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# Gas chromatographic–mass spectrometric approach to the problem of characterizing binding media in paintings

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**Abstract**

A GC–MS method is proposed for the characterization of binding media in paint works of art. The basic methodology relied on the determination of amino acids and fatty acids after hydrolysis with HCl and derivatization with 2-propanol and trifluoroacetic anhydride, and on the comparison with reference materials. The use of fused-silica capillary columns coated with methylphenylsilicone phases allowed to separate amino acid and fatty acid derivatives in a single analytical run. The method was applied to identify binding media used in ground and paint layers of polychrome sculptures of the eighteenth century.

*Keywords:* Binding media; Art analysis; Derivatization, GC; Amino acids; Fatty acids

## 1. Introduction

The analysis of paintings provides important and needed information to art historians and art restorers. For the first ones, the knowledge of pigments and binding media used in the creation of a work of art can be useful in painting technique and also in provenance and authentication studies. Painting restorers need to know the materials used in a painting, before beginning the cleaning or restoration procedure, in order to plan a working strategy aimed at avoiding any damage to the work of art. Particularly, painting media are all organic substances and can include drying oils, waxes, gums, resins, and proteinaceous materials such as egg, milk and animal glues. Most of these substances are inherently complex natural products and, moreover, a number of artists used mixed binding media techniques [1–3].

The identification of binding media in paintings is a difficult issue for an analytical chemist. The basic problem lies in the small quantity of sample generally available, in its complexity and low purity [4]. However, the latest advances in analytical instrumentation and methodology have allowed to focus on the identification of painting media [5,6]. The proposed main analytical techniques include thermal analysis [7–9], Fourier transform infrared spectroscopy [10,11], and above all chromatographic techniques.

A protocol for the identification of different proteinaceous binding media in paintings was developed by means of HPLC analysis with fluorescent detection of 9-fluorenylmethyl chloroformate deriva-

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tives of amino acids [12]. Moreover, the Waters Pico-Tag method (Millipore, Milliford, MA, USA) has been employed to identify proteinaceous binding media in panels by Cosimo Tura [13], and also in some polychrome sculptures, stuccoes and gesso grounds [14]. A method for the identification of proteins from the gas chromatographic pattern of their amino acid derivatives, after hydrolysis by a protonated cation exchanger, was applied by Kenndler et al. to identify media used in priming and paint layers of easel and wall paintings of the 16th and 18th century [15]. Samples from the paintings of the Baptistery in Parma (Italy), executed by Byzantine masters around 1250, were analyzed by GC and GC–MS. The obtained results indicated that these paintings were executed by the tempera technique, using proteinaceous binding media such as animal glues. The possibility of dating by using amino acid racemization ratios was also investigated by means of chiral columns [16]. Another paper reports on a subsequent series of analyses carried out on new samples taken from the Baptistery in Parma. Binding media were investigated by GC and GC–MS, and multivariate chemometric techniques were used to facilitate the recognition of the protein source on the basis of chromatographic data [17]. Although pyrolysis–gas chromatography (Py–GC) is a very powerful technique for characterization of non-volatile materials and although it has been widely used in forensic science [18] and in industrial laboratories [19,20], its application to works of art has been limited due to the compositional complexity and extremely limited sample size of the materials presented for examination. The use of Py–GC in the study of materials has been reviewed by Shedrinsky et al. [21] to provide an introduction to application of this technique in art and archaeology.

The general picture of the fatty and dicarboxylic acid content in binding media has been confirmed and developed by several groups of workers [22,23]. Particularly, Mills [22] has proposed a method of analysis by gas chromatography based on the ratio of palmitic acid to stearic acid, as oils contain saturated fatty acids that are so stable that their concentration hardly changes on ageing. He demonstrated that this ratio remains constant in time and is specific for each oil.

In the present paper an analytical methodology to identify binding media in paintings is proposed. Binding media have been studied through the identification of their constituent chemical components, as their pattern is characteristic for the natural material as a whole. The methodology is based on the simultaneous determination of amino acid and fatty acid content by using capillary GC–MS. In fact, amino acids and fatty acids determination is useful for the recognition of proteinaceous and oil-based media, respectively; moreover, the identification of fatty material in a proteinaceous matrix may be diagnostic of the protein source and then of the artistic recipe. A preliminary acid hydrolysis is required to transform proteins into amino acids, and oils into free fatty acids; the subsequent derivatization procedure gives derivatives of these compounds suitable for gas chromatographic analysis. The use of the GC–MS technique, combining the high resolution of capillary columns with the reliability of an MS detector, allows to obtain a good separation, identification and quantitation of analytes. Moreover, the high sensitivity, the low detection limit, and the high speed of the analysis offer a great advantage when very small amounts of samples are available.

The proposed method has been applied to a polychrome sculptural group from the eighteenth century, kept in the Basilica of Santa Maria di Campagna in Piacenza (Italy). The work, representing the Crucifixion, is signed by the Flemish sculptor Jan Geernaert and made of four statues in poplar heartwood. Pictorial technique papers report that a coat of white ground was laid on the wooden substrate before the application of the real paint. It is well-known, for instance, that the most widespread kind of ground in Medieval and Renaissance Italian painting was obtained by mixing gypsum (sometimes calcium carbonate) with a proteinaceous binder (above all animal glue). The true application of paint followed the rules of oil painting technique. The most used drying oils in western European painting practice are linseed, walnut, and poppyseed [24].

Proteinaceous binding materials, selected on the basis of the information provided by historical texts [1,2], were analyzed, as reference media, to identify the binding media used in ground; moreover, fresh oils, historically used in painting, were selected to characterize the oil-based medium probably used to make the paint layers [25].

## 2. Experimental

### 2.1. Materials

The amino acids were purchased from Sigma (St. Louis, MO, USA), the saturated fatty acid lauric (C12:0) from Fluka Chemie (Buchs, Switzerland), and myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) from Carlo Erba (Milan, Italy). The azelaic acid (Azel) was purchased from Merck (Darmstadt, Germany), the unsaturated palmitoleic acid (C16:1) and linoleic acid (C18:2) from Sigma, and oleic acid (C18:1) from Merck. A solution of amino acids and fatty acids in 6 *M* HCl at a concentration of 0.1 mg/ml each was prepared.

Reference materials were selected following historical written sources on painting techniques. Casein, egg and animal glue are materials used as binding media. Analytical products and commercially available goods were both used. Analytical products included cow milk casein (Fluka). Goods available on the market were used to obtain two hen eggs, some rabbit-skin glue, and also undried fresh oils, as linseed oil, walnut oil, and poppyseed oil.

In cooperation with the restorer, samples of paint layers were collected before the beginning of conservation works. Since a work of art of considerable value was involved, sample collection required extreme care: thus, small amounts of samples were collected either by taking them from raised areas or by scraping the surface of the painting with a scalpel blade, and transferring the material into a glass vial. The paint sample mass ranged from a few to 20 mg (only when paint drops have been sampled on the back of the sculptures).

### 2.2. Hydrolysis

An amount of 10–20 mg of reference material or 1–20 mg of paint layer sample was dissolved in 2 ml of 6 *M* HCl and hydrolyzed for 5 h at 100°C in an oil bath, under nitrogen atmosphere. Reference materials were spiked with an amount of norleucine corresponding to one tenth of the total protein content, and with an amount of norvaline corresponding to one hundredth of the total protein content on the basis of the literature data, while paint sample hydrolyzates were spiked with 10 $\mu$l of a 1 g/l norleucine solution (10 $\mu$g) and 10 $\mu$l of a solution of 0.1 g/l norvaline (1 $\mu$g) per 1 mg of weighted sample, which were used as internal standards. During hydrolysis, proteins are transformed into amino acids and fats into fatty acids.

### 2.3. Derivatization procedure

After evaporation to dryness, the sample hydrolyzed was dissolved in 3 ml 2 *M* HCl in 2-propanol (p.a. grade, Carlo Erba) in a screw-cap tube by maintaining at 90°C for 1 h. After evaporation of the solvent, the residue was dissolved in 2 ml dichloromethane (p.a. grade, Carlo Erba) and treated with 0.2 ml trifluoroacetic anhydride (Fluka) in a screw-cap tube at 60°C for 1 h. After cooling, the tube was opened, and the solvent evaporated. The residue of reference materials was then dissolved in 5 ml dichloromethane, while the residue of paint sample was dissolved in 0.2 ml dichloromethane and both the solutions were used for chromatographic injection (1 $\mu$l). This derivatization procedure simultaneously transforms the amino acids into their N-trifluoroacetyl-O-2-propyl esters, and the fatty acids into their 2-propyl esters. A mixture of standard amino acids and fatty acids was derivatized following the same procedure used for paint and reference samples.

### 2.4. GC–MS analysis

Analysis was performed on a HP-5890 Series II gas chromatograph coupled to a HP-5971A mass selective detector (Hewlett-Packard, Palo Alto, CA, USA). GC separations were achieved on an SE52 (25 m×0.32 mm I.D.) fused-silica capillary column with a 0.25-$\mu$m film methylphenyl silicone (5% phenyl groups) coating from Mega (Legnano, Milan, Italy), under the following temperature program: isothermal conditions at 50°C for 3 min, and then, with a rate of increase of 25°C/min, up to the final temperature of 280°C. The injector was kept at 280°C, while the helium gas flow-rate was approximately 1.4 ml/min. The injection mode was splitless (30 s).

MS conditions were as follows: interface temperature, 280°C; ion source temperature, ca. 190°C; electron impact mode, 70 eV; scan rate of the mass

spectrometer 1.8 scan/s in the range of $m/z$ 40–450. In the selected-ion monitoring (SIM) mode two target ions for each analyte were selected and the sampling time was set at 50 or 100 ms per mass.

## 3. Results and discussion

The derivatization method involves the esterification of the carboxylic groups with acid 2-propanol followed by the acetylation of the amino groups with trifluoroacetic anhydride [26]. In this way the amino acids were quantitatively transformed into N-trifluoroacetyl-O-2-propyl esters and, at the same time, the fatty acids into 2-propyl esters. The amino acid pattern, as a fingerprint of the proteinaceous material, is made up of alanine (Ala), glycine (Gly), leucine (Leu), proline (Pro), aspartic acid (Asp), glutamic acid (Glu), and phenylalanine (Phe) derivatives. Under the adopted working conditions, these amino acids are suitable for quantitative GC analyses. Hydroxyproline (Hpro) and valine (Val) also are inserted in the pattern of amino acids, but only for qualitative evaluation. In fact, the N,O-bis trifluoroacetyl hydroxyproline 2-propyl ester is unstable as it easily loses the O-trifluoroacetyl group. Moreover, the high volatility of the valine derivative might cause relevant losses during the final concentration step required for the preparation of the samples. In addition, norvaline (Nval) and norleucine (Nleu) derivatives have been considered, with the role of internal standard. They were added to the unknown samples in different amounts to cover a wide range of concentrations.

The use of an SE-52 column gives a complete separation of both amino acid and saturated fatty acid derivatives. Only the two unsaturated fatty acid derivatives, oleic acid (C18:1) and linoleic acid (C18:2), are partially unresolved, but they are not taken into consideration for an identification study of ancient fat materials, as over time they have been 'transformed' into their oxidative degradation products. In Fig. 1, a scan-mode total ion current chromatogram (TIC) of a standard mixture of amino acid and fatty acid derivatives (each at 0.1 $\mu g/\mu l$) is shown. The identification of the chromatographic signals was performed either by the combined use of computerized library searches or through mass spectral interpretations based on established fragmentation of all the eluting components, injected singularly.

Each recognized analyte is shown in Table 1, together with the expected retention time, the fragments selected as target ions, and the absolute detection limits of each analyte reached both by the TIC mode and the SIM mode. The unsaturated acids were detected using only the scan mode, as their spectra did not show useful target ions. The SIM

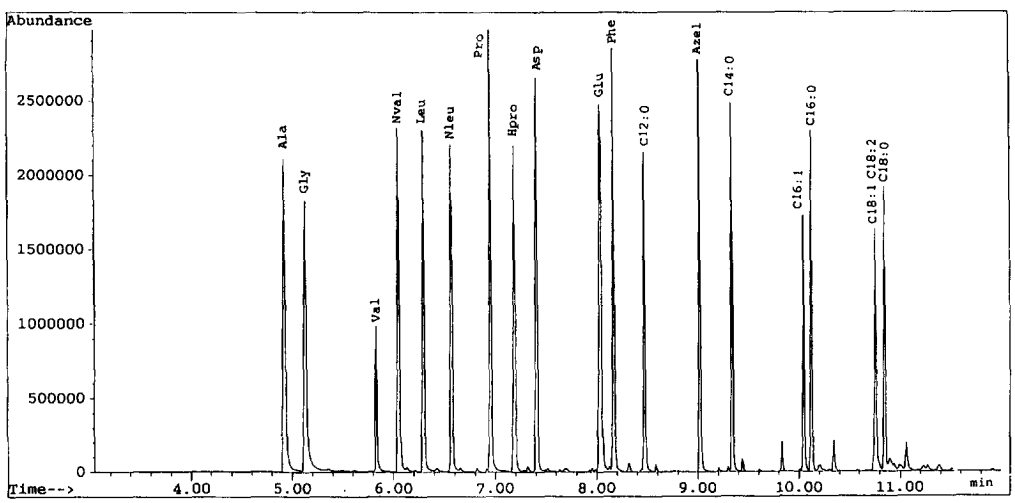


Fig. 1. Total ion chromatogram of a standard mixture of amino acids as their N-trifluoroacetyl-O-2-propyl esters, and fatty acids as their 2-propyl esters (each at 0.1 $\mu g/\mu l$), on an SE-52 capillary column. Other experimental conditions and abbreviations are reported in the text.

Table 1
Expected retention time, principal ions (*m/z*) for the detection and identification of amino acid and fatty acid derivatives and their detection limit (signal-to-noise ratio=3) by TIC and SIM mode data acquisition, on an SE-52 capillary column

| Analyte | Expected retention time (min) | *m/z* | TIC mode detection limit (ng) | SIM mode detection limit (pg) |
|---|---|---|---|---|
| Ala | 4.93 | *140*; 168 | 0.6 | 2.0 |
| Gly | 5.14 | *126*; 154 | 0.4 | 4.3 |
| Val | 5.83 | *168*; 153 | 2.5 | 6.2 |
| Nval | 6.05 | *168*; 126 | 0.5 | 3.4 |
| Leu | 6.30 | *182*; 140 | 0.5 | 4.7 |
| Nleu | 6.57 | *182*; 126 | 0.5 | 2.8 |
| Pro | 6.96 | *166*; 253 | 0.5 | 0.8 |
| Hpro | 7.19 | *164*; 279 | 0.4 | 1.3 |
| Asp | 7.41 | *184*; 139 | 0.6 | 3.4 |
| Glu | 8.04 | *198*; 180 | 0.4 | 3.0 |
| Phe | 8.17 | *91*; 190 | 0.6 | 4.0 |
| C12:0 | 8.48 | *102*; 200 | 0.6 | 13.0 |
| Azel | 9.02 | *171*; 152 | 0.7 | 3.8 |
| C14:0 | 9.34 | *102*; 228 | 0.7 | 5.6 |
| C16:1 | 10.05 | *237*; 296 | 1.7 | n.d. |
| C16:0 | 10.13 | *102*; 256 | 0.5 | 2.4 |
| C18:2 | 10.76 | *279*; 322 | n.d. | n.d. |
| C18:1 | 10.76 | *265*; 324 | 1.0 | n.d. |
| C18:0 | 10.85 | *102*; 284 | 0.7 | 10.0 |

The ions with *m/z* in italics were the most commonly used for determination.

mode allows the monitoring of all the amino acids, and also of azelaic, myristic, palmitic and stearic acids at the picogram level. In particular, the detection limits reached acquiring the data by the SIM mode are 50–500 times better than the ones obtained by the scan mode. In general, the data acquisition by the SIM mode is necessary when the amount of binding media in a real sample is lower than 10 $\mu$g, and it is preferable when the complexity of the sampled organic material generates interfering peaks in the chromatographic profiles, which complicate the identification and quantitation of the sample components.

TIC chromatograms for animal glue, whole egg and an artificial mixture obtained from 2 mg of rabbit-skin glue and 2 mg linseed oil are shown in Fig. 2. The reproducibility of the method was verified on this artificial mixture by repeating the sample preparation procedure (hydrolysis and derivatization steps) in quadruplicate and by injecting an amount of the derivatized solutions, corresponding to 1 $\mu$g of the initial material, in duplicate. The identified substances, their mean values, determined by the peak ratio of each analyte peak to the internal standard base peak, together with their standard deviations are reported in Table 2, both by using scan mode and SIM mode. It appears that data acquisition by SIM mode provides a better reproducibility for almost all the analytes, except for Leu and Azel, which are present at trace level.

Several paint samples of ancient polychrome sculptures were analyzed to evaluate the amino acid and fatty acid content. Samples were hydrolyzed, spiked with norleucine and norvaline as internal standards, and derivatized. The use of both norleucine and norvaline was due to the fact that it is difficult to foresee the chromatographic behaviour of a real sample. Moreover, the relative amounts of each analyte require again the use of the two internal standards at different concentrations. In this way, one can obtain quantitative data using the internal standard that presents its peak intensity approximately in the range of that of the other signals. All paint samples showed the presence of amino acids and fatty acids differently related between them. In fact, undercoat samples showed higher content of amino acids, while paint topcoat layer samples showed mainly the presence of fatty acids. TIC chromato-

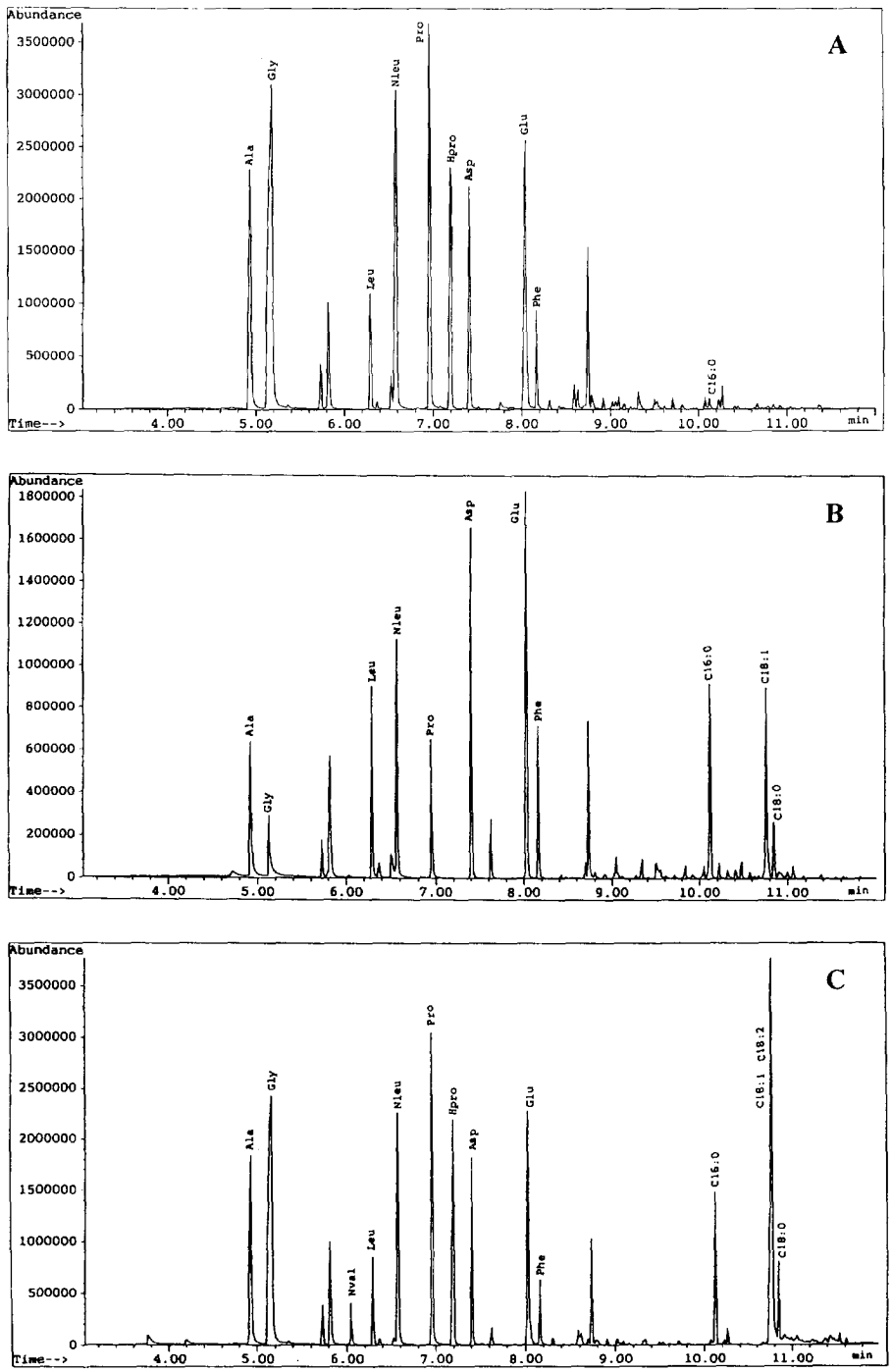


Fig. 2. Total ion chromatograms obtained from reference materials, after hydrolysis and derivatization procedure, on an SE-52 capillary column: (A) rabbit-skin glue, (B) whole egg, (C) synthetic medium obtained mixing rabbit-skin glue and linseed oil. Other experimental conditions and abbreviations are reported in the text.

Table 2
GC–MS data (TIC and SIM mode acquisition) of amino acids and fatty acids from a synthetic medium obtained mixing rabbit-skin glue and linseed oil, after acid hydrolysis and derivatization

| Analyte | TIC mode | | | SIM mode | | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ [a] | S.D. | R.S.D. (%) | $\bar{x}$ [a] | S.D. | R.S.D. (%) |
| Ala | 58.63 | 6.86 | 11.71 | 122.08 | 13.69 | 11.21 |
| Gly | 179.63 | 11.24 | 6.25 | 193.54 | 9.05 | 4.68 |
| Val | n.d. | – | – | 6.34 | 0.51 | 8.10 |
| Leu | 16.72 | 0.35 | 2.11 | 27.66 | 0.84 | 3.03 |
| Nleu | 100.00 | – | – | 100.00 | – | – |
| Pro | 112.28 | 2.25 | 2.00 | 212.60 | 1.55 | 0.73 |
| Hpro | 99.53 | 2.83 | 2.84 | 117.20 | 2.22 | 1.90 |
| Asp | 47.38 | 1.47 | 3.11 | 38.23 | 0.65 | 1.70 |
| Glu | 92.31 | 6.87 | 7.44 | 47.28 | 1.33 | 2.82 |
| Phe | 10.99 | 0.66 | 6.04 | 13.57 | 0.46 | 3.35 |
| C12:0 | n.d. | – | – | n.d. | – | – |
| Azel | 1.59 | 0.50 | 31.18 | 1.69 | 0.55 | 32.88 |
| C14:0 | n.d. | – | – | 0.50 | 0.01 | 2.35 |
| C16:1 | n.d. | – | – | n.d. | – | – |
| C16:0 | 44.26 | 6.08 | 13.73 | 17.32 | 0.82 | 4.71 |
| C18:1+C18:2 | 335.13 | 58.60 | 17.49 | 3.08 | 0.17 | 5.52 |
| C18:0 | 21.07 | 3.24 | 15.36 | 8.91 | 0.53 | 5.89 |

[a] Average from eight chromatograms. The mean has been determined by the peak ratio of each analyte peak area to the internal standard (Nleu) base peak area. In all cases 0.05 mg of Nleu per 1 mg reference material has been added. Means and standard deviations have been multiplied by 100.

grams of one ground sample and one paint sample are shown in Fig. 3A and Fig. 3B, respectively.

Amino acid data, given as area percentage values, corrected using response factors, are summarized in Table 3, for both reference proteinaceous materials and ground paint samples. Response factors have been applied to compensate for differences in detector response to the analyte and the internal standard. Moreover, they should be calculated for each series of experiments, by injecting a standard mixture, as they are influenced by instrumental parameters. One can note that all the reference materials are characterized by a particular amino acid composition. Particularly, glutamic acid is the most abundant amino acid in egg components and the same is true for casein (from 24 to 37% of the determined amino acids). In addition, casein contains about 23% of proline, while egg components are characterized by 23% aspartic acid and 7% proline, 12% leucine and 16% alanine. As for the rabbit-skin glue composition, glycine and proline are the most abundant amino acids (45% and 20%, respectively, of the determined compounds), and alanine can be up to 14%, while leucine, aspartic acid and phenylalanine never exceed 7%. Moreover, hydroxyproline – which has not been included in the table – is present in the animal glue only. Table 3 shows that the undercoat samples mainly contain glycine, which represents from 21 to 41% of the determined amino acids: it reaches up to 41% in sample PC2, while only two samples (PC1 and PC7) have a content as low as 26%. Glutamic acid (from 17 to 31%) – it is the most abundant amino acid in samples PC1 and PC7 – and proline (from 17 to 27%) are the other most abundant amino acids. Moreover, all the chromatographic profiles of undercoat samples show the presence of hydroxyproline – not inserted in the table – which can come only from animal glue. The presence of hydroxyproline and the high contents of glycine and proline in nearly all the samples give an indication that animal glue seems to have been used as a binder in a generalized way by the artist involved in the decoration of these sculptures. Moreover, all the chromatographic profiles of undercoat samples show the presence of a fatty acid content, suitable only for qualitative evaluation. In fact, a general survey of the analytical data can all the same add useful information to that collected from the

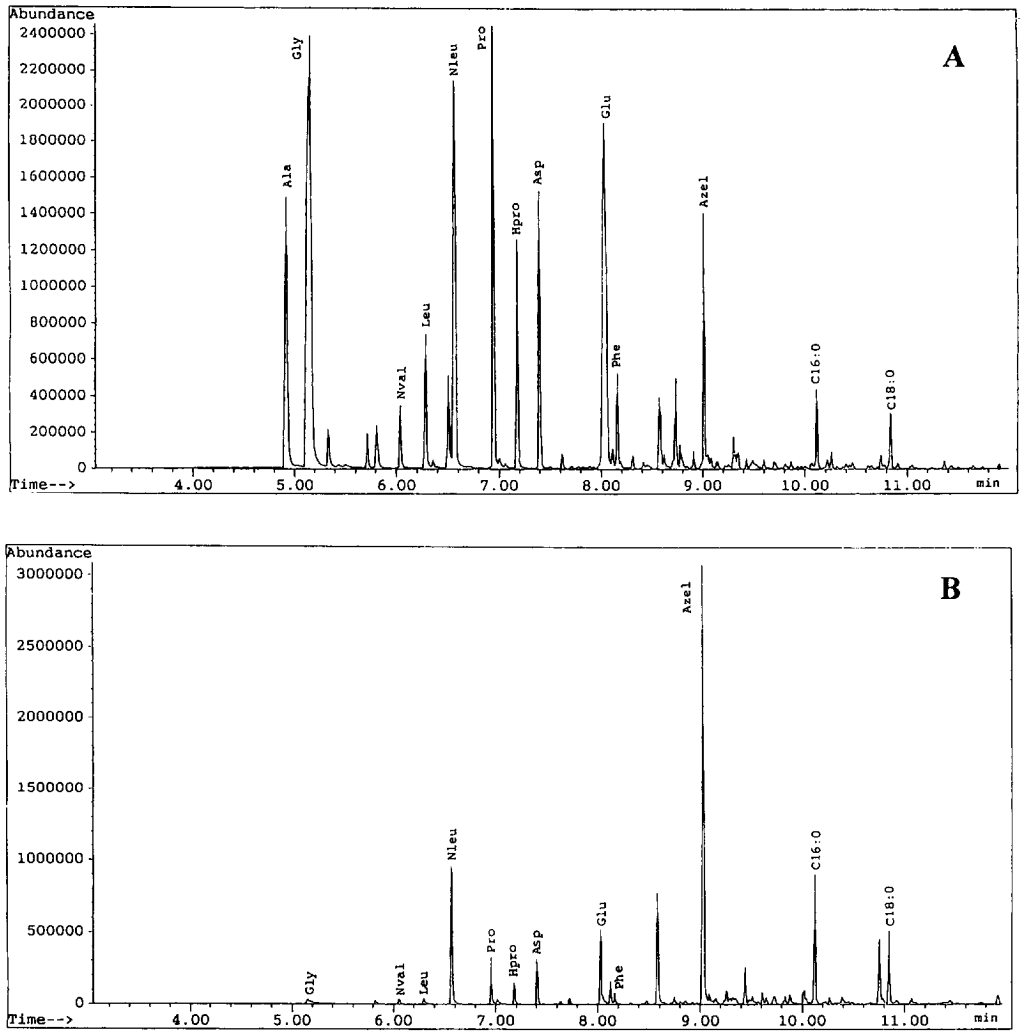


Fig. 3. Total ion chromatograms obtained from paint samples, after hydrolysis and derivatization procedure, on an SE-52 capillary column: (A) ground sample (PC2), (B) paint layer sample (PC8). Other experimental conditions and abbreviations are reported in the text.

amino acids analysis. This concerns particularly the presence of myristic acid, detected by SIM mode in some of the ground samples, which could be due to the contribution of milk fatty acids. As most of these samples also contain a relevant amount of glutamic acid and proline, one might infer the presence of milk or milk derivatives. This could lead to conclude, for example, that milk might have been added to animal glue to paint the sculptures. However, how exactly this combination of materials was used as a medium remains unclear.

Chromatographic data obtained from the paint film samples show the predominant presence of fatty acids with amino acids present as minor components. All the samples examined show high amounts of azelaic acid, whose determination is important because the high amount of this fatty acid indicates the presence of drying oils. Now, the drying power of a

Table 3
Relative peak areas, corrected with response factors, (normalized 100%) of the main amino acid derivatives from the reference proteinaceous materials and paint samples

| | Ala | Gly | Leu | Pro | Asp | Glu | Phe |
|---|---|---|---|---|---|---|---|
| *Reference* | | | | | | | |
| Whole egg | 16 | 10 | 12 | 7 | 23 | 24 | 8 |
| Casein | 6 | 3 | 11 | 23 | 13 | 37 | 7 |
| Rabbit-skin glue | 14 | 45 | 3 | 20 | 7 | 10 | 1 |
| *Samples* | | | | | | | |
| PC 1 | 1 | 21 | 2 | 27 | 15 | 31 | 3 |
| PC 2 | 11 | 41 | 3 | 17 | 8 | 17 | 2 |
| PC 6 | 3 | 28 | 3 | 24 | 11 | 28 | 3 |
| PC 7 | 2 | 26 | 3 | 26 | 13 | 28 | 2 |
| PC 12 | 5 | 33 | 3 | 26 | 10 | 21 | 2 |
| PC 15 | 5 | 37 | 3 | 21 | 11 | 21 | 2 |

vegetable oil is related to the concentration of polyunsaturated fatty acids in such an oil; their concentration should reach at least 65%. Particularly, the presence of linolenic acid is essential for rapid drying. In fact, it was determined that the order of reaction rates of fatty acids is the following one: linolenic>linoleic>oleic≫saturated acids. Moreover, it was found that no linolenic or linoleic acids can be detected in paint films older than a few weeks [22]. The product obtained from the oxidative degradation of long-chain unsaturated fatty acids is azelaic acid. In fact, some of the reactions which take place during the drying step lead not to cross-linking but to bond breaking and to the formation of low-molecular-mass degradation products. This is most likely happening through alternative reaction paths of hydroperoxide intermediates [25]. The chain length of the possible oxidative products depends on the exact position of the hydroperoxy group on the $C_{18}$ fatty acid chain. Usually, it is somewhere more or less in the middle of the chain and consequently the major products are the half esters of the $C_9$ dicarboxylic acid, azelaic acid, and the $C_8$ and $C_{10}$ compounds, with still smaller amounts of the short-chain compounds [25].

Fatty acid data obtained from fresh substances are not comparable with expected data obtained by analyzing aged binding media, for the degradation of unsaturated acids. For this reason, only the palmitic/stearic acid ratios have been calculated. The data obtained from the analyzed fresh oil are listed in Table 4, together with the data obtained from the paint samples. One can note that the ratio values calculated for oil materials characterize each oil. Moreover, one can see that for the three reference oils the P/S ratios are so widely spaced that they would be expected not to overlap, and therefore single determinations alone would suffice to identify them with a fair degree of probability. The question then arises as to how constant these ratios are for different samples of the same type of oil, but of different provenance. All the results for the paint samples do fall within a narrow range, from 1.3 to 2.3. As a consequence it can be supposed that linseed

Table 4
Palmitic/stearic ratios (P/S) obtained from fresh oils and paint samples

| | P/S |
|---|---|
| *Oil* | |
| Linseed | 1.8–2.4 |
| Walnut | 2.5–3.3 |
| Poppyseed | 7.2–8.0 |
| *Paint sample* | |
| PC3 | 1.8 |
| PC4 | 2.2 |
| PC5 | 1.5 |
| PC8 | 2.1 |
| PC9 | 2.3 |
| PC10 | 1.3 |
| PC11 | 1.4 |

oil was used as a medium for the paint film forming. The differences in the values found for the paint samples, with respect to fresh oils, can be due to ageing.

## 4. Conclusions

A GC–MS methodology was proposed for the characterization of binding media in paintings through the determination of amino acids and fatty acids after hydrolysis and derivatization. This method is promising for the study of paint media, because it requires a sample of small size, and provides a diagnostic fingerprint of the material.

The good detection limit of the SIM technique allows a satisfactory characterization of very small amounts of binding samples (1 mg).

The method, applied to the identification of binding media from ground and paint layers of polychrome sculptures from the eighteenth century, allowed to distinguish between animal glue mixed with milk derivatives to form the undercoat, and other linseed oil to form paint film. These results are consistent with the available historical information [27,28], and may help to shed light on the actual technique used in paintings.

These preliminary results indicate that the proposed method could be effective and highly selective in the determination of binding media in complex matrices.

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## References

[1] C. Cennini, Il Libro dell'Arte, Neri-Pozza, Vicenza, 1982.
[2] G. Vasari, Le Vite dei più Eccellenti Pittori, Scultori e Architettori, Sansoni, Firenze, 1967.
[3] L. Masschelein-Kleiner, Ancient Binding Media, Varnishes and Adhesives, ICCROM, Rome, 1985.
[4] R. White, Natl. Gallery Techn. Bull., 8 (1984) 5.
[5] L. Masschelein-Kleiner, PACT Sci. Exam. Easel Paintings, 13 (1986) 185.
[6] D.C. Stulik, in D. Littejohn and D. Thorburn Burns (Editors), Reviews on Analytical Chemistry – Euroanalysis VIII, The Royal Society of Chemistry, Cambridge, 1994, p. 214.
[7] M. Odlyha, J. Thermal Anal., 37 (1991) 1431.
[8] A. Burmester, Stud. Conserv., 37 (1992) 73.
[9] M. Odlyha and R.P.W. Scott, Thermochim. Acta, 234 (1994) 165.
[10] R.J. Meilunas, J.G. Bentsen and A. Steinberg, Stud. Conserv., 35 (1990) 33.
[11] H. Schulz and B. Kropp, Fresenius' J. Anal. Chem., 346 (1993) 114.
[12] C.M. Grzywacz, J. Chromatogr. A, 676 (1994) 177.
[13] S.M. Halpine, Stud. Conserv., 37 (1992) 22.
[14] F. Ronca, Stud. Conserv., 39 (1994) 107.
[15] E. Kenndler, K. Schmidt-Beiwl, F. Mairinger and M. Pohm, Fresenius' J. Anal. Chem., 342 (1992) 135.
[16] A. Casoli and G. Palla, Sci. Technol. Cultural Heritage, 3 (1994) 177.
[17] A. Casoli, P. Mirti and G. Palla, Fresenius' J. Anal. Chem., 351 (1995) 1829.
[18] J.M. Challinor, For. Sci. Int., 21 (1983) 269.
[19] J.M. Challinor, J. Anal. Appl. Pyrolysis, 16 (1989) 323.
[20] J.M. Challinor, J. Anal. Appl. Pyrolysis, 18 (1991) 233.
[21] A.M. Shedrinsky, T.P. Wampler, N. Indictor and N.S. Baer, J. Anal. Appl. Pyrolysis, 15 (1989) 393.
[22] J.S. Mills, Stud. Conserv., 11 (1966) 92.
[23] F. Rasti and G. Scott, Stud. Conserv., 25 (1980) 145.
[24] G. Perusini, Il Restauro dei Dipinti e delle Sculture Lignee, Del Bianco, Udine, 1989.
[25] J.S. Mills and R. White, The Organic Chemistry of Museum Objects, Butterworths–Heinemann, Oxford, 1994.
[26] I. Gandolfi, G. Palla, L. Delprato, F. De Nisco, R. Marchelli and C. Salvadori, J. Food Sci., 57 (1992) 377.
[27] F.R. Pesenti, Le Tecniche Artistiche, Mursia, Milano, 1973.
[28] C. Merucci, Preparazione e Finitura delle Opere Pittoriche, Mursia, Milano, 1993.