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Analysis of plant gums and saccharide materials in paint samples: comparison of GC-MS analytical procedures and databases

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Abstract

Background: Saccharide materials have been used for centuries as binding media, to paint, write and illuminate manuscripts and to apply metallic leaf decorations. Although the technical literature often reports on the use of plant gums as binders, actually several other saccharide materials can be encountered in paint samples, not only as major binders, but also as additives. In the literature, there are a variety of analytical procedures that utilize GC-MS to characterize saccharide materials in paint samples, however the chromatographic profiles are often extremely different and it is impossible to compare them and reliably identify the paint binder.

Results: This paper presents a comparison between two different analytical procedures based on GC-MS for the analysis of saccharide materials in works-of-art. The research presented here evaluates the influence of the analytical procedure used, and how it impacts the sugar profiles obtained from the analysis of paint samples that contain saccharide materials. The procedures have been developed, optimised and systematically used to characterise plant gums at the Getty Conservation Institute in Los Angeles, USA (GCI) and the Department of Chemistry and Industrial Chemistry of the University of Pisa, Italy (DCCI). The main steps of the analytical procedures and their optimisation are discussed.

Conclusions: The results presented highlight that the two methods give comparable sugar profiles, whether the samples analysed are simple raw materials, pigmented and unpigmented paint replicas, or paint samples collected from hundreds of centuries old polychrome art objects. A common database of sugar profiles of reference materials commonly found in paint samples was thus compiled. The database presents data also from those materials that only contain a minor saccharide fraction. This database highlights how many sources of saccharides can be found in a paint sample, representing an important step forward in the problem of identifying polysaccharide binders in paint samples.

Keywords: GC-MS of saccharide materials, Saccharide materials database, Paint binders, Cultural heritage

Introduction

Plant gums have been used in a variety of applications such as in food emulsifiers, stabilisers, and thickeners, pharmaceuticals, cosmetics, textiles, and in art. Plant gums have been used for centuries as binding media, to paint, write and illuminate manuscripts and to apply metallic leaf decorations [1]. Gums and other kind of saccharide materials, such as honey, fig milk or starch,

are known to have been used as binding media, sizing agents or mummification materials since antiquity. Actually, carbohydrates are contained in a variety of materials used as support, binders and varnishes in painted objects [2]. Wood and paper are common paint supports, and carbohydrates, both free and bound, can be encountered as minor fractions in a variety of paint materials, such as proteinaceous binders, as well as plant and animal terpenoid resins.

GC-MS analytical techniques are routinely used to determine the sugar composition of polysaccharide materials, and are among the best suited for the identification of natural organic materials used in the field of

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Cultural Heritage. GC-MS is a sensitive technique and highly suitable for the analysis of natural organic substances when the resolution and determination of the molecular profile is essential in order to identify the materials present and the ageing pathways [3]. In general the GC-MS analysis of polysaccharide materials requires a chemolysis step, followed by derivatisation [4,5]. Moreover the analysis of saccharide materials in paint samples needs an additional step of purification, in order to remove pigments and fillers. There are many different chemolysis procedures used to study plant gums [6], based on: the methanolysis [7,8], on the hydrolysis [9-13], and hydrolysis assisted by microwaves of the polysaccharide [14-16]. Rates of degradation differ for each monosaccharide during hydrolysis. The sugars are released in the order of ease of bond fission: furanosidic > pyranosidic,6-deoxyhexosidic > hexosidic > and neutral hexosidic > uronosidic [17]. Hydrolysis is complicated if there are proteins or polyphenols in the gum sample, and therefore interaction with the reducing sugars may take place. If the polysaccharide has a limited solubility, this also increases the difficulties. Derivatisation is fundamental in the GC-MS analysis of saccharides, due to the high number of polar moieties present in each molecule [4,18,19]. Thermally assisted hydrolysis and methylation [20] and on-line pyrolysis/silylation [21,22] can also be used, but the interpretation of the data in samples from Cultural Heritage is more complex and needs further investigation. Most sugars in saccharide materials used in the field of Cultural Heritage occur as three types: aldoses (e.g. glucose), ketoses (e.g. fructose), or uronic acids (e.g. glucuronic acid). It is very difficult to analyze all three types of sugars in one quantitative analysis without creating multiple derivatives of each sugar [23]. To avoid the formation of multiple derivatives, which occur because sugars have different isomeric forms in solution [24], various derivatisation procedures have been proposed, such as the reduction of carbonyl moieties followed by acetylation [25,26] the conversion of monosaccharides into acyclic oximes, followed by silylation [11] or acetylation [9,10], or the formation of diethyl mercaptal derivatives followed by silylation [13,27]. Each of these methodologies has its own advantages and drawbacks [1], but none of them is able to derivatise aldoses, ketoses and uronic acids at the same time without inducing any degradation, or producing chromatograms too complex to be unequivocally interpreted.

Because of these inherent difficulties, those analyzing carbohydrates in the field of cultural heritage are not able to fully compare data obtained from a variety of different analytical methodologies, because each of them produce extremely different chromatographic profiles

and multiple databases [13,28,29]. Moreover, in most cases the results of the quantitative analyses are not given, so that evaluating the sugar profiles of the paint samples are even more complex.

In this paper we present a comparison between two different analytical procedures that have been independently developed, optimised and systematically used to characterise plant gums in samples collected from works of art [22,27,29-34]. The research was carried out independently at two laboratories: the Getty Conservation Institute in Los Angeles, USA (GCI) and the Department of Chemistry and Industrial Chemistry of the University of Pisa, Italy (DCCI). The GCI procedure is based on the methoxylamine acetate derivatisation of neutral sugars (aldoses and ketoses) obtained from saccharide materials after hydrolysis. The DCCI procedure is based on the analysis of the mercaptal derivatives of the parent aldoses and uronic acids obtained after hydrolysis assisted by microwaves. Both procedures are used to quantitatively determine the sugar profile obtained by each polysaccharide gum after chemolysis. The data obtained with the two procedures are compared in order to understand if the chromatographic profiles are influenced by the analytical protocols and are affected differently by the sample composition. Given the proven homogeneity of the data obtained with the two techniques, a common database of sugar profiles of organic materials which can be encountered in paint samples, has been developed and is presented here.

Methods
Reagents, raw materials and reference solutions
Monosaccharides and uronic acids D-(+)-galactose, l-(−)-fucose, l-(+)-arabinose, l-(−)-ramnose, l-(−)-mannose, D-(+)-xylose, D-(+)-glucose, D-glucuronic acid, D-galactu ronic acid monohydrate, d-allose, D-glucuronic acid, 2-deoxy-d-ribose, D-psicose, D-tagatose, myo-inositol and mannitol, used as an internal standard, purity 99%, were obtained from Sigma–Aldrich (Milan, Italy).

Trifluoroacetic acid 99% purity, and anhydrous pyridine were from Fluka (Milan, Italy), ethanthiol (ETSH) 99.5%, sodium azide (NaN_3) 99.5% and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with and without 1% trimethylchlorosilane (TMCS), were from Sigma–Aldrich. Pyridine Sequanal Grade and Trifluoroacetic Acid Sequanal Grade were from Thermo Scientific. Acetic anhydride was from Supelco Inc. Carbohydrates 1, 31 standards and Methoxyamine Hydrochloride were from Sigma Aldrich. Ethyl Alcohol, Absolute, 200 Proof was from Spectrum Quality Products, Inc. Water, high purity, Chloroform, Burdick & Jackson, for GC&GC-MS analysis, 4 L were from VWR Scientific.

Standard solutions of monosaccharides with concentrations of about 100 ppm were prepared in bidistilled water and 1% sodium azide was added to prevent microbial growth. The solutions were stored at 4°C.

Amberlite MB 6113 mixed bed ion exchange resub (with color indicator) was purchased from Fluka Analytical.

Samples

The raw materials and reference solutions used in this study are detailed in Table 1. The paint samples analysed were collected from:

– Huaca de La Luna, Peru. Polychromy on wood, 10th century. Sample P-c (white, calcite, 0.9 mg).
– Nefertari Tomb, Luxor, Egypt. Mural painting, 13th century BC. Samples: Nef-y (yellow, 0.7 mg); Nef-r (red, 1.0 mg).

Analytical procedures

Analytical procedure used at GCI based on the conversion of aldoses and ketoses into acyclic methoximes, followed by acetylation

1. Samples are weighed on the ultramicrobalance and placed in a conical reaction vial. A solution of allose as internal standard is added to give a final concentration of 20 ppm in the injection volume.
2. 100 μl of 1.2 M trifluoroacetic acid is added, oxygen is evacuated under a stream of nitrogen for 30 s. Hydrolysis is performed in the closed vials at 125°C for 1 h. After hydrolysis, the vials are removed from the heat and left to stand until cool.
3. To remove the insoluble inorganic matter, vials are centrifuged, and the supernatant liquid is transferred to a 2 ml autosampler vial. The contents are evaporated using a nitrogen stream while warming the vial to 50°C. Vials are rinsed with 40 μl water, and evaporated under a stream of nitrogen. 40 μl of ethanol is added and evaporated to dryness under a stream of nitrogen.
4. 200 μl of a solution of O-methoxyamine hydrochloride in pyridine and methanol (300 mg/2 ml/1 ml) are added to the vial, and the cap is replaced. The vial is heated at 70°C for 20 min, and is then removed from the heat and left to stand until cool. The solution is slowly evaporated for 10 min to syrup.
5. 400 μl of acetic anhydride in pyridine (3 ml/1 ml) is added and the cap is replaced. The vial is kept at 70°C for 20 min; it is then removed from the heat, left to stand until cool, and the solution is evaporated using a nitrogen stream to syrup or dryness. The contents are reconstituted in 400 μl of chloroform.

6. To remove salts and pyridine the chloroform solution is rinsed twice with 500 μl 0.1 M hydrochloric acid and 500 μl of deionised water. The chloroform solution is removed between rinsing and reduced under nitrogen stream to 50 μl of chloroform. 1 μl of the chloroform solution containing the acetylated methoximes of the parent sugars is injected into the GC-MS.

Analytical procedure used at DCCI based on the conversion of aldoses and uronic acids into diethyl mercaptal derivatives followed by silylation

1. A weighed amount of the sample is admixed with 200–400 μL of 2.5 M NH_3 to the sample in a conic glass vial, and is placed in an ultrasonic bath at 60°C for 120 min twice, to remove insoluble organic and inorganic matter. The ammonia solution is transferred into a PTFE closed vial and is dried under a nitrogen stream.
2. 0.5 ml of trifluoroacetic acid 2 M is added, and subjected to microwave-assisted acid hydrolysis (power 500 W, temperature 120°C, duration 20 min).
3. After hydrolysis, the sample is filtered with a PTFE membrane filter and then dried in the rotatory evaporator.
4. Once reconstituted in 100 μl of bidistilled water, the freed sugars are purified on a Amberlite MB 6113 mixed bed ion exchange resin, packed on a 0.5 cm diameter glass column. Sugars are eluted with 1 ml of bidistilled water. Soluble inorganic salts are thus retained in the stationary phase.
5. An aliquot of the solution containing monosaccharides and uronic acids is added with the derivatization internal standard solution, evaporated to dryness in the rotary evaporator and subjected to mercaptalation. By admixing 25 μl of ethanethiol/trifluoroacetic acid (2/1, v/v), and keeping the resulting solution at room temperature for 10 min and shaking it sporadically, the corresponding diethyldithioacetals and diethyl dithioacetal lactones are formed. The mercaptalation mixture is then subjected to silylation prior to GC/MS analysis. The silylation is performed in two steps. In the first step, 100 μl of BSTFA is added to the mercaptalation mixture and the mixture is kept for 15 min at 60°C. The solution is then dried under a nitrogen flow, and subsequently added with 50 μl of BSTFA (1% TMCS) as a derivatising agent and 100 μl pyridine as a solvent, and kept at 60°C for 45 min.
6. The reaction mixture is then dried under a nitrogen flow and reconstituted in 50 μl of hexane: 2 μl of this

Table 1 Description of the raw materials analyzed in the study

Common name	Family and species	General origins of plant family	Origin
Acacia sp.	*obtained from the sap of Acacia giraffe trees*	Africa (India has several spp.)	L.A. County Arboretum, USA
Angra (acacia)	*obtained from the sap of Acacia karoo trees*	South Africa	University of Oxford, UK
Tahla (acacia)	*obtained from the sap of Acacia seyal trees*	Senegal to Sudan, Africa	University of Oxford, UK
Gum Arabic	*obtained from the sap of trees Acacia senegal*	Tropical Africa	Sigma
Mesquite	*obtained from the plant Prosopis sp.*	North and South America	Mexico
Almond	*exuded by Prunus amygdalus trees*	Eurasia, North Africa	Cal State Polytechnic, USA
Apricot	*exuded by the trees Prunus armeniaca*	Armenia, India, Greece	Cal State Polytechnic, USA
Cherry	*exuded by the trees Prunus serrulata*	Northern Hemisphere	Cal State Polytechnic, USA
Cherry	*exuded by the trees Prunus Cerasus*	Northern Hemisphere	Opificio delle Pietre Dure, Florence
Peach	*exuded by the trees Prunus persica*	China/Persia	Mogao China, Peach Tree Orchard
Plum	*exuded by the trees Prunus*	Eurasia	Czechoslovakia, Prague Opificio delle Pietre Dure, Florence
Tragacanth	*obtained from the sap of the* *plants Astragalus*	Eurasia and Africa	Sigma Kremer Local market Egypt Local market Persia Local market Anatolia
Ghatti	*exuded by the trees Anogeissus latifolia*	India	Sigma
Karaya	*exuded by the plant Sterculia sp.*	India	Sigma
Angico	*exuded by the trees Piptadenia sp.*	Brazil	University of Parana, Brazil.
Cashew	*exuded by the plants Anacardium sp.*	India, South America, Southeast Asia, Africa	EMBRAPA, Brazil
Carageean	extracted from Seaweed	China	Serva Feinbiochemica, Germany
Locust bean	*exuded by the trees Ceratonia sp.*	Mediterranean	Sigma
Guar	*is the endosperm of guar beans, legumen of* *the plants Cyamopsis tetragonolobus*	India	Sigma
Orchid	*obtained from the fruit of the plants Orchis sp.*	World Wide	Australia
Frankincense	*exuded by the trees Boswellia*	Arabic pensinsula and Africa	Oman Somalia
Myrrh resin	*sap of the plants Commiphora*	Arabic pensinsula and Africa	Local market Zecchi (Italy)
Mastic	*sap of the trees of the genus: Pistacia;* *species: Pistacia lentiscus*	Mediterranean region	Zecchi (Italy)
Elephant apple	*Dillenia indica*	India	India, local market
Escobilla	*Sida rhombifolia*	North America	Mexico
Rice Powder	*seed of the plants Oryza sativa*	World Wide	Aiko's Art Materials, Japan
Mangosteen fruit	*obtained from the fruit of* *Garcinia mangostana plants*	Southeast Asia	Jahan, India
Nopal Cactus	*obtained from the Opuntia-fiucs indica*	South America	California, USA
Honey	*produced by Apis honey bees*	World Wide	Local market, USA Acacia, Italy Millefiori, Italy Chesnut, Italy
Beeswax	*produced by Apis honey bees*	World Wide	local market, Italy
Propolis	*produced by Apis honey bees*	World Wide	local market, Italy
Cochineal dye	*produced from the scale insects Cochineal* *(Dactylopius coccus)*	primarily tropical and subtropical South America and Mexico	Mexico, Local market

Table 1 Description of the raw materials analyzed in the study (Continued)

Henna dye	*produced from the leaves of the plants Lawsonia inermis*	tropical and subtropical regions of Africa, southern Asia, and northern Australasia in semi-arid zones	Kremer
Indigo dye	*extracted from the Indigofera plants*	originally from Pakistan, Indigofera plants can be found in tropical and subtropical regions of the world	Mexico, local market
Redwood dye			Kremer
paper/ wood (average)		World Wide	Unknown
wood	*Beech* *Oak* *Pine* *Fir*	hardwood hardwood softwood softwood	Fagus sylvatica Quercus roburs Pinus sylvestris Abies alba
White fluffy fungus	*obtained from Acremonium* spp.		Mogao Cave, China
Luohanguo	*water extract fruit of the of Siraiti trees; species: Siraitia grosvenorii*	China	local market, China
fig latex	*obtained from Common Fig trees (Ficus carica), when the fruit is detached from the branch*	Mediterranean region, Iran, Pakistan and northern India, and also in other areas of the world with a similar climate	from fig tree, Italy
Hen's Egg		World Wide	local market, Italy
Animal glue	*obtained from the cartilagenous parts of rabbits*	World Wide	Zecchi (Italy)
Cow's Milk		World Wide	local market, Italy

solution, containing diethyl-dithioacetal trimethylsilyl derivatives of the parent sugars, is then injected into the gas chromatograph.

Blank evaluation

Environmental contamination must be taken into account especially when samples are collected from the field of cultural heritage. In particular, xylose and glucose are widespread and can show very high contamination levels. To avoid misinterpreting the chromatograms obtained, blank evaluations are thus fundamental to assess whether a sugar is really present in the sample, or whether it belongs to the environmental or laboratory blanks. An environmental blank is a taken from the object, or nearby, where it is expected not to contain gum, and provides an indication of the levels of carbohydrates in the environment; this is especially important when testing samples from mural paintings or outdoor paintings. At DCCI, laboratory blanks are periodically run to determine the detection limit (LOD) and quantitation limit (LOQ) of the analytical procedures for each sugar and to assess whether a sugar is present in a sample or is due to environmental contamination. At GCI a different approach is used: the relative sugar content of a sample, determined as the sum of the absolute amount of detected sugars in a sample, must be above 0.1% of the sample weight, and must be 5X times as concentrated when compared to the environmental blank and have a significantly different profile

in order to assess whether or not a sample contains a saccharide binder.

Results and discussions

Analytical procedures - a comparison

The GC-MS analysis of polysaccharide materials requires a hydrolysis step, followed by derivatisation. A comparison of each analytical step in the two procedures is presented in Figure 1. The two procedures here presented are the evolution of analytical protocols previously reported in the literature [27,35]

As it can be seen in Figure 1, there are three main steps that are required in order to analyse sugars by GC-MS: hydrolysis, derivatisation and purification from inorganic materials. Below, the main steps are discussed and the optimised steps in respect to the published literature are highlighted.

Hydrolysis

The hydrolysing agent used in both procedures is trifluoracetic acid (TFA) because it shows a good compromise between reaction efficacy and degradation of most labile sugars. Trifluoroacetic acid is also relatively volatile, meaning that it can be removed from the reaction mixture under a stream of nitrogen. At DCCI microwave assisted hydrolysis was used, as it was proven to significantly shorten reaction time, giving good reaction yields and a reduced decomposition of labile sugars [14]. At GCI hydrolysis is performed at

Figure 1 A comparative scheme of the two analytical procedures.
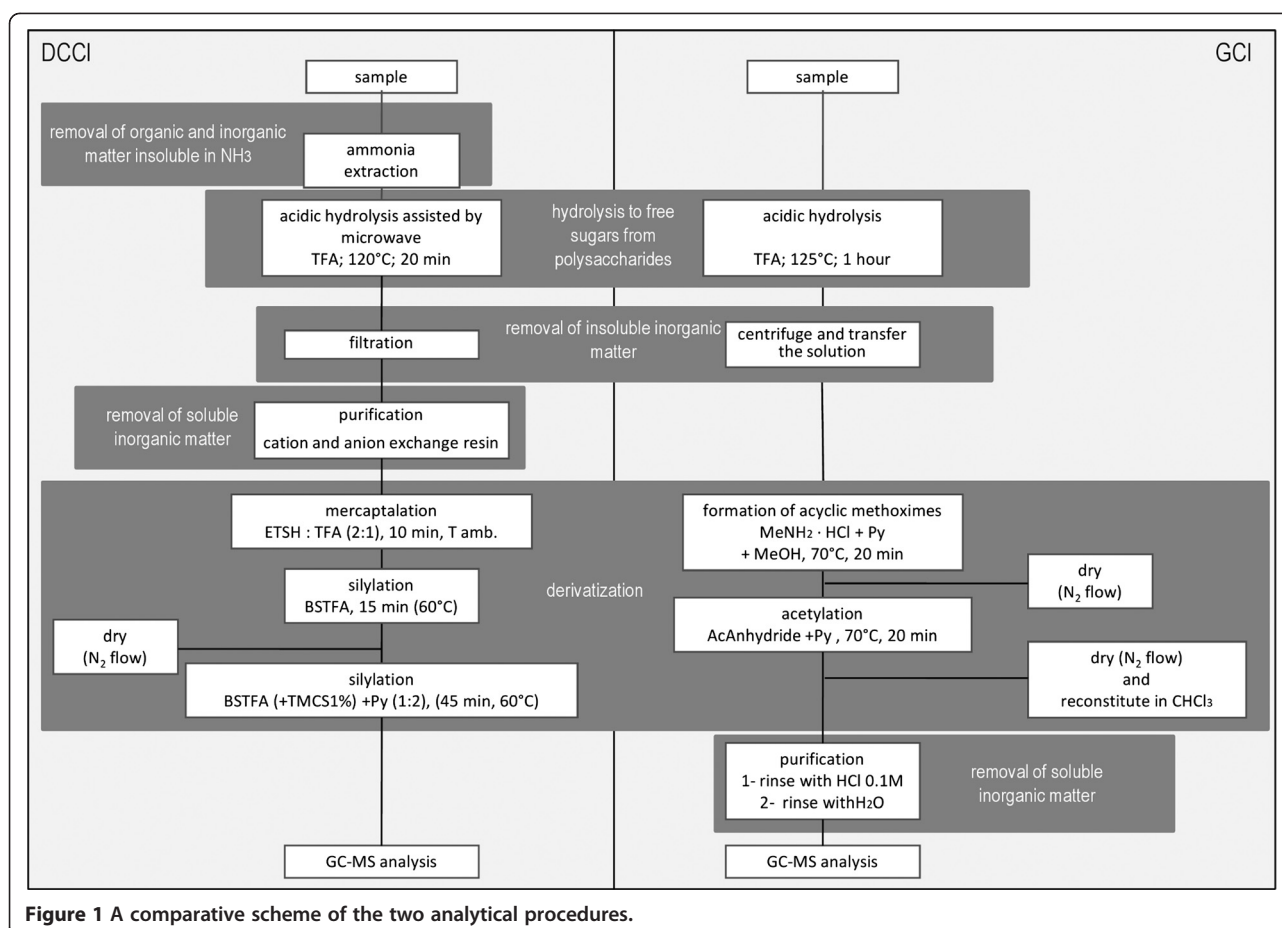
the same temperature than at DCCI, but using conventional heating.

Optimization of hydrolysis condition (GCI)

Hydrolysis time was optimized with respect to the literature work [35], in order to find the right compromise between the reaction yield and decomposition of labile sugars which can be encountered in plant gums used in the field of cultural heritage. In particular, 1, 2, 4 and 6 h hydrolysis of arabic gum were tested showing the same behavior for all sugars: the sugars were compared to the yield at 1 h of hydrolysis, and they decreased at 2 h, increased at 4 h, and decreased again at 6 h. This is shown for arabic gum in Figure 2.

The behavior observed at 6 h with respect to 4 h can be explained considering that sugars are degraded after a prolonged heating. The decrease observed after 2 h, followed by an increase at 4 h might be ascribed to a degradation of the sugars freed in the first stage of the hydrolysis, which is subsequently recovered by higher efficacy of the hydrolysis. On the basis of these results, the best hydrolysis duration would be 4 h, but considering that in a sample from works of art free fructose might be present, this is not possible, as

fructose is degraded after 2 h exposure to TFA. As a result 1 h hydrolysis was chosen, as it appeared to be the best compromise.

Derivatisation

The GCI procedure is based on the conversion of aldoses and ketoses into acyclic methoximes, followed by acetylation. Two peaks are generated in reproducible ratios in the chromatogram for each sugar: the syn and
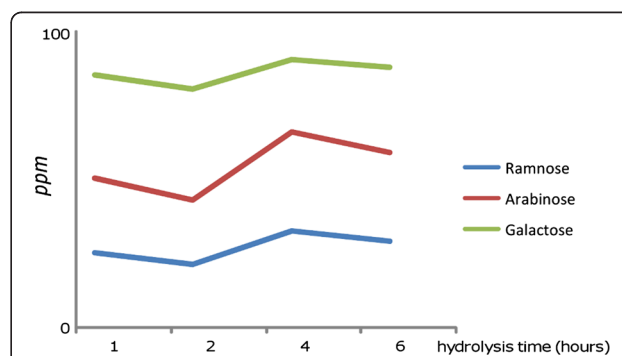

Figure 2 Content of the sugars freed from the hydrolysis of arabic gum, expressed in ppm as a function of the hydrolysis time.

anti form, and uronic acids are not derivatised. The chromatogram of a standard solution of aldoses and ketoses is reported in Figure 3. The DCCI procedure is based on the conversion of aldoses and uronic acids into diethyl mercaptal derivatives followed by silylation. One chromatographic peak is obtained for each sugar. Ketoses are not quantitatively derivatised producing several peaks in the chromatogram, since ketoses undergo decomposition reactions during mercaptalation. The chromatogram of a stardard solution of aldoses and uronic acids is reported in Figure 3.

Given the high reproducibility of the derivatisation procedures (RSD < 10% for both DCCI and GCI), quantitative analyses are possible.

Purification from inorganic materials

The two analytical procedures provide more than one step to remove both soluble and insoluble inorganic materials. Purification from inorganic materials is extremely important in the analysis of paint samples, as pigments and fillers are often major constituents of the paint layers, and may interfere at different levels during the analytical procedure. In order to investigate the possible negative effects of pigments on the GCI derivatization process, a mixture of gum arabic and gum

tragacanth were added to 21 different pigments and were analyzed for sugars. Most of the pigments had little to no effect on the sugars: hematite (Fe_2O_3), lead white (($PbCO_3$)$_2$·$Pb(OH)_2$), viridian green (Cr_2O_3·$2H_2O$), yellow ochre (Fe_2O_3·H_2O), vermillion (HgS), milori blue ($C_{28}H_{14}N_2O_4$), bone black (C), cadmium red (CdS+CdSe), malachite ($Cu_2CO_3(OH)_2$), ultramarine ($Na_{8-10}Al_6Si_6O_{24}S_{2-4}$), orpiment ($As_2S_3$), azurite ($Cu_3(CO_3)_2(OH)_2$) all showed extremely reproducible chromatograms. A measure of the extent of the reproducibility of the sugar profiles obtained from the pigmented systems with respect to the unpigmented ones can be expressed by calculating the correlation coefficients. For the above mentioned pigments values between 0.99-1.0 were obtained. Several of the pigments showed small effects: zinc white (ZnO), verdigris ($Cu(CH_3COO)_2$·CuO·$6H_2O$; $2Cu(CH_3COO)_2$·CuO·$6H_2O$; $Cu(CH_3COO)_2$·H_2O), red lead ($2PbO$·PbO_2), and vine black (C) all gave good matching with the profiles of the reference gums (correlation coefficients were between 0.96-0.98). Some pigments gave poorer matching with the reference gums mainly due to slightly altered quantitative profiles: burnt umber (clay containing oxides of Fe and Mn) (correlation coefficient: 0.93), French ochre (correlation coefficient: 0.93), chalk ($CaCO_3$)(correlation coefficient: 0.90), chrome, yellow
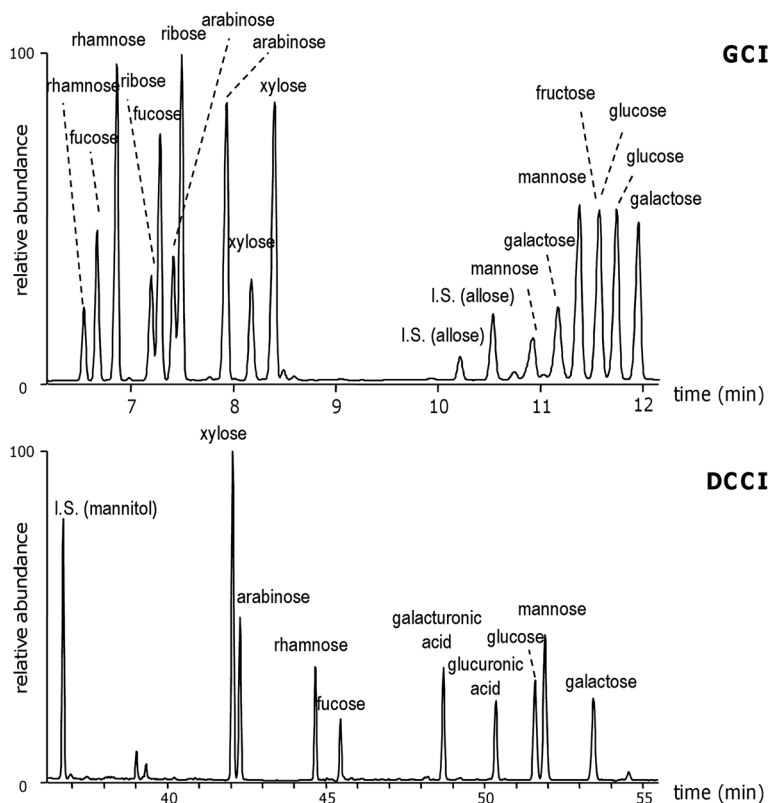

Figure 3 GCI- chromatograms of a standard solution of aldoses and ketoses; DCCI – chromatogram of a standard solution of aldoses and uronic acids.

($PbCrO_4$) (correlation coefficient: 0.90), and calcium sulphate ($CaSO_4$) (correlation coefficient: 0.87).

Optimization of the purification step (DCCI)

An ammonia extraction step was introduced in the DCCI analytical procedure as a further optimisation of a previously published procedure [29] as it was observed that certain pigments can strongly interfere in the hydrolysis step, leading to chromatographic profiles different from those obtained from the reference materials. Table 2 shows the sugar profiles obtained for a paint replica containing arabic gum and red lead ($2PbO \cdot PbO_2$).

The sugar profile obtained without the ammonia extraction is extremely different from that of the reference gum. This suggests that the gum, during hydrolysis, can react with the pigment, maybe with the aid of the microwaves, leading to the formation of unpredictable reaction products, and resulting in chromatographic profile that cannot be related to the literature data. The introduction of the ammonia step completely solves this problem: the same samples, when subjected to ammonia extraction, gave chromatographic profiles that were perfectly in agreement with those of the reference materials.

In addition, for the same reasons, ammonia extraction enabled higher amounts of saccharide material to be recovered from the sample, as indicated by the weight% calculated as the relative weight of the saccharide material (determined as the sum of the quantified sugars) with respect to the sample weight. This is particularly important in painting samples where the amount of organic material can be very low.

Comparison of the data obtained with the two analytical procedures

In order to compare the data obtained with the two analytical procedures, the relative sugar percentage composition will be used when analysing paint samples, as the absolute amount of organic content cannot be determined by GC-MS, because the amount of material that has undergone degradation, oxidation and cross-linking reactions cannot be determined. Conversely, saccharide materials are identified on the basis of the sugar profiles [3]. Additional file 1: Table S1 shows the relative sugar percentage content obtained with the two analytical procedures on samples of unpigmented and pigmented arabic, cherry and tragacanth gums. Data presented are the average of triplicate analyses. The average composition and corresponding confidence interval, at a confidence level of 99%, of the tree gums according to both of the procedures is reported in Table 3.

In order to evaluate whether or not the data obtained with the two procedures are comparable, the mean values of the relative sugar content of each gum were compared by the *t* test., The F-test was used to compare the variances between the two procedures and for the sugars[a] in each gum, in order to define which formula to use to perform the *t* test. At a confidence level of 99%, the variances obtained for all sugars with the two procedures were not significantly different, with the exception of the relative percentage content of xylose in cherry gum. The unpaired *t* test was, thus, chosen for comparing the mean values of independent samples and equal variance (homoscedastic *t*-test). The confidence levels considered were at 99% and 99.9% level. The calculated values of the t student for each sugar in each gum, and the theoretical values are reported in Table 4.

The results indicate that at a confidence level of 99%, 55% of the data belong to the same statistical population, while, if we widen the confidence interval by choosing a confidence level of 99.9%, over 75% of the data appear to belong to the same statistical population. These percentages are surprisingly good if we consider that we have been comparing data available from two independent sets of samples analysed with two procedures, and the experiments were not originally designed to perform this comparison. Moreover, plant gums are characterised by a high intrinsic variabilities. It is a known fact that parts of the same plant or segments of the plant growing in different conditions, seasons, or maturity, may give products that vary in composition and structure [36].

To further investigate the comparability of the data, Principal Component Analysis was performed on the dataset obtained by combining the DCCI and GCI data, and the score plot is reported in Figure 4 a. The figure clearly shows that the samples are grouped in three clusters, one for each gum. Moreover the data of DCCI and GCI were separately used to build two databases: the data obtained with one procedure was subjected to PCA with the dataset obtained from the other procedure. The resulting score plots are reported in Figure 4b and Figure 4c. The results show that if we compare the sugar profile of a sample obtained with one procedure with the dataset obtained with the other procedure, we obtain a correct assignment of the sample to the corresponding cluster.

All this concur to conclude that the data obtained with the two procedures on reference samples are highly comparable.

Finally, results obtained from the analysis of ancient paint samples were compared. In particular two works of art were analysed: a mural painting from Egypt, 13th century BC, and a polychromy of wood coming from Perù, 10th century AD. Two samples from Egypt were analyzed with the the two procedures, sample Nef-y (containing yellow ochre [37] analzed at the GCI) and Nef-r (containing red ochre [37] analyzed at the DCCI). One sample from a Peruvian wooden painting, (P-c

Table 2 Sugar profile obtained from the paint replicas containing arabic gum and red lead using the procedure with and without ammonia extraction

Sample		Relative percentage content (%)									Weight%
		xylose	arabinose	rhamnose	fucose	galacturonic acid	glucuronic acid	glucose	mannose	galactose	
paint replica with arabic gum and red lead	without ammonia extraction	9	11	1	0	0	7	1	0	71	0.7*****
	with ammonia extraction	0	34	15	0	0	12	0	0	39	1.4*****
Reference arabic gum		0	26	13	0	0	12	0	0	49	65.2*****

*UC note that the paint replica gave a much lower recovery of arabic gum, as the sample weigh is dominated by the read led.

Table 3 Average relative sugar percentage content of arabic, cherry and tragacanth gums obtained with the DCCI and GCI procedures, and corresponding confidence interval at a confidence level of 99%

Gum	Relative percentage content (%)										Analytical procedure
	xylose	arabinose	rhamnose	fucose	galacturonic acid	glucuronic acid	fructose	glucose	mannose	galactose	
arabic	0	28 ± 4	14 ± 2	0	0	12 ± 3	-	0	0	45 ± 5	DCCI
	0	37 ± 3	18 ± 3	0	-	-	0	0	0	44 ± 5	GCI
cherry	6 ± 3	46 ± 10	1 ± 1	0	0	7 ± 7	-	0	3 ± 2	34 ± 14	DCCI
	11 ± 1	50 ± 6	2 ± 1	0	-	-	0	1 ± 1	2 ± 2	34 ± 6	GCI
tragacanth	22 ± 5	34 ± 6	2 ± 1	8 ± 2	8 ± 9	1 ± 1	-	12 ± 6	0	12 ± 2	DCCI
	24 ± 3	45 ± 5	1 ± 0	11 ± 3	-	-	1 ± 0	8 ± 3	0	9 ± 2	GCI

containing calcite) was split into two aliquots and analysed at DCCI and GCI. The samples were collected from paintings with different geographical origins (Peru and Egypt) made with different pigments, were several centuries old, underwent severe ageing conditions, due to temperature and humidity variations, and were subject to different conservation campaigns. The comparison of this data may allow us to understand if the data obtained from the two procedures are still comparable when such complex matrixes are analysed. Given the dimensions and uniqueness of the samples from the paintings, it was not possible to perform replicate analysis on different sample aliquots, and thus it was not possible to perform the *t* test. Data obtained are presented in Table 5.

Table 5 clearly shows that the sugar profiles obtained for each paint sample using the two analytical procedures are similar. In order to measure this similarity, the correlation coefficients were calculated, resulting 0.98 for the Egyptian samples and 0.90 for the Peruvian one. These results are acceptable if we consider the variability associated with these analytical procedures and the intrinsically inhomogeneous nature of the samples. This is another indication that the GCI and DCCI protocols for the GC-MS analysis of polysaccharide materials produce highly comparable data[b].

However, the sugar profiles obtained from the paint samples do not match the reference arabic,

fruit tree and tragacanth gums, rending problems with PCA results, and underlining the limitations of databases that contain the sugar profiles that we have at our disposal.
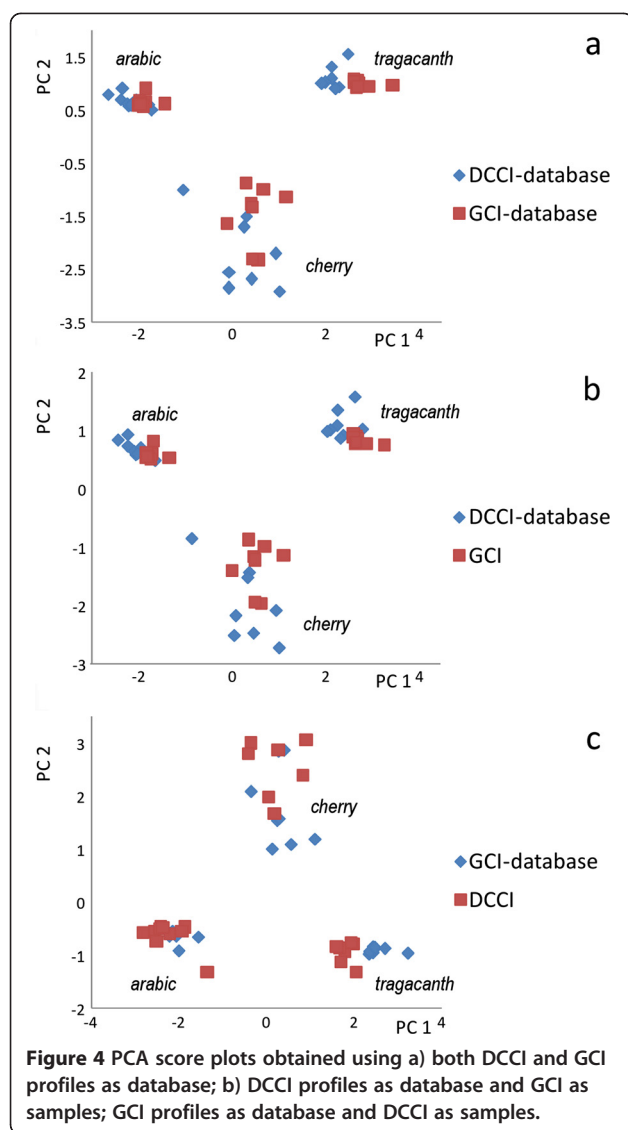
Databases

Given the fact that the GCI and DCCI procedures gave highly comparable data, it has been possible to build a common database of sugar profiles obtained by analysing materials, containing a major or minor saccharide fraction, which can be commonly found in a paint samples. A subset of this database obtained from the analysis of the raw unpigmented reference materials is shown in Table 6.

The table reports the sugar profiles of different plant gums of different geographical origins, such as a variety of arabic, fruit tree and tragacanth gums, as well as other plant gums which are not known to be used as paint materials, but are never the less produced by plants widespread in areas where arabic, fruit tree and tragacanth are not present. Moreover the table presents the sugar content of materials that were commonly added, by artists or manufacturers, to plant gums in order to modify their physical properties, such as honey, sugar or starch [28]. The sugar profile of "non-saccharide materials" that may be present in works of art is also reported. Organic colorants, proteinaceous binders, and gum

Table 4 Calculated values of t student for each sugar in each gum, and corresponding theoretical values

Gum	t student	xylose	arabinose	rhamnose	fucose	mannose	galactose
arabic	t theoretical *confidence level: 99%*			2.86093			
	t theoretical *confidence level: 99.9%*			3.8834			
	t calculated	-	−3.3	−2.0	-	-	2.9
cherry	t theoretical *confidence level: 99%*			2.97684			
	t theoretical *confidence level: 99.9%*			4.1405			
	t calculated	−4.3	−0.1	−0.4	-	2.0	1.1
tragacanth	t theoretical *confidence level: 99%*			3.05454			
	t theoretical *confidence level: 99.9%*			4.3178			
	t calculated	0.5	−3.1	7.3	−2.0	-	4.7

Figure 4 PCA score plots obtained using a) both DCCI and GCI profiles as database; b) DCCI profiles as database and GCI as samples; GCI profiles as database and DCCI as samples.

chromatogram profiles that are difficult to interpret when the overall composition of the organic materials is unknown. In these cases the knowledge of the artists' technique, the general artistic practice of the period and which other organic materials are present in the sample can be extremely useful to interpret the results [28,38]. Finally, the table reports the sugar content of saccharide materials that could be source of contamination of the paint sample such as fungi and bacteria, or more importantly wood [39,40].

The analysis of artworks that have been applied to wood, or other cellulose based supports, such as canvas or paper, is especially difficult as the wood degrades overtime, and the sugars can migrate to the painted surface causing contamination. In addition, it seems reasonable to suppose that the dust that normally accumulates on works of art, particularly in objects that are not stored in protected environments, might contain non negligible amounts of wood particles. This may be an explanation for the high content of xylose and glucose found in many samples of works of art, rather than any transformation of the sugars originally present in the sample [28,29,41].

In the light of this new database, the sugar profiles obtained from the analysis of the samples collected from the mural paintings of the Nefertari tomb may be ascribed to an arabic gum obtained from the sap of *Acacia giraffe*, *Acacia karoo*, or *Acacia seyal* trees. The sample from the polychrome wood in Peru shows a sugar profile similar to that of fruit tree gum. Despite this, this hypothesis must be rejected, as fruit trees are not native of the area. As a general rule, the geographical origin of a work of art may help to limit the number of the possible sources of plant materials that were available to the artist. It is thus important to collect reference plant gums from the surrounding area to broaden the database available. Also, knowing when the artwork was created may help to isolate species of plants to the geographical origin. This type of information is especially important for ancient works of art, or when cultures were geographically isolated. Making the hypothesis that the wood of the support of the Peruvian painting might have degraded and

resins all contain sugars that contribute to the saccharide content of a sample. Therefore, the detection of sugars in a painting sample can be ascribable not only to a plant gum, but also to different other binders, and as a result, a mixture of organic materials may lead to

Table 5 Sugar composition of the painting samples collected from the paintings in El Brujo (Peru) and in the Nefertari tomb (Egypt)

Sample	Analytical procedure	Sugar relative content	Sugar composition (%)								
			Xylose	arabinose	rhamnose	fucose	galacturonic acid	glucuronic acid	mannose	galactose	glucose
P-c	GCI	0.15%	24	25	4	0	-	-	9	37	Yes
P-c	DCCI	0.12%	17	15	5	1	0	0	14	44	Yes
Nef-y	GCI	2.04%	0	70	1	0	-	-	0	29	No
Nef-r	DCCI	1.54%	0	61	0	0	0	0	0	39	No

Table 6 Database of the average relative percentage sugar composition of raw unpigmented reference materials

Common name	Family and species	General origins of plant family	Relative percentage content										Total Sugars %	Procedure
			xylose	arabinose	rhamnose	fucose	galacturonic acid	glucuronic acid	glucose	mannose	galactose	fructose		
Acacia sp.	*obtained from the sap of Acacia giraffe trees*	Africa (India has several spp.)	0	30	4	0	-	-	0	1	65	0	*22*	GCI
Angra (acacia)	*obtained from the sap of Acacia karoo trees*	South Africa	0	59	2	0	-	-	0	0	39	0	*66*	GCI
Tahla (acacia)	*obtained from the sap of Acacia seyal trees*	Senegal to Sudan, Africa	0	63	0	0	-	-	0	0	36	0	*71*	GCI
Gum Arabic	*obtained from the sap of trees Acacia senegal*	Tropical Africa	0	38	17	0	-	-	0	0	45	0	*65*	GCI
			0	26	13	0	0	12	0	0	49	no	*90*	DCCI
Mesquite	*obtained from the plant Prosopis sp.*	North and South America	0	71	1	0	-	-	0	0	29	0	*77*	GCI
Almond	*exuded by Prunus amygdalus trees*	Eurasia, North Africa	14	49	1	0	-	-	0	1	34	0	*56*	GCI
Apricot	*exuded by the trees Prunus armeniaca*	Armenia, India, Greece	7	44	1	0	-	-	1	4	43	0	*60*	GCI
Cherry	*exuded by the trees Prunus serrulata*	Northern Hemisphere	8	57	1	0	-	-	1	2	31	0	*67*	GCI
Cherry	*exuded by the trees Prunus Cerasus*	Northern Hemisphere	5	35	2	0	0	4	0	2	52	no	*95*	DCCI
Peach	*exuded by the trees Prunus persica*	China/Persia	14	44	2	0	-	-	1	1	38	0	*73*	GCI
			15	44	1	0	0	4	0	2	34	no	*34*	DCCI
Plum	*exuded by the trees Prunus*	Eurasia	11	43	4	0	-	-	0	3	40	0	*75*	GCI
			12	31	3	0	0	13	0	4	37	no	*12*	DCCI
Tragacanth	*obtained from the sap of the plants Astragalus*	Eurasia and Africa	22	43	2	9	-	-	10	0	12	0	*57*	GCI
			19	31	2	6	17	1	12	0	12	no	*50*	DCCI
			19	43	5	7	0	0	12	0	14	no	*24*	GCI
			24	20	2	19	7	0	18	0	10	no	*14*	DCCI
			14	40	6	7	0	0	12	0	21	no	*27*	DCCI
			9	38	5	4	3	0	20	0	21	no	*24*	DCCI
Ghatti	*exuded by the trees Anogeissus latifolia*	India	1	60	2	1	-	-	1	1	34	0	*55*	GCI
			0	47	3	0	0	11	0	2	37	no	*65*	DCCI
Karaya	*exuded by the plant Sterculia sp.*	India	0	1	40	0	-	-	0	0	58	0	*37*	GCI
			0	0	25	0	7	4	0	0	64	no	*41*	DCCI
Angico	*exuded by the trees Piptadenia sp.*	Brazil	0	61	11	0	-	-	0	0	27	0	*75*	GCI

Table 6 Database of the average relative percentage sugar composition of raw unpigmented reference materials (Continued)

Material	Source	Origin												Method
Cashew	exuded by the plants Anacardium sp.	India, South America, Southeast Asia, Africa	0	28	1	0	-	-	1	0	70	0	*30*	GCI
Carageean	extracted from Seaweed	China	1	0	0	0	-	-	4	0	95	0	*33*	GCI
Locust bean	exuded by the trees Ceratonia sp.	Mediterranean	1	8	0	0	-	-	3	63	21	5	*84*	GCI
			0	0	0	0	0	0	0	62	38	no	*30*	DCCI
Guar	is the endosperm of guar beans, legumen of the plants Cyamopsis tetragonolobus	India	0	2	0	0	-	-	2	58	37	1	*91*	GCI
			0	2	0	0	0	0	0	63	35	no	*75*	DCCI
Orchid	obtained from the fruit of the plants Orchis sp.	World Wide	0	0	1	0	-	-	25	72	1	0	*62*	GCI
Frankincense	exuded by the trees Boswellia	Arabic pensinsula and Africa	3	28	10	2	-	-	11	1	40	5	*1*	GCI
			0	21	9	0	0	6	0	1	62	no	*5*	DCCI
			0	23	1	1	0	3	0	1	72	no	*12*	DCCI
Myrrh resin	sap of the plants Commiphora	Arabic pensinsula and Africa	2	30	0	3	-	-	0	8	58	0	*16*	GCI
			3	43	1	1	0	2	0	4	46	no	*7*	DCCI
Mastic	sap of the trees of the genus: Pistacia; species: Pistacia lentiscus	Mediterranean region	0	51	2	1	0	2	0	3	40	no	*1*	DCCI
Elephant apple	Dillenia indica	India	5	46	0	0	-	-	0	0	49	0	*57*	GCI
Escobilla	Sida rhombifolia	North America	13	6	19	0	-	-	36	2	20	4	*14*	GCI
Rice Powder	seed of the plants Oryza sativa	World Wide	0	0	0	0	-	-	100	0	0	0	*58*	GCI
Mangosteen fruit	obtained from the fruit of Garcinia mangostana plants	Southeast Asia	2	2	1	0	-	-	4	1	2	87	*18*	GCI
Nopal Cactus	obtained from the Opuntia-fiucs indica	South America	1	23	4	0	-	-	3	0	69	0		GCI
Honey	produced by Apis honey bees	World Wide	0	0	0	0	-	-	31	0	0	69	*56*	GCI
			0	0	0	0	0	0	100	0	0	yes	*20*	DCCI
			0	0	0	0	0	0	100	0	0	yes	*20*	DCCI
			0	0	0	0	0	0	100	0	0	yes	*20*	DCCI
Beeswax	produced by Apis honey bees	World Wide	0	0	0	0	0	0	100	0	0	no	*0*	DCCI
Propolis	produced by Apis honey bees	World Wide	9	16	13	0	0	1	40	13	8	yes	*2*	DCCI
Cochineal dye	produced from the scale insects Cochineal (Dactylopius coccus)	primarily tropical and subtropical South America and Mexico	0	0	0	0	-	-	69	14	1	11	*4*	GCI

Table 6 Database of the average relative percentage sugar composition of raw unpigmented reference materials (Continued)

Material	Description	Origin													Method
Henna dye	produced from the leaves of the plants Lawsonia inermis	tropical and subtropical regions of Africa, southern Asia, and northern Australasia in semi-arid zones	7	9	3	0	-	-	61	1	8	10	8	GCI
Indigo dye	extracted from the Indigofera plants	originally from Pakistan, Indigofera plants can be found in tropical and subtropical regions of the world	1	6	24	1	-	-	33	2	14	4	2	GCI
Redwood dye			76	3	2	0	-	-	13	3	4	0	7	GCI
paper/ wood (average)		World Wide	70	2	1	0	-	-	20	3	1	1	8	GCI
wood	Beech	hardwood	75	2	1	0	0	0	16	1	5	no	7	DCCI
	Oak	hardwood	72	2	0	0	0	0	21	3	2	no	13	DCCI
	Pine	softwood	24	7	0	0	1	0	27	32	9	no	7	DCCI
	Fir	softwood	22	4	0	0	0	0	33	36	5	no	7	DCCI
White fluffy fungus	obtained from Acremonium spp.		1	1	0	0	-	-	22	16	59	0	15	GCI
Luohanguo	water extract fruit of the of Siraiti trees; species: Siraitia grosvenorii	China	0	0	0	0	0	0	100	0	0	no	nd	DCCI
fig latex	obtained from Common Fig trees (Ficus carica), when the fruit is detached from the branch	Mediterranean region, Iran, Pakistan and northern India, and also in other areas of the world with a similar climate	3	7	37	4	0	0	23	17	9	no	nd	DCCI
Hen's Egg		World Wide	0	0	0	0	0	0	18	70	12	no	<1	DCCI
Animal glue	obtained from the cartilageneous parts of rabbits	World Wide	2	0	0	0	0	0	35	2	61	no	<1	DCCI
Cow's Milk		World Wide	0	0	0	0	0	0	39	3	59	no	12	DCCI

its sugars might have migrated into the paint layers, then xylose, glucose and mannose could be ascribed to the wood contamination and the sugar profile observed to a gum from mesquite tree or cashew gum, which are native of the geographical area.

Conclusions

This paper compares two GC-MS analytical procedures used for the quantitative determination of sugars in polysaccharide materials at the Getty Conservation Institute in Los Angeles, USA (GCI) and the Department of Chemistry and Industrial Chemistry of the University of Pisa, Italy (DCCI). The optimisations of the analytical steps were necessary in order to obtain a reliable sugar profile of polysaccharides in paint samples. The comparison of the independently obtained data of the raw materials, reference paint layers and paint samples showed that the two procedures produce highly comparable results, even when extremely complex systems are taken into consideration.

Given these results, a common database of sugar profiles was built by analysing materials containing a major or minor saccharide fraction, which can be commonly found in a paint sample. The database represents an important step forward in the problem of identifying saccharide materials in art objects, as it highlights the variety of sources of saccharides that can be encountered and the necessity of understanding their contributions when analysing the sugar profile of a paint sample. This was shown by two examples of paintings which were painted with a saccharide binder, whose sugar profile did not match those of the reference arabic, tragacanth and fruit tree gums, or could not be attributed to any of them for geographical reasons. Based on the new knowledge built on the new database available, a potential interpretation of the sugar profiles of the paint samples is suggested.

Endnotes

[a]In this comparison only xylose, arabinose, fucose, rhamnose, mannose and galactose were taken into consideration. The uronic acids and fructose were in fact not quantitated with both procedures, and glucose is subject to high environmental contamination levels and for this reason is not used in the literature to identify the source of the plant gum in paint samples.

[b]To note that the also the sugar contents of the analysed samples are highly comparable.

Additional file

Additional file 1: Table S1. Average relative sugar percentage content pigmented and unpigmented reference samples of arabic, cherry and tragacanth gums obtained with the DCCI and GCI procedures.

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