from *Pinus sylvestris* were prepared using a
70 procedure from experimental archaeology. Varying proportions of beeswax were added to the
71 replicas and studied by a multi-analytical approach comprising the use of gas chromatography/mass
72 spectrometry (GC/MS) and thermoanalytical techniques (DSC, TG and TG-FTIR). DSC and TG
73 analysis have already been successfully used to investigate archaeological woods [18,19], and have
74 been used to highlight interactions among pigments and fluid binders such as proteins, linseed oils
75 and alkyd resins [20-24]. Consequently, we believe that they could also help us to understand the
76 interaction between pine pitch and beeswax and to give an idea about the relative proportion of pine
77 pitch and beeswax in unknown archaeological adhesives.

78 The pine pitch used in this study was produced using an autothermal method [12]. GC/MS was used
79 to assess the molecular composition of pitch replica and to identify a series of species acting as
80 markers of technological manipulation and possible degradation. In addition, GC/MS analysis was
81 applied to pine pitch heated up to 300°C under nitrogen and air flow.

82 By studying the mixtures of pine pitch with different amounts of additive by thermal analysis we
83 can extend our knowledge of the techniques used in the past. The thermo analytical results of the
84 samples obtained by adding different amounts of beeswax to the pine pitch compared with those of
85 the pure pine pitch and beeswax highlight how beeswax affects the pitch and the results also reveal
86 the presence of possible pine pitch/beeswax interactions.

87 The same approach was also used to study a mixture of pitch from *Pinus sylvestris* L. and beeswax
88 "Zopissa" (furnished by Dr. Andreas Kurzweil, Arbeitsgruppe Teerschwele in Museumsdorf
89 Düppel, Berlin, Germany), and two archaeological adhesives collected from glass *opus sectile*
90 fragments found in the northern necropolis of Antinoopolis (Egypt, 4th-5th century AD) to establish
91 a correlation with the mixtures obtained by experimental archaeology.

2. Materials and Methods

2.1 Chemicals

95 All solvents were Carlo Erba (Milan, Italy) pesticide analysis grade. *n*-Hexadecane (internal
96 standard, IS_1), tridecanoic acid, (internal standard, IS_2), hydrochloric acid (HCl), potassium

hydroxide (KOH), and N,O-bis(trimethyl)silyltrifluoro-acetamide (BSTFA) containing 1% trimethylchlorosilane, were purchased from Sigma–Aldrich (Milan, Italy).

2.2 Samples

Following traditional procedures, reference pitch was obtained after two days of pyrolysis of pine wood (*Pinus sylvestris* L. Roots) according to the "Pile (autothermal)" process (carried out by Andreas Kurzweil, Arbeitsgruppe Teerschwele in Museumsdorf Düppel, Berlin, Germany). The pitch was produced using an autothermal method where resinous wood is piled in a wide pit covered in the centre with clay. The firewood heats the resinous wood from the upper part of the pile and inside the pile the temperature ranges from 400 to 700°C [12]. Varying amounts of beeswax were added to the pine pitch after heating at 40-50°C. Four mixtures with different proportions were thereby obtained with 10%, 33%, 50% and 70% (in weight) of beeswax, respectively.

The mixture of pitch from *Pinus sylvestris* L. and beeswax ("Zopissa"), whose relative proportions were unknown, was prepared by Andreas Kurzweil (Arbeitsgruppe Teerschwele in Museumsdorf Düppel, Berlin, Germany). Two archaeological adhesives (Samples 2 and 3) collected from glass *opus sectile* fragments found at the northern necropolis of Antinoopolis (Egypt, 4th-5th century AD), whose chemical composition (mixture of pine pitch and beeswax) was already obtained by GC/MS [15], were also used in this study.

2.3 DSC

DSC measurements were performed by a Perkin Elmer Pyris Diamond Differential Scanning Calorimeter in the temperature range 10-550°C, with a heating rate of 10°C/min and using air as purging gas. The sample masses of about 3 mg were prepared in aluminium pans.

2.4 TG-FTIR

Thermogravimetric (TG) analysis were performed using a TA Instruments Thermobalance model Q5000IR equipped with an FT-IR Agilent Technologies spectrophotometer model Cary 640 for Evolved Gas Analysis (EGA). TG measurements were performed at a rate of 10 °C/min, from 50 °C to 600 °C under air and nitrogen flow (25 ml/min). TG-FTIR measurements were performed at a rate of 20 °C/min, from 50 °C to 600 °C under nitrogen flow (90 ml/min), from 600 to 3000 cm^{-1} with a slit (4 cm^{-1} in width). To reduce the strong background absorption from water and carbon dioxide in the atmosphere, the optical bench was usually purged with nitrogen. In additional, a background spectrum was taken before the beginning of each analysis in order to zero the signal in

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the gas cell and to eliminate any contribution from ambient water and carbon dioxide. The amount of sample in each TG and TG-FTIR measurement varied between 2.4 and 3.8 mg.

2.5 GC/MS

Sample (1-3 mg) was subjected to alkaline hydrolysis by adding 1 mL of methanolic KOH (KOH in CH_3OH (10 wt%)/KOH in H_2O (10 wt%), 2:3), and heating at 60°C for 3h. After hydrolysis, neutral organic components were extracted with *n*-hexane (3× 500 µL) and, after acidification with hydrochloric acid (10 M; to pH 2), the acidic organic components were extracted with diethyl ether (3× 500 µL). Aliquots of both fractions were evaporated to dryness under a gentle stream of nitrogen and subjected to trimethylsilylation. This was achieved by mixing the dried aliquots with an internal standard solution (5 µL of tridecanoic acid solution, 140 µg g^{-1}) and derivatising with 20 µL of BSTFA (at 60°C, 30 min), using 150 µL isooctane as the solvent. After adding 10 µL of *n*-hexadecane solution (80 µg g^{-1}) as an internal standard for the injection, 2 µL of the solution were analyzed by GC/MS.

The Gas Chromatograph System 6890N (Agilent Technologies, Palo Alto, CA, USA) was coupled with a 5973 Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA) single-quadrupole mass spectrometer. For the gas chromatographic separation, an HP-5MS fused silica capillary column (5% diphenyl-95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., J&W Scientific Agilent Technologies, USA) with a de-activated silica pre-column (2 mx 0.32 mm i.d., J&W Scientific Agilent Technologies, USA) was used.

The split-splitless injector was used in splitless mode at 320°C. The GC/MS parameters for the analysis of the various fractions were as follows: 80°C isothermal for 2 min, 10°C min^{-1} up 200°C and isothermal for 4 min, 6°C min^{-1} up 280°C and isothermal for 40 min. The carrier gas (He, purity 99.9995%) was used in the constant flow mode at 1.2 mL min^{-1}.

Peaks assignments were performed using mass spectra interpretation, comparisons with mass spectral libraries (NIST 2.0) and with published mass spectra.

3. Results and discussion

3.1 Characterization of the pure pine pitch

Figure 1 (a, b) shows the thermal decomposition of pine pitch under nitrogen and air flow, respectively. Figure 2 (a, b) shows the FTIR spectra of the gas evolved by the pine pitch during its

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main mass losses under nitrogen flow. Tables 1 and 2 summarize the temperature and the percentage mass loss of each degradation step under nitrogen and air flow, respectively.

Table 1

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Table 2

The thermal oxidative and the pyrolytic profiles of pine pitch (Figure 1a, b) are very similar. Three different steps can be seen in Fig. 1 a, b: an initial broad mass loss at about 80°C, the main mass loss in the range 180-205°C, and a final loss in the range 490-530°C.

The FTIR spectra of the evolved gasses from the pine pitch during the mass loss at 81°C under nitrogen flow (Figures 2 a) show many signals associated with C=O stretching vibrations at 1725, 1743, 1780 and 1799 cm^{-1}. The absorptions at 1780 and 1799 cm^{-1} suggest the formation of 5 or 6-member lactones, and the peak at 1190 cm^{-1} confirms the presence of these species. These compounds could be formed from the lipid extractives (free fatty acids, triglycerides, waxes) or from the polysaccharides present in the pine wood during the thermal treatment used to obtain the pitch and then entrapped in the pine pitch matrix [25-27]. The peaks at 2326 and 2366 cm^{-1} are due to the formation of carbon dioxide from the decarboxylation of the lactones initially formed. The peaks in the range 2889-2970 cm^{-1} are related to the stretching vibrations of C-H aliphatic groups, and the peaks at 1386 and 1460 cm^{-1} with the deformation vibrations (rocking and scissoring respectively) of the C-H bonds. The FTIR spectrum of the evolved gasses from the pine pitch during the mass loss at 183°C under nitrogen flow (Figures 2 b) could be due to diterpenoid acids which might have been in a high state of oxidation (double C=O stretching vibration in the range 1700-1765 cm^{-1} due to ketones, carboxylic acids and methyl esters, and the characteristic band at 3572 cm^{-1} due to the stretching of O-H groups of acid and alcohols both in gas phase) [28]. The spectrum also shows signals associated with alkenes: a small band at 3078 cm^{-1} due to stretching vibrations of =C-H groups, the out-of-plane bending of =C-H at 820 and 890 cm^{-1}, and the =C-H rocking vibration at 1250 cm^{-1}. The broad band at 1606-1650 cm^{-1} is due to the overlapping of signals, probably related to C=C stretching (typically at 1640 cm^{-1}) and to β-diketones.

In addition, there are also signals associated with C-H stretching (2865 and 2933 cm^{-1}), C-H bending (1383 and 1460 cm^{-1}) and C-O stretching (1250 cm^{-1}). The signals related to carbon dioxide (2326 and 2366 cm^{-1}) are due to the decarboxylation process of carboxylic acids.

The mass loss at higher temperatures (531°C) may be the result of the pyrolysis/combustion of the high-polymerized (oxidized) terpenoid fraction of the pine pitch. The quantity of the evolved gas at 530°C is below the FTIR detection limit and for this reason the FTIR spectra at 530°C are not reported in Figure 2.

Fig. 1.

Fig. 2.

Pine pitch pyrolysis differs from pine pitch thermoxidative degradation mostly in terms of the mass losses in the range 180-205°C and 490-530°C. Pine pitch pyrolysis produces a mass loss of 80% at 183°C, and a mass loss of 4.5% at 531°C. On the other hand, pine pitch thermoxidative degradation produces a mass loss of 70% at 206°C and a mass loss of 16.3% at 491°C. Almost 10% of the substance, which is degraded at 183°C under nitrogen flow, is degraded at ~500°C under air flow. This behavior suggests the occurrence of oxidative processes (low-temperature oxidation, LTO) on the pine pitch during the TG run under air flow.

To confirm this hypothesis, we analyzed a pine pitch sample that had been kept under air flow at 80°C for 5000 min. We found that the mass loss at 500°C increased by 3% and the mass loss at 200°C decreased by the same quantity.

In addition, we also performed GC/MS investigations on pine pitch, on the residues of pine pitch heated up to 300°C under nitrogen flow and air flow. The chromatographic profile of the reference pine pitch is similar to that reported in the literature [1-234,29] since the main compounds detected were di-dehydroabietic, dehydroabietic, abietic, and 7-oxo-dehydroabietic acids along with neutral tricyclic terpenes, such as retene, nor-abietatrienes and methyl-dehydroabietate.

Figure 3 shows the TIC chromatograms of the residue left by the pine pitch after it had been heated to 300°C under nitrogen and air flow, respectively. Table 3 lists the compounds identified along with their relative (%) abundance in the two samples. The data mainly demonstrate that the two residues have a different degree of oxidation. In particular, the pine pitch residue under air flow

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shows a higher content of oxidized compounds, thus confirming the evidence obtained by TG. The residue obtained under air flow is richer in 7-oxo-dehydroabietic acid and also contains other diterpenes at a higher degree of oxidation (compounds 12 and 14) which are not present in the residue obtained under nitrogen flow. On the other hand, the pine pitch residue heated to 300°C under nitrogen flow shows the presence of abietic acid and its isomer, thus demonstrating that in the absence of oxygen, isomerization reactions are prevalent. In addition, the residue obtained under nitrogen flow shows a more abundant content of methyl-dehydroabietate and does not contain methyl-7-oxo-dehydroabietate. In fact, the latter compound is present only in the residue obtained under air flow, indicating that its formation is due to an oxidation reaction undergone by methyl-dehydroabietate.

Note that tricyclic terpenes such as retene, nor-abietatrienes and norabietatetraenes are practically absent in both residues. In fact as they are quite volatile, they were not preserved during heating at 300°C.

Figure 4 shows the oxidation undergone by some diterpenoid compounds of pine pitch due to the incorporation of one or more oxygen atoms. The relative degree of oxidation is indicated by the position of the various compounds. The graph highlights that the pine pitch residue under air flow has a higher degree of oxidation (III level of oxidation) due to the major abundance of highly oxidized diterpene acids.

Fig. 3.

Table 3

Fig. 4.

The DSC curves of pine pitch under air flow show three (266°C, 410°C, 520°C) exothermic processes, which correlate with the main TG decomposition steps at ~ 260°C and ~ 500°C, respectively (see Fig. 5). Although the mass loss associated with the step at ~ 260°C observed by

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TG is higher than that at~ 500°C, the thermal effect observed by DSC and related to the peak at ~ 500°C is much greater than that at ~ 260°C. The degradation step at ~ 500°C is a combustion, which is strongly exothermic, whereas the peak at ~ 260°C most likely involves a non oxidative degradation (pyrolytic process).

It is reasonable to assume that at around 260 °C the combustion is only a minor event, although strongly exothermic, and the endothermic pyrolitic degradation is the prevailing process. The exothermic peak observed at 260 °C most likely arises from the overlapping of an endothermic and an exothermic process, wherein the exothermic signal is predominant. We found almost the same behavior in the thermo oxidative degradation of proteinaceous materials [23]. The broad exothermic peak in the range 370-470 °C can be similarly explained as the composition of evaporation and combustion of terpenoid. There are no endothermic effects due to melting, thus revealing that the pitch has an amorphous structure.

Fig. 5.

3.2 Characterization of the pure beeswax

The pyrolysis of beeswax (Figure 6) is characterized by a broad mass loss in the range 200-250°C followed by a mass loss with a maximum in DTG at 306°C. The IR spectrum (Figure 7) recorded during the mass loss at a lower temperature shows asymmetrical (2930 cm^{-1}) and symmetrical (2860 cm^{-1}) CH$_2$ stretching and CH$_2$ scissoring (1460 cm^{-1}) bands, thus suggesting that this first mass loss is due to the evaporation of the n-alkanes, which represent 21.5% of the beeswax. There is also a small band associated with C=O stretching (1780 cm^{-1}) in the spectrum, which is typical of a saturated lactone species. These species may have been formed by the thermal degradation of the hydroxy fatty acids in the beeswax.

The second mass loss at about 300°C can be attributed to the beeswax lipidic fraction. In fact, the IR spectrum registered during the mass loss shows almost the same bands reported for the gas evolved at ~200° and the C=O stretching absorptions (broad band in the range 1730-1710 cm^{-1}, with a maximum at 1720 cm^{-1}) is due to esters and carboxylic acids from the thermal degradation of esters (confirmed by the band at 3572 cm^{-1} due to O-H stretching of carboxylic acids and alcohols in the gas phase).

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Long-chain esters make up about 70% of the beeswax and the above results are consistent with literature [30]. The thermo-oxidation of beeswax produces a main mass loss at 225°C, followed by a shoulder at 280°C, and a small mass loss at 460°C (Figure 6b). The comparison of the beeswax degradation under nitrogen and under air (Figure 6) shows that the pyrolysis of beeswax is complete at about 350°C, while beeswax losses the 91.9 % below 300°C and a 8.1% at 457°C when heated under air flow. The presence of oxygen highlights that there may be a portion of material more oxidized an/or polymerized which degradates at higher temperatures. In addition, oxygen decreases the thermal stability of the lipidic portion of beeswax. We can hypothesize that the presence of hydrocarbons and oxygen triggers the degradation of lipids, shifting the mass loss at lower temperatures.

Fig. 6.

Fig. 7.

The DSC curve of beeswax under air flow in Figure 8 shows an endothermic process below 100°C, two exothermic partially overlapping processes in the range 200-400°C, and an exothermic process at ~ 500°C. The exothermic processes correlate with the main TG decomposition steps. The endothermic process is reversible and represents the melting of beeswax. The characteristic shape of two partially resolved peaks is due in the first part of the peak (40-60°C) to the heat absorbed by free fatty acids and hydrocarbons, and in the last part (60-70°C) to the sharp heat absorption by long chain palmitate esters [31,32]. The onset temperature, which represents the beginning of the melting is 54°C, the temperature of the maximum is 63°C, and the total melting and enthalpy is 153J/g. The results for the temperature parameters and the phase transition enthalpy are comparable with the literature [31-33].

Fig. 8.

3.3 Characterization of pine pitch samples with added beeswax

Figure 9 (a-h) shows the TG and DTG curves of pine pitch and beeswax mixtures under nitrogen (a, c, e, g) and air flow (b, d, f, h), respectively. Figure S1 (a, b) shows the FTIR spectra of the gas evolved by the mixture containing 50% of beeswax during its main mass losses under nitrogen flow. Tables 1 and 2 summarize the temperature and the percentage mass loss of each degradation step of the pine pitch and beeswax mixtures under nitrogen and air flow, respectively.

The thermal degradation of pine pitch/beeswax mixtures under nitrogen flow shows five main mass losses, as reported in Table 1 and Figure 9 (a, c, e, g): one below 100°C, one at ~200°C, one in the range 270-290°C, one in the range 365-375°C and the last one above 470°C. Increasing the beeswax content of the mixture, whereby decomposition of the beeswax becomes the major process, causes the following:

- the mass loss at ~200°C decreases and moves to a higher temperature;

- the mass loss in the range 269-291°C increases and moves to a higher temperature;

- the mass loss in the range 365-375°C increases and moves to a higher temperature;

- the mass losses below 100°C and above 470°C, due to the pitch, become less evident and the temperatures of their inflection point are less precise.

The mass losses below 100°C and above 470°C can be attributed to the pine pitch portion, while the mass losses at ~200°C and in the range 269-291°C are due to both pine pitch and beeswax degradation. The small mass loss in the range 365-375°C is only present in the pine pitch/beeswax mixtures and could be due to some kind of adduct between pine pitch and beeswax.

The FTIR analysis of the evolved gasses of the mixture with 50% of beeswax (Figure S1a) at 200°C shows the characteristic profiles of the pitch materials and the wax. The asymmetrical (2931 cm^{-1}) and symmetrical (2853 cm^{-1}) CH_2 stretching and CH_2 scissoring (1461 cm^{-1}) bands can be attributed to the n-alkanes of the beeswax. The presence of ketones, carboxylic acids and methyl esters from the pitch and beeswax is confirmed by the peaks at 1724, 1747, 1128 and 3572 cm^{-1}. There are also signals due to carbon dioxide (2326 and 2366 cm^{-1}) from the decarboxylation of carboxylic acids.

The FTIR analysis of the evolved gasses of the mixture with 50% of beeswax (Figure S1b) at 290°C shows signals associated with a mixture of esters, ketones and carboxylic acids (double C=O stretching at 1702 and 1735 cm^{-1}) [28], which are present in beeswax and pitch.

12

Fig. 9.

Four different steps can be observed in the thermal degradation of pine pitch/beeswax mixtures under air flow: one at ~86°C, one in the range 200-210°C, one in the range 260-271°C and the last one above 440°C (see Table 2 and Figure 9 (b, d, f, h)).

Increasing the beeswax content of the mixture:

- the mass losses at ~86°C decrease and the temperature is almost constant;

- the mass loss in the range 200-212°C decreases and moves to a higher temperature;

- the mass loss in the range 267-271°C increases, but the temperature is almost constant;

- the mass loss in the range 460-478°C decreases, but the temperature is almost constant.

The mass losses at ~86°C can be still attributed to the pine pitch portion only, as also supported by the fact that almost the same mass losses are present both under air and under nitrogen.

The decrease in the mass loss in the range 200-210°C, from 67.5 % to 46.5%, with the increase in the beeswax content in the mixtures is quite strange. In fact, since the mass loss of pure beeswax and pine pitch is the same (70%) at 225°C and 205°C, respectively, the mass losses of the mixtures should be constant (70%). On the other hand, the increase in the mass losses of the mixtures in the range 269-291°C is higher than that it would be if only the mass loss of pure beeswax at 270°C contributed to it. A portion of pine pitch thus probably forms a more stable adduct with beeswax, as also previously suggested by the results obtained under nitrogen.

In the mixture containing 70% of beeswax, the mass loss in the range 200-210°C moves to 237°C, and the mass loss in the range 267-271°C moves to 325°C. To sum up both, under air and nitrogen all the pine pitch in the mixture containing 70% beeswax seems to be involved in the formation of the adduct with beeswax.

Comparing the DSC curves of the pine pitch/beeswax mixtures with those of pure beeswax and pine pitch in the range 200-550°C under air flow (see Figure 10) reveals that the DSC profile of the pine pitch/beeswax mixtures is not just a simple combination of the pure beeswax and pine pitch DSC curve, thus confirming the presence of interactions between the beeswax and pine pitch.

When the beeswax content of the mixtures is decreased, the beeswax melting peak is shifted to a lower temperature, but it maintains the shape of double partially-resolved peaks until the beeswax content is reduced to 10% when only a single broad peak is visible (see Figure S2).

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Fig. 10.

3.4 "Zopissa" and archeological adhesives

Table 4 reports the experimental temperatures and the percentage mass losses of the corresponding degradation steps under nitrogen flow of the studied samples: an unknown mixture of pine pitch and wax ("Zopissa") and two archaeological adhesives (Samples 2 and 3) collected from glass *opus sectile*. The two archaeological samples had been previously studied by GC/MS and their chemical composition, a mixture of pine pitch and beeswax, was known [15].

Figure 11 shows the thermal decomposition of Zopissa, Samples 2 and 3 under nitrogen flow. Figure S3 and Figure S4 show the FTIR spectra of the gas evolved by Zopissa and Sample 2 during their main mass losses under nitrogen flow.

Table 4

Due to the small quantities of the samples, we did a TG-FTIR analysis of Samples 2 and 3, but not a simple TG. As a consequence, the results reported in Table 4 and Figure 12 were carried out at 20°/min rather than at the temperature for all the other samples (10°C/min). The increase in the heating rate can produce a shift to a higher temperature of the mass losses and a loss of resolution in consecutive steps. Thus the temperature of Samples 2 and 3 could be higher and the mass losses less resolved than they would have been if they had been recorded at a heating rate of 10°C/min.

The degradation of Samples 2 and 3 under nitrogen flow shows mass losses below 400°C. These losses are due to the presence of pine pitch and beeswax. There was also a mass loss at about 700°C, which could be associated with the presence of an inorganic material. The FTIR of the mass loss at about 700°C shows that the main evolved gas at this temperature is CO_2.

We can thus hypothesize the presence of $CaCO_3$ both in Samples 2 and 3. Actually, a previous investigation carried out by FTIR analysis on similar samples had revealed the presence of calcite [15]. Calcite, in the form of grains or powder, was used in ancient times as a filler for the organic adhesive. Taking into account the quantity of CO_2 evolved we can calculate a content of $CaCO_3$ of 50% in Sample 2 and 28% in Sample 3. The residue at 900°C of Samples 2 and 3 is higher that it would have been if $CaCO_3$ had been the only inorganic material in the samples. Both in Samples 2

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and 3, we can assume the presence of a 10% of another inorganic material, almost inert in the investigated temperature range. Taking into account that the samples were collected from the back of glass plaques, the inert material is probably silica. In any case, contamination during burial or an intentional addition cannot be ruled out. To sum up the pine pitch / beeswax content is almost 40% and 60% in Samples 2 and 3, respectively.

By comparing the mass losses in the range 190-250°C and 290 400°C of Zopissa (Samples 2 and 3) with those of the mixtures previously discussed, Zopissa seems to be a pine pitch/beeswax mixture made up of 50% beeswax, while Samples 2 and 3 seem to have a lower beeswax content between 10 and 30%. To confirm these data we did a DSC scan between 0-100°C of Sample 3. The melting peak of the beeswax, reported in Figure S5, had an area of 40.5 mJ, and the mass of Sample 3 was 3.09 mg. Considering that the pine pitch/beeswax mixture made up 60% of Sample 3, and that the melting enthalpy of the beeswax is 153J/g, as discussed above, we found that the beeswax content of Sample 3 was 14%. Thermoanalytical data are completely in line with those obtained by GC/MS analyses [15].

Fig. 11

The FTIR spectra of the evolved gasses by Zopissa during thermal degradation under nitrogen flow (Figure S3a,b) are similar to the spectra of the pine pitch based mixture containing 50% beeswax collected in the same temperature range . In the spectrum of gas evolved at about 200°C signals are visible that are associated with the presence of carboxylic acids in gas phase (3574, 1752, 1724 and 1257 cm^{-1}) and with the n-alkanes of the beeswax (2935, 2854, 1460 and 1383 cm^{-1}). On the other hand, the FTIR analysis of the evolved gasses at about 295°C shows signals associated with a mixture of esters, ketones and carboxylic acids (double C=O stretching at 1700-1732 cm^{-1}) which are present in pitch and beeswax.

The FTIR spectra of the evolved gasses by Sample 3 during the main mass losses under nitrogen flow (Figure S4a) reveal the predominance of pine pitch in the sample. In fact, the spectra show IR absorption due to pine pitch: carboxylic acids in gas phase (3573, 1761, 1730 and 1250 cm^{-1}), to alkene species (3074, 1630, 889 and 821 cm^{-1}), and to C-H vibrations (stretching at 2940 and 2873 cm^{-1} and bending at 1381 and 1460 cm^{-1}). There are also signals related to carbon dioxide (2324 and

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2366 cm^{-1}) and small quantities of carbon monoxide at higher temperatures (2180 and 2104 cm^{-1}) (Figure S4b).

The high intensity of the signals associated with carbon dioxide (2324 and 2366 cm^{-1}) due to the decarboxylation of carboxylic acids are likely due to a higher amount of free fatty acids in the archaeological sample. In fact, in archaeological beeswax, the partial hydrolysis of the long-chain esters is known to lead to the formation of free palmitic acid and long chain alcohols [1,30].

4. Conclusions

In this work varying proportions of beeswax were added to pine pitch replicas from *Pinus sylvestris* prepared following procedures from of experimental archaeology. These replicas were then studied using gas chromatography/mass spectrometry (GC/MS) and thermoanalytical techniques (DSC, TG and TG-FTIR). The same multi-analytical approach was also used to study a mixture of pine pitch and beeswax ("Zopissa"), whose relative proportions were unknown, and two archaeological adhesives collected from glass *opus sectile* fragments found in the northern necropolis of Antinoopolis (Egypt, 4th-5th century AD). The comparison between TG data recorded under air and nitrogen atmosphere suggested the occurrence of oxidative processes (low-temperature oxidation, LTO) on the pine pitch during the TG run under air flow. GC/MS investigations on the residues obtained after the pine pitch had been heated to 300°C under nitrogen flow and air flow confirmed TG data, highlighting that the pine pitch residue under air flow had a higher content of highly oxidized diterpenoid compounds.

The analysis of the pine pitch/beeswax mixtures with a beeswax content from 10% to 70% showed the presence of pine pitch/beeswax interactions with the formation of a new adduct. All the pine pitch in the mixture containing 70% beeswax seems to have been involved in the formation of the adduct with beeswax. In particular, the comparison of the DSC curves of the pine pitch/beeswax mixtures with those of the pure components, reveals that the DSC profile of the mixture is not just a simple combination of the DSC curves of the components. In addition, the thermal degradation of pine pitch/beeswax mixtures under nitrogen shows a small mass loss in the range 365-375°C which was not present in the degradation curve of the single components and can be related to the formation of a new adduct. Moreover, the thermal degradation of pine pitch/beeswax mixtures in air shows that the increase in the mass losses of the mixtures in the range 269-291°C is higher than that it would have been if only the mass loss of pure beeswax at 270°C had contributed to it.

The detailed study TG-FTIR of pine pitch/beeswax mixtures provided some insights into Zopissa and Samples 2 and 3. Zopissa was revealed to be a simple pine pitch/beeswax mixture with a beeswax content of 50%, however Samples 2 and 3 also contained $CaCO_3$ and other inorganic inert materials.

The pine pitch/beeswax mixture represents almost 40% of Sample 2 and 60% of Sample 3. TG data indicate that the beeswax content in the pine pitch/beeswax mixtures of Samples 2 and 3 was between 10 and 30%. DSC analysis of the beeswax melting peak (available only for Sample 3), revealed a beeswax content in the mixture of 14%.

Acknowledgments

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Figure Captions

Fig. 1. Thermogravimetric curves (left axis) and their derivatives (right axis) under a) nitrogen, and b) air flow at $10°min^{-1}$ heating rate of pine pitch.

Fig. 2. FTIR spectra of the gas evolved by pine pitch at a) 81°C and b) 183°C during the TG experiment performed under nitrogen.

Fig. 3. TIC Chromatograms of a) pine pitch residue heated to 300°C under nitrogen flow, and b) pine pitch residue heated to 300°C under air flow obtained by GC/MS. * Contamination.

Fig. 4. Oxidation reaction paths of diterpenoid compounds of pine pitch with the relative state of oxidation (0, I, II, II) linkable to the numbers of carbon atoms incorporated. The numbers () represent the compounds identified in the chromatograms.

Fig. 5. DSC curves of pine pitch under air flow at $10°min^{-1}$ heating rate.

Fig. 6. Thermogravimetric curves (left axis) and their derivatives (right axis) under a) nitrogen, and b) air flow at $10°min^{-1}$ heating rate of beeswax.

Fig. 7. FTIR spectra of the gas evolved by beeswax at a) 260°C, and b) 326°C during the TG experiment performed under nitrogen.

Fig. 8. DSC curves of beeswax under air flow at $10°min-1$ heating rate. Inset: DSC heating and cooling scan of beeswax melting peak.

Fig. 9. TG (left axis) and DTG (right axis) of pine pitch/beeswax mixtures containing 10% (a, b), 33% (c, d), 50% (e, f), and 70% (g, h) of beeswax at $10°min^{-1}$ under nitrogen (a, c, e, g) and air (b, d, f, h) flow, respectively.

Fig. 10. DSC curves in the temperature range 200-550°C under air flow at 10°/min of pure beeswax, pure pine pitch and pine pitch/beeswax mixtures containing 10%, 30% and 50% of beeswax.
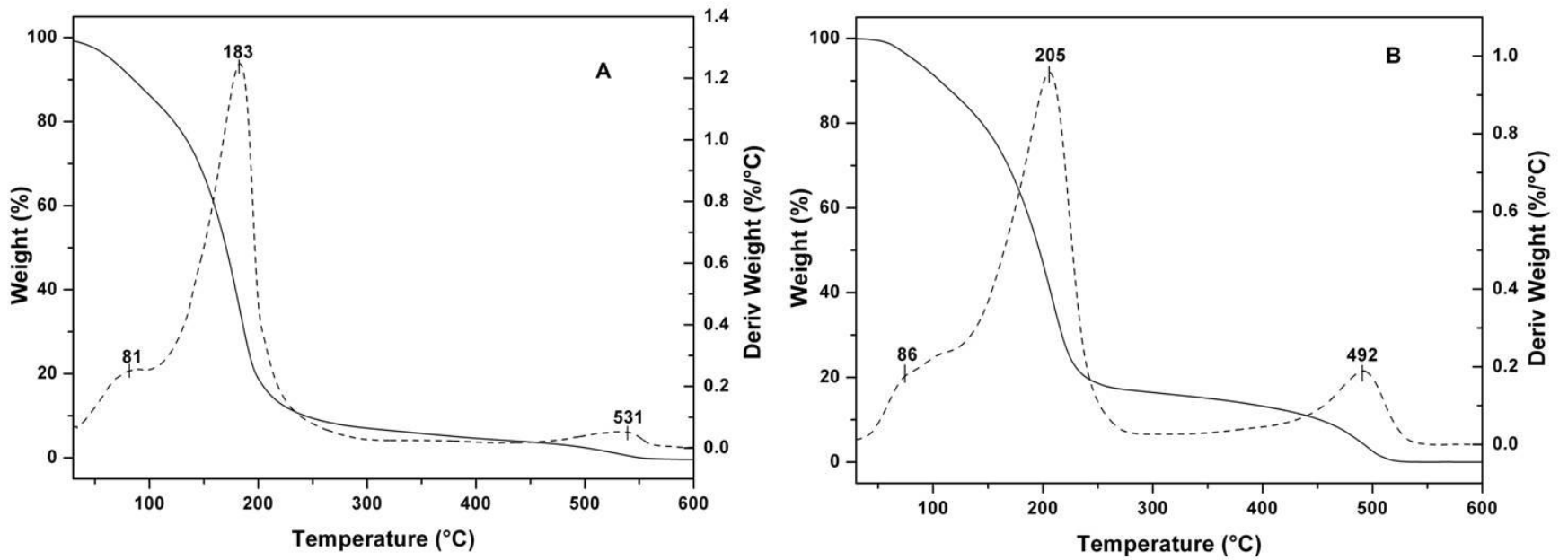
Fig. 11. TG curves (left axis) and DTG (right axis) of Zopissa sample at (a) $10°min^{-1}$; samples 2 (b) and 3 (c) at $20°min^{-1}$ under nitrogen flow.

Highlights

1. We evaluated the effect of the beeswax addition on the properties of pine pitch.

2. A multi-analytical approach (DSC, TG, TG-FTIR and GC/MS) was used to study replicas.

3. The same approach was used to study unknown mixtures and archaeological adhesives.

4. DSC and TG are useful to determine the relative proportion of pine pitch/beeswax

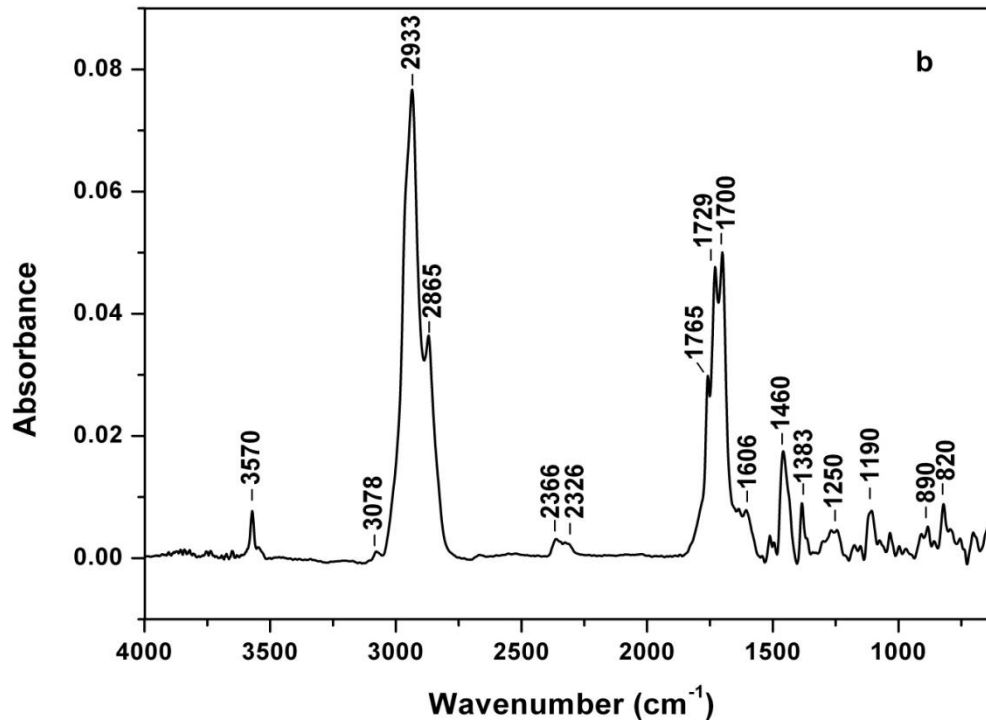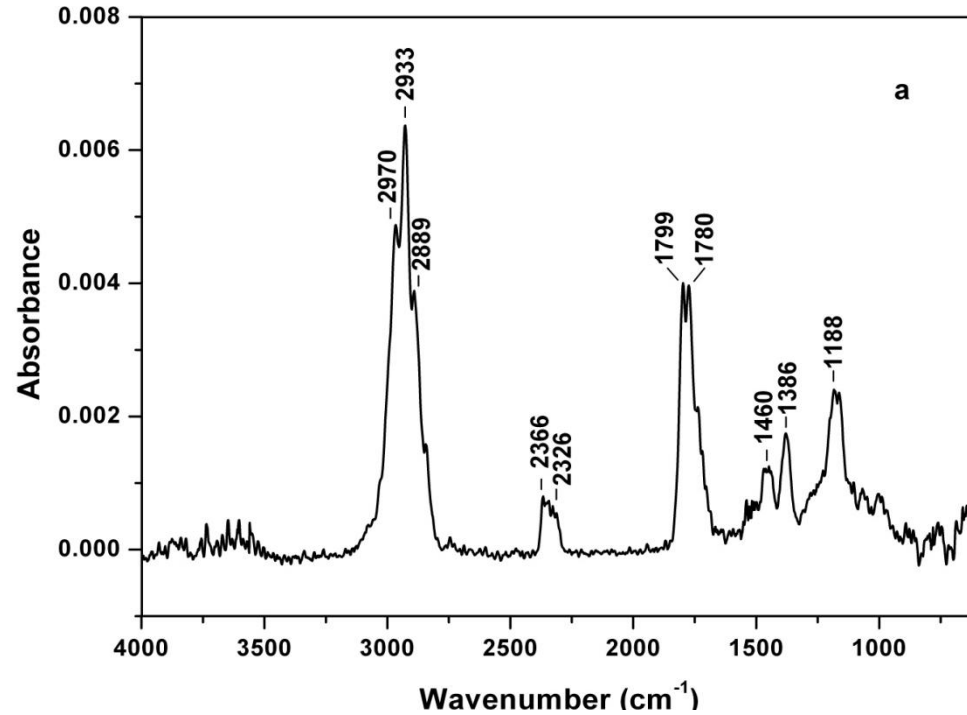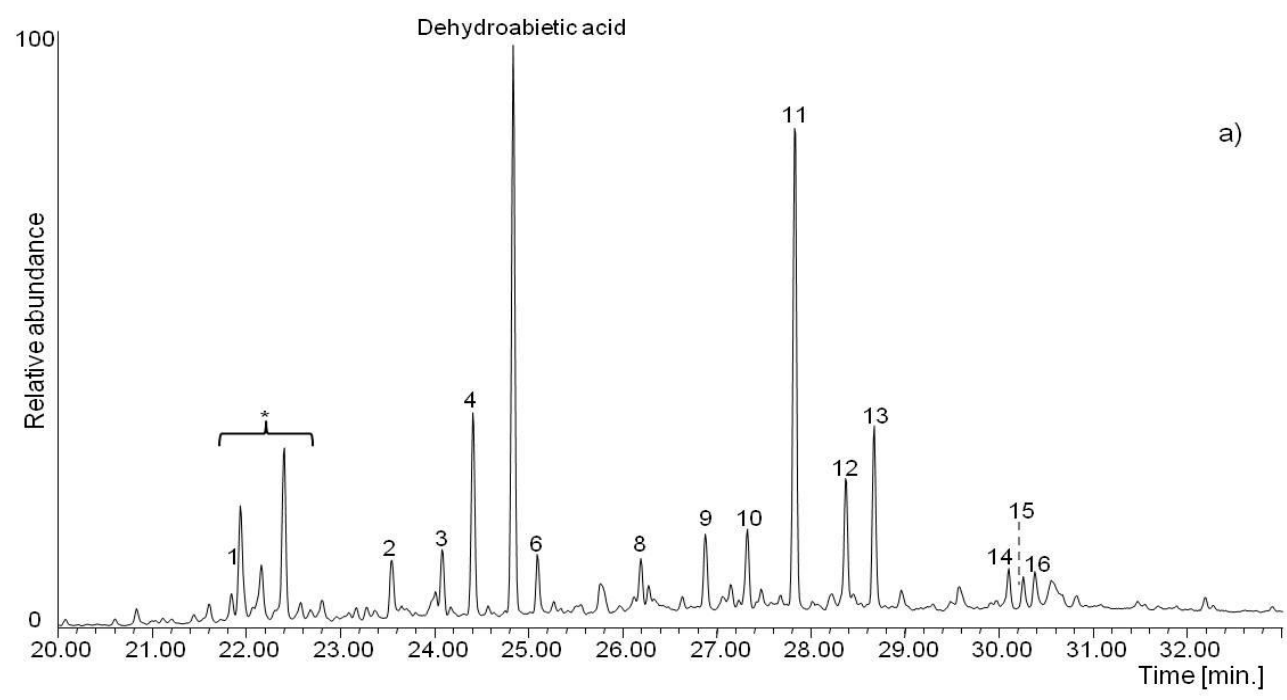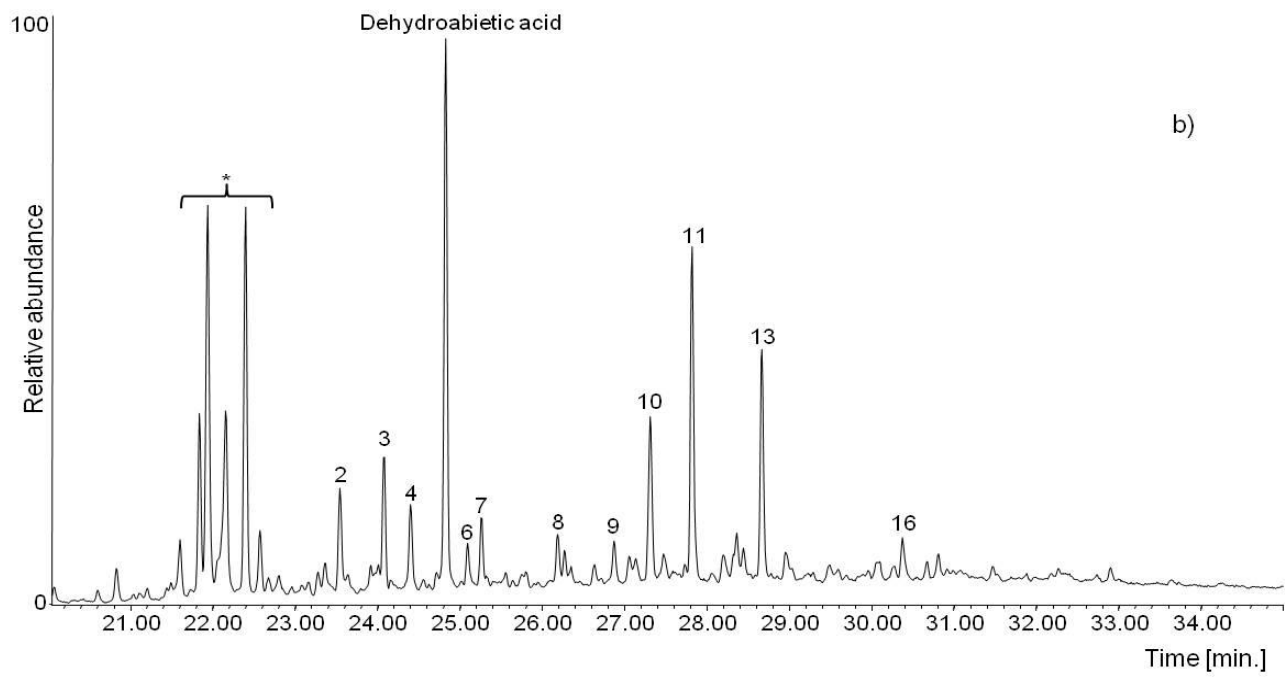5. Interactions between pitch and beeswax were highlighted

Figure(1)

Fig.1.

Figure(2)

Fig.2.

Figure(3)

Fig.3 .

Figure(4)

0 oxidation degree

abietic acid (**7**) and its isomers (neoabietic, palustric, levopimaric acids)

DHA methyl (**3**)

DHA acid (**5**)

diDHA acid(**4**)

I oxidation degree

7-oxo-DHA methyl (**15**)

7-oxo-DHA acid (**11**)

15-hydroxy-DHA acid (**10**)

15-hydroxy-diDHA acid (**9**)

II oxidation degree

15-hydroxy-7-oxo-DHA acid (**16**)

and other oxidized compounds (**12**, **13** and **14**)

III oxidation degree
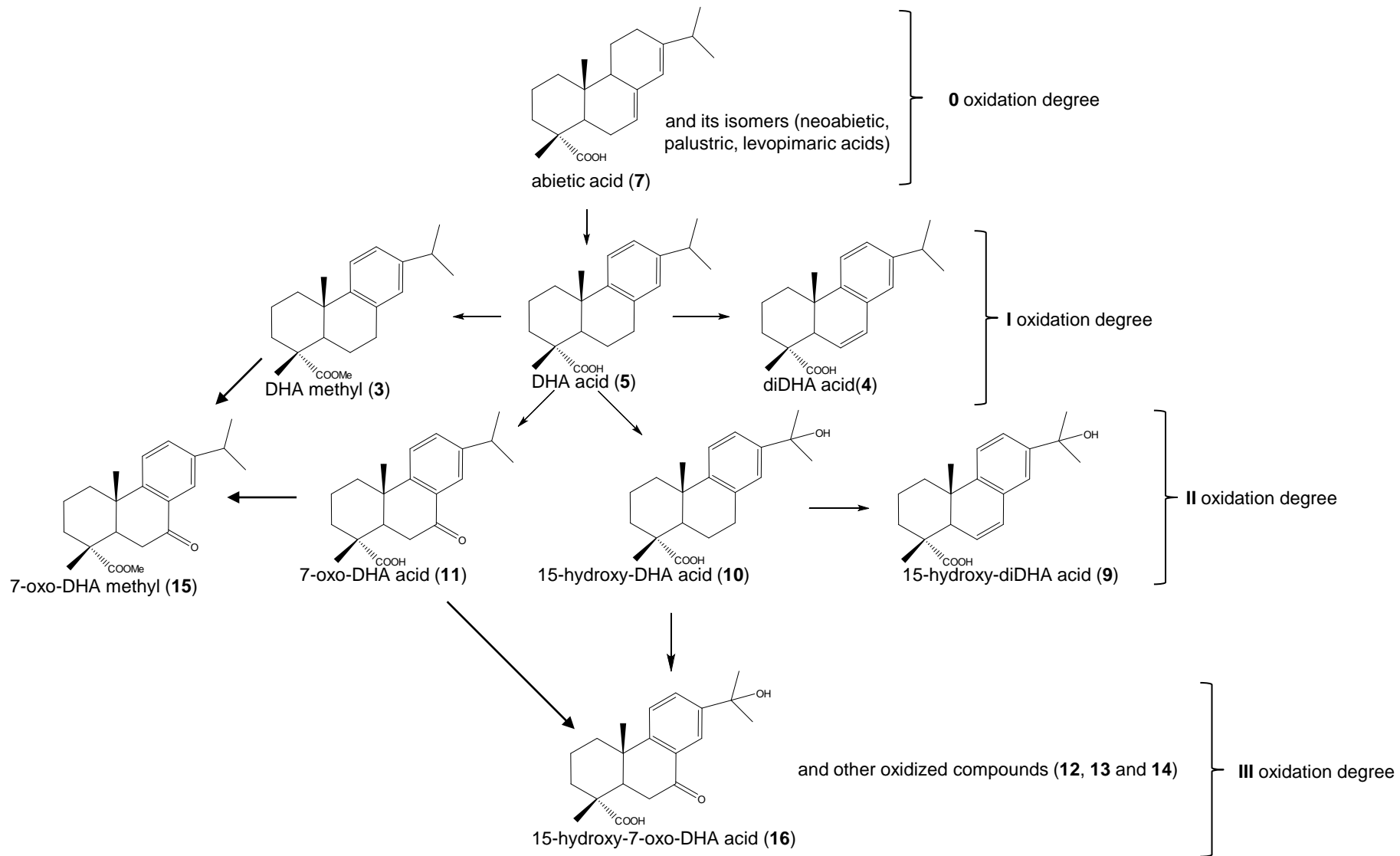
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Fig.5.
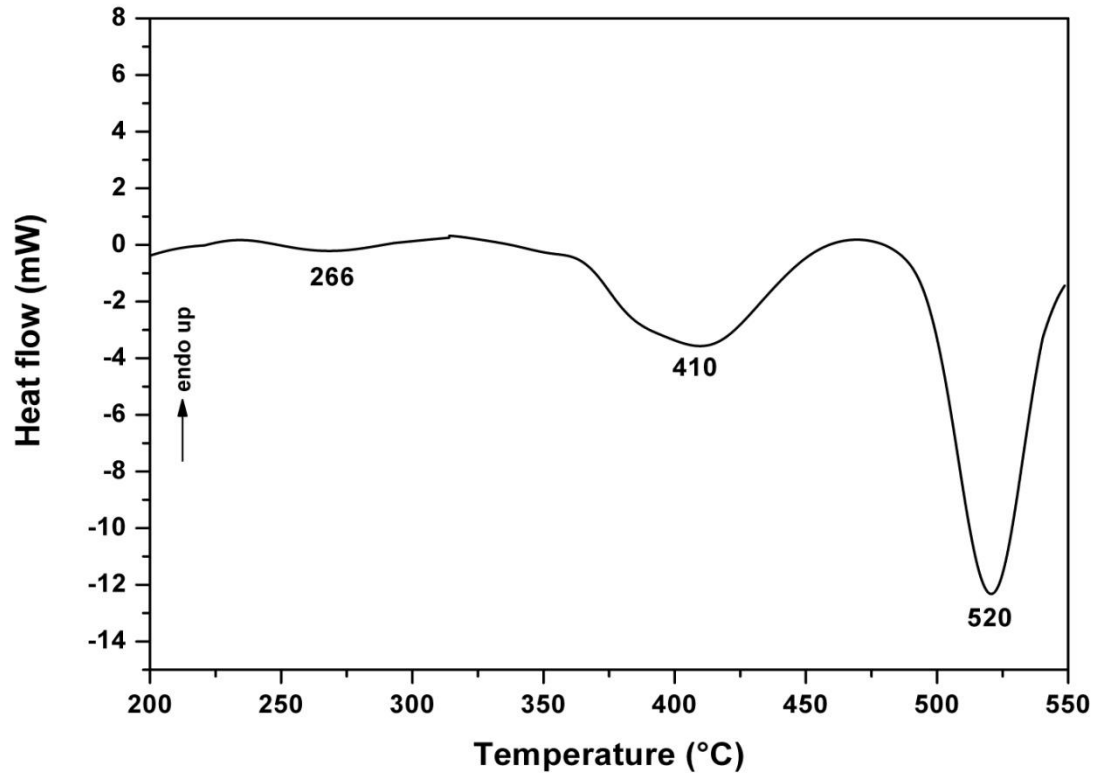
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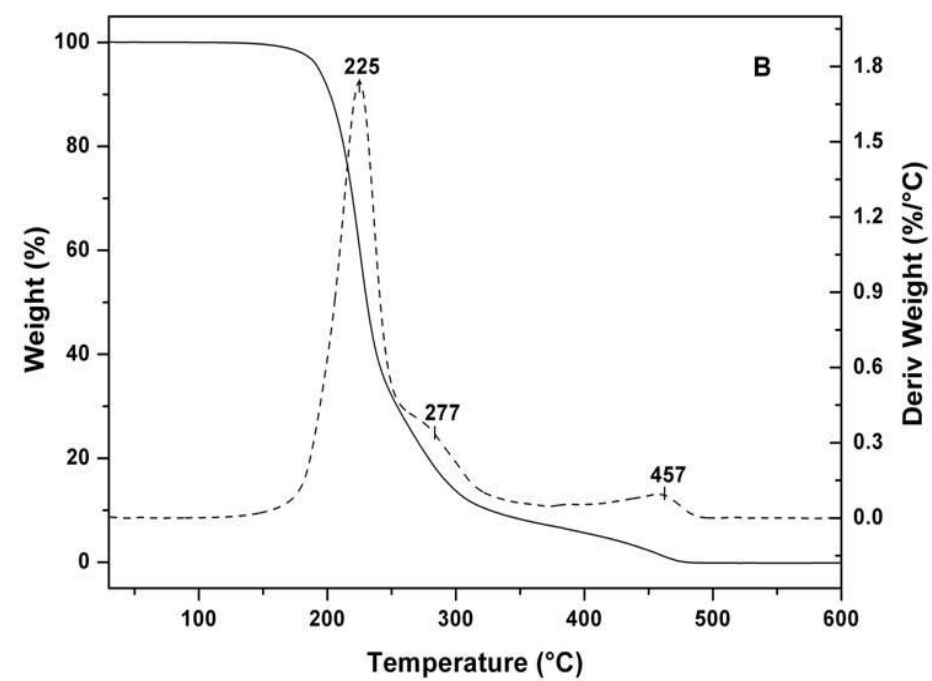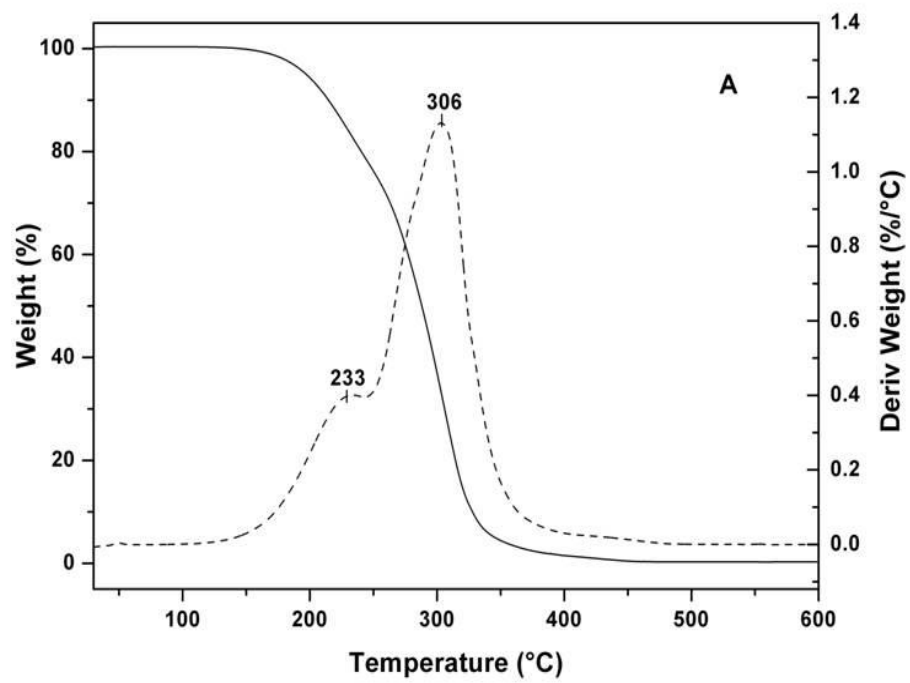
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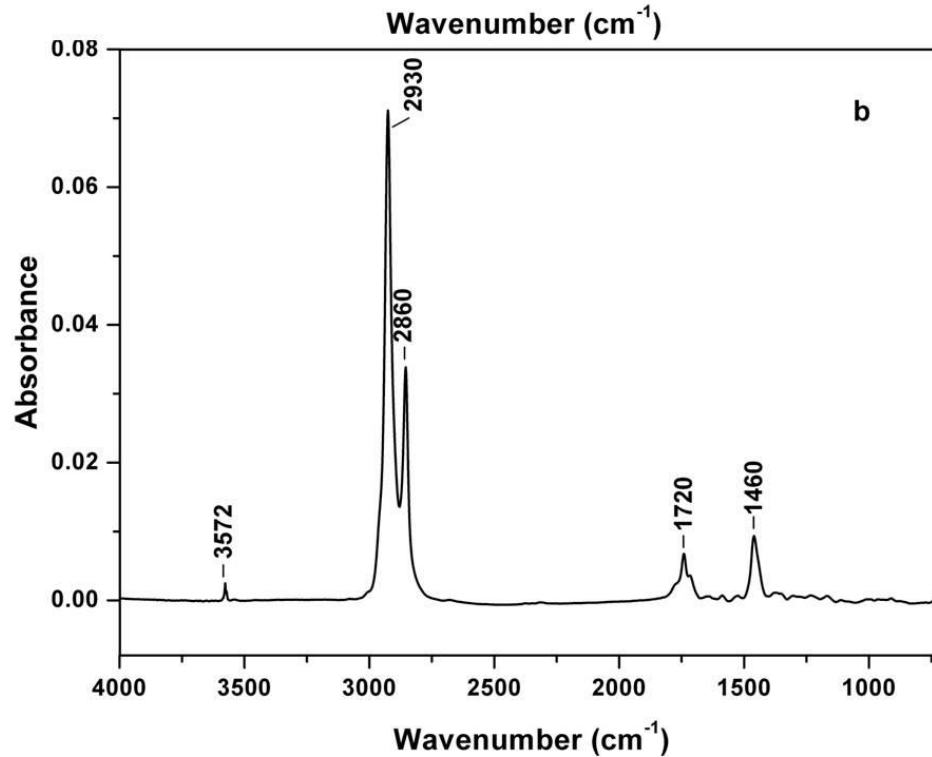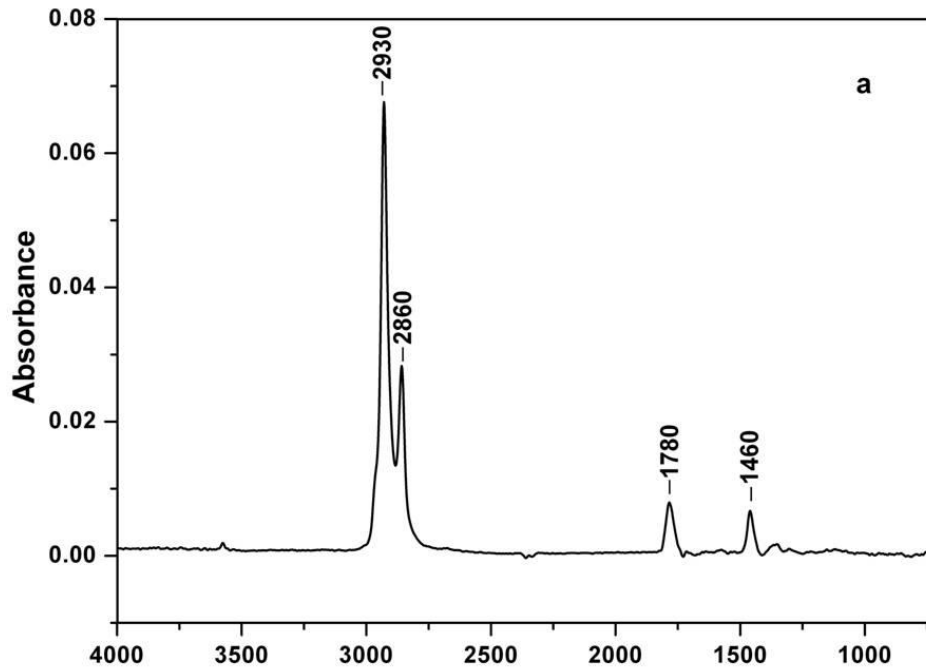
Fig.7.

Figure(8)

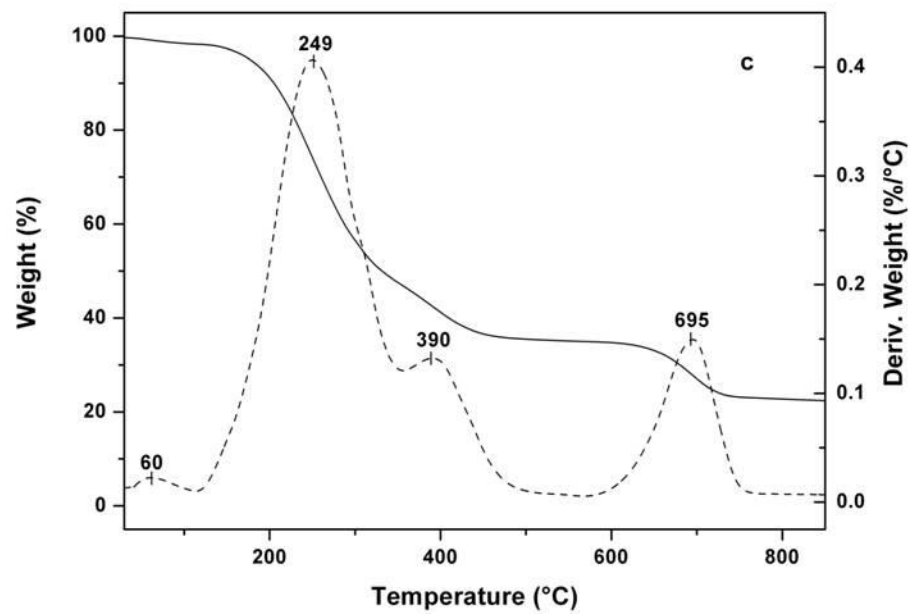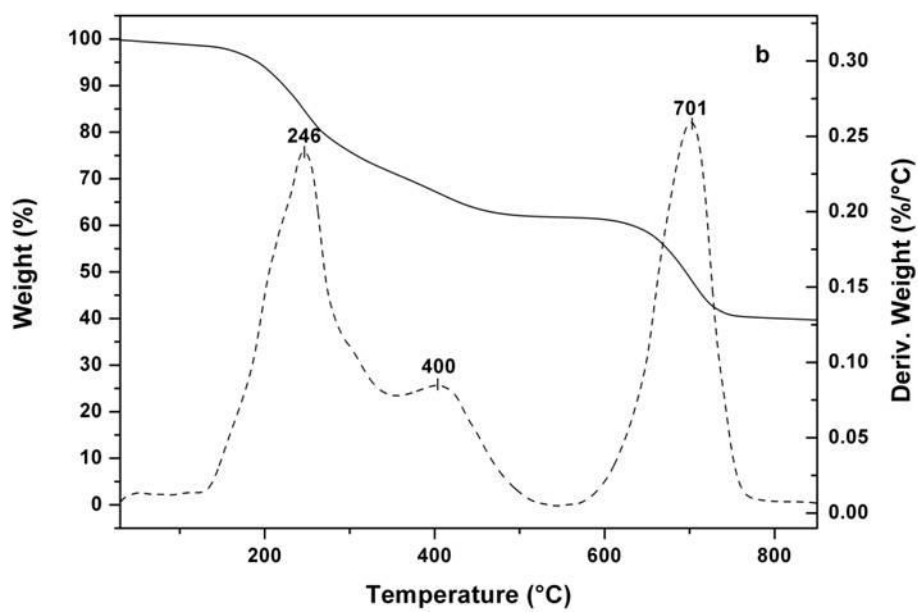Fig.8.
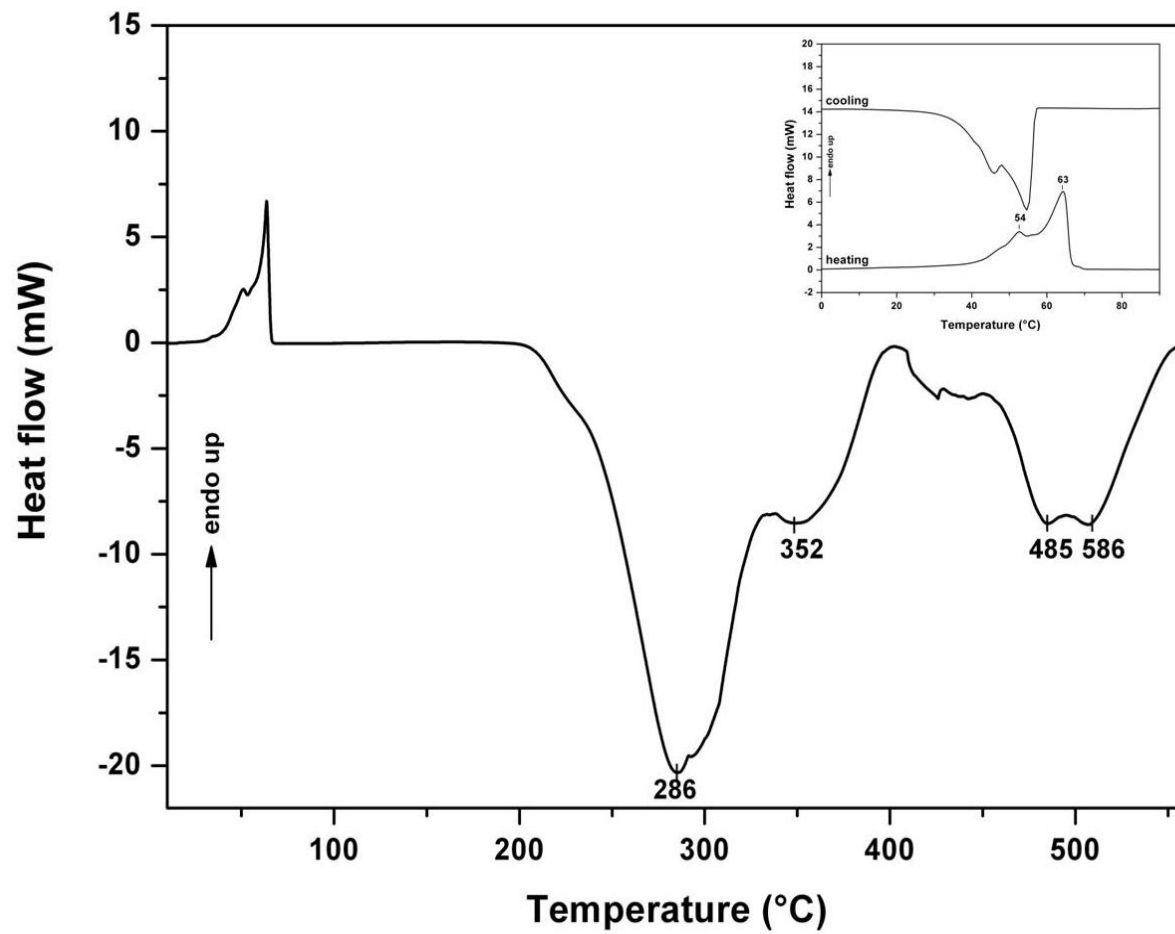
Figure(9)

Fig.9.

Figure(10)

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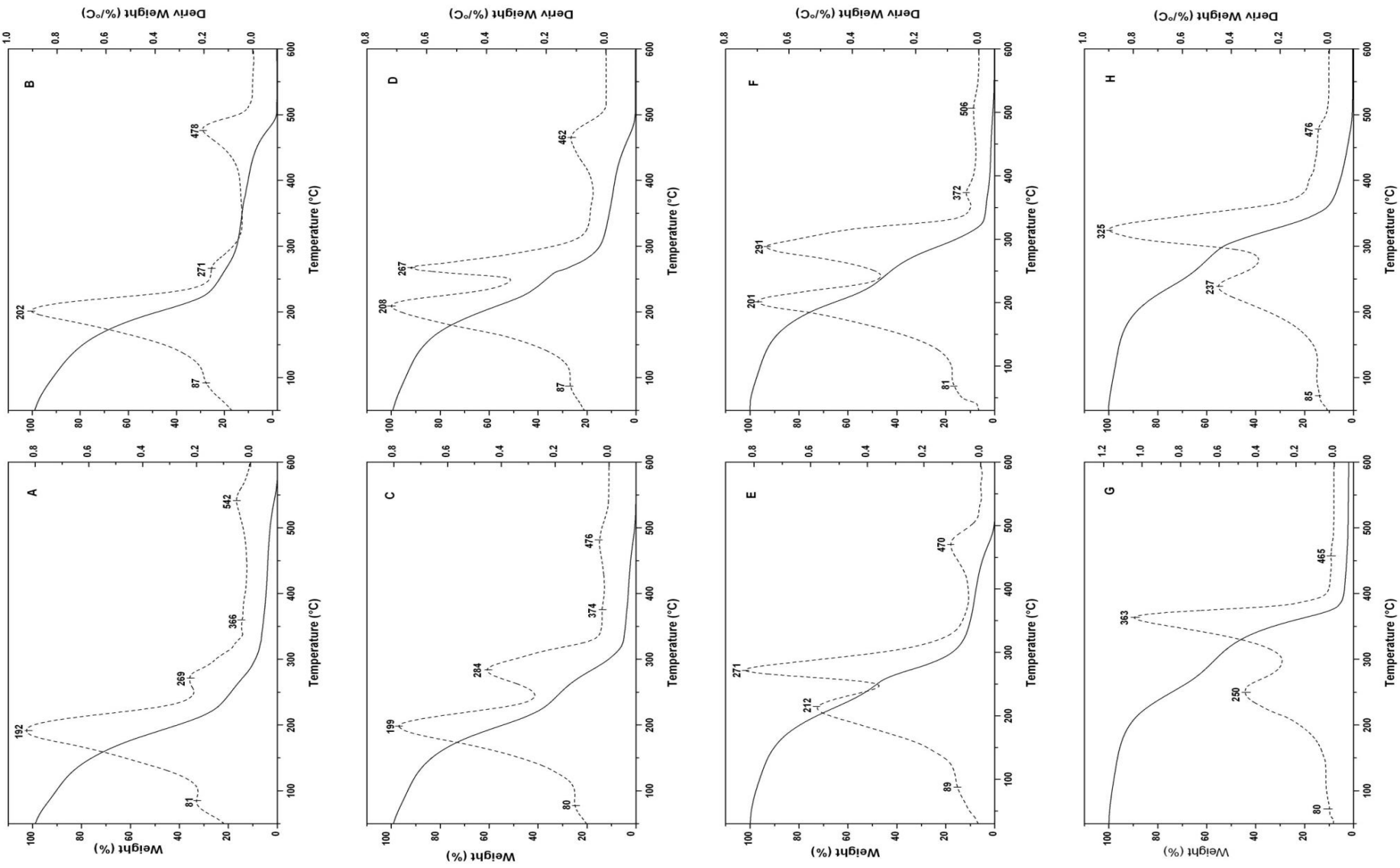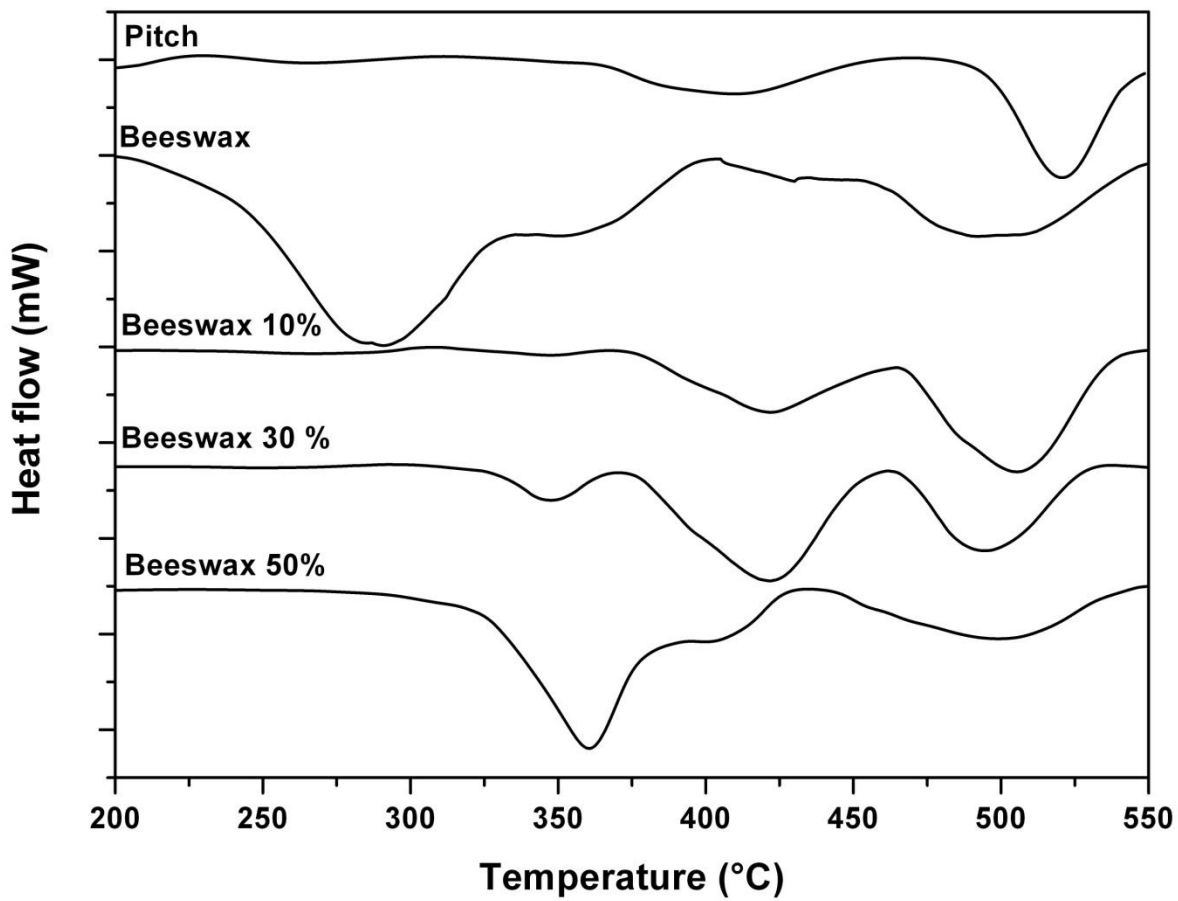
Figure(11)

Fig.11.
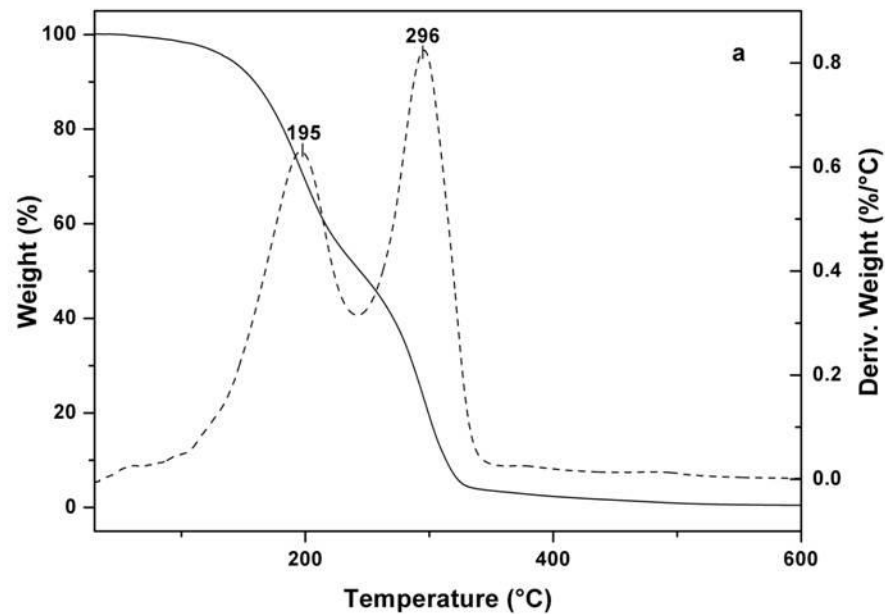
Tables

Table 1

Experimental temperatures and the percentage mass losses of the corresponding degradation steps under nitrogen flow of pure pine pitch, pure beeswax and their mixtures.

Step No.	Temperature of the step and (mass loss %)					
	pine pitch	beeswax	beeswax 10%	beeswax 33%	beeswax 50%	beeswax 70%
1	81.2°C (13.1%)		81°C (10.2%)	80°C (6.9%)	81°C (4.3%)	80°C (2.8%)
2	183°C (80.4%)	233°C (21.5%)	192°C (70.4%)	199°C (60.8%)	201°C (50.7%)	250°C (40.6%)
3		306°C (78.5%)	269°C (13.2%)	284°C (28.2%)	291°C (41.5%)	
4			366°C (0.8%)	374°C (1%)	372°C (1.9%)	363°C (54.8%)
5	531°C (6.1%)		542°C (5.4%)	476°C (2.9%)	506°C (1.4%)	465°C (1.2%)
Residue at 600°C	0.4%	0	0	0.2%	0.2%	0.6%

Table 2

Experimental temperatures and the percentage mass losses of the corresponding degradation steps under air flow of pure pine pitch, pure beeswax and their mixtures.

Step No.	Temperature of the step and (mass loss %)					
	pine pitch	beeswax	beeswax 10%	beeswax 33%	beeswax 50%	beeswax 70%
1	86°C (13.7%)		87°C (9.9%)	87°C (6.4%)	89°C (4.6%)	85°C (3.1%)
2	205°C (70%)	225°C (70.6%)	202°C (66%)	208°C (56.4%)	212°C (46.5%)	237°C (36.9%)
3		277°C (21.3%)	271°C (9.2%)	267°C (26.1%)	271°C (40.3%)	325°C (55.5%)
4	492°C (16.3%)	457°C (8.1%)	478°C (14.9%)	462°C (10.8%)	470°C (8.5%)	476°C (4.0%)
Residue at 600°C	0	0	0	0.3%	0.1%	0.5%

[a]Total mass loss in the range 100-350°C.

Table 3

Compounds identified by GC/MS, their molecular weight (MW), the most abundant fragments observed in the EI mass spectrum (m/z, the base peak is highlighted in bold), and their relative percentage abundance determined in the residues left by the pine pitch heated to 300°C under nitrogen and air flow. Numbers refer to the peak numbers in the chromatograms shown in Figure 3.

n.	Compound identified	MW	m/z	Air flow	Nitrogen flow
1	Retene	234	234, **219**, 204, 189	0.5	0
2	Isomer of abietic acid-TMS ester	374	374, 359, **253**, 239	3.0	5.9
3	Dehydroabietic acid-methyl ester	314	314, 299, **239**	0.0	3.3

4	Didehydroabietic acid-TMS ester	370	370, 355, 252, **237**	2.9	6.6
5	Dehydroabietic acid- TMS ester	372	372, 357, 255, **239**	10.8	4.5
6	Diterpenoid acid-TMS ester	354	354, 339, **237**	26.2	28.9
7	Abietic acid-TMS ester	374	374, 359, **256**, 241	2.8	2.1
8	oxidized terpenoid compound	418	418, 403, **285**	2.0	2.8
9	7-hydroxy-didehydroabietic acid-TMS ester, -TMS ether	458	458, 443, **325**	4.0	2.1
10	7-hydroxy-dehydroabietic acid-TMS ester, -TMS ether	460	460, 445, **327**	4.8	9.8
11	7-oxo-dehydroabietic acid-TMS ester	386	386, 371, 327, 268, **253**	23.8	18.0
12	oxidized terpenoid compound	412	412, 397,372, 357, 255, **239**	1.6	0.0
13	oxidized terpenoid compound	432	432, 417, **299**	5.6	0.0
14	oxidized terpenoid compound	446	446, 431, 356, **313**, 247	8.4	13.6
15	7-oxo-dehydroabietic acid-methyl ester	386	386, 371, **268**, 253	1.5	0.0
16	7-oxo-15-hydroxy-dehydroabietic acid-TMS ester	474	474, 459, **357**, 341	2.1	2.4

Table 4

Experimental temperatures and percentage mass losses of the corresponding degradation steps under nitrogen flow of the studied archaeological samples: an unknown mixture of *Pinus sylvestris* L. and wax ("Zopissa") and two archaeological adhesives collected (Samples 2 and 3) from glass *opus sectile*.

Step No.	Temperature of the step and (mass loss %)		
	Zopissa	Sample 2*	Sample 3*
1			60°C (1.3%)
2	195°C (48.5%)	246°C (28.5%)	249°C (51.5%)
3	296°C (51.5%)		
4		400°C (9.2%)	390°C (11.6%)
5		701°C (22%)	695°C (12.4%)
Residue at 900°C		39.3%	23.2%

*Heated at 20°C/min

Supplementary Material(s)
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