DETERMINATION OF NATURAL ORGANIC COMPONENTS IN ARTISTIC PLASTERS AND GESSO GROUNDS (INTRINSIC COMPONENTS VERSUS POLLUTANTS)

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Abstract

The knowledge of the presence and the nature of organic compounds in artistic plasters and gesso grounds may contribute to clarify the technologies utilized by the masters of works of art, to study pollutants and degradative processes and to help in future restoration. The organic compounds in paintings have been extensively investigated while few data are available on the organic compounds in plasters and gesso grounds.
In a previous research we studied proteinaceous compounds (extraction, determination, aminoacid composition) in polychromed stone sculptures, stuccoes and gesso grounds as well as in plasters (F. Ronca, Studies in Conservation, 39,107-120, 1994). We have now extended the determination to lipids and saccharides. Several artificial specimens were prepared by mixing sand, calcium carbonate and/or calcium sulphate with different amounts of lipids (linseed oil, olive oil ...), saccharides (can sugar, meals, algae and fungi cell walls...), proteins (gelatin, casein ...), and of some natural products (lemon juice, wine vinegar...).
The recovery of the organic compounds with different extractive procedures (n-pentane; chloroform-methanol, 1:1; NaOH 0.1N;...) was determined. Methods which allow the determination of sugars directly in the samples, were also investigated.
Proteins, saccharides and lipids were determined in gesso grounds and plasters from Refettorio S. Spirito (Florence), Cappella della Rovere, S. Maria del Popolo (Rome), S. Chiara in Colle Paganica (Monreale, Aosta), Chiesa Rossa, Castel S. Pietro (Chiasso, Switzerland) and Oratorio S. Cita (Palermo). All the samples were characterized from a mineralogical point of view. The extracted organic material ranged from >0.02 to 7.4% for proteins; from 0.05 to 1.6% for

saccharides; from 0.1to 6.0% for lipids. In some cases the organic materials were further examined by FTIR, mass spectrometry, thin-layer chromatography.... Intrinsic organic components and organic pollutants were identified.

Introduction

The knowledge of the presence and the nature of organic compounds in artistic plasters and gesso grounds may contribute to clarify the technologies utilized by the masters of works of art, to study pollutants and degradative processes and to help in future restoration. The organic compounds in paintings have been extensively investigated while few data are available on the organic compounds in plasters and gesso grounds. Proteinaceous substances such as gelatin, casein, glair (egg white), egg yolk, gliadins and glutelins from wheat have been used as binding media, either alone or mixed with animal fats, vegetal oils, gums, meals, sugars, milk and other biological materials in plasters, stuccoes and gesso grounds (Forbes [1], Znachko-Iavorskii [2]).

In a preceeding paper one of us (Ronca [6]) has performed the determination of proteinaceous materials in artistic specimens using a general alkaline method of extraction followed by colorimetric determination of proteins or direct hydrolysis with HCl and aminoacid analysis. The aim of the present work was to extend this kind of determination to lipids and sugars which may be present in the artistic specimens. In this way more details on the technology utilized and on biological and chemical pollutants can be obtained.

To this purpose we have utilized various methods of extraction of lipids and sugars followed by quantitative determination using specific and sensitive procedures in order to detect even low quantities of lipids and sugars as well of proteins in these types of art objects. Ideally the extraction steps should produce a low solubilization of inorganic matrix and interfering substances such as salts and metallic ions. The determination method should allow the rapid screening of a large number of samples so as to evaluate the presence and the amount of organic compounds.

We have prepared artificial samples containing sand, calcium carbonate and/or calcium sulfate and definite amounts of several biological materials which have been utilized or may be present as pollutants in plasters and gesso grounds. Extractive methods for lipids have been tested followed by determination of the extracted lipids with enzymatic and colorimetric methods, thin layer chromatography (TLC), column chromatography or mass spectrometry analysis. Several plasters and gesso grounds from various artistic and not artistic works of different age have been investigated for lipid content with the same methodology experimented for artificial samples. On the same samples we have determined the presence and the quantity of sugars by extraction with alkaline solutions followed by colorimetric determination, by colorimetric method without extraction and by trifluoroacetic acid (TFA) hydrolysis followed by TLC of monosaccharides.

The methods that we have used allow good determination of lipids and sugars in samples in which the organic compounds are a low fraction of the total material and they are almost unaffected by the inorganic matrix.

Materials and methods

Reagents

All the reagents were of analytical grade. Olive oil, bee wax, gums, casein, gelatin, glue (colla caravella) were commercial samples. Row cell walls from Euglena gracilis, from baker yeast and from some lichen species were prepared by cell homogenization in 0.9% NaCl with an Ultraturrax for 3 min at maximal speed followed by filtration through a cheese cloth. The filtrate was centrifuged at 3000xg for 5 min. The pellet was resuspended in 0.9% NaCl, again homogenized with Ultraturrax and centrifuged. This procedure was repeated 3 times.

Samples

Artificial samples (AS) Several artificial specimens were prepared by mixing 20% sand and 70% calcium carbonate with 10% linseed oil (AS1) or row olive oil (AS2), lard (AS3), row olive oil soap (AS4), row bee wax (AS5), arabic gum (AS6), tragancanth gum (AS7), dehydrated wheat meal glue (AS8), row can sugar (AS9), algae cell walls (AS10), yeast cell walls (AS11), lichen cell walls (AS12), row casein (AS13), dehydrated hen egg yolk (AS14), gelatin (AS15). Some of the above organic materials were also mixed at 10% concentration with 60% calcium sulfate and 30% calcium carbonate. These samples have been numbered in the same order from 101 to 115. Sample 116 (AS116) was prepared mixing 90% lime with 10% row olive oil. After the addition of the same weight of water the sample was mantained for 16 hours at 90°C to favour saponification. Two other samples containing 10% lemon juice (AS 117) or 10% wine vinegar (AS118) were prepared with 90% calcium sulfate. All the artificial samples were carefully mixed with water and then dehydrated at 45°C up to constant weight. In some cases reagent grade ethanol was added to facilitate mixing. Reagent grade calcium carbonate (AS119), calcium sulfate (AS120), building lime (AS121) and gesso (AS122) were also examined.

Non artistic plasters (NAP) Two external plasters are made of lime and coarse sand without visible biological contamination (NAP1, XV century; NAP2, XVIII century). Two external plasters are made of lime and coarse sand with relevant biological contamination (NAP3 and 4, both about 70 years old). NAP5 and 6 are internal plasters made of lime, fine sand and calcium sulfate (XIX century). NAP7 and 8 come from a cellar (XV century), are made of lime and coarse sand. NAP8 shows evident mould contamination. NAP9 and 10 are internal plasters made of calcium sulfate (XIX century).

Artistic works (AW) The artistic samples were characterized from a petrographic and mineralogic point of view using several methods (elementary analysis, Gandolfi camera, scanning electron microscopy,...). The place of origin and the composition of the samples are reported below. Two samples come from the Cathedral of S. Martino (Lucca, Italy), XII-XIII century. One sample (AW1) is mainly composed of calcium carbonate, the other (AW2) of calcium sulfate. They were used to seal external architectonic elements and

sculptures. The age of the two samples is unknown. AW3 and AW4 are plasters of calcium carbonate and sand from external frescoes of Case Mazzanti (Verona, Italy) by A. Cavalli (XVI century). A plaster of the inside polychromed decorations from the Church of S. Chiara in Colle Paganica (L'Aquila, Italy) (AW5) is made of lime with abundant heterogeneous inert and is covered by two layers: a red layer and a white layer. The white layer contains an oil medium. AW6-10 come from Chiesa Rossa, XIV century, (Castel San Pietro, Switzerland). They correspond to an ancient plaster made of lime and fine sand (AW6), a tardive plaster made of lime containing mica and very fine sand (AW7). The surface plaster (AW8) is made of lime and organic binder. Two pictorial layers are made of yellow ocher (AW9) and Italian green (AW10). AW11-13 come from Cappella della Rovere S. Maria del Popolo (Rome). They are multi-layer samples. AW11 is mainly composed of a sulfated plaster coated with one layer of calcium sulfate and a proteinaceous glue and with the bole armeniac containing a oily resinous medium. AW12 is mainly made of calcium carbonate coated with calcium sulfate and proteinaceous glue and with the bole. AW13 is mainly composed of calcium carbonate coated with white lead containing proteinaceous glue and with the bole. AW14-15 are stuccoes made of calcium carbonate and calcium sulfate with trace amounts of magnesium carbonate. AW16-17 are the plasters, made of calcium carbonate and fine sand, foun under AW14-15 stuccoes. AW18 is a stucco, similar to samples AW14-15, coated with a brown waxy layer. All these samples derive from the artistic works of G. Serpotta in Palermo, Italy (XVIII century). For comparison two superficial deposits, layered over the mural paintings of B. Poccetti (1597) in the ex-Refettorio of the Church of Santo Spirito (Florence), are included (AW19-20).

Methods

Extraction with organic solvents Finely powered samples (10-500 mg) were extracted in pirex tubes with teflon screw taps. The extraction was performed with 2:1 (v/v) chloroform-methanol or 1:1 (v/v) chloroform-methanol at 40°C for 3 hours with constant agitation. After centrifugation the solvent was carefully transferred in a weighted tube, evaporated and re-weighted. The extracted material was further analysed for free fatty acids, triglycerides, phospholipids and waxes.

Lipid determination Free fatty acids were converted to cupric soap, extracted with chloroform-heptane-methanol and determined colorimetrically after reaction of copper with diethyl sodium carbammate. Triglycerides and phospholipids have been quantitatively determined after enzymatic hydrolysis on the basis of glycerol or choline content, respectively (Pasquinelli [4]). Many TLC or gas chromatography methods allow to analyse lipid mixture extracted from natural sources (Stahl [5]).

Carbohydrate determination Sugars were determined colorimetrically by sulfuric acid-phenol method after extraction from finely powered samples with hot water or 0.1N NaOH. Direct determination by the same method was also performed. Hydrolysis of standard polysaccharides, artificial samples, artistic works and not artistic plasters was also carried out in 1.0M TFA under nitrogen at 100°C for 5 hours. TFA was removed under vacuum. Quantitative determinations of sugars in the hydrolysates were carried out with sulfuric acid-phenol method and TLC. For TLC, precoated plates of Silica Gel G with

or without sodium acetate impregnation were used, the developing system was propanol-water-ammonia hydrate (80:19:1; v/v) or ethyl acetate-65% isopropanol (65:35; v/v). Sugars were visualized with anisaldehyde-sulfuric acid or with p-anisidine phthalate (Stahl [5]; Kharbade [6]).

Protein determination Protein content and aminoacid composition were determined as previously described (Ronca [3]).

Results and Discussion

Table 1 reports the recovery of organic materials from some artificial samples. n-Pentane is very effective for the extraction of neutral fats (triglycerides, waxes...) while chloroform-methanol (2:1) is also effective for the extraction of polar lipids such as phospholipids of egg yolk and soap fatty acids. Chloroform-methanol (2:1) solubilizes some sugars too (see also Table 2). With chloroform-methanol (1:1) the extraction of not lipidic materials further increases.

Table 1

Extraction with organic solvents from some artificial samples

	% recovery of the organic materials included in AS*		
Sample No.	n-pentane extraction	chloroform-methanol (2:1) extraction	chloroform-methanol (1:1) extraction
AS1	84.4±6.1	88.5±5.7	80.9±6.3
AS3	73.8±5.5	73.5±5.0	72.0±4.6
AS4	7.5±0.60	84.5±7.4	80.3±8.1
AS9	0.4±0.03	2.0±0.3	11.6±2.8
AS14	51.0±3.8	71.9±5.0	72.5±5.4
AS116	5.1±0.50	87.5±6.6	83.7±7.2

* gravimetric method

mean of at least 4 determinations± Standard Deviation

Similar results have been obtained when the organic compounds are mixed with calcium sulfate (data not shown).

The determination of lipids, sugars and proteins in some artificial samples is reported in Table 2.

Table 2

Determination of organic materials in some artificial samples

	% recovery of the organic materials included in AS			
Sample No.	n-pentane extraction(1)	chlor.-meth. (2:1)extr.(1)	Sugars(2)	Proteins(3)
AS5	89.1	92.2	1.4	0.5
AS7	0.5	3.2	84.7	0.2
AS8	—	1.2	71.4	7.6
AS10	2.2	3.0	77.4	2.1
AS12	1.5	2.7	73.9	3.4
AS13	2.1	3.6	ND	68.3
AS104	7.2	85.3	ND	ND
AS107	—	—	84.7	ND
AS108	—	1.0	70.6	6.9
AS115	0.8	1.5	0.1	78.5

(1) gravimetric determination (2) direct sulfuric acid determination

(3) colorimetric determination after extraction with 0.1N NaOH
ND= not detectable —= not determined

The results obtained well correspond to the organic materials utilized to make the artificial samples. In many cases in fact lipids, sugars and proteins are present in the organic materials used. The presence of calcium carbonate does not interfere with the determination of sugars with sulfuric acid-phenol method (direct method). Whereas only a small decrease (lower than 10%) of the color is obtained in the presence of high concentration of calcium sulfate (more than 50% of the weight of the sample). We have confirmed the gravimetric determinations with further analysis. In particular we have determined triglycerides, phospholipids, free fatty acids, sugars and proteins in the extracts. We have also tried to extract sugars with the same procedure used for proteins (1N NaOH at 80°C for 1 hour). The extraction of monosaccharides and oligosaccharides was almost quantitative while no more than 50% of polysaccharides (starch, gums) was obtained. The hydrolysis with an excess of TFA under nitrogen may be carried out also in presence of high content of calcium carbonate and/or sulfate. This procedure in many case allowed the identification of the polysaccharides present in the samples. We have also tested that when the amount of the samples is small (a few mg) the following procedure should be used on the same sample: extraction with n-pentane, extraction with chloroform-methanol (2:1), extraction with 1N NaOH for the determination of proteins and solubilized sugars, direct determination (on the sample) of the not solubilized sugars. We have applied these methods to some not artistic plasters since we would like to test not artificial samples without problems concerning the quantity of the materials. The results we have obtained are reported in Table 3.

Table 3
Determination of organic materials in some not artistic plasters

	% of the organic materials included in NAP			
Sample No.	n-pentane extraction(1)	chlor.-meth. (2:1)extr.(1)	Sugars(2)	Proteins(3)
NAP1	0.7	0.7	0.9	0.3
NAP2	0.4	0.4	0.2	ND
NAP3	1.1	1.3	3.5	1.0
NAP4	1.0	1.1	3.7	0.7
NAP5	2.2	4.1	1.6	0.5
NAP6	3.1	5.8	1.6	1.1
NAP7	ND	0.8	3.7	0.3
NAP8	ND	1.3	5.2	1.1
NAP9	1.3	1.4	0.3	1.7
NAP10	1.1	0.9	0.4	2.0

(1)gravimetric determination (2)direct sulfuric acid determination
(3) colorimetric determination after extraction with 0.1N NaOH
ND= not detectable

In all the samples the presence of organic materials was determined. In some samples (NAP1 and 2) the organic material was low (less than 1.9%) and they were not further examined. In other samples the sugars were the main organic material present. In these samples biological colonizations (NAP3, 4

and 8) were present or could be suspected (NAP7). The TLC analysis of monosaccharides showed the presence of glucose, mannose, galactose, fucose, N-acetylglucosamine, glucuronic acid..., suggesting the presence of fungal and microbial polysaccharides. NAP5 and 6 sugar analysis showed the presence of arabic gum. In the same samples the presence of triglycerides and free fatty acids (soaps) was demonstrated. This was also suggested from the difference in the n-pentane and chloroform-methanol extraction. The analysis of fatty acids indicated that animal lipids rich in saturated fatty acids has been utilized. Animal glue was found in NAP9 and 10.

Table 4

Determination of organic materials in some artistic works

% of the organic materials included in AW				
Sample No.	n-pentane extraction(1)	chlor.-meth. (2:1)extr.(1)	Sugars(2)	Proteins(3)
AW1	0.5	0.6	0.9	ND
AW2	1.4	1.5	1.6	0.5
AW3	ND	ND	ND	0.1
AW4	ND	0.1	1.3	0.8
AW5	—	3.7	ND	ND
AW6	1.1	2.5	ND	0.7
AW7	0.9	1.7	0.4	0.9
AW8	3.2	6.0	0.3	2.1
AW9	1.3	3.0	ND	4.3
AW10	1.8	4.2	0.4	7.0
AW11	2.2	2.3	ND	3.3
AW12	1.5	1.8	0.7	2.6
AW13	2.3	2.7	0.5	4.4
AW14	1.0	2.5	1.2	ND
AW15	1.2	3.0	1.0	ND
AW16	0.6	0.5	0.8	ND
AW17	0.5	0.5	0.6	ND
AW18	5.1	3.6	1.1	6.1
AW19	2.4	2.7	1.2	6.4
AW20	3.0	3.1	1.3	7.4

(1) gravimetric determination (2) direct sulfuric acid determination

(3) colorimetric determination after extraction with 0.1N NaOH

ND= not detectable —= not determined

Table 4 reports the materials extracted with organic solvents, sugars and proteins present in artistic plasters, gesso grounds and superficial deposits. In all the samples organic materials were present. n-pentane extractable material varied from a not detectable value to 5.1%. In samples AW6-10 and AW14-15 the extraction with chloroform-methanol gave higher values than that with n-pentane suggesting the presence of polar lipids. The chromatographic analysis demonstrated the presence of soaps in these samples, of triglycerides in samples AW18-20 and of triglycerides resins in AW11-13. In AW5 welnut-oil (triglycerides) penetrated from the pictorial layer into the plaster. The content of proteinaceous materials appeared to be very high in the plasters covered with pictorial layers (AW9-13) and in superficial deposits (AW18-20). In some

of these samples (AW9,10,10) calcium oxalates (weddellite and whewellite) were present. It is noteworthy that in Serpotta plasters no proteinaceous material was used. In several samples the sugar content was higher than 1%. However it was always lower than lipidic and proteinaceous materials. Only in AW2 sugars came from biological colonization as shown by TLC. In the other samples sugars were present in the row natural organic materials used directly or constitute a part of the recipe utilized by artists.

In conclusion, the quantitative determination of all the natural organic compounds that may be present in artistic plasters, together with petrografic and stratigrafic analyses, gives an important contribute to the knowledge of the materials and the technologies utilized. The methods we have employed are very simple, cheap and do not require specialized technician or apparatus; they require small amounts (2-10 mg) of sample and are the starting point for more complex analyses (TLC, gas chromatography, HPLC, mass spectrometry, amino acid analysis...) which may be carried out on the solvent extracts (for lipids), on the alkaline extracts (for proteins and solubilized sugars) and on the sample after sequential extraction with n-pentane, chloroform-methanol (2:1) and 0.1N NaOH, for not solubilized sugars.

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