

Multivariate analysis of pyrolysis-GC/MS data for identification of polysaccharide binding media

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The method of Thermally assisted Hydrolysis and Methylation (THM) was applied to the analysis of plant gums that can be found in artistic and archaeological objects. The main products obtained by THM of gums arabic, cherry, tragacanth, ghatti and karaya are permethylated and partially methylated 3-deoxyaldonic acids, characteristic products obtained from alkaline hydrolysis of monosaccharides. These markers allowed only a preliminary distinction between the different gums because they are not representative of the entire monosaccharides profile. As classification on the basis of sugars quantification is impossible, multivariate data analysis was employed, using the peak areas of THM products as variables. Calculations were performed on several different sets of data, enhancing the capability of the method to differentiate gums, also when submitted to accelerated ageing treatments. A case study on samples coming from ancient Egyptian *cartonnage* funerary masks is presented.

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1 Introduction

Plant gums are biopolymers of botanical origin, obtained from plants by exudation. The term “gum” is used in the nomenclature of these polysaccharide materials to avoid confusion with resins, which are also obtained by exudation from plants, but are terpenic compounds.

The similarity of these materials to cell wall constituents gives rise to the suggestion that they are derived from modification of polysaccharides produced in normal metabolic processes.¹ The primary function of gums appears to be the retention of water because exudation does not occur in the wet season or in cold weather. Gum tears or ribbons form whenever the bark is damaged, therefore the largest quantities of gums are produced when the plant grows under adverse conditions.^{2,3}

Plant gums are employed in several different applications ranging from the food industry to the pharmaceutical industry, to cosmetic production and art. In artistic applications plant gums have been used as binding media since antiquity, due to their solubility and swelling in water. In Ancient Egypt they were employed for decoration of funerary masks⁴ and as embalming materials. In the Middle Ages plant gums were used as binders for inks and in miniated codes, mixed with proteinaceous binding media to enhance pigment brightness.^{5–7} Many different kinds of plant gums were used in the making of British watercolor cakes⁸ and are nowadays used in watercolors and drawing pencils.

The most used analytical technique for the characterization of plant gums in cultural heritage is chromatography coupled to

mass spectrometry. Analysis of polysaccharides with GC/MS requires an initial decomposition of the polymeric chains to obtain free sugars. Different chemolysis methods have been employed to achieve this purpose. One of these methods is methanolysis^{9–11} which has proved to allow the best recovery of neutral sugars and glucuronic acids.¹² Acidic hydrolysis is also a procedure largely employed, in particular with trifluoroacetic acids.^{8,13–15} Other authors suggested an improvement of the hydrolysis procedure by coupling with microwave treatment.^{14,16,17}

Another technique that is widely employed in the field of cultural heritage is pyrolysis coupled to GC/MS and several studies on identification of plant gums have been reported. Simple pyrolysis without derivatization showed that different plant gums give different pyrograms, but no precise identification is possible^{18–20} as the markers for each different sugar present in the polysaccharide chain are not specific. Important improvements can be achieved using on-line derivatizations, for example silylation with hexamethyldisilazane (HMDS) or with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA).^{18,19}

In the present work the method used was Thermally assisted Hydrolysis and Methylation (THM). THM has been applied to polysaccharides and some kinds of plant gums^{21–24} and combines alkaline hydrolysis of tetramethylammonium hydroxide (TMAH) with high temperatures under pyrolysis conditions to achieve decomposition of polysaccharide chains. The main products obtained by THM of plant gums are permethylated and partially methylated 3-deoxyaldonic acids, which are used as markers for monosaccharides. Uronic acids are not detectable and neutral monosaccharides that are epimers on C-2 give the same markers.²⁵ For these reasons a complete monosaccharide profile is not obtainable and

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classification on the basis of percentage of sugars is not possible by THM. Also classification by use of main peak ratios was attempted in a previous work²⁴ but this can be used only with data obtained under identical experimental conditions. In fact, as reported in the referenced work, analytical parameters (e.g. pyrolysis temperature and derivatizing agent concentration) deeply affect the peak area ratios. Ageing of the materials may also affect pyrolysis products and must be taken into account.

Therefore a different approach, based on the use of chemometric multivariate data analysis, was followed to recognize and classify plant gums. The data submitted to chemometric calculations were obtained under different analytical conditions (pyrolysis temperature, reagent concentration, reaction time) and the analyzing samples were unaged and aged with different treatments. Also, samples analyzed in a range of two years were considered to determine the variability introduced by chromatographic column degradation and change of column.

Agglomerative hierarchical Cluster Analysis (CA) and Principal Components Analysis (PCA), two of the most common methods applied for multivariate analysis of experimental results, were performed. The application of this type of data treatment is useful for comparing chromatographic profiles in a convenient way to highlight differences and similarities between the samples. Furthermore, PCA allows correlation between sample differences and chemical features.

This approach was also applied to the characterization of organic materials contained in *cartonnage* funerary masks from the Museum of Egyptian Antiquity in Turin. The term *cartonnage* refers to a particular kind of material in use in Ancient Egypt for decoration and protection of an embalmed body. Cartonnage was made by several layers of tissue of linen (or recycled papyrus) and gesso, with the outer layer of gesso being painted.²⁶ A few studies are present in the literature about *cartonnage*, most of them^{27,28} investigating inorganic components of this material with different techniques, including optical microscopy, XRD and XRF. However, for the recognition of organic materials, gas chromatography is the best choice and combination with pyrolysis allows discrimination of macromolecular components such as binding media. In the present work the chemometric interpretation of pyrolysis-GC/MS data made possible the identification of fruit-trees gum as a binder in *cartonnage* samples.

2 Materials and method

2.1 Materials

Several plant gums from different provenances were analyzed: gum arabic (Phase-Prodotti per il Restauro, Italy and Sigma-Aldrich, Italy), gum tragacanth (Phase-Prodotti per il Restauro, Italy and Sigma-Aldrich, Italy), cherry gum (Zecchi, Italy), gum karaya (Sigma-Aldrich, Italy) and gum ghatti (Sigma-Aldrich, Italy).

Several pigments were added to reference gum samples: Sheele's green (CuHAsO_3 , no. 10870 by Kremer, Germany), Cadmium Yellow (CdS , no. 21020 by Kremer, Germany), Chromium Green (Cr_2O_3 , no. 44200 by Kremer, Germany), and Red Mars (Fe_2O_3 , no. 793 by Maimeri, Italy).

The derivatizing agent used was TMAH in aqueous solution at a concentration of 25% by weight (Sigma-Aldrich, Italy).

The real samples analyzed in the present study were collected from two funerary *cartonnage* masks coming from the Museum of Egyptian Antiquity of Turin. These masks were sampled before restoration and exposition. Preliminary results on binding media analysis (not reported here) showed that polysaccharides could be contained in the paint layer, but precise identification was not achieved, therefore new analyses using THM were performed. Archive numbers of the two masks, discovered by Ernesto Schiaparelli in an excavation campaign between 1911 and 1913 in Assiut, are S14721 and S14722 (Fig. 1a and b). Both objects are dated to the 2nd century AD and are in a bad conservation state.²⁹

The mask S14721 is plastered both in the exterior and in the interior sides. It presents lacunae in the pictorial layers and appears laterally compressed, showing the fragility of *cartonnage*. The false beard is lost and the hole where it was originally placed is visible. The back side of the interior of the mask is dark, probably because of absorption of organic materials derived from the mummy that was buried in the supine position.

The mask S14722 has preserved its original shape, but the pictorial layer appears damaged by an extensive area of brown materials, probably due to bituminous substances used for waterproofing of the wooden external sarcophagus and dropped on the face of the mummy. The interior of the mask is not plastered and shows the presence of the linen cloth used for *cartonnage* fabrication.

2.2 Artificial accelerated ageing

Reference samples of plant gums were submitted to different types of accelerated artificial ageing.

Photooxidation of cherry gum, gum arabic and gum tragacanth was done by dissolving weighed amounts of the gums in deionized water. Solutions were maintained under magnetic stirring for about 1 hour, then filtered with a cotton cloth to simulate ancient preparation of binding media, then pigments were added following recipes suggested by the technical literature.³⁰ Selected pigments were chosen because of their possible influence on the ageing of plant gums.³¹ Pure gums and gums with pigments were layered on microscope glass slides and after



Fig. 1 Egyptian *cartonnage* masks S14721 (a) and S14722 (b).

drying they were put in a solar box for 1000 hours. The apparatus used was the Q-SUN Xe1 Chiller (Q-LAB Corporation, USA) with an outdoor filter. The temperature was 40 °C and irradiation of the Xenon lamp was fixed at 0.68 W m⁻².

Gum arabic and gum tragacanth were also submitted to thermoxidation. The two gums were prepared with the same procedure as that used for photooxidation experiments, but without pigments. Glass slides with reference materials were put in a forced-air circulation oven at 60 °C for about 1000 hours.

For ozonolysis 1 g of each gum (*i.e.* arabic and tragacanth) was dissolved in deionized water under magnetic stirring in a two necked Erlenmeyer bulb with a volume of 100 ml. Ozone was fluxed for about 20 hours keeping the solution under stirring at room temperature. At the end of the treatment solutions were dried and gum samples were recovered.³²

In the enzymatic treatment 1 g of each gum was dissolved in 40 ml of deionized water under strong magnetic stirring in an Erlenmeyer bulb of 100 ml. 1 ml of 10% solution of cellulose, 10 mg of lipase and 10 mg of laccase were added; the same additions were done 4 times until 12 h of reaction. At the end of the treatment the products were recovered by extraction.³²

2.3 THM-GC/MS analysis

Samples for pyrolysis were prepared according to two different procedures. On one hand the solid sample was loaded in a quartz tube closed with two small pieces of quartz wool, then 5 µl of TMAH in aqueous solution was added to the sample using a micro-syringe. On the other hand, a weighed sample was loaded in a vial and dissolved in aqueous solution of TMAH, then the mixture was spread into quartz wool inserted in a quartz tube for pyrolysis. The sample weight and the volume of derivatizing agent may vary in the different methods.

Samples coming from Egyptian *cartonnages* were powdered and loaded in microvials. Because the sample weight is about 5–10 mg a larger amount of TMAH was added, using 10 µl instead of 5 µl. After addition of TMAH the samples were kept in an oil bath at 90 °C for about 30 minutes, and then analyzed.

Pyrolysis was performed with a CDS Pyroprobe 1500 (Analytical Inc., USA) filament pyrolyzer directly connected to a GC/MS system. Different pyrolysis temperatures were used.

The GC is a 6890N Network GC System (Agilent Technologies, USA) gas chromatograph with a methyl-phenyl-poly-siloxane cross-linked 5% phenyl methyl silicone (30 m, 0.25 mm i.d., 0.25 µm film thickness) capillary column. The temperature program was: 50 °C for 2 minutes, then a temperature ramp to 300 °C (heating rate 10 °C min⁻¹ to 130 °C, 5 °C min⁻¹ to 180 °C min⁻¹ then 15 °C min⁻¹ to 300 °C, held for 5 minutes). The temperature of the injector and of the Py-GC interface was kept at 280 °C. The carrier gas was helium (1.0 ml min⁻¹) and the split ratio was 1/20 of the total flow.

The mass spectrometer coupled to the GC apparatus was a 5973 Network MASS Selective Detector (Agilent Technologies, USA). Mass spectra were recorded under an electron impact at 70 eV and a scan range 40–600 *m/z*. All the analyses were performed in TIC (Total Ion Count), but where necessary the SIM (Single Ion Monitoring) profile of the ion at *m/z* 129, the most

intense of monosaccharide markers, was extracted by software. The interface was kept at 280 °C, the ion source at 230 °C and the quadrupole mass analyzer at 150 °C.

All instruments were controlled by Enhanced Chem Station (ver. 9.00.00.38) software. The mass spectra assignment was done with the Wiley 138 and NIST1992 libraries and by comparison with literature data.

2.4 Multivariate data analysis

The software used is XL-Stat 2009 (Addinsoft™, Paris, France) for Microsoft Excel®. Peaks integration was done with Enhanced Chemstation (Agilent).

3 Results and discussion

Pyrograms of the five reference gums (see Fig. 2) were preliminarily compared to highlight differences and similarities. As previously commented, pyrolysis with TMAH is not able to give a chromatographic profile representative of the exact sugar composition, but only some monosaccharide markers and other polysaccharide pyrolysis products (listed in Table 1). In all the pyrograms four peaks with identical mass spectra are present, labeled as “unknown” because assignment has not been possible. On the basis of tests performed on standard polysaccharides and monosaccharides (not reported here) these peaks, which are absent in monosaccharide pyrograms but always present in polysaccharide pyrograms, must be considered useful to discriminate polysaccharides from a simple monosaccharides mixture. Another compound common to all gums is 1,2,4-trimethoxybenzene (peak 7), a typical pyrolysis product of carbohydrates.²² By observing the pyrograms it is possible to see that gum tragacanth (Fig. 2a) and gum karaya (Fig. 2b) are well discernible from other gums because only in gum karaya rhamnose markers are visible (peaks 3 and 6) and arabinose/xylose markers (peaks 1 and 2) are absent, and only in gum tragacanth fucose markers (peaks 4,5) are shown. The other gums (arabic, cherry and ghatti, Fig. 2c–e) are not differentiable because pyrograms have a comparable profile. In the literature a decisional scheme for gums classification on the basis of sugar content is proposed¹⁴ and suggests that arabic gum may be distinguished by the absence of mannose, and gum ghatti by the absence of xylose, which are present in fruit tree gums. Mannose markers are not identified in pyrograms and xylose markers are identical to those of arabinose, as they are C-2 epimers and react under alkaline conditions giving identical products.²⁵ The above decisional scheme is not usable to identify gums, and therefore multivariate data analysis was applied.

The particular results obtained from the aged samples need some comments. Samples photooxidated with and without pigments and those thermoxidated give pyrograms (not reported here) perfectly superimposable with the unaged pure reference materials. On the other hand, gum arabic and gum tragacanth submitted to ozonolysis and enzymatic treatments show differences. With ozonolysis, which is a quite strong oxidation process, changes in ratios between chromatographic

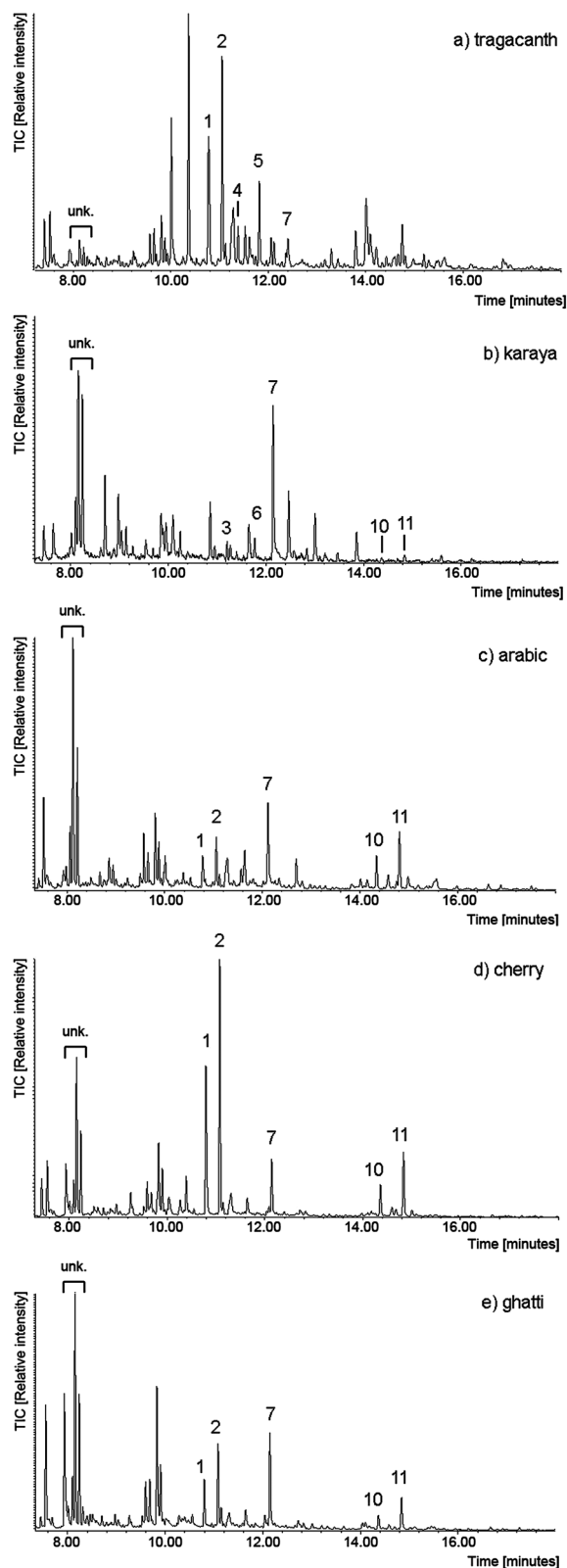


Fig. 2 Pyrograms obtained from the different kinds of gums. For assignments see Table 1.

peaks are observable. In particular there is an increment of monosaccharide markers and a decrement of the unknown compounds related to polysaccharide chains, suggesting that

ozonolysis causes depolymerization.³³ With enzymatic treatment two new markers appear, which are assigned to glucose. Detailed investigation of the enzyme effect on plant gums is beyond the scope of the present work, but one hypothesis is that glucuronic acid present in the gums can undergo decarboxylation forming glucose. This suggestion needs further experimental support to be confirmed.

Agglomerative hierarchical cluster analysis and analysis of principal components were applied to the various sets of THM-GC/MS data. The data are not homogeneous because peak area values are affected by differences of sample weight, different analytical conditions, ageing of materials and imperfect reproducibility of measurements over a long time period.

The first step of multivariate data analysis was the choice of variables. For plant gums the variables selected are the markers of monosaccharides, in particular permethylated markers of arabinose/xylose, rhamnose, fucose and galactose. The choice of variables was done purely on the chemical criterion, as the variable is representative of polysaccharide chain composition. Also the four peaks labeled as “unknown” were considered useful for plant gum identification, following the previously explained hypothesis that they are related to the polymeric nature of gums. Initially all the four variables were used, but the second time only the two most intense were selected, rejecting the others because they were too deeply affected by background noise.

The raw data were subjected to “row scaling”, meaning that each variable (the peak areas) is normalized by the sum of all the variables (sum of all peak areas). This kind of data pre-treatment is commonly used when variables are the amounts of the analytes in the sample and it is not possible to estimate the absolute concentration but the relative quantities only.

Cluster analysis of all datasets was performed with an *average linkage agglomerative hierarchical method* using Euclidean matrix distances. Analysis of principal components was done with a Pearson correlation coefficient matrix, meaning that data are subjected to autoscaling, to put variables in the same scale. Due to low repeatability of pyrolysis, without autoscaling two repeated measurements on the same sample may be wrongly classified as two different samples.

The first set of data contains 85 samples that include:

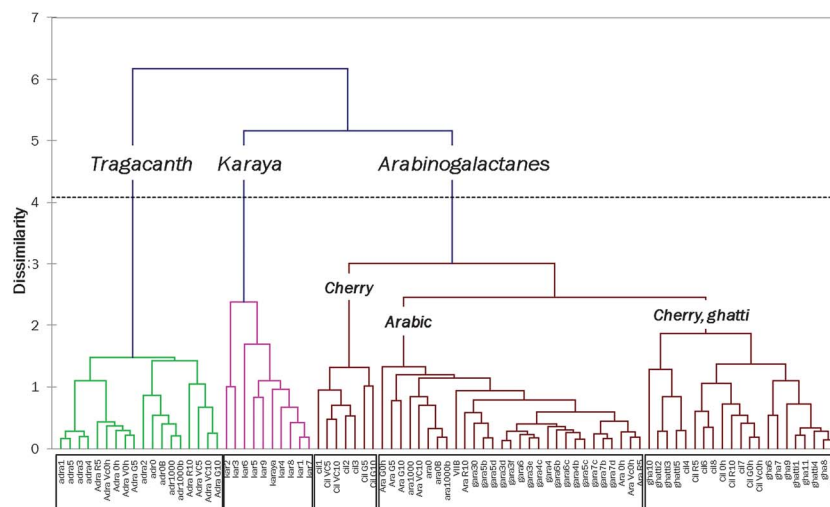
- gums arabic, tragacanth and cherry unaged and aged with photooxidation, with and without pigments;
- gum arabic samples analyzed with different pyrolysis temperature (300 °C to 700 °C);
- gum karaya
- gum ghatti

The dendrogram (Fig. 3) obtained from CA shows three principal groups that contain gum tragacanth, gum karaya and the three gums whose main polymeric component is arabinogalactane. Within this group sub-groups are formed, reflecting the similarity between gum arabic, cherry gum and gum ghatti. In particular gum arabic samples are correctly grouped, but it is not possible to differentiate cherry gum and gum ghatti.

The scores plot obtained by principal components analysis confirms the discrimination of gum arabic, gum tragacanth and gum karaya with cluster analysis. Gum ghatti and cherry gum clusters are partially overlapped (Fig. 4a), confirming that they are

Table 1 Assignments of main monosaccharide markers

No	Rt	Assignment	Marker
1	11.43	Tri- <i>O</i> -methyl-3-deoxy- <i>D</i> -threo-pentonic acid, methyl ester	Ara-1
2	11.72	Tri- <i>O</i> -methyl-3-deoxy- <i>D</i> -erythro-pentonic acid, methyl ester	Ara-2
3	11.93	Tetra- <i>O</i> -methyl-3,6-deoxy- <i>D</i> -arabino-hexonic acid, methyl ester	Rha-3
4	12.06	Tetra- <i>O</i> -methyl-3,6-deoxy- <i>D</i> -lyxo-hexonic acid, methyl ester	Fuc-4
5	12.41	Tetra- <i>O</i> -methyl-3,6-deoxy- <i>D</i> -xylo-hexonic acid, methyl ester	Fuc-5
6	12.45	Tetra- <i>O</i> -methyl-3,6-deoxy- <i>D</i> -ribo-hexonic acid, methyl ester	Rha-6
7	12.57	1,2,4-Trimethoxybenzene	—
8	14.04	Tetra- <i>O</i> -methyl-3-deoxy- <i>D</i> -arabino-hexonic acid, methyl ester	Glu-8
9	14.55	Tetra- <i>O</i> -methyl-3-deoxy- <i>D</i> -ribo-hexonic acid, methyl ester	Glu-9
10	15.17	Tetra- <i>O</i> -methyl-3-deoxy- <i>D</i> -xylo-hexonic acid, methyl ester	Gal-10
11	15.66	Tetra- <i>O</i> -methyl-3-deoxy- <i>D</i> -lyxo-hexonic acid, methyl ester	Gal-11

**Fig. 3** Dendrogram obtained from a set of 85 samples of various kinds of gums.

very similar in composition,⁹ but separation between the two groups is more appreciable than that obtained with cluster analysis.

Comparison between the scores plot and the loadings plot (Fig. 4b) aids in the interpretation of PCA results. As previously described, rhamnose (Rha-3 and Rha-6) and fucose (Fuc-4 and Fuc-5) variables are related respectively to gum karaya and gum tragacanth samples.

Arabinose markers (Ara-1 and Ara-2) are present in all the gums (excluded gum karaya) in different amounts and the related variables are useful to separate gum clusters on PC2.

Galactose variables (Gal-10 and Gal-11) are related to the samples containing arabinogalactanes as the main polysaccharide chain.

The unknown variables (unk4 and unk3) are not determinant in the discrimination between the gums, as proved by calculations done discarding these variables (not here reported) but are considered important to differentiate polysaccharide materials from a simple mixture of sugars, as they are totally absent in the monosaccharide pyrograms. Mixtures of monosaccharides to simulate gum arabic composition were prepared, analyzed and added to the data table for PCA calculations: in the

obtained scores plot (not reported here) it was observed that these samples were grouped into a separate cluster. The unknown peaks must be therefore used to avoid the error of classifying a simple mixture of sugars as real gums.

It is important to remark that both CA and PCA are capable of grouping the different kinds of gums correctly, notwithstanding the fact that they use data coming from different analytical conditions.

The last set of data considers all the samples previously discussed with addition of the samples of gum arabic and gum tragacanth aged with thermoxidation, ozonolysis and enzymatic treatment, and contains 113 different samples. In this case also the two markers of glucose (Glu-8 and Glu-9) are considered as variables, in order to take into account the difference caused by the enzymatic treatment. In a real case study glucose should be excluded, because usually it is present in a large amount as a contaminant, but in a laboratory investigation it is important to consider all variables to understand their influence for samples discrimination. The cluster analysis result is depicted in Fig. 5: four big families are defined, grouping respectively gum karaya, arabinogalactane gums, samples treated with enzymes (gum arabic and gum tragacanth, divided into sub-groups 2 and 3)

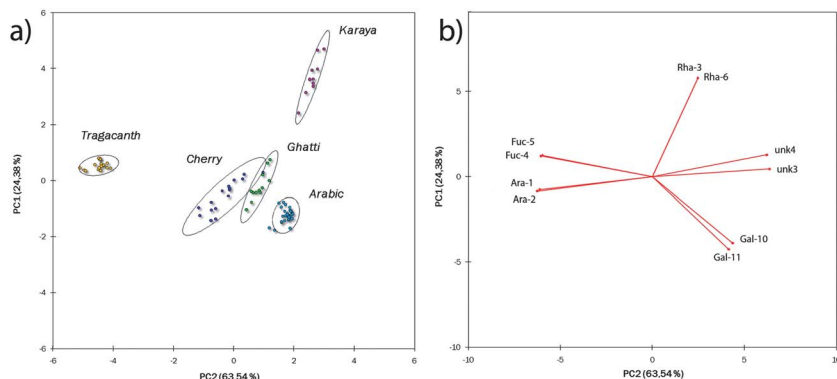


Fig. 4 Scores (a) and loadings (b) plots obtained from PCA of 85 samples. For variables assignment see Table 1.

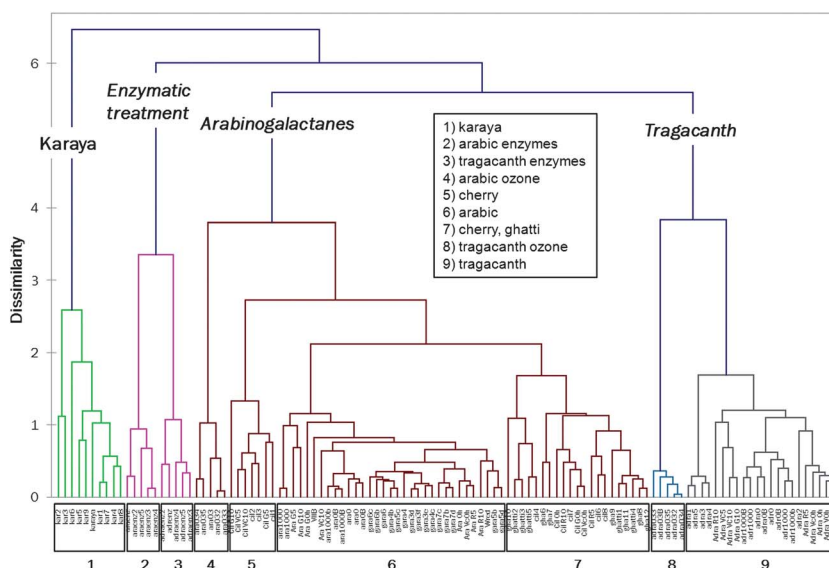


Fig. 5 Dendrogram obtained from a set of 113 samples of various kinds of gums.

and gum tragacanth (subdivided into ozonized ones and not treated, groups 8 and 9). Within the arabinogalactanes group, all gum arabic samples (unaged, photooxidized with and without pigments, analyzed with different pyrolysis temperatures) are correctly grouped (group 6). Cherry gum samples are divided into two groups (groups 5 and 7), one of them (group 7) also containing gum ghatti samples.

PCA confirms the clusters identified by CA, but for correct separation of ozonized gum arabic and that treated with enzymes it is also necessary to consider the third principal component (PC3), because on PC1 and PC2 there is superimposition of these two groups (Fig. 6 and 7). Analysis of clusters on PC1 and PC3 is important because PC3 collects a significant amount of variance (about 20%). As in the previous set of data, in comparison to CA, PCA allows a better distinction between cherry gum and gum ghatti samples.

The samples from the two *cartonnage* masks were analyzed with THM-GC/MS according to the procedure described in the Experimental section (paragraph 2.3).

Fig. 8 shows the pyrogram of one of the samples (TIC curve) and the extracted SIM profile of the ion at 129 m/z , characteristic

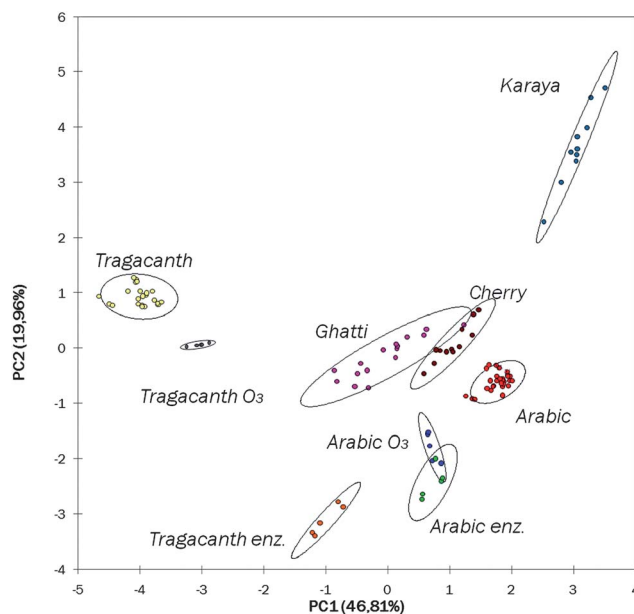


Fig. 6 Scores plot on PC1 and PC2 obtained from PCA of 113 samples.

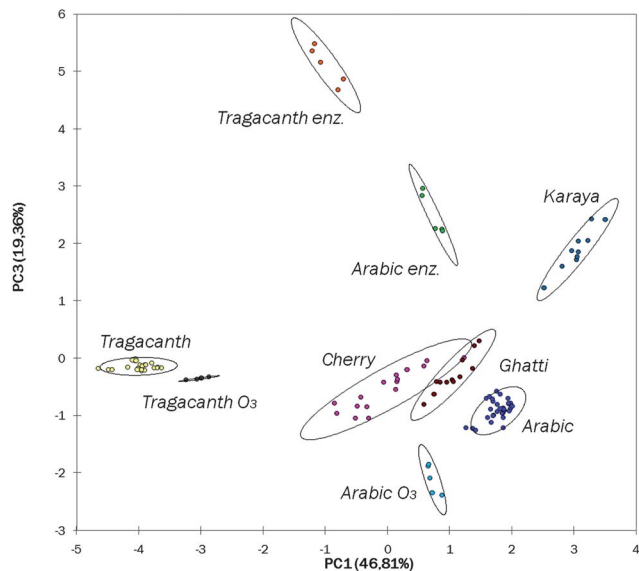


Fig. 7 Scores plot on PC1 and PC3 obtained from PCA of 113 samples.

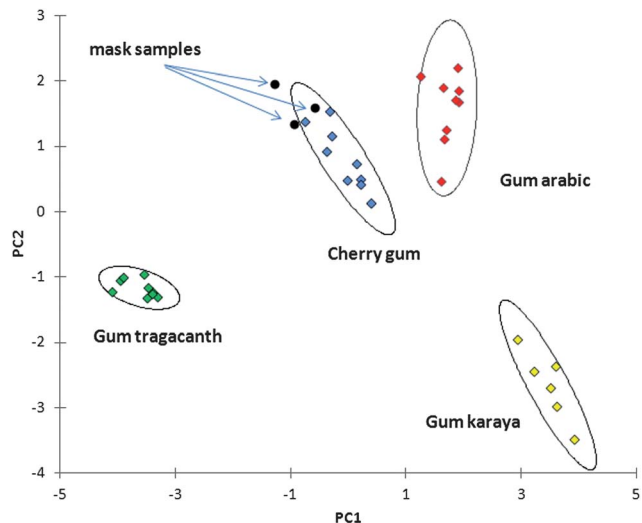


Fig. 9 Scores plot obtained from PCA of various reference samples and 3 real samples from cartonnage masks.

of monosaccharide markers.^{22–24} All samples gave identical and superimposable pyrograms therefore only one is reported here (sample 3). The part of the pyrogram at a higher retention time (not reported) shows the presence of two intense peaks which could be attributed to methyl esters of palmitic and stearic acids. Dimethyl esters of C7, C8 and C9 fatty acids indicate the presence of an aged lipidic material probably derived from contamination of the paint layer by substances used in body embalming processes.

In the pyrogram the markers of monosaccharides could be clearly identified, especially in SIM mode. The four unknown peaks associated with polysaccharides are present, even though with low intensity. As previously discussed these peaks are indicative of the presence of polysaccharide chains and their low intensity may be attributed to the polymer decomposition caused by ageing. Arabinose marker peaks are intense and appear both in permethylated (peaks 1 and 2) and in partially methylated (1a and 2a) forms. Peaks of the permethylated marker of galactose have been also identified (peaks 10 and 11). The relatively high intensity of the arabinose/xylose marker suggests that in these samples the plant gum could be cherry gum or another fruit tree gum. Peak 9, assigned to a permethylated marker of glucose, may be not only due to the

ageing of the gum¹⁴ but also due to the presence of linen fibers, that contain cellulose. This hypothesis is supported by the presence of peaks a and a1, assigned to partially methylated isosaccharinic acids, indicative of the 1 → 4 glycosidic bond²⁵ that characterizes the cellulose polymeric chain.

Peak integrations were done, even if the gum markers have very low intensity, in order to obtain data for chemometric calculations. References used for this calculation are the set of samples unaged and aged in a solar box, with and without metallic pigments. Principal component analysis results are plotted in Fig. 9 where it is possible to observe that samples 1 and 3 are comprised in the cherry gum area. Sample 2 is shifted on PC2 axis, but its position suggests that also this sample can be classified as cherry gum, or in general as a gum derived from some *Prunus* species.

4 Conclusions

The survey of THM-GC/MS data with agglomerative hierarchical clustering and principal component analysis offers a solution for deeper characterization of plant gums and more precise classification, while the simple analysis of pyrograms is not sufficient to provide information about the type of gum. The reference set

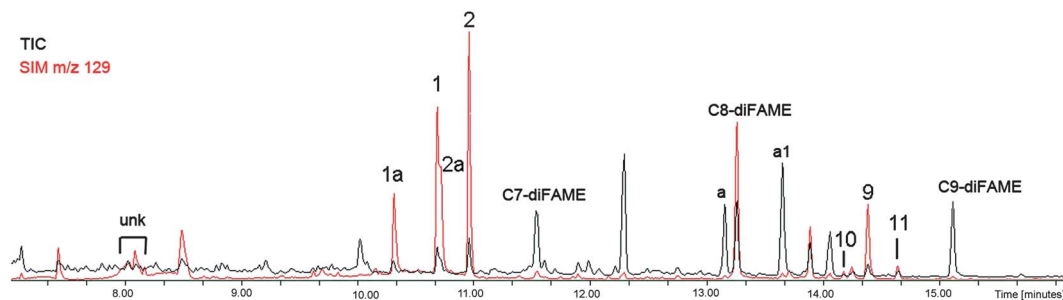


Fig. 8 Pyrograms of one of the samples collected from the funerary masks.

of data we have organized allows good identification of gums. In the absence of aged samples, all the gums are grouped into a well-defined cluster. The only exception is the partial overlap of gum ghatti with cherry gum. This is not a severe limitation because gum ghatti is not widely employed outside of India,³⁴ and obviously an aid to proper assignments also derives from the knowledge of the objects' geographical origin.

A different framework was observed considering simultaneously aged and unaged samples. Gums are not affected by thermoxidation and photoxidation treatments, but samples aged with ozonolysis and enzymatic degradation appear different from unaged ones. In particular with enzymatic treatments new markers were detected, related to glucose content and in ozonized materials a different ratio between principal markers was observed. These differences were highlighted by submitting chromatographic data to CA and PCA analyses: as observed in Results and discussion, samples treated with ozone and with enzymes always were separated from unaged materials. This feature may cause a problem when real ancient samples are considered, because natural ageing processes are not as strong as the artificial ones tested in this work. For real samples it is more suitable to perform multivariate data analysis calculation by considering only unaged and photoxidated reference samples. Samples coming from Egyptian funerary masks were introduced in the data matrix with the reference samples and PCA was performed. The resulting scores plot indicates that the binding medium contained in the paint layers was plant gum from fruit trees.

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