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A diagnosis of the yellowing of the marble high reliefs and the black decorations in the chapel of the tomb of Saint Anthony (Padua, Italy)

Alessia Andreotti, Ilaria Bonaduce*, Maria Perla Colombini[1], Francesca Modugno, Erika Ribechini

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126 Pisa, Italy

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ABSTRACT

This paper focuses on the chemical characterization of samples of black decorations and inscriptions, and of yellow-brown patinas collected from various panels of the ark from the chapel of Saint Anthony (Padua, Italy), using three analytical procedures based on gas chromatography/mass spectrometry (GC/MS). Analytical pyrolysis in the presence of hexamethyldisilazane followed by gas chromatographic–mass spectrometric analysis (Py-GC/MS) and two procedures based on gas chromatography/mass spectrometry after wet-chemical treatment of the samples (GC/MS) were chosen for the recognition of the organic substances (used originally or in the course of restoration) and of their degradation products. In terms of the two GC/MS procedures, one was used to characterize proteins, lipids, resins, waxes, bituminous materials and their degradation products, and the other to characterize saccharide materials.

The three analytical procedures used enabled us to obtain information on the presence of egg proteins, lipid materials (beeswax, siccative oil and animal fatty material), saccharide materials and Pinaceae resin.

Beeswax, animal fat, egg and saccharide materials have been used in the past as restoration materials, and pine resin and siccative oil were the main ingredients in the black decorations and inscriptions.

1. Introduction

One of the most significant effects of atmospheric pollution is the deterioration of historic statues and monuments. Volatile hydrocarbons, nitrogen oxides, sulphur dioxide, aromatic hydrocarbons and suspended particulate matter, affect both outdoor and indoor surfaces. Air pollutants reach the surfaces either by wet deposition (dissolved in rain or fog droplets or in candle smog) or by dry deposition in particle form, leading to permanent corrosion and damage [1,2]. As a result, black crusts and coloured patinas are often observed on stone in urban environments. Coloured patina, together with organic matter from bioactivity [3] and/or from past protective coatings [4,5], can be found on several mainly outdoor marble monuments. Maintenance aimed at refreshing the brightness of the stone surface has been documented in the literature [6]: organic substances such as egg, milk, animal fats, beeswax, natural resins, oils, plant gum, and molasses were commonly applied in the past. In the last 20 years synthetic coatings based on acrylic or silicone polymers have been widely used but tend to be of short durability and subject to yellowing [7,8].

Despite considerable scientific interest, the origin of patina remains an open issue, particularly regarding marble objects stored indoors. Investigating the formation of the yellow-brown patina formed on indoor marble objects involves not only understanding the phenomenon in itself, but also drawing on the conservation history of the object, in terms of decay linked to atmospheric weathering, bio-colonization and past restoration. Moreover, an understanding of such processes can help to guide future conservation initiatives, by suggesting the best possible restoration methods and how to remove the patina.

High reliefs, i.e., sculptures where the background is carved away to such an extent that the figures are almost free-standing, have appeared in all ages and places, modelled in clay, carved in stone or cast in bronze. Since ancient times, marble high reliefs have been widespread in Greece and Italy and their conservation deserves great attention.

In 2008 a conservation intervention started on the chapel of the tomb of Saint Anthony in Padua (Italy). The chapel containing the "Ark" – the Saint's tomb – is located in the northern part of the Basilica of Saint Anthony in Padua. Since the time it was built in 1300, the humidity has caused severe damage so that in the 1500s the original frescoes were replaced with marble high reliefs and bronzes.

* Corresponding author. Tel.: +39 0502219252; fax: +39 0502219260.
E-mail addresses: alessia@dcci.unipi.it (A. Andreotti), ilariab@dcci.unipi.it (I. Bonaduce), perla@dcci.unipi.it (M.P. Colombini), frances@dcci.unipi.it (F. Modugno), erika@dcci.unipi.it (E. Ribechini).
[1] Tel.: +39 0502 219305; fax: +39 0502 219260.

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Fig. 1. Panel 1, *The miracle of the speaking newborn*, by Antonio Lombardo; 2 m wide by 3 m long. Basilica of Saint Anthony from Padua, Italy.

Nine high reliefs representing the Saint's miracles and life, created by Sansovino, Lombardo and other 16th century masters, decorate the walls behind the tomb. The panels represent the following:

Panel 1: *The miracle of the speaking newborn*, by Antonio Lombardo (1505), Tullio's brother (Fig. 1);
Panel 2: *The miracle of the glass thrown by a misbeliever*, begun by Giovanni Maria Mosca, and completed by Paolo Stella (1529);
Panel 3: *The miracle of the reattached foot,* by Tullio Lombardo (1504);
Panel 4: *The miracle of the usurer*, by Tullio Lombardo (1525);
Panel 5: *The resuscitated child,* by Antonio Minello, with retouches by Sansovino (1536);
Panel 6: *The resurrected young girl,* by Jacopo Sansovino (1563);
Panel 7: *The young man resurrected by the Saint*, by Danese Cattaneo, completed by Girolamo Campagna (1573);
Panel 8: *The jealous husband stabs his wife*, begun by Giovanni Rubino (known as il Dentone), and completed by Silvio Cosini (1536);
Panel 9: St. Anthony receiving the Franciscan habit, by Antonio Minello (1517).

Some of the panels are decorated with black painted details as shown in Fig. 2, and black inscriptions. The white marble of most of the panels presents large areas covered with a yellow to dark-brown coloured patina (see the details of the high relief in Fig. 3).

Fig. 2. Particular of panel 8 with black painted decorations on the Basilica of Saint Anthony from Padua, Italy.

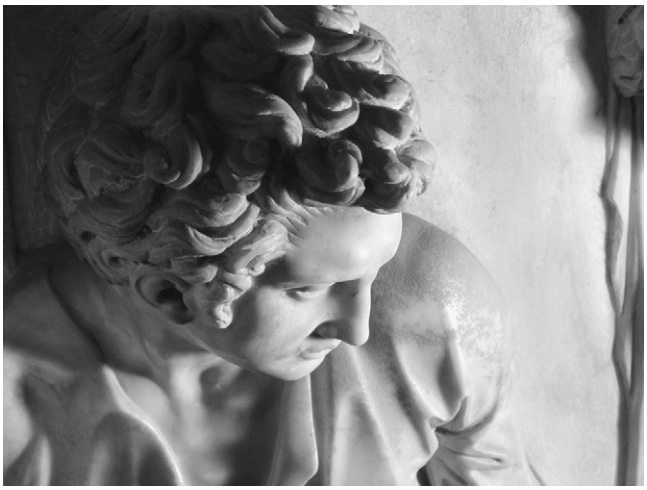

Fig. 3. Particular of panel 9 in which on the shoulder of the man is clearly visible an inhomogeneous area corresponding to the yellow-brown patina. Basilica of Saint Anthony from Padua, Italy.

The phenomenon is less pronounced in the upper parts of the high reliefs, while being heavily present in the lower parts. The conservation was undertaken to clean the high reliefs in order to remove the coloured film on the surface and to regain the original brightness without altering the porosity of the stone. As previously mentioned, it is fundamental for accurate restoration, to characterize the materials that need to be removed and to understand their origin in order to prevent or reduce their occurrence in the future.

This paper deals with the chemical characterization of samples of black decorations, a black inscription and yellow/brown patinas collected from various panels of the chapel, using gas chromatographic–mass spectrometric techniques.

The aim of the study was to set up a reliable restoration approach, by studying the materials used as binders in the black painted areas and the materials applied to the marble in past treatments that have generated the coloured patina with ageing.

Analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS), and gas chromatography/mass spectrometry after wet-chemical treatment of the samples (GC/MS) [9] were chosen for the recognition of materials and their degradation products. Hexamethyldisilazane (HMDS) was used in Py-G/MS for in situ thermally assisted derivatization of pyrolysis products.

As both methods are micro-invasive, they can be successfully adopted for the analysis of tiny samples from cultural heritage objects. Indeed, these methods lead to the identification of materials at a molecular level using a few micrograms of sample for Py-GC/MS and less than one milligram in the case of GC/MS. In addition, when applied to different aliquots of the same sample they provide complementary information and enable the simultaneous detection of natural and synthetic materials. The Py-GC/MS method [10–12] in particular, reveals the composition of polymers and the presence of molecular markers related to specific materials; the GC/MS procedure [13,14] leads to the identification of proteins, lipids, resins, waxes, bituminous materials and their degradation products; another GC/MS procedure applied on another aliquot of the sample was used to characterize the saccharide materials [15].

The application of these three procedures to the samples from the Saint Anthony chapel revealed various materials as the main components of the patina and the black paint. The most significant results are presented and discussed in the following sections.

Table 1
Description of the samples.

Panel	Title	Sample	Sample location	Colour	Weight (mg)
1	*The miracle of the speaking newborn*	10		Yellow-brown	0.7
		11		Yellow-brown	0.3
2	*The miracle of the glass thrown by a misbeliever*	9		Yellow-brown	0.5
		8		Black	0.6
3	*The miracle of the reattached foot*	7		Black	1.8
		6		Black	0.5
7	*The young man resurrected by the Saint*	4		Black	0.5
9	*St. Anthony receiving the Franciscan habit*	3		Black	0.8
		2		Yellow-brown	1.2
		1		Yellow-brown	0.3

2. Experimental

2.1. Reagents

2.1.1. GC/MS procedure for the analysis of proteinaceous, lipid and resinous materials

N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane, N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) with 1% trimethylchlorosilane, triethylamine, norleucine, tridecanoic acid, hexadecane were purchased from Sigma–Aldrich (USA). All the solvents were Baker HPLC grade. Amino acid standard solutions in HCl 0.1N from Sigma–Aldrich (USA), containing 12.5 μmol/ml of proline (Pro) and hydroxyproline (Hyp), and 2.5 μmol/ml of aspartic acid (Asp), glutamic acid (Glu), alanine (Ala), arginine, cysteine, phenylalanine (Phe), glycine (Gly), hydroxylysine, isoleucine (Ile), histidine, leucine (Leu), lysine (Lys), methionine (Met), serine (Ser), tyrosine (Tyr), threonine, valine (Val) were prepared. Fatty acid standard solution in acetone from Sigma–Aldrich (USA), containing lauric acid (0.24 mg/g), suberic acid (0.27 mg/g Su), azelaic acid (0.28 mg/g A), myristic acid (0.25 mg/g My), sebacic acid (0.3 mg/g Se), palmitic acid (0.25 mg/g P), oleic acid (0.51 mg/g, O), stearic acid (0.51 mg/g, S) were also prepared.

2.1.2. Py-GC/MS procedure

Hexamethyldisilazane (HMDS) was purchased from Sigma–Aldrich (USA).

2.1.3. GC/MS procedure for the analysis of saccharide material

Trifluoroacetic acid, and anhydrous pyridine were from Fluka (Milan, Italy), ethanethiol (ETSH), sodium azide (NaN_3), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and the cationic exchange resin Dowex-50W-8X, 8% cross linking grade, and granulometry comprised between 100 and 200 meshes, were supplied by Sigma (USA).

2.2. Sample descriptions

The samples are listed in Table 1, and were taken by scalpel from: the coloured patina on panel 9 (samples 1 and 2), panel 2 (sample 9) and panel 1 (samples 10 and 11); the black painted decorations from panel 9 (sample 3), panel 7 (sample 4), panel 3 (sample 6) and panel 2 (sample 8); and the black inscription from panel 3 (sample 7).

2.2.1. Py-GC/MS analytical procedure

The sample was placed in a quartz tube, admixed with 5 μl of hexamethyldisilazane (HMDS) and pyrolysed at 550 °C for 20 s. The pyrolyser (CDS Pyroprobe 5000 series) was coupled online with a 6890N GC System Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled with a 5973 Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA) single quadrupole mass spectrometer. The pyrolyser interface was kept at 180 °C, the transfer line at 300 °C, and the valve oven at 290 °C. For the gas chromatographic separation a HP-5MS fused silica capillary column (5%diphenyl-95%dimethylpolysiloxane, 30 m × 0.25 mm i.d., J&W Scientific Agilent Technologies, USA) with a de-activated silica pre-column (2 m × 0.32 mm i.d., J&W Scientific Agilent Technologies) was used. The split–splitless injector was used in the split mode (split ratio range between 1:10 and 1:20) at 300 °C, with a split ratio dependent on the sample dimensions. The chromatographic conditions were: 30 °C isothermal for 8 min, 10 °C/min up to 240 °C and isothermal for 3 min, 20 °C/min up to 300 °C and isothermal for 30 min. The carrier gas (He, purity 99.995%) was used in the constant flow mode at 1.0 ml/min.

2.2.2. GC/MS analytical procedure for the analysis of proteinaceous, lipid and resinous materials

The analytical procedure based on GC/MS and used for the analysis of lipids, terpenoid resins and proteinaceous materials has already been reported in the literature [13]. The sample is subjected to ammonia extraction in order to solubilize proteins and to separate the proteinaceous matter from insoluble inorganic salts. The extracted ammonia solution is evaporated to dryness under a stream of nitrogen and then subjected to acidic hydrolysis assisted by microwave in vapour phase. After the hydrolysis, bidistilled water is added to the acidic hydrolyzate which is then extracted with diethyl ether. The ethereal extracts are then mixed with the residue of the ammonia extraction. The free acids and any other organic compounds extracted by ammonia together with the proteins can thus be retrieved. The residue of the diethyl ether extraction is an acidic solution of amino acids. An aliquot of the amino acidic solution is evaporated to dryness under a stream of nitrogen and is subjected to derivatization with N-methyl-N-(t-butyldimethylsilyl)trifluoroacetamide) (MTBSTFA), 2 μl are analyzed by GC–MS. The residue of the ammonia extraction added with the ethereal extract of the hydrolyzed solution, is subjected to saponification assisted by microwave. After saponification the hydroalcoholic solution is diluted in bidistilled water and an extraction with hexane followed by an extraction with diethyl ether is performed. Finally, an aliquot of this extract is evaporated to dryness under a nitrogen stream and subjected to derivatization with N,O-bistrimethylsilyl-trifluoroacetamide (BSTFA) and 2 μl of the resulting solution is injected onto the GC/MS system.

2.2.3. GC/MS analytical procedure for saccharide materials

The sample is subjected to microwave assisted acid hydrolysis. After hydrolysis, the sample is filtered with a PTFE membrane and then purified on a cation-exchange resin. Aldoses and uronic acids are silylated after being converted into the corresponding diethyl-dithioacetals and diethyl-dithioacetal lactones. Details of the analytical procedure are reported in the literature [15]. The gum was identified as already outlined in the literature [15].

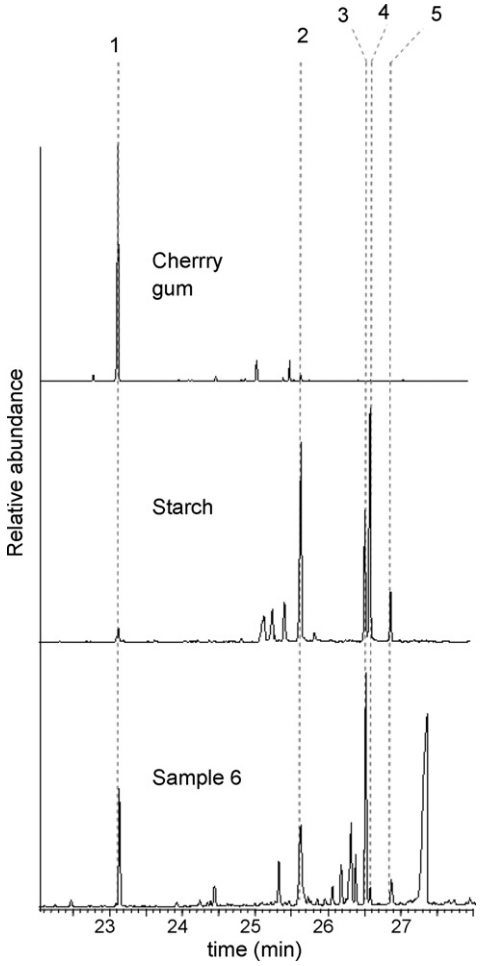

Fig. 4. Extracted ion Py-GC/MS chromatograms for *m*/*z* 217 of cherry gum, starch and sample 6.

3. Results and discussion

To identify the organic materials that make up the patinas and black decorations, the samples were submitted to both Py-GC/MS and GC/MS. The following subsections report the most significant results achieved with the three methods. Some patina samples were also examined beforehand by infrared spectroscopy in order to highlight the inorganic components present. This analysis (spectra not reported) showed that the content of sulphates was quite low and other salts such as oxalates were not present.

3.1. Py-GC/MS

All the samples showed the presence of organic materials. The main classes of observed materials are discussed separately below.

a) Pyrolysis products deriving from saccharide materials were observed in the Py-GC/MS analysis of samples 3, 4, 6, 10 and 11. It is critical to identify all the peaks in the Py-chromatograms of carbohydrate materials, due to the high number of isomers and pyrolysis products formed. Thus, a tentative identification of saccharide materials in pyrolysis can be achieved essentially by comparing chromatograms of standard materials. However, possible effects on the chromatographic profiles due to the simultaneous presence of other materials can occur and cannot be anticipated. In this case, all samples showed the presence of tri-(O-TMS)-levoglucosan, a typical pyrolysis product of glucose containing polysaccharides, such as starch and dextrins. Moreover, most of the samples contained 1,2,3,5-tetrakis-(O-TMS)-xylofuranose, which is the main pyrolysis product of polysaccharidic plant gums, such as arabic gum and fruit tree gums [16,17]. In Fig. 4, an extracted ion pyrogram of *m*/*z* 217 from sample 6 is compared with those of cherry gum and starch. The ion *m*/*z* 217 is the fragment ion $[H_2C=CHC(OTMS)=OTMS]^+$ arising from the interaction between two TMS derivatized moieties in the same molecule [18], and is abundant in all the mass spectra of saccharide material pyrolysis products. Arabic and tragacanth gum show a very similar profile of ion *m*/*z* 217, and for this reason are not reported. At the moment it is not possible to discriminate between fruit tree, arabic and tragacanth gums using Py-GC/MS, and a GC/MS analytical procedure was used, as will be shown in the next section. Starch was chosen as being representative of a glucose based polymer. Dextrine showed a very similar profile of ion *m*/*z* 217, and is thus not reported.

As expected with glucose based polymers, starch showed tri-(O-TMS)-levoglucosane as one of the main pyrolysis products (peak 3). Peaks 2, 4 and 5 were unidentified. Cherry gum showed 1,2,3,5-tetrakis-(O-TMS)-xylofuranose (peak 1) as the main pyrolysis product, a compound that is absent in the pyrogram of starch. On this basis, sample 3 may have contained both a polysaccharide gum, such as fruit tree, arabic or tragacanth gum, and a material made of glucose such as starch or dextrin. Mass spectrum of 1,2,3,5-tetrakis-(O-TMS)-xylofuranose (peak 1), and those corresponding to peaks 2, 4,

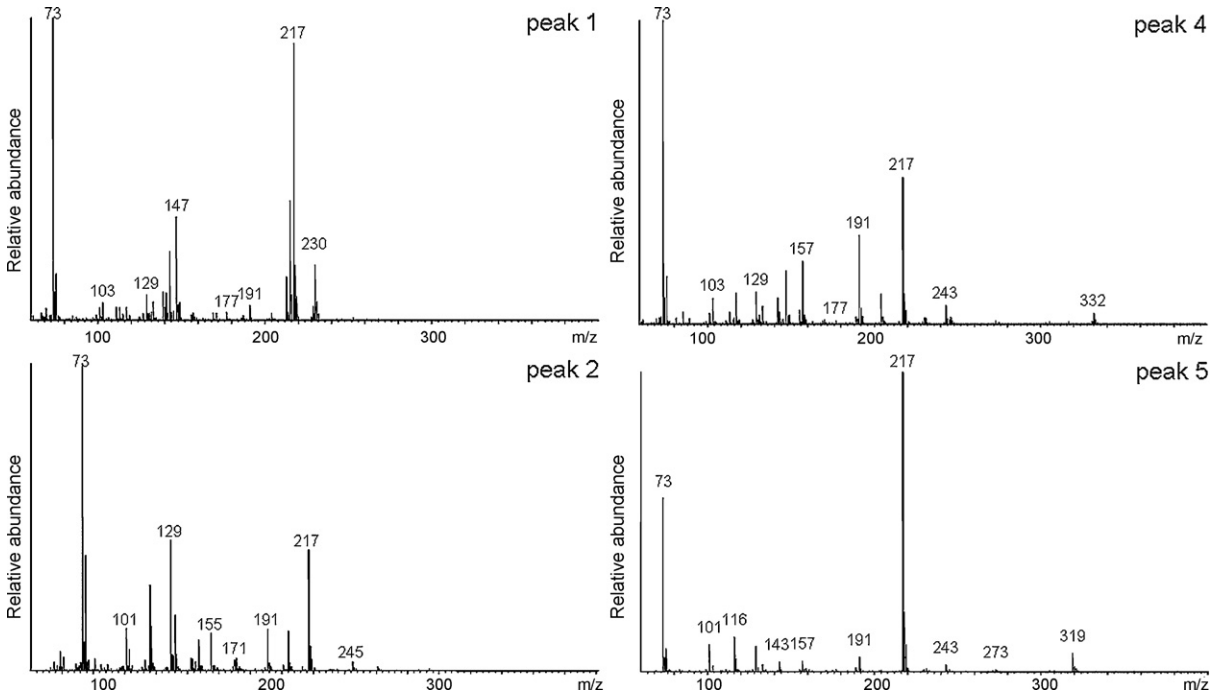

Fig. 5. Mass spectra of peaks labelled between 1 and 5 in Fig. 4.

and 5 are reported in Fig. 5, since the mass spectrum of 1,2,3,5-tetrakis-(O-TMS)-xylofuranose is not reported in the literature and compounds corresponding to peaks 2, 4, and 5 are unknown. CI and MS/MS experiments could be used to help in their identification.

b) A drying oil was identified in samples 3, 4, and 6. Drying oils can be identified on the basis of the presence in the chromatograms of monocarboxylic fatty acids (palmitic and stearic acids being the most abundant) and dicarboxylic acids (azelaic being the most abundant). From the literature, it is known that drying oils are characterized by a high content of azelaic acid, which arises from the oxidation processes undergone by polyunsaturated fatty acids originally present in the triglycerides [19–22]. A mature drying oil shows a higher azelaic content or at least equal to palmitic acid. Given that saturated fatty acids are less subjected to degradation upon ageing, the ratio of palmitic acid over stearic acid can be used to identify the source of the oil [20,21]. All these aspects can be easily evaluated with GC/MS based procedures, after the hydrolysis of glycerides. However, the behaviour in pyrolysis of these materials is complex, especially when an in situ silylating agent is used, because the pyrolysis side effects of fragmentation and isomerization of double bonds alter the observed fatty acid profile. In addition, the simultaneous presence of other materials, organic and inorganic, can lead to changes in the yields of pyrolysis, hydrolysis and derivatization. For these reasons it is not possible to evaluate the source of the drying oil on the basis of the palmitic over stearic ratio. In Fig. 6 the extracted ion chromatogram of m/z 129 of sample 3 is shown in comparison to a linseed oil aged sample. m/z 129 is a fragment ion ($[H_2C=CHCOOTMS]^+$) typical of mono and dicarboxylic acid TMS esters [18]. The high content of dicarboxylic acid clearly indicates a drying oil.

c) Beeswax was identified in the pyrograms of samples 6, 7, 8, 9, 10, 11 on the basis of the presence of fatty acids with a higher number of carbons than 20, small amounts of long chain alcohols, hydrocarbons, and 15-hydroxyhexadecanoic acid [12,23].

d) A Pinaceae resin was detected in samples 3,4, 7, and 8, on the basis of the presence, in the pyrograms, of characteristic diterpenoid species such as didehydroabietic, dehydroabietic and 7-oxo-dehydroabietic acids, together with some pimaradienic acids, such as pimaric and isopimaric acids [24].

Fig. 7 reports the pyrogram of sample 3, where between 31 and 34 min the markers of beeswax and a Pinaceae resin are highlighted.

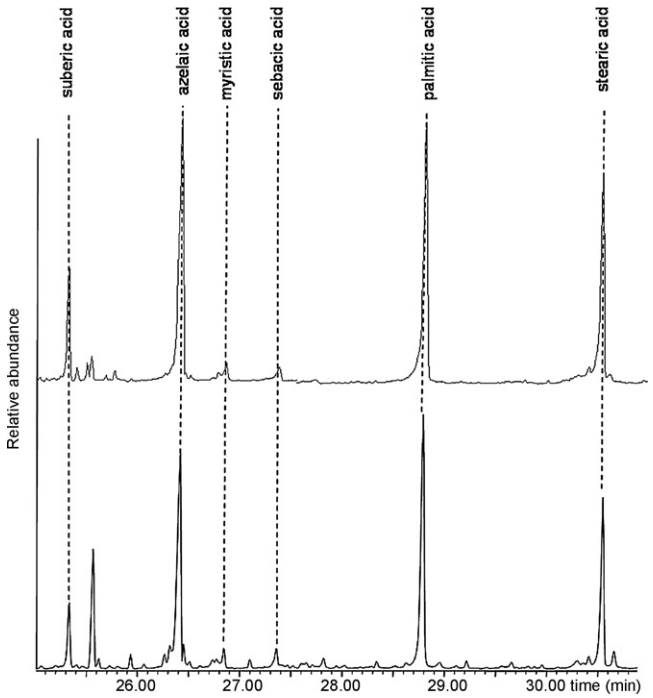

Fig. 6. Extracted ion chromatogram of m/z 129 (fragment ion typical of mono and dicarboxylic acids TMS esters) of sample 3 (upper chromatogram) is shown in comparison to that of a linseed oil aged sample (lower chromatogram).

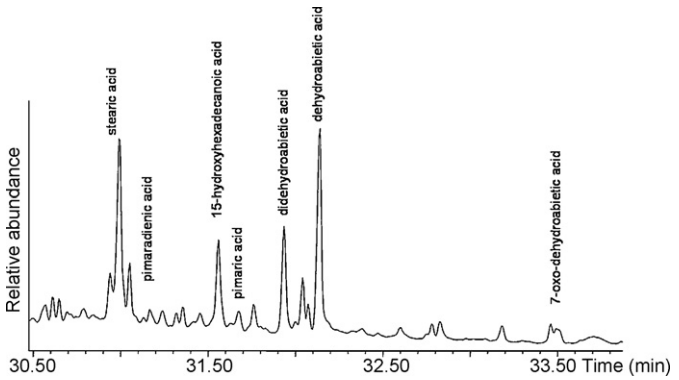

Fig. 7. Pyrogram of sample 3 with highlighted the markers of beeswax and *Pinaceae* resin.

e) Egg (whole or egg yolk) was clearly identified in samples 2, 7, and 9 on the basis of the presence in the pyrograms of hexadecanenitrile and octadecanenitrile [10].

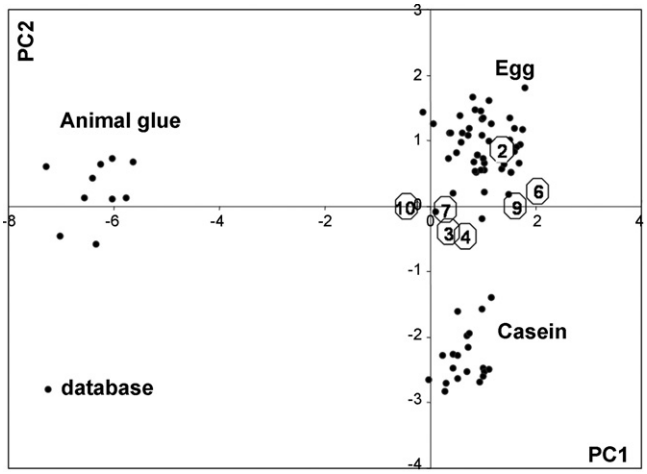

Fig. 8. PCA score plot relative to samples 2, 3, 4, 6, 7 and 9. The relative amino acid percentage content of the samples is reported in Table 2.

3.2. GC/MS

3.2.1. GC/MS analytical procedure for the analysis of proteinaceous, lipid and resinous materials

The combined analytical procedure based on GC/MS for the simultaneous characterization of proteinaceous materials, drying oils, animal and plant terpenoid resins and natural waxes was used for the characterization of samples 2, 3, 4, 6, 7, 8 and 9. The analysis of the amino acids enabled us to assess the presence of proteinaceous materials in all the samples, except for sample 8. For each sample, the percentage amino acid content is reported in Table 2. The absence of hydroxyproline, a marker of collagen, enabled us to rule out the presence of animal glue. The chromatographic profile showed that the samples contained egg protein. This was inferred on the basis of a principal component analysis (PCA) of the amino acid profiles of the samples together with those of the reference paint materials. In fact, proteinaceous material (egg, casein or animal glue) in unknown samples can be identified by PCA of the percentage amino acidic content data, using a reference data set of 101 reference samples containing egg, casein and animal glue [25]. The PCA was performed on the correlation matrix of the data and the first two components accounted for 95.3% of the data variance. The relative score plot for the first two principal components, where all the samples were located very close to the egg cluster, is reported in Fig. 8.

As far as the lipid and terpenoid fraction is concerned, the results showed that all the samples, excluding sample 2, contained a substantial amount of fatty acids and terpenoid compounds. As an example, Fig. 9 reports the chromatogram of sample 7.

The presence of bicyclic diterpenoid compounds with an abietane skeleton, namely di-dehydroabietic (DDA), dehydroabietic (DA) and 7-oxo-dehydroabietic acids (7ODA), evident in all the samples, highlighted the presence of a diterpenoid resin obtained from a tree of the *Pinaceae* family. Moreover, GC/MS analyses of the lipid fractions revealed a quite complex fatty acid composition, highlighting the presence of several compounds related to the use of a siccative oil, beeswax, and lipids of animal origin:

- Beeswax was present in all the samples, except for sample 3. Its presence was ascertained by the presence of linear saturated fatty acids (indicated in Fig. 9 as $A_{X:Y}$ where X is the chain length and Y is the degree of insaturation) containing 14–34 carbon atoms, with palmitic, stearic and lignoceric acids as the most abundant, long-chain *n*-alcohols (indicated in Fig. 9 as OHX where X is the chain length) containing 24 to 34 carbon atoms and long-chain hydroxyacids, namely 14-hydroxyhexadecanoic acid, 15-hydroxyhexadecanoic acid [12].
- Siccative oil was recognized in samples 3, 4 and 6. On the basis of a quantitative analysis of monocarboxylic fatty acids (indicated in Fig. 9 as $A_{X:Y}$ where X is chain length and Y is the degree of insaturation) and dicarboxylic fatty acids (indicated in Fig. 9 as diA_X where X is the chain length), the following parameters were established for samples 3 and 4, respectively: A/P (azelaic over palmitic ratio) = 2.4, P/S (palmitic over stearic ratio) = 0.6 and $\Sigma D\%$ (% sum of dicarboxylic acids, namely suberic azelaic and sebacic acids) = 51.3; A/P = 1.7, P/S = 1.7 and $\Sigma D\%$ = 54.9. The A/P value together with $\Sigma D\%$ can be used to evaluate the presence/absence of a siccative oil, whereas since saturated fatty acids are less reactive, the P/S value can be used to identify the source of the oil [20,21]. If A/P > 1 and $\Sigma D\%$ > 40, a drying oil is present, and its identification can be based on the following P/S values: linseed oil: P/S < 2, walnut oil: 2 < P/S < 3 and poppy seed oil: P/S > 3 [26]. In samples 3, 4 and 6, the A/P ratio >1 and a high content of dicarboxylic acid suggested that a drying oil had been used. It was not possible to use the characteristic parameters to determine the kind of siccative oil due to the presence of beeswax and egg lipids, since both materials contain high amounts of palmitic acid.

Table 2
Relative amino acid percentage content of the samples determined by GC/MS analysis.

Sample	Ala	Gly	Val	Leu	Ile	Ser	Pro	Phe	Asp	Glu	Hyp
2	9.0	14.4	12.0	19.6	9.1	8.6	0.1	7.5	6.7	13.0	0.0
3	9.0	11.3	12.8	14.1	8.9	6.9	6.8	4.3	8.6	17.4	0.0
4	8.5	14.6	10.5	16.9	8.9	4.7	5.0	7.0	7.6	16.3	0.0
6	8.0	16.8	12.8	21.1	11.8	2.3	4.6	10.5	5.3	6.9	0.0
7	8.6	16.0	9.0	16.4	7.8	5.6	4.0	5.9	9.5	17.3	0.0
9	8.5	9.8	11.8	19.8	12.3	4.6	2.3	3.3	11.0	16.7	0.0

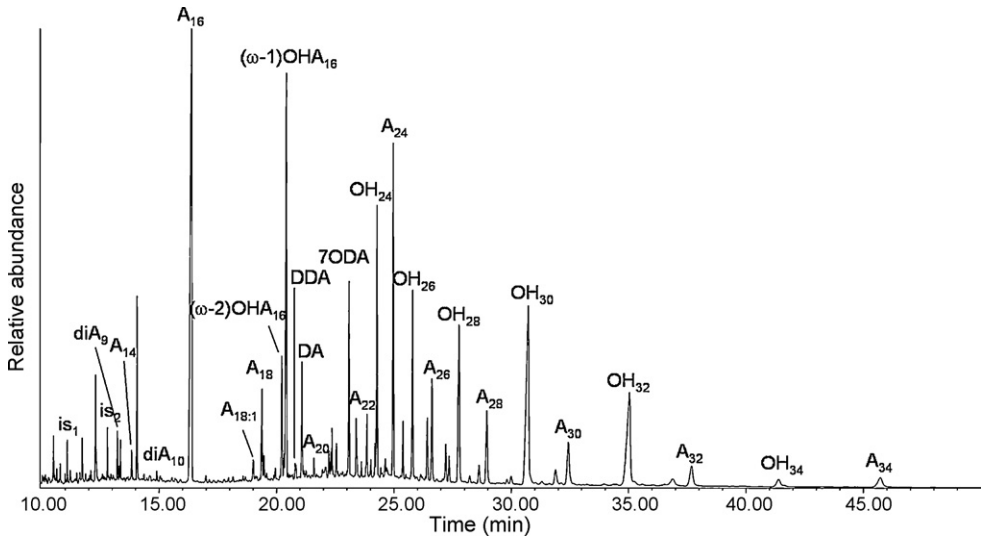

Fig. 9. Total ion current chromatogram of the trimethylsilylated lipid fraction of sample 7 obtained by GC/MS. $A_{X:Y}$ are fatty acids of chain length X and degree of unsaturation Y; diA_X are α,ω-dicarboxylic fatty acids of chain length X; OHX are n-alkanols of chain length X; DDA is didehydroabietic acid; DA is dehydroabietic acid; 7ODA is 7-oxo-didehydroabietic acid; IS_1 and IS_2 are the n-tridecanoic acid and n-hexadecane internal standards.

- In samples 6, 7, 8 and 9 the presence, at trace levels, of a series of linear and branched monocarboxylic fatty acids with 15 and 17 carbon atoms together with the presence of cholesterol possibly indicates the use of a fatty material of animal origin [27]. The extracted ion chromatogram (m/z 129) relative to sample 9 is reported in Fig. 10 ($A_{X:Y}$ are fatty acids of chain length X and degree of unsaturation Y; diA_X are α,ω-dicarboxylic fatty acids of chain length X).

3.2.2. GC/MS analytical procedure for saccharide materials

An aliquot of sample 3 was submitted to the GC/MS procedure to determine the polysaccharide materials. The chromatogram is reported in Fig. 11. Glucose is the main peak in the chromatogram, suggesting that its source cannot be ascribed to a polysaccharide gum. In fact, among the gums used as binding media including arabic, karaya, ghatti, guar, locust bean and tragacanth, cherry, plum and peach gums, only tragacanth has glucose in its composition, but its relative content is much smaller than that found in the sample [15]. The high content of glucose can be ascribed to the presence of a glucose-based material, such as starch or dextrine, as already hypothesized on the basis of the pyrolysis results. In addition to glucose, xylose, arabinose, ramnose, glucuronic acid, mannose and galactose were present. As outlined in a previous paper, fruit tree gum can be identified as the other source of the sugars present in the sample [15].

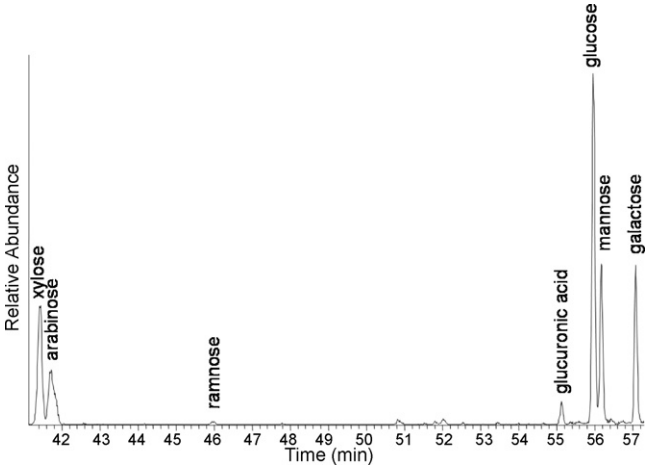

Fig. 11. Chromatogram of sample 3, relative to the saccharide material.

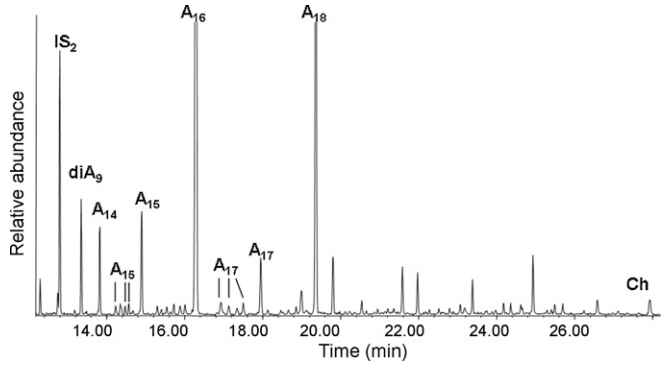

Fig. 10. Extracted ion chromatogram (m/z 129) of the trimethylsilylated lipid fraction of sample 9 obtained by GC/MS. $A_{X:Y}$ are fatty acids of chain length X and degree of unsaturation Y; diA_X are α,ω-dicarboxylic fatty acids of chain length X; Ch is cholesterol; IS_2 is the n-tridecanoic acid internal standard.

4. Conclusions

The Py-GC/MS procedure is a very fast technique for characterizing the organic material of a micro-sample. Since no sample pre-treatment is necessary, possible losses and contamination are reduced. GC/MS procedures are certainly more laborious than pyrolysis, but enabled us to confirm the materials found by pyrolysis and to obtain detailed results on material composition, such as quantitative amino acid profiles, and to identify the source of the drying oil and the saccharide material. Despite the presence of beeswax and egg, in some samples the P/S and A/P ratios highlighted the presence of linseed oil. In addition a series of odd linear and branched monocarboxylic fatty acids together with the presence of cholesterol indicated the use of an animal fat. Fruit three gum was also identified in one of the samples.

The application of both procedures on the same sample allowed us to assess the presence of the materials summarized in Table 3.

Table 3
Summary of materials found by Py-GC/MS and GC/MS.

Panel	Sample	Drying oil	Saccharide material	Animal fat	Beeswax	Pine resin	Egg
1	10 (patina)	No	Yes	Traces	Yes	No	Yes
	11 (patina)	No	Traces	Traces	Yes	No	Traces
2	8 (black decoration)	No	No	Yes	Yes	Yes	No
	9 (patina)	No	No	Yes	Yes	No	Yes
3	6 (black decoration)	Yes	Yes	Yes	Yes	Traces	Yes
	7 (black inscription)	No	No	Yes	Yes	Yes	Yes
7	4 (black decoration)	Yes	Yes	No	Traces	Yes	Yes
9	1 (patina)	No	No	Yes	No	No	Traces
	2 (patina)	No	No	Yes	No	No	Yes
	3 (black decoration)	Yes	Yes	No	No	Yes	Yes

Examination of the data enabled us to draw the following conclusions:

1. The black decorations were obtained using two techniques. One entailed using a drying oil, saccharide material and pine resin (samples 3, 4 and 6 in panels 9, 7 and 3 respectively, by Girolamo Campagna, Antonio Minello and Tullio Lombardo). The other technique, used for the black inscription, involved the use of pine resin and beeswax (samples 7 of panel 3 and 8 of panel 2 by Tullio Lombardo and Giovanni Mosca). In fact, resins were often mixed with waxy materials in order to harden and colour the material;
2. The presence of egg and animal fat in all the patinas suggests that these materials were once used to polish the marble. Though nothing has been written about this practice, old Venetian restorers refer to it in recipes that have been passed down orally from generation to generation (Prof. Giorgio Torraca, personal communication);
3. The presence of beeswax may be related to further restoration work to make the marble shiny, which has been a well known practice since the Egyptians and is still widespread in Europe. In panel 9, beeswax was not found because in the recent past there was an attempt to lightly clean the marble and only the first layer of patina was eliminated: all the other materials, i.e., egg and animal fats, were not removed because oxidation reactions and cross-linking caused a quite hard and insoluble film;
4. Patina samples from panel 1 contained saccharide materials; this means that they may also have been used in past restorations;
5. A small amount of sulphates was detected, highlighting that the marble had degraded slightly; the high reliefs were covered by a very compact man-made layer of patina which was a yellow brown colour especially in the more exposed parts. Oxidation of the organic materials found was responsible for the yellowing, plus the fact that inorganic materials (such as iron oxides together with clays) and combustion particles from lit candles could also have been deposited on the surface and have gradually been adsorbed by the fatty materials contributing to the final colour. Notwithstanding this, oxalates were not detected, suggesting that the organic materials had not yet reached the highest degree of oxidation 4.

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