GC–MS detection of plant pigments and metabolites in Roman Julio-Claudian wall paintings

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ABSTRACT

For the first time, plant pigments were detected in Roman *frescoes*. Indeed, only sporadic papers proved the application of natural hues in ancient paintings and none of them relative to Roman art. Therefore, eight differently coloured fragments of Julio-Claudian wall paintings, collected from *Terme degli Stucchi Dipinti* archaeological site (Rome, Italy), were subjected to GC–MS analysis, obtaining detailed chemical profiles. Vegetal dyes (ie. Indigotin, Neronine, Alizarin) or colourless metabolites ascribable to plant species (ie. *Basella rubra*, *Sesamum radiatum*, *Lamium purpureum*) were detected. Plant waxes, oils (ie. Brassicaceae) and resins (ie. Pinaceae) were found to be preferred as binders or protective coatings instead of animal glues (ie. collagen, egg). Several natural molecules, typically associated to plant organisms (ie. Lamiaceae and *Citrus* species), were also identified. These *data* suggest the predominant role of plant extracts in ancient art and increase the knowledge about organic materials of Roman *frescoes*, facilitating modern restorers' activities.

1. Introduction

In the last decades, modern urban activities led to the identification of numerous archaeological sites in the South-Eastern suburbs of Rome (Italy), an area between *Colli Albani* volcanic hills and the ancient border of the city. Among them, remains of *villae*, funerary edifices, pit burials, water pipelines and agricultural fields were detected (Ashby, 1991; Avilia, 2007; Coletti and Pacetti, 2007; Ciceroni, 2008; Gioia and Volpe, 2009). These findings gave back a wealth of data about antique Roman communities which lived outside the old walls. Above all, great attention was paid to a residential complex found, in June 2013, by the Soprintendenza Speciale per i Beni Archeologici di Roma, near the University of Rome 'Tor Vergata' (coordinates: 41.837485 N; 12.627976 E) (Bonanno et al., 2017, 2018). The building was dated back to Late Republican period (2nd-1st century B.C.), although traces of successive architectural transformations to the original plan were associated to Julio-Claudian Age (1st century A.D.). The complex, placed near a still visible paved Roman road, included a large agricultural plot, as suggested by the existence of parallel tuff trenches which could be linked to grapevine or cereal cultivations in rows, and a thermal zone, named *Terme degli Stucchi Dipinti* (Fig. S1A and B). This last archaeological area, of about 500 m², was made up of, at least, 13

different rooms. Indeed, starting from a *praefurnium*, located in the Northern side of the structure and characterized by the presence of a wood-fired furnace which operated at high temperature (400–700 °C), a sequence of adjacent rooms was arranged, according to a rectangular apsidal plan, in order to favour a sophisticated circulation system of heat and steam. In particular, during the excavations, a *laconicum* or *sudatio* (similar to a modern sauna), a *calidarium* equipped with hot tubs and tegulae mammatae, a *tepidarium* where temperature decreased, a *frigidarium* and a small covered *natatio* for cold baths were discovered and identified (Fig. S1A) (Bonanno et al., 2017). In this context, pottery, coins, glass and metal objects, bones and shells were unearthed, as well as interior decorations (ie. mosaic floors, fragments of painted stuccoes and frescoes).

Wall paintings of *Terme degli Stucchi Dipinti* were spectacularly coloured and represented both geometric motifs, as evidenced in *laconicum* area, and more complex and realistic images (ie. in *calidarium*). This evidence clearly confirmed the well-known propensity of Roman artists in decorating the interior of edifices (Bragantini, 2004; Malgieri, 2013).

Literature reporting *data* about composition and structure of wall paintings is not abundant. In general, it demonstrates that colours used for antique paintings derived from inorganic compounds, containing

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metal, minerals and salts, while organic matrixes (ie. eggs, waxes, plant resins) were essentially applied as binders or protecting coatings (Andreotti et al., 2006; Daniilia et al., 2007; Herrera et al., 2009; Duran et al., 2010; Cuní, 2016). Indeed, although plant extracts contain natural dyes (Barnett et al., 2006; Eastaugh, 2008; Colombini and Modugno, 2009; Orna, 2012) and were the first colouring substances employed by human civilizations for their art creations, only sporadic scientific works proved the presence of plant pigments in ancient paintings (Leona et al., 2006; Casadio et al., 2010; Abdel-Ghani et al., 2012; López-Montalvo et al., 2017) and none of them was related to Roman wall paintings. For these reasons, in order to support the theory that Romans actually used plant extracts as colours for their paintings, a qualitative gas-chromatographic mass-spectrometry (GC–MS) analysis was performed on Julio-Claudian *fresco* fragments, found in *Terme degli Stucchi Dipinti* archaeological site, obtaining the profiles of organic molecules detectable in these samples. However, the inorganic composition of these ancient materials was not studied in the current work because a lot of information about it already exists in literature (Edreira et al., 2001; Edwards et al., 2003; Edreira et al., 2003; Mazzocchin et al., 2003a,b, 2004; Mazzocchin et al., 2007; Edwards et al., 2009).

2. Materials and methods – experimental

Eight different monochromatic fragments of Julio-Claudian *fresco* paintings were collected, using specific restoration tools (i.e. trowel, sieve, scalpel), in *Terme degli Stucchi Dipinti* archaeological site (Rome, Italy) (Fig. S1C), during the excavations 2013–2014. Samples were named with a letter, which indicated the respective room (reported in Fig. S1A) where they were found, and a number, discriminating fragments of different colour. All methodological procedures were carried out paying attention to avoid exogenous environmental contamination, following the same guidelines reported in Gismondi et al. (2016) for ancient DNA analysis. In particular, under a sterile vertical laminar flow hood (Heraeus HERAsafe HS12 Type), samples were subjected to UV-light for 10 min and washings with 5% sodium hypochlorite, sterile water and 100% ethanol. After drying, the surface of each sample was strongly scraped by a scalpel, producing a coloured powder. Four mg of paint powder were solubilized in 1 mL of 100% ethyl acetate and put in agitation overnight. Then, the sample was centrifuged at 13.000 rpm for 10 min and the relative supernatant was recovered, filtered (0.45 µm) and completely dried out on a vacuum concentrator (Eppendorf – AG 22331 Hamburg, Concentrator Plus). Finally, the pellet was resuspended with 50 µL of 100% ethyl acetate and analyzed by a GC–MS instrument (QP2010, Shimadzu, Japan). Two µL of extract were injected in each run. Gas chromatograph was equipped with a Rtx-5MS column (length: 30 m; diameter: 0.25 mm; thickness: 0.25 µm) and an oven set according to the following program: initial temperature at 150 °C for 1 min, gradient of 5 °C/min up to 300 °C, final step at 300 °C for 10 min. Helium was used as carrier gas at a constant column flow of 1.0 mL/min. Mass spectrometer presented the next parameters: electron impact (EI) at 0.70 eV; scanning from 50 to 600 *m/z*; ion source temperature at 300 °C; interface temperature at 280 °C; solvent cut time 2 min. To produce the molecular profiles of each sample, all compounds detected by GC–MS analysis were identified comparing their mass spectra to those registered in the NIST library (v. 14), a mass spectrometry database associated with the instrument. Only the molecules which showed a similarity value higher than 85% with NIST library standards were considered acceptable. Each sample was analyzed, at least, in triplicate and no significant difference among replicates was revealed. Relative quantitation of the molecules detected in each sample was performed by measuring the percentage area (relative abundance percentage) of the chromatographic peak of each compound, with respect to the total area (considered as unit, 100%) of all chromatographic peaks identified in the same extract, as reported in Giovannini et al. (2016). All data were expressed as mean value of the independent replicates and the maximum deviation for each of them

was always calculated to be lower than 5% of the respective molecule peak area. As negative controls, five fragments of wall of the same archaeological context, which were not painted and did not constitute portions of *fresco*, were sampled, decontaminated, as previously described, and subjected to the analysis. None of them revealed significant chromatographic peaks; rarely, GC profiles showed infinitesimal traces of generic hydrocarbons which could represent degradation forms of organic environmental contaminants preserved to the cleaning procedures. Pure ethyl acetate was also injected in GC–MS, as blank control: no signal was detected in this case, except that those relative to this specific molecule.

3. Results and discussion

In plant tissues, colours are linked to the presence of secondary metabolites, such as phenols and alkaloids. These molecules play a crucial role in protecting plants from biotic and abiotic environmental factors and promoting their propagation. In particular, the attraction of pollinators and animals responsible for seed dispersal is essentially due to these pigments (Gismondi et al., 2017). Although plant extracts are natural dyestuffs, the application of these substances in antique artworks is quite rare, as documented by the limited amount of literature works reporting it (Leona et al., 2006; Casadio et al., 2010; Abdel-Ghani et al., 2012; López-Montalvo et al., 2017). Indeed, it was well-known that they are easily prone to chemico-physical degradation and not persistent over time (Edwards et al., 2000).

According to this evidence, the main purpose of the present work was the scientific confirmation of the hypothetical use of plant dyes in ancient Roman art. To do it, eight fragments of Roman Julio-Claudian *fresco* paintings were collected from *Terme degli Stucchi Dipinti* archaeological site (Rome, Italy) (Fig. S1A, B). Two of them were yellow-ochre (e1 and d3), three samples appeared on red tonalities (d1, a3 and a4), another one seemed to be between dark grey and violet (a2) and the last two were light blue (d2 and a1) (Fig. S1C). The coloured surface of the specimens was adequately cleaned up, isolated, solubilized, purified and analyzed by GC–MS (Fig. S2), in order to identify the organic compounds present in the original matrixes and measure their concentrations. The choice of such type of chemical approach was due to the fact that, firstly, we did not have any idea of the compounds which made up these materials; therefore, the use of an instrument able to identify both polar and apolar molecules, by using molecular database (NIST library), represented the best option. Similarly, the application of ethyl acetate extraction method, as described in *Materials and Methods* section, was due to the fact that such type of solvent could be able to solubilize a wide variety of chemical compounds. The molecular profile of each sample was reported, in detail, in Table S1. The relative abundance percentage of each substance was also shown (Table S1). Negative and blank controls were performed, as reported in experimental section, to confirm our data.

To better characterize the composition of the wall paintings, all detected molecules per *fresco* fragment were clustered in chemical classes (Table 1). Among samples, total amount and nature of the substances revealed by chromatographic technique were very variable, starting from the poorest specimens, such as a1 and a2 which presented only 20 different compounds, arriving to the richest ones, as in the case of d2 and e1. In general, the most abundant groups of molecules were alkanes/alkenes, alcohols and fatty acids, while the less representative were sugars, derivatives of aminoacids and aldehydes.

In all *frescos*, a great number of alkanes/alkenes (ie. tetradecene, octadecene, tetracontane derivatives, cycloalkanes) was found. Surely, they derived from the degradation of organic materials (ie. plant cells, animal products) employed for the preparation of basal matrixes (Ribechini et al., 2008, 2009; Duran et al., 2010).

Similarly, the detection of a large variety of alcohols could be associated to degradation, transformation and oxidation processes which occurred on *fresco* molecules over time (Andreotti et al., 2006).

Table 1

Chemical groups identified in fresco samples. For each samples, all molecules detected by GC–MS were clustered in principal chemical classes.

Chemical groups	Fresco sample								Tot
	e1	d1	d2	d3	a1	a2	a3	a4	
Alkanes/Alkenes	7	13	13	5	5	2	6	6	57
Alcohols	5	1	7	3	4	1	7	9	37
Fatty acids	7	2	7	5	5	9	5	7	47
Benzene derivatives	11	2	0	3	0	2	2	0	20
Terpens	2	1	6	6	3	1	1	0	20
Sugars	4	0	2	0	0	0	0	0	6
Derivatives of aminoacids	0	0	1	2	0	1	0	0	4
Other plant metabolites	1	1	5	2	2	1	3	2	17
Aldehydes	1	0	0	0	0	0	1	1	3
Others	1	2	3	1	1	3	1	11	23
Total molecules	39	22	44	27	20	20	26	36	

A significant portion of the extracts was represented by fatty acids, as evidenced in Table S1. In particular, Erucic acid, a monounsaturated omega-9 fatty acid (22:1 ω9) also known as brassic acid, was one of the most reachable molecules in the samples. It clearly demonstrated that this compound represented one of the principal ingredients of the analyzed frescos. Moreover, since Erucic acid is produced in plants, mainly in species of Brassicaceae family, the detection of this molecule strongly confirmed the habit of Roman artists to add vegetable preparations to their painting materials. In fact, the semi-siccative properties of brassica oil were already well-known to ancient Romans which used it to solubilize pine resins, a natural binder widely used in wall paintings, obtaining a more malleable adhesive (Ribechini et al., 2009). In support for this hypothesis, GC–MS analysis also revealed different terpenic molecules, such as Dihydroabietic acid, Abietic acid and Retene (detected in half of the specimens), which are peculiar indicators of Pinaceae glues (Andreotti et al., 2006; Ribechini et al., 2009; Osete-Cortina, 2011). Only d1 extract, which also presented the lower number of fatty acids among all specimens (Table 1), did not contained Erucic acid, replacing it with another plant fatty acid, the *trans*-11-Icosenamide.

The ubiquitous identification of other typical vegetal fatty acids, such as derivatives of Hexadecanoic acid (Palmitic acid), Dodecanoic acid (Lauric acid), Octadecenoic acid (Oleic acid), Tetradecanoic acid (Myristic acid) and *cis*-Vacenic acid (Table S1), further corroborated the application of plant oils and waxes in the preparation of the samples (Andreotti et al., 2006; Ribechini et al., 2008b; Katsibiri and Howe, 2010; Osete-Cortina, 2011; Corso et al., 2012; Cuni et al., 2012). In addition, as fresco extracts did not reveal any specific markers (ie. Hydroxyproline, Cholesterol) for animal proteinaceous binders (ie. egg, collagen, casein) (Andreotti et al., 2006; Daniilia et al., 2007), except that d2, d3 and a2 fragments where Hydroxyproline was detectable, it could be assumed that plant glues played a fundamental role in the production of Roman wall paintings, compared to animal ones.

Different secondary metabolites were also found in the samples, validating again the use of plant derivatives in Roman painting techniques. Among all, indole and indole epoxide resulted very interesting, being the basal chemical structures of various alkaloids, including indigotin (Table S1). Indigotin is a blue dye, naturally produced in leaves of *Isatis tinctoria* and *Indigofera* species, whose extraction method was known by Egyptians, Romans (Abdel-Ghani et al., 2012) and Greeks (Brysbaert and Vandenabeele, 2004). It was curious to notice that these indolic compounds were present only in light blue samples (d2 and a1), suggesting that the colour of these frescoes could be probably due to that specific plant pigment. In the same context, it was also observed that only d2 extract contained a Neronine derivative, an yellowish alkaloid synthesized in *Galanthus panchratium longiflorum* and *Nerine krigelii* bulbs (Amaryllidaceae family), which maybe could have been used to soften indigotin dark blue. Although this hypothesis can be reasonable,

it is important to consider the possibility that this effect might be due to the interaction of the indigotin with clay and calcareous components present in the inorganic matrix of the samples (Doménech-Carbó et al., 2013; Doménech-Carbó et al., 2014).

Another important result was obtained by studying the reddish wall paintings d1 and a3, where the Alizarin (1,2-dihydroxyanthraquinone) and its derivative, 1-Methyl-alizarin, were identified (Table S1). Indeed, the famous colouring properties of these anthraquinones, extracted from roots of Madder genus (ie. *Rubia tinctorum*, *Rubia peregrina* and *Rubia cordifolia*), easily justified the red shades of these fresco samples (Mazzocchin et al., 2003a; Casadio et al., 2010). On the other hand, with great surprise, the GC–MS profile of a4 specimen, whose hue was likewise dark red, did not show such type of pigments. However, the detection of two other molecules, Tetrahydro-22-desoxy-tomatillidine and 3-Eicosyne (Table S1), testified the possible addition of ruddy plant extracts to the matrix of this fresco. In particular, the Tomatillidine derivative, a steroidal alkaloid with a pyrrolidinyl side-chain, indicated the potential presence of juice of African spiny Solanaceae plants (the same family of tomato and *Sanguinaria* genus) (Ripperger and Schreiber, 1981; Southon and Buckingham, 1989; Vorontsova et al., 2013; Ng and Smith, 2016), while the second compound supported the possible use of *Basella rubra* tissues (Malabar spinach), being abundant in this species which has stems and fruits rich of betalains, purple and scarlet anthocyanins (FoodDB, 2013; Kumar et al., 2015).

About a2 wall painting, the chromatographic analysis revealed three different plant metabolites which could be linked to the grey-violet colour of this fragment. Two of them, a Secoyohimban derivative, typically produced by *Sesamum radiatum* (black sesame), and the Benzaldehyde, copious in almond oil and seeds, would explained the origin of the grey tone (Table S1). Indeed, dark varieties of sesame might be potential sources of deep pigments and the peach-black, obtained by calcination of *Prunus* sp. seeds, was already widely employed as dye at Roman times (International Correspondence Schools, 2008; Ogunlesi et al., 2010). The third molecule, the Germacrene (Table S1), was a sesquiterpene deriving from degradation of Germacrene, a secondary metabolite abundant in *Lamium purpureum*. Since flowers and even pollen of this last species are light purple, the hypothesis that the extract of this plant could be used as dyestuff in a2 fresco may not be excluded.

No specific natural dye was detected in yellow-ochre samples (e1 and d3). Nevertheless, only these samples and d2 extract showed terpenic compounds typical of Lamiaceae species and/or *Citrus* fruits (ie. Citronellol epoxide, Citronellyl isobutyrate, *o*-Menth-8-ol, *D*-Limonene, hexahydro-Nerolidol, 1,2-Dihydrolinalool) (Table S1). Obviously, these compounds cannot be considered responsible for the peculiar colour of these fragments.

Curiously, only two samples (d2 and d3) revealed in their biochemical spectra the presence of Phytol, an acyclic diterpenic alcohol constituent of chlorophylls (Peisker et al., 1989). On the contrary, all fresco extracts contained at least a basal precursor, *o*-degraded form, of phenolic or alkaloid compounds (ie. Hydrocinnamic acid derivatives, Piperidinone derivatives, Benzyl sydnone, Benzene derivatives) (Table S1).

Finally, GC–MS analysis detected some sugars (ie. Xylose, Glucitol and Mannitol derivatives) in d2 and e1 specimens. Particular attention should be paid to the identification of a Galactose derivative in the wall painting e1 (Table S1), suggesting the use of milk or plant sugary extracts, as binder or solvent for pigments (Daniilia et al., 2007; Mazzocchin et al., 2010; Lluveras-Tenorio et al., 2012).

It is important to remark how the current results do not exclude the possibility that also inorganic substances, undetectable by GC–MS, could have been employed for the preparation of *Terme degli Stucchi Dipinti* wall paintings. Surely, traces of cinnabar, hematite, Egypt blue (cuprorivaite), red or yellow ochre might be surely found in the present samples, being widely applied in Roman art (Edreira et al., 2001; Edwards et al., 2003; Edreira et al., 2003; Mazzocchin et al., 2003a;

Mazzocchin et al., 2003b; Mazzocchin et al., 2004; Mazzocchin et al., 2007; Edwards et al., 2009). According to it, further investigations should be performed on these ancient remains, although our preliminary research opens new perspectives on such type of art materials.

4. Conclusions

In conclusion, eight differently coloured fragments of Julio-Claudian *frescoes* were subjected to GC–MS analysis, in order to determine all their organic components. This research: i) produced a detailed biochemical profile for each sample; ii) confirmed the key function of plant waxes, oils (ie. Brassicaceae) and resins (ie. Pinaceae) as binders or protective coatings; iii) revealed a low use of animal glues (ie. milk); iv) scientifically demonstrated, for the first time, the use of plant pigments (ie. alizarin) as dyestuffs in Roman wall paintings; v) detected a great variety of other natural molecules (ie. terpenic, phenolic and alkaloid derivatives) ascribable to several plant species. All these *data* strongly suggest the wide, essential and predominant role of plant extracts in the production of Roman *frescoes*. However, the most important goals of the present research were the increase in knowledge regarding raw organic materials employed in antique wall paintings and the facilitation that it could provide to modern restorers during their preservative activities on Roman *frescoes*.

Contribution and authorship

AG and AC designed the study; GR, MP, AG and MB collected samples; AG and LC performed the analysis; AG wrote the paper; all authors performed the interpretation of data and approved the version of the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.phytol.2018.03.016>.

References

- Abdel-Ghani, M., Stern, B., Edwards, H.G.M., Janaway, R., 2012. A study of 18th century Coptic icons of Ibrahim Al-Nasekh using Raman microscopy and gas chromatography–mass spectrometry: indigo as an organic pigment in Egyptian panel paintings. *Vib. Spectrosc.* 62, 98–109.
- Andreotti, A., Bonaduce, L., Colombini, M.P., Gautier, G., Modugno, F., Ribechini, E., 2006. Combined GC/MS analytical procedure for the characterization of glycerolipid, waxy, resinous, and proteinaceous materials in a unique paint microsample. *Anal. Chem.* 78, 4490–4500.
- Ashby, T., 1991. *Gli acquedotti dell'antica Roma*. Quasar, Roma.
- Avilia F., 2007. Nuovi dati sulla viabilità antica nell'ambito tuscolano. II Dati archeologici preliminari, in *Tusculum. Storia Archeologia Cultura e Arte di Tuscolo e del Tuscolano*, a cura di F. Arietti, A. Pasqualini, La Moderna Roma, pp. 183–188.
- Barnett, J.R., Miller, S., Pearce, E., 2006. Colour and art: a brief history of pigments. *Opt. Laser Technol.* 38, 445–453.
- Bonanno, M., Ghelli, A., Pisani, M., Rocco, G., 2017. La Villa romana delle “Terme degli stucchi dipinti” tra scavo, ricerca e didattica. *Università Ed. Roma*, pp. 978–988 ISBN 978-88-3293-068-9.
- Bonanno, M., Rocco, G., Pisani, M., Ghelli, A., Felici, F., 2018. La villa delle “Terme degli Stucchi dipinti” nel suburbio di Roma. *Atti del XXIII Convegno AISCOM. Associazione Italiana per lo Studio e la Conservazione del Mosaico*, Narni, pp. 81–90.
- Bragantini, I., 2004. Una pittura senza maestri. La produzione della pittura parietale romana. *J. Roman Archaeol.* 17, 131–145.
- Brysbaert, A., Vandenabeele, P., 2004. Bronze Age painted plaster in Mycenaean Greece: a pilot study on the testing and application of micro-Raman spectroscopy. *J. Raman Spectrosc.* 35, 686–693.
- Casadio, F., Leona, M., Lombardi, J.R., Van Duyn, R., 2010. Identification of organic colorants in fibers, paints, and glazes by surface enhanced Raman spectroscopy. *Acc. Chem. Res.* 43, 782–791.
- Ciceroni, M., 2008. La villa A204, in: P. Gioia (a cura di), *Torre Spaccata. Roma S.D.O. Le indagini Archeologiche*, Roma, pp. 173–221.
- Coletti, C.M., Pacetti, F., 2007. La villa della Piscina, in: R. Volpe (a cura di), *Centocelle II: Roma S.D.O. le indagini archeologiche*, Roma, pp. 317–387.
- Colombini, M.P., Modugno, F., 2009. *Organic Mass Spectrometry in Art and Archaeology*. John Wiley & Sons, New York.
- Corso, G., Gelzo, M., Sanges, C., Chambery, A., Di Maro, A., Severino, V., et al., 2012. Polar and non-polar organic binder characterization in Pompeian wall paintings: comparison to a simulated painting mimicking an a secco technique. *Anal. Bioanal. Chem.* 402, 3011–3016.
- Cuní, J., Cuní, P., Eisen, B., Savizky, R., Bové, J., 2012. Characterization of the binding medium used in Roman encaustic paintings on wall and wood. *Anal. Methods* 4, 659–669.
- Cuní, J., 2016. What do we know of Roman wall painting technique? Potential confounding factors in ancient paint media analysis. *Heritage Sci.* 4, 44.
- Daniilia, S., Tsakalof, A., Bairachtari, K., Chrysoulakis, Y., 2007. The Byzantine wall paintings from the Protaton Church on Mount Athos, Greece: tradition and science. *J. Archaeol. Sci.* 34, 1971–1984.
- Doménech-Carbó, M.T., Ossete-Cortina, L., Doménech-Carbó, A., Vázquez de Agredos-Pascual, M.L., Vidal-Lorenzo, C., 2014. Identification of indigoid compounds present in archaeological Maya blue by pyrolysis-silylation-gas chromatography–mass spectrometry. *J. Anal. Appl. Pyrol.* 105, 355–362.
- Doménech-Carbó, A., Valle-Algarra, F.M., Doménech-Carbó, M.T., Domine, M.E., Ossete-Cortina, L., Gimeno-Adelantado, J.V., 2013. Redox tuning and species distribution in Maya blue-type materials: a reassessment. *ACS Appl. Mater. Interfaces* 5, 8134–8145.
- Duran, A., Jimenez De Haro, M.C., Perez-Rodriguez, J.L., Franquelo, M.L., Herrera, L.K., Justo, A., 2010. Determination of pigments and binders in pompeian wall paintings using synchrotron radiation–high-resolution x-ray powder diffraction and conventional spectroscopy–chromatography. *Archaeometry* 52, 286–307.
- Eastaugh, N., 2008. *Pigment Compendium: a Dictionary and Optical Microscopy of Historical Pigments*. Routledge, London.
- Edreira, M.C., Feliu, M.J., Fernández-Lorenzo, C., Martín, J., 2001. Roman wall paintings characterization from Cripta del Museo and Alcazaba in Mérida (Spain): chromatic energy dispersive X-ray fluorescence spectroscopic, X-ray diffraction and Fourier transform infrared spectroscopic analysis. *Anal. Chim. Acta* 434, 331–345.
- Edreira, M.C., Feliu, M.J., Fernandez-Lorenzo, C., Martín, J., 2003. Spectroscopic analysis of roman wall paintings from Casa del Mitreo in Emerita Augusta Mérida, Spain. *Talanta* 59, 1117–1139.
- Edwards, H.G.M., Newton, E.M., Russ, J., 2000. Raman spectroscopic analysis of pigments and substrata in prehistoric rock art. *J. Mol. Struct.* 550, 245–256.
- Edwards, H.G.M., Middleton, P.S., Villar, S.J., De Faria, D.L.A., 2003. Romano-British wall-paintings II: Raman spectroscopic analysis of two villa sites at Nether Heyford, Northants. *Anal. Chim. Acta* 484 (2), 211–221.
- Edwards, H.G.M., Middleton, P.S., Hargreaves, M.D., 2009. Romano-British wall paintings: Raman spectroscopic analysis of fragments from two urban sites of early military colonisation. *Spectrochim. Acta A* 73, 553–560.
- FoodDB, 2013. Food Database, The Metabolomics Innovation Centre (TMIC). Version 1.0. <http://foodb.ca/> (Accessed 10 May 17).
- Gioia, P., Volpe, R., 2009. Archeologia nel Parco di Centocelle, in: D. Manacorda, R. Santangeli Valenzani (a cura di), *Arch.it.Arch. Dialoghi di archeologia e architettura: seminari 2005–2006*, Roma, pp. 100–115.
- Giovannini, D., Gismondi, A., Basso, A., Canuti, L., Braglia, R., Canini, A., Mariani, F., Cappelli, G., 2016. *Lavandula angustifolia* Mill. Essential oil exerts antibacterial and anti-inflammatory effect in macrophage mediated immune response to *Staphylococcus aureus*. *Immunol. Invest.* 45, 11–28.
- Gismondi, A., Di Marco, G., Martini, F., Sarti, L., Crespan, M., Martínez-Labarga, C., Rickards, O., Canini, A., 2016. Grapevine carpological remains revealed the existence of a Neolithic domesticated *Vitis vinifera* L. specimen containing ancient DNA partially preserved in modern ecotypes. *J. Archaeol. Sci.* 69, 75–84.
- Gismondi, A., Di Marco, G., Canuti, L., Canini, A., 2017. Antiradical activity of phenolic metabolites extracted from grapes of white and red *Vitis vinifera* L. cultivars. *Vitis* 56, 19–26.
- Herrera, L.K., Montalbani, S., Chiavari, G., Cotte, M., Solé, V.A., Bueno, J., et al., 2009. Advanced combined application of μ -X-ray diffraction/ μ -X-ray fluorescence with conventional techniques for the identification of pictorial materials from Baroque Andalusia paintings. *Talanta* 80, 71–83.
- International Correspondence Schools, 2008. *A Treatise on Architecture and Building Construction* (1899). Version III. The Colliery Engineer Co, Scranton, Pennsylvania.
- Katsibiri, O., Howe, R.F., 2010. Characterisation of the transparent surface coatings on post-Byzantine icons using microscopic, mass spectrometric and spectroscopic techniques. *Microchem. J.* 94, 14–23.
- Kumar, S.S., Manoj, P., Shetty, N.P., Prakash, M., Giridhar, P., 2015. Characterization of major betalain pigments–gomphrenin: betanin and isobetanin from *Basella rubra* L. fruit and evaluation of efficacy as a natural colourant in product (ice cream) development. *J. Food Sci. Technol.* 52, 4994–5002.
- López-Montalvo, E., Roldán, C., Badal, E., Murcia-Mascarós, S., Villaverde, V., 2017. Identification of plant cells in black pigments of prehistoric Spanish Levantine rock art by means of a multi-analytical approach. A new method for social identity

- materialization using chaîne opératoire. PLoS One 12, e0172225.
- Leona, M., Stenger, J., Ferloni, E., 2006. Application of surface-enhanced Raman scattering techniques to the ultrasensitive identification of natural dyes in works of art. J. Raman Spectrosc. 37, 981–992.
- Lluveras-Tenorio, A., Mazurek, J., Restivo, A., Colombini, M.P., Bonaduce, I., 2012. The development of a new analytical model for the identification of saccharide binders in paint samples. PLoS One 7, e49383.
- Malgieri, A., 2013. *La bottega delle immagini parietali, Fecit te, Mondadori, Milano.*
- Mazzocchin, G.A., Agnoli, F., Mazzocchin, S., 2003a. Investigation of a Roman age bulk pigment found in Vicenza. Anal. Chim. Acta 475, 181–190.
- Mazzocchin, G.A., Agnoli, F., Mazzocchin, S., Colpo, I., 2003b. Analysis of pigments from Roman wall paintings found in Vicenza. Talanta 61, 565–572.
- Mazzocchin, G.A., Agnoli, F., Salvadori, M., 2004. Analysis of Roman age wall paintings found in Pordenone, Trieste and Montegrotto. Talanta 64, 732–741.
- Mazzocchin, G.A., Rudello, D., Murgia, E., 2007. Analysis of Roman wall paintings found in Verona. Ann. Chim.-Rome 97, 807–822.
- Mazzocchin, G.A., Vianello, A., Minghelli, S., Rudello, D., 2010. Analysis of Roman wall paintings from the Thermae of 'Iulia Concordia'. Archaeometry 52, 644–655.
- Ng, J., Smith, S.D., 2016. How to make a red flower: the combinatorial effect of pigments. AoB Plants 8, plw013.
- Ogunlesi, M., Okiei, W., Osibote, E.A., 2010. Analysis of the essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor, by gas chromatography-mass spectrometry. Afr. J. Biotechnol. 9, 1060–1067.
- Orna, M.V., 2012. The Chemical History of Color. Springer Science & Business Media, Heidelberg.
- Ossete-Cortina, L.O., 2011. Analytical study of the wall paintings in the Borgherini chapel in the Sant Pietro in Montorio church (Rome, Italy) by Sebastiano del Piombo. Arch. Instituto Universitario de Restauración del Patrimonio de la UPV, pp. 391–398.
- Peisker, C., Düggelin, T., Rentsch, D., Matile, P., 1989. Phytol and the breakdown of chlorophyll in senescent leaves. J. Plant Physiol. 135, 428–432.
- Ribechini, E., Modugno, F., Colombini, M.P., Evershed, R.P., 2008. Gas chromatographic and mass spectrometric investigations of organic residues from Roman glass unguentaria. J. Chromatogr. A 1183, 158–169.
- Ribechini, E., Modugno, F., Baraldi, C., Baraldi, P., Colombini, M.P., 2008b. An integrated analytical approach for characterizing an organic residue from an archaeological glass bottle recovered in Pompeii (Naples, Italy). Talanta 74, 555–561.
- Ribechini, E., Orsini, S., Silvano, F., Colombini, M.P., 2009. Py-GC/MS: GC/MS and FTIR investigations on LATE Roman-Egyptian adhesives from opus sectile: new insights into ancient recipes and technologies. Anal. Chim. Acta 638, 79–87.
- Ripperger, H., Schreiber, K., 1981. *Solanum* steroid alkaloids. The Alkaloids: Chemistry and Physiology. Elsevier, Amsterdam, pp. 81–192.
- Southon, I.W., Buckingham, J., 1989. Dictionary of Alkaloids. CRC Press, Boca Raton, Florida.
- Vorontsova, M., Stern, S., Bohs, L., Knapp, S., 2013. African spiny *Solanum* (subgenus *Leptostemonum*, solanaceae): a thorny phylogenetic tangle. Bot. J. Linn. Soc. 173, 176–193.