Pigments and binders in Pompeian four styles wall paintings
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1. Introduction
Pompeii’s archaeological site is of utmost importance for the study of ancient remains of great historical and scientific relevance [Saiz-Jimenez C., 2003]. However, very few studies have been performed on the constituent materials of ancient paintings whose characterization is fundamental for the understanding of the artists painting techniques. Moreover, these analyses should provide useful suggestions for the selection of suitable conservation treatments to assure their preservation and transfer to future generations. Given the complexity of their molecular components, the analysis of ancient painting samples is a very challenging task and requires the integration of several scientific and humanistic disciplines. In this contest, the study of wall painting samples from a relevant archaeological site might furnish such opportunity. In particular, the comparison of binders composition employed by artists over the time might be helpful to understand changes in the painting technique. Here, we have applied an experimental strategy for the analysis of pigments and binders (polar and non-polar) of Pompeian mural painting unearthed in the archaeological area of Marcus Fabius Rufus house, Insula Occidentalis, and classified as representative of the first, second, third and fourth painting styles.

1.1. The Marcus Fabius Rufus House
The house represents one of the most outstanding examples of Pompeii’s architecture. It is an example of “villa in the city”. The garden (1,581 square meters wide) is located west of the house close to the city walls made of square work in Sarno limestone. Marcus Fabius Rufus house area was first explored during the Bourbon period with the purpose to remove some decorations that were then placed in the National Archaeological Museum of Naples [Grimaldi M., 2006]. The house plan made by La Vega brothers (1787-1809) was not accurate and only the upper floor was surveyed; for instance, the circular exedra was shown too far to the south, highlighting one of the plan’s inconsistencies. Later investigations of Amedeo Maiuri in 1961 [Maiuri A., 1961] served to immediately restore the house that, except for the central circular exedra, had clearly borne the full brunt of the destructive impact of the 79 AD eruption. At the end of the 1970s, a new series of excavations uncovered the outer garden on the west slope and restorations were carried out [Cerulli Irelli G., 1981]. Because the inaccuracy of previous research, from 2004 to the pre-
sent, Marcus Fabius Rufus house and associated areas have been subjected to new investigations [Ciardiello R., 2006; Grimaldi M., 2006; Varriale I., 2006]. We have undertaken excavations in the garden area of the house and also conducted an architectural survey of the house [Grimaldi M., 2007; Grimaldi M., 2008] (Fig. 1). In particular, we have also studied and published the wall paintings found within the house as well as its neighboring buildings [Aoyagi M. and Pappalardo U., 2006]. These studies aimed to reconstruct the phases of urbanization for this part of ancient Pompeii that we believe is an ideal model for the analysis of the phases of occupation of the Insula Occidentalis. The decoration of the villa was almost completely restored and re-executed after the damage that the architecture had to suffer for the great earthquake of 62 AD that invested the Campania coast. The necessary static renovations led to the restoration or destruction of the previous decorations of the 4th, 3rd and 2nd style present in the rooms. All the discarded material of such an operation was used to raise the floor level of the garden of the house. In the last excavation, other data emerged that helped to understand the phases of the plant life of the previous house [Grimaldi M., 2008]. In fact, it reached the levels of the late Republican period characterized by a large presence of painted plaster of the first style. Therefore, the collected wall painting specimens were never subjected to any restoration and resulted also preserved from...
possible deterioration caused from the subsequent eruption of the Vesuvius in 79 AD. Under this consideration, these pieces of decorations were considered appropriate for comparative analysis of the binder ingredients utilized by the artists in a large spanning time from 200 BC up to 79 AD.

2. Experimental investigations and results

2.1. Samples preparation

Pompeii sample powder (1st style, yellow; 2nd style, light red; 3rd style, dark red, and 4th style, black) (Fig. 2) was scraped off from the painting surface with a blade and about 100, 86, 40 and 80 mg of the four samples were collected. Equal amounts (40 mg) of wall painting powder were then used for the extraction of polar and non-polar compounds for chemical analysis as previously described by us [Corso G. et al., 2012-A; Corso G. et al., 2012-B].

2.2. Raman Spectroscopy analyses

Raman spectroscopy was used to first characterize the nature of main pigments present in the powder scraped from the paint layers of the four samples. 1st style sample was characterized by Raman features at 1161, 391 and 280 cm$^{-1}$. These frequencies and the relative intensities suggest an organic saffron-like pigment [Aguayo T. et al., 2011]. 2nd style provided very strong signals at 253 and 344 cm$^{-1}$, easily assigned to cinnabar (HgS), indicating a red vermillion, as already reported in other Roman wall paintings [Clark R.J.H. et al., 1997]. 3rd style was characterized by 289, 406, 495, 605 and 661 cm$^{-1}$, indicating a mixture of two iron oxides (hematite and magnetite), typical of red ochre pigment [Clark R.J.H. et al., 1997]. 4th style showed two weak and broad bands around 1338 and 1589 cm$^{-1}$ that can be assigned to stretching C-C modes of amorphous carbon [Baraldi P. et al., 2007]. These results were consistent with those described by S. Augusti [Augusti S., 1967] with the exception for the 1st and 4th styles sample where no inorganic components were so far detected by Raman spectroscopy. Colors of organic nature such as those based on carotenoids used for decorative purpose have been already described in roman paintings [Maguregui M. et al., 2012].

2.3. FT-IR spectro-microscopy analysis

FT-IR analysis of the polar fraction (water/methanol) showed the presence of several major bands characterizing the presence of organic compounds, such as carbohydrates components, instead the region corresponding to amide I signals was weakly represented or not detectable thus suggesting the absence of proteinaceous materials. FT-IR spectra profile of non-polar fraction of the 1st style sample was significantly different than those of other three styles. In fact, the spectra showed the presence of bands similar to those characterizing the presence of terpenoid derivatives [Tarantilis P.A. et al., 1998]. Spectra of the 2nd, 3rd, and 4th style showed instead a profile typical of oily compounds characterized by the presence of a functional groups region, a region of double bond’s stretching, and a region of double bond deformation due to the C-H bending [Derrick M., 1989]. All the major bands observed in the spectra together with peak assignments are in agreement to that reported in the literature.
2.4. Chromatography and mass spectrometry analysis

Because natural binders are mainly composed of polysaccharides, proteinaceous media, oils, waxes, and resins [Colombini M.P. and Modugno F., 2004] a combined strategy based on gas-chromatography (GC) coupled to flame-ionization detector (GC-FID) or mass spectrometry (GC-MS), liquid chromatography coupled to MS techniques (LC-Q/q/Q and LC-Q/q/TOF) were employed for the analysis of polar and non-polar components extracted from paint powders. In all samples analyzed we found and characterized the profiles of polysaccharides and free amino acids in the polar fraction, and fatty acids from complex lipids in the non-polar fraction. In table 1 and Fig. 3 are reported the total amounts of amino acids, fatty acids, and sugars found in the four styles of Pompeii paintings as mg/Kg and percentage. We also used a recent developed procedure for the identification of protein binders by LC-Q/q/TOF mass spectrometry [Chambery A. et al., 2009].

2.4.1. Gas chromatography-mass spectrometry

Sugars analysis by GC-FID and GC-MS, performed after methanolysis and trimethylsilyl derivatization, of Pompeii’s sample powders showed the presence of several peaks identified as sugars. The total amounts of sugars of 1st and 3rd styles were markedly richest compared to that found in the 2nd and 4th styles. The results showed the presence of 7 sugars, such as arabinose, fucose, xylose, galactose, glucose, galacturonic acid, and myo-inositol, the xylose was the most abundant sugar found in all samples. The percentage of sugar composition was quite closer by comparison between the four styles analyzed. The analysis of the non-polar fraction was performed after transesterification by GC-FID and GC-MS of all Pompeii’s painting styles show mainly the presence of five fatty acids (FA) identified by mass spectrometry as C16:1, C16:0, C18:2, C18:1, and C18:0. To note, an abundant peak detected

Table 1 - Total amounts and percentages of free amino acids, fatty acids, and sugars

<table>
<thead>
<tr>
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<th>1st Style</th>
<th>2nd Style</th>
<th>3rd Style</th>
<th>4th Style</th>
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</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>mg/Kg</td>
<td>%</td>
<td>mg/Kg</td>
<td>%</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>162.2</td>
<td>24.0</td>
<td>94.3</td>
<td>42.0</td>
</tr>
<tr>
<td>Sugars</td>
<td>509.8</td>
<td>75.3</td>
<td>121.5</td>
<td>54.2</td>
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</tbody>
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Fig.3 - Total amounts and percentages of free amino acids, fatty acids, and sugars
in the 1st style sample showed a mass spectrum identified as saffron like compound, the result was in agreement with that observed by FT-IR and Raman spectroscopy. As shown in Fig. 3, the 1st style was richest in lipids compared to the samples of 2nd, 3rd, and 4th styles. The percentage of lipid composition was quite closer by comparison between the four samples, C16:0 and C18:0 fatty acids were the most abundant accounting on average for 42 and 44%, respectively.

2.4.2. Tandem mass spectrometry (LC-Q/q/Q)
The polar fraction was analyzed by MS/MS to detect the presence of free amino acids using a mass spectrometry technique largely employed to analyze the profiles of amino acids and other compounds from complex matrices [Corso G. et al., 2012-A]. All recorded signals were integrated, and their concentrations, expressed as mg/Kg of powder, were calculated using the abundances of labeled internal standards. The results showed the presence of 19 amino acids, on average the most abundant were Glu, Asp, Phe, Ile, Leu, Val, Pro, Ala, Ser and Gly. The free amino acid profile resulted proportionally similar to that found previously by us [Corso G. et al., 2012-A; Corso G. et al., 2012-B]. The total amounts of AA showed that the more ancient samples, 1st and 2nd styles, are markedly less rich of amino acids compared to the richest sample of 3rd style, instead the sample of 4th style show an intermediate total amount of total amino acids.

2.4.3. Shotgun tandem mass spectrometry (LC-Q/q/TOF)
The presence of proteinaceous material was evaluated by the sensitive LC-Q/q/TOF method on the tryptic digest of the paint powder samples as previously described [Chambery A. et al., 2009]. In this case, no proteinaceous material was detected in all samples. This result was also confirmed by SDS-PAGE analysis. However, the presence of proteinaceous materials is one of the most intriguing aspects in Pompeii’s wall painting. The doubts arising from the use of materials of animal and/or plant origin in the art works of roman ages are still present. Nevertheless, following the method described by Casoli et

![Fig.4 - PCA score plot of amino acids from the four Pompeian samples and from 15 reference samples proteins from wheat flour, egg, animal glue and milk](image-url)

1395
al. [Casoli A. et al., 2012], we have tried to match the free amino acid profiles obtained from four styles studied in this work with that of different proteins of natural origin. The result obtained from the principal component analysis (PCA), by comparing the AA percentage composition of Pompeii paint four styles with that of wheat flour, egg, glues and milk showed that the composition of both 3rd and 4th styles matched with wheat flour protein cluster, whereas the composition of both 1st and 2nd styles did not grouped with any other protein clusters (Fig. 4). Furthermore, the percentage of amino acid cluster selected by PCA was submitted to Swiss-Prot and/or TrEMBL AACompIdent to allow the identification of proteins from its amino acid composition. The search did not match any protein. Therefore, we confirm that Pompeii’s wall paint was probably made of pigments dissolved in a liquid medium and organic binders not of animal but from vegetable and plant origin. Under this aspect, we will continue our investigation in order to elucidate this question.

3. Conclusions
Our analysis of Pompeii’s wall paintings of different historical time showed similar patterns with few differences thus indicating a quite similar chemical composition among them. These data were also comparable with those obtained from sporadic Pompeii’s samples taken at “Villa Imperiale, Insula Occidentalis” [Corso G. et al., 2012-A] and from a sporadic sample from Liternum Archaeological Park [Corso G. et al., 2012-B] even though in the latter case, proteins of animal origin were identified only in the red color. These findings suggest that the painting binders generally contained similar ingredients that were down over years. Obviously, to our opinion, it is very difficult to determine with accuracy the true composition of the different organic compounds composing the paint mixtures even though their presence has been constantly detected in all samples so far investigated also by other authors. The differences observed might depend by i) the deterioration of the paint layer due to their aging [Regert M. et al., 2001]; ii) the qualitative and quantitative choice made by the artists in preparing their own paints. Under this aspect, it is worth to note that the richness of the 3rd style sample composition with respect to the 2nd and 4th styles could be linked to the historical contest. In fact, in the second half of the first century B.C., during the Augustan age (27 B.C. - 14 A.D.), there was a new innovative impulse toward architecture, sculpture, and painting. This aspect could be relevant for future archaeological investigations dedicated to the study of past human habits and diet [Kuckova S. et al., 2009].

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