

FT-NIR spectroscopy for non-invasive identification of natural polymers and resins in easel paintings

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Received: 10 June 2009 / Revised: 7 September 2009 / Accepted: 9 September 2009 / Published online: 29 September 2009
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Abstract In the present study, the analytical strengths and limitations of near-infrared (NIR) spectroscopy to non-invasively characterize organic components in painting materials have been investigated. In spite of the increased amount of information available today from advanced modern analytical instrumentations dedicated to cultural heritage, the non-invasive identification of materials belonging to the wide class of organic compounds historically used in paintings is still a challenging task. Near-infrared spectroscopy offers several attractive features that make this technique particularly suitable to this purpose. In fact, it is non-invasive, allows for non-contact measurements in reflectance mode, gives molecular information on complex macromolecules, and can be performed on-site by means of portable devices. First-derivative transformation of reflectance spectroscopic data has been applied to provide a simple and fast way to deduce more information from NIR spectra. This approach has allowed spectral features to be identified that can be useful to distinguish different compounds belonging to the classes of lipids, proteins, and resins. To this purpose, at first, a spectral database of pure standard has been collected. Our analytical approach was then successfully validated on pictorial models reproducing

the typical stratigraphy of an easel painting. As final step, the study of a real painting has been attempted and a drying oil, animal glue, and a terpenic natural resin, as well as an earth pigment were clearly identified, as cross-validated by GC-MS analysis.

Keywords Near-FTIR · Paintings · Natural organic compounds · Portable equipments

Introduction

Over the last 25 years, near-infrared spectroscopy has been developed into a fast and cost-effective method of analysis on account of increasing applications of mathematical tools for processing spectra and application of fiber optic probe, useful for monitoring a variety of processes [1]. In fact, the low molar absorbance of NIR bands allows measurements in reflectance mode recording spectra of solid samples with minimal or no pre-treatment [2]. These attractive features have increased the interest towards NIR spectroscopy in a variety of fields: pharmaceutical, foods, polymers, etc [2–6]. Conversely, only few applications have been reported in the field of cultural heritage [7–10] despite the fact that NIR spectroscopy may address the requisites necessary for an analytical method to be applied for the study of precious and unique artworks. It allows for non-invasive and contactless measurements, gives molecular information on complex macromolecules, and can be performed on-site by means of portable devices. On the other hand, the main drawback of NIR spectroscopy is the difficult band assignment due to the fact that signals in this spectral region are originated by NH, CH, OH, CO, and CC combination and overtone modes, generally broader and less resolved than the fundamental bands. For this, reason it

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is less specific than mid-infrared spectroscopy and in the case of mixtures of organic substances, as commonly encountered in painting materials, single components cannot be distinguished.

In this work, we present FT-NIR spectra of 16 natural organic materials (used in ancient paintings as binding media and varnishes), first as pure films and then in paint-layered models mixed with pigments. To highlight significant differences inside each class of compounds (lipids, proteins, and terpenes) first-derivative mathematical function was applied to the spectra [11, 12]. The first derivative was also applied to spectra collected on pictorial models in order to resolve band overlapping due to the multi-layer structure. An original seventeenth-century oil paint was used as a case study to assess the feasibility of the first-derivative approach to analyze NIR spectra with the purpose of identifying organic components in the paint. Gas-chromatography mass-spectrometry (GC-MS) analysis was carried on a selected micro-sample in order to validate the results obtained from the FT-NIR non-invasive study.

Experimental

Materials and reagents

Siccative oils, animal glues, casein, and terpenic resins (larch Venice turpentine, pine colophony, sandarac, Manila copal, mastic, and dammar) were provided by Zecchi, Florence, Italy. The inorganic materials analyzed in this work are lead white ($2\text{PbCO}_3 \cdot \text{Pb}(\text{OH})_2$), gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), calcium carbonate (CaCO_3), and the earth pigment burnt umber, made of a clay rich in iron and manganese oxides. While lead white and gypsum were provided by Sigma-Aldrich, calcium carbonate and burnt umber were provided by Zecchi, Florence.

Pure organic materials have been individually applied as thick film on glass slides and left to completely dry in open air before measurements for 1 year at least. Pigments were analyzed as loose powder. Laboratory samples simulating easel painting were prepared using wood support coated with a gypsum/animal glue ground. Single layers of cinnabar (purchased by Zecchi, Florence, Italy), chosen because of its low absorbance in the near-infrared spectral region, were spread on the prepared support using egg or oil, in a pigment to binder ratio suitable to obtain a workable paint. A small portion of the oil painting model was coated with a turpentine varnish.

Chemicals used for the GC-MS quantitative analyses of proteinaceous and lipid materials are reported in the following.

Standard amino acids solution in 0.1 N HCl containing $2.5 \mu\text{mol/mL}$ of proline (Pro), aspartic acid (Asp), glutamic

acid (Glu), alanine (Ala), arginine, cysteine, phenylalanine (Phe), glycine (Gly), hydroxylysine, isoleucine (Ile), histidine, leucine (Leu), lysine (Lys), methionine (Met), serine (Ser), tyrosine (Tyr), threonine (Threo), and valine (Val) was purchased from Sigma-Aldrich (Germany). A solution of $2.5 \mu\text{mol/mL}$ hydroxyproline (Hyp) was separately purchased from the same company and added to the amino acid mixture.

A fatty and dicarboxylic acids standard solution of lauric, suberic, azelaic (A), myristic, sebacic, palmitic (P), oleic, stearic (S) acids was prepared in acetone using single standards purchased from Sigma-Aldrich (Germany).

Both amino acids and fatty acids standard solutions were used to derive calibration curves in the range of about $1\text{--}15 \mu\text{g/g}$. Norvaline and heptadecanoic acid (Sigma-Aldrich, Germany) were used as internal standards respectively for the two classes of compounds. Hexadecane (Fluka, Switzerland) was used as injection standard.

All the solvents were HPLC-grade and used without any further purification. Derivatization reagents *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) were purchased from Sigma-Aldrich (Germany).

Analytical methods and instrumentation

Reflectance FT-NIR spectra were recorded using a compact portable JASCO VIR 9600 spectrophotometer equipped with a near-infrared fiber optic sampling probe.

The optical bench is made up of a halogen lamp as source, a Michelson interferometer equipped with a CaF_2 beam splitter and a not cooled InGaAs detector. The instrumentation weighs about 35 kg and has an overall dimension of $50 \times 50 \times 50 \text{ cm}^3$. The spectrophotometer is equipped with a Y-shaped silica-glass fiber optic probe containing 14 fibers, seven of which carry infrared radiation from the source to the sample, while the other seven collect the radiation reflected off the surface. Correction for background absorption was performed by recording the reflectance spectrum of a metal mirror plate as reference. The fiber optic probe was kept perpendicular to the sample surface. The spatial resolution is determined by the probe diameter and is about 12 mm^2 . In this work, the spectra were collected by 400 interferograms covering a spectral range from $12,000$ to $4,000 \text{ cm}^{-1}$, at a resolution of 4 cm^{-1} and with acquisition times of few minutes.

The first-derivative transformation of near-infrared spectra was made by the Spectra Manager software from Jasco using the Savitzky–Golay algorithm [13]. The derivative algorithm employed in this study permits a simultaneous smoothing of spectra (11 data points were used for pure standards and model paintings, whereas 25 data points were used for the real case study) which reduces the noise produced from derivative transformation.

The organic materials present in the seventeenth-century painting were characterized by GC-MS according to a multi-step sample pre-treatment adapted from [14] that allows to analyze proteinaceous, resinous, glycerolipidic, and waxy components in a single micro-sample. The analytical protocol is based on the extraction from the sample of an aqueous and two (acid and neutral) organic fractions containing the different classes of compounds prior GC-MS analysis.

The adopted procedure consists of the following steps:

1. Proteins are extracted from the sample by sonication in 400 μL of an aqueous ammonia solution 2.5 M at 45°C for 60 min for three times.
2. The extracted solution is evaporated to dryness under a stream of nitrogen and then subjected to acidic hydrolysis in 400 μL of HCl 6 N at 100°C for 24 h in N_2 atmosphere.
3. Pure water is added to the acidic hydrolysate, and then free fatty acids and other compounds extracted with proteins in step 1 are recovered for the analysis of the organic fraction by extraction with diethyl ether (400 μL , three times).
4. The aqueous solution containing amino acids is evaporated to dryness under a stream of nitrogen and then subjected to derivatization by addition of 50 μL of MTBSTFA at 60°C for 60 min in presence of 10 μL of DMF. Norvalina is added in this step as internal standard (final concentration 3.5 $\mu\text{g/g}$). After silylation, 240 μL of isooctane-containing hexadecane are added and 1 μL of the final solution is then used for the chromatographic run. The analytical protocol allows for the quantitative determination of 14 amino acids (Pro, Hpro, Asp, Glu, Ala, Phe, Gly, Ile, Leu, Met, Ser, Tyr, Threo, and Val).
5. The residue of step 1 combined with the ether extract of step 3 is subjected to saponification (1.5 h at 80°C) with hydroalcoholic KOH (10% KOH in EtOH/ H_2O 3:1);
6. After saponification, 100 μL of H_2O are added and the unsaponifiable fraction is extracted with 400 μL of *n*-hexane for three times (neutral fraction); the residual solution is kept for step 8;
7. The *n*-hexane extract is spiked with heptadecanoic acid (final concentration 3.2 $\mu\text{g/g}$), evaporated to dryness under nitrogen stream, and then, subjected to silylation for GC-MS analysis with 50 μL of BSTFA at 60°C for 30 min. After the addition of 250 μL of hexadecane in isooctane solution, 1 μL of the final solution is analyzed by GC-MS.
8. The residual solution of the *n*-hexane extraction is acidified and acidic fraction is extracted with 500 μL of diethyl ether for three times to recover fatty and resin

acids; after solvent evaporation acidic fraction is then subjected to the same procedure of derivatization described for neutral fraction.

Chromatographic analyses were carried out by a 6890N gas-chromatograph (Agilent Technologies) with split/splitless injector, interfaced to a model 5973 quadrupole mass spectrometer. Injector was used in splitless mode at a temperature of 230°C, while transfer line was kept at 280°C. MS ion source temperature was 230°C, while the mass quadrupole was maintained at a temperature of 150°C. The mass spectrometer operated in the EI mode at 70 eV. Helium was used as gas carrier at a constant flow of 1 ml min^{-1} . The gas-chromatograph is equipped with a fused silica capillary column, model HP-5MS, with a 5% diphenyl/95% dimethyl-polysiloxane stationary phase, of 30 m length with internal diameter of 0.25 mm and 0.25 μm film thickness (J&W Scientific, Agilent Technologies, Palo Alto, CA).

The amino acids analysis has been carried out programming the oven temperature as follows: initial temperature 80°C, which is held for 1 min and then ramped at 8°C/min to 280°C, where it is held for 2 min. The temperature program of the oven adopted for the analysis of the neutral and acidic fractions has an initial temperature of 80°C, which is held for 1 min and then ramped at 10°C/min to 200°C, which is held for 3 min and then ramped at 10°C/min to 280°C, which is held for 3 min and then ramped at 20°C/min to 300°C which is held for 20 min.

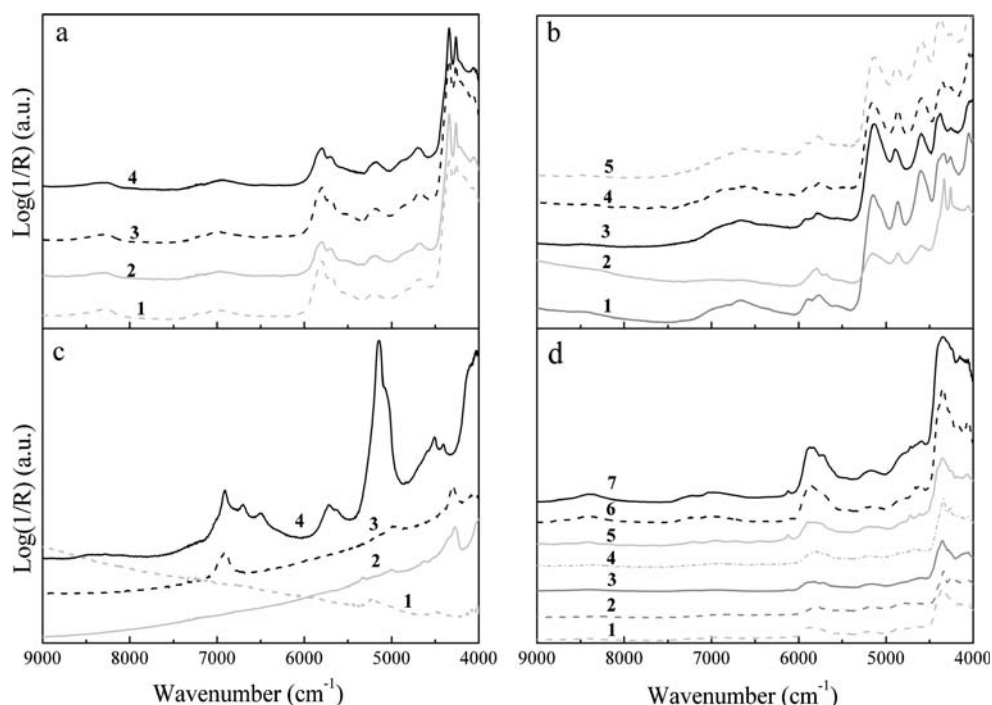
Chromatograms have been acquired in both single-ion monitoring (SIM) and total ion current (TIC) modes. The SIM acquisition mode was used for quantitative analysis of amino and fatty acids.

Results and discussion

Pure standards

Near-infrared reference spectra of siccative oils, proteinaceous materials, natural resins, and some inorganic materials commonly used in the ground layer are reported in Fig. 1 as collected and before the derivative transformation. Concerning drying oils (Fig. 1a) the assignment for the most relevant NIR bands is given in Table 1 [15]. A region with combination of methylenic C–H stretching and bending between 4,250 and 4,350 cm^{-1} is identified. First and second CH_2 stretching overtone bands occur in the 5,700–5,800 cm^{-1} spectral range and at 8,290 cm^{-1} , respectively. The first overtone of ester carbonyl stretching mode is observed at 5,176 cm^{-1} whereas the combination of ester C=O and methylenic C–H stretching modes occurs at 4,690 cm^{-1} . Raw spectra of the four lipidic standards do not show significant spectral differences, but when consid-

Fig. 1 Reflectance NIR spectra of: **a** siccative oils (1 stand oil, 2 walnut oil, 3 poppy oil, 4 linseed oil); **b** proteinaceous materials (1 casein, 2 egg, 3 animal glue, 4 egg white, 5 strong glue); **c** some pigments used on the paint ground layer (1 burnt umber, 2 calcium carbonate, 3 lead white, 4 gypsum); **d** natural terpenic resins (1 dammar, 2 shellac, 3 colophony, 4 copal, 5 sandarac, 6 mastic, 7 turpentine)



ering their derivative transformation (Fig. 2), some distinctive features clearly appear especially in the spectral range between 4,000 and 4,600 cm^{-1} . In particular, the derivative bands show different relative intensity ratios and wavenumber shifts, which can be helpful for diagnostic purposes. These differences are remarkably evident in the case of standoil; however, no information could be inferred about the effect of the heating process.

Concerning proteinaceous materials, the assignments of the main bands observed for the reflectance spectra of whole egg, animal glue, casein, and egg white (Fig. 1b) are reported in Table 2 [16]. The characteristic signals of proteins are the combination band of amide II and the first overtone of carbonyl stretching at about 4,600 cm^{-1} and the combination band of the stretching and bending modes of NH at 4,880 cm^{-1} . The broad shape of these bands hinders

discrimination of the different proteinaceous binders with the exception of whole egg due to the lipid content of yolk that explains why the methylenic C–H combination bands and their overtones in the ranges 4,250–4,350 cm^{-1} and 5,600–5,800 cm^{-1} are much more evident with respect to the other proteins. However, when considering real painting materials, these spectral features cannot be considered reliable for egg recognition; in fact, the simultaneous presence of proteinaceous and lipidic components, as, for example, in *tempera grassa*, may induce misleading interpretation of the NIR reflectance spectra.

More information can be derived by derivative transformations of the spectra (Fig. 3a) that show differences in

Table 1 Experimental NIR wavenumbers (cm^{-1}) of siccative oils and tentative assignment

Experimental wavenumber (cm^{-1})	Band assignment
4,261	$\nu_s(\text{CH}_2) + \delta(\text{CH}_2)$
4,340	$\nu_a(\text{CH}_2) + \delta(\text{CH}_2)$
4,690	$\nu_a(\text{CO}) + \nu_a(\text{CH}_2)$
5,176	2nd overtone $\nu(\text{C}=\text{O})$ ester
5,700	1st overtone $\nu_s(\text{CH}_2)$
5,790	1st overtone $\nu_a(\text{CH}_2)$
6,940	2C–H stretch + C–H def.
8,290	2nd overtone $\nu(\text{CH}_2)$

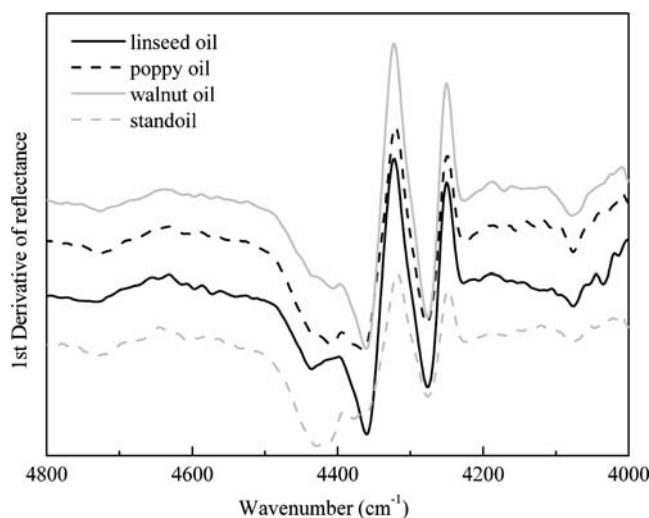


Fig. 2 Derivative NIR spectra of siccative oils

Table 2 Experimental NIR wavenumbers (cm^{-1}) of proteinaceous materials and tentative assignment

Samples	Experimental wavenumber (cm^{-1})	Band assignment
Animal glue	4,260	$\nu_s(\text{CH}_2)+\delta(\text{CH}_2)$
	4,375	$\nu_a(\text{CH}_2)+\delta(\text{CH}_2)$
	4,595	1st overtone $\nu(\text{CO})$ amide I+amide II
	4,880	$\nu_s(\text{NH})+\delta(\text{NH})$
	5,140	$\nu(\text{OH})+\delta(\text{OH})$
	5,780	1st overtone $\nu(\text{CH}_2)$
	6,640	1st overtone $\nu(\text{NH})$
	6,910	1st overtone $\nu(\text{OH})+\delta(\text{OH})$
	8,460	2nd overtone $\nu(\text{CH}_2)$
	11,390	3rd overtone $\nu(\text{CH}_2)$
	Casein	4,335
4,600		1st overtone $\nu(\text{CO})$ amide I+amide II
4,865		$\nu(\text{NH})+\delta(\text{NH})$
5,145		$\nu(\text{OH})+\delta(\text{OH})$
5,760		1st overtone $\nu_s(\text{CH}_2)$
5,890		1st overtone $\nu_a(\text{CH}_2)$
6,270		$\nu(\text{NH})$ +1st overtone amide II
6,680		1st overtone $\nu(\text{NH})$
6,830		1st overtone $\nu(\text{OH})+\delta(\text{OH})$
8,400		2nd overtone $\nu(\text{CH}_2)$
Egg white		4,260
	4,590	1st overtone $\nu(\text{CO})$ amide I+amide II
	4,860	$\nu(\text{NH})+\delta(\text{NH})$
	5,150	$\nu(\text{OH})+\delta(\text{OH})$
	5,750	1st overtone $\nu_s(\text{CH}_2)$
	5,890	1st overtone $\nu_a(\text{CH}_2)$
	6,620	1st overtone $\nu(\text{NH})$
	8,420	2nd overtone $\nu(\text{CH}_2)$
Egg	4,260	$\nu_s(\text{CH})+\delta(\text{CH})$
	4,330	$\nu_a(\text{CH})+\delta(\text{CH})$
	4,595	1st overtone $\nu(\text{CO})$ amide I+amide II
	4,860	$\nu(\text{NH})+\delta(\text{NH})$
	5,150	$\nu(\text{OH})+\delta(\text{OH})$
	5,675	1st overtone $\nu_s(\text{CH}_2)$
	5,790	1st overtone $\nu_a(\text{CH}_2)$
	6,635	1st overtone $\nu(\text{NH})$
	6,940	1st overtone $\nu(\text{OH})+\delta(\text{OH})$
	8,260	2nd overtone $\nu(\text{CH}_2)$

the range 4,000–6,500 cm^{-1} on band shape, wavenumber position, and intensity ratio. These features make it possible to discriminate amongst animal glue, casein, and egg white. Strong glue is distinguished for the peculiar signal at around 7,250 cm^{-1} (see inset of Fig. 3a) that corresponds to a small shoulder between 7,000 and 7,500 cm^{-1} in the reflectance spectrum.

Notably, in the case of whole egg, the shape and position of the methylenic combination bands from the lipid component are different with respect to those from siccative oils, thus providing distinctive features for egg yolk recognition. In

particular, we observed a shift of the minima shown by siccative oils at about 4,360 and 4,277 cm^{-1} to lower wavenumbers in egg, respectively, at 4,344 and 4,270 cm^{-1} .

Regarding near-infrared spectra of natural resins (Fig. 1d, Table 3), they can be distinguished according to two mean classes: diterpenes (turpentine, colophony, sandarac, and copal), showing the methylenic stretching and bending overtone bands in the range 5,600–6,000 cm^{-1} as a doublet, and triterpenes (mastic and dammar) for which these signals are not resolved and result in a unique convoluted band. Shellac, an animal resin, and copal are

distinguished for a well-resolved doublet in the range $4,000\text{--}4,500\text{ cm}^{-1}$ corresponding to the methylenic stretching and bending combination bands. In derivative spectra (Fig. 3b), several new differences relatively to band shapes are visible both in the spectral regions $4,000\text{--}4,500\text{ cm}^{-1}$ and $5,600\text{--}6,000\text{ cm}^{-1}$, which help for resin identification within the same terpenic class and also for distinguishing shellac resin from the others.

On account of the susceptibility of organic compounds to aging that may induce important compositional changes of painting materials, the effect of these degradation processes on infrared spectral data has been considered.

While proteins are quite stable when exposed to prolonged aging, as shown by the occurrence of limited spectral modification in the mid-infrared region [17], siccative oils and natural resins are much more affected by oxidative degradation [18–20]. A preliminary evaluation of these effects on NIR spectra has been achieved by

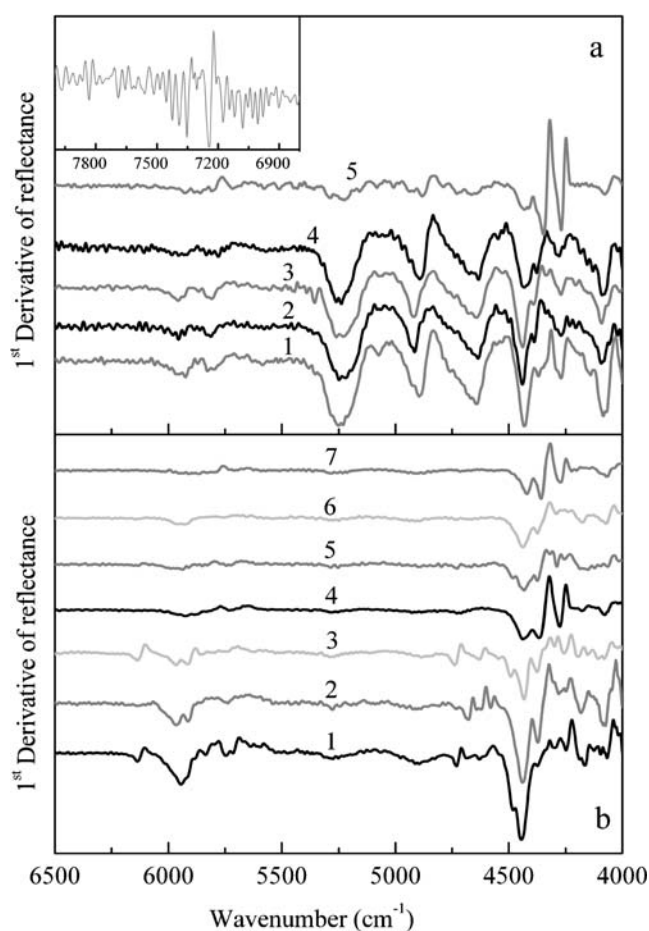


Fig. 3 Derivative NIR spectra of: **a** proteinaceous materials (1 casein, 2 animal glue, 3 strong glue, 4 egg white, 5 egg) and **b** natural varnishes (1 turpentine, 2 mastic, 3 sandarc, 4 copal, 5 colophony, 6 dammar, 7 shellac)

measurements carried out on artificially aged standards of oils and resins. Layers of the pure materials have been prepared on glass slides as described in the “Experimental” section and aged at $40\text{ }^{\circ}\text{C}$ for more than 1 year before measurements. Figure 4 shows the NIR spectra obtained for aged linseed oil, mastic, and sandarc resins, as representative of the three classes of compounds: siccative oils, triterpenic, and diterpenic resins, respectively. The thermal treatment was aimed at accelerating kinetics of their curing process, even though it cannot be equated to natural aging that occurs in paintings during the time span of centuries. The results obtained for the artificially aged materials provide a first important indication that, apart from a worsening of noise, no remarkable NIR spectral modifications are present after the thermal treatment (also in the derivative transforms, data not shown). This is mainly due to the limited specificity of NIR spectroscopy which makes this technique less sensitive to structural changes of materials with respect to mid-infrared spectroscopy. This intrinsic limit of NIR spectroscopy turns out in the possibility of undertaking NIR spectroscopic investigation of ancient painting materials also using, in first analysis, non-aged standard organic compounds as reference.

In regards to inorganic pigments, a general description of the spectral features distinguishing the most important ones in the NIR region has been provided by Bacci [21] and applied on real cases for the identification of artists’ palettes [22]. Pigments strongly absorbing in the near-infrared are those presenting OH combination bands (i.e., hydrate minerals) or low-energy electronic transitions (i.e. Co- and Fe-based compounds).

With this work being aimed at evaluating strength and limitations of the FT-NIR spectro-analytical method for the detection of organic materials in paintings, the possible spectral overlapping with absorption bands of pigments had to be taken into account. In particular, we considered those pigments that are commonly used in preparation layers. In fact, the near-infrared radiation is rather penetrating through painting layers and typically it can reach the ground layer. Here, spectral derivative treatment has been applied to lead white, gypsum, calcium carbonate, and burnt umber for the characterization of the painting models and for the investigation of the case study. Lead white and gypsum were typically used in Italian paintings, calcium carbonate, is frequently encountered in northern European paintings while earth pigments were commonly employed to enhance dark areas. NIR reflectance spectra of these pigments are shown in Fig. 1c and the relative band assignment is reported in Table 4.

Concerning lead white, the band observed at $4,300\text{ cm}^{-1}$ is attributed to the combination of OH stretching and the deformation modes of Pb–OH [21, 23], while the band at around $6,900\text{ cm}^{-1}$ is due to OH stretching overtone modes.

Table 3 Experimental NIR wavenumbers of natural resins and tentative assignment

Samples	Experimental wavenumber (cm ⁻¹)	Band assignment	
Copal	4,060	3rd overtone $\delta(\text{CC})$	
	4,262	$\nu_s(\text{CH}_2)+\delta(\text{CH}_2)$	
	4,340	$\nu_a(\text{CH}_2)+\delta(\text{CH}_2)$	
	4,670	$\nu(\text{CO})+\nu(\text{CH}_2)$	
	5,185	$\nu(\text{OH})+\delta(\text{OH})$	
	5,700	1st overtone $\nu_s(\text{CH}_2)$	
	5,800	1st overtone $\nu_a(\text{CH}_2)$	
	6,930	1st overtone $\nu(\text{OH})$	
	8,340	2nd overtone $\nu(\text{CH}_2)$	
	Shellac	4,055	3rd overtone $\delta(\text{CC})$
		4,260	$\nu_s(\text{CH}_2)+\delta(\text{CH}_2)$
		4,340	$\nu_a(\text{CH}_2)+\delta(\text{CH}_2)$
		4,673	$\nu(\text{CO})+\nu(\text{CH}_2)$
4,792		$\nu(\text{CO})+\nu(\text{OH})$	
5,164		$\nu(\text{OH})+\delta(\text{OH})$	
5,513		$\nu(\text{OH})+\nu(\text{CH})$	
5,700		1st overtone $\nu_s(\text{CH}_2)$	
5,778		1st overtone $\nu_a(\text{CH}_2)$	
6,874		1st overtone $\nu(\text{OH})$	
8,273		2nd overtone $\nu(\text{CH}_2)$	
Dammar, Mastic	4,057	3rd overtone $\delta(\text{CC})$	
	4,164	$\nu_s(\text{CH}_2)+\delta(\text{CH}_2)$	
	4,345	$\nu_a(\text{CH}_2)+\delta(\text{CH}_2)$	
	4,632	$\nu(\text{CO})+\nu(\text{CH}_2)$	
	4,830	$\nu(\text{CO})+\nu(\text{OH})$	
	5,190	$\nu(\text{OH})+\delta(\text{OH})$	
	5,737	1st overtone $\nu_s(\text{CH}_2)$	
	5,864	1st overtone $\nu_a(\text{CH}_2)$	
	8,410	2nd overtone $\nu(\text{CH}_2)$	
	Colophony, Turpentine	4,348	$\nu(\text{CH}_2)+\delta(\text{CH}_2)$
4,588		$\nu(\text{CO})+\nu(\text{CH}_2)$	
5,166		$\nu(\text{OH})+\delta(\text{OH})$	
5,718		1st overtone $\nu_s(\text{CH}_2)$	
5,840		1st overtone $\nu_a(\text{CH}_2)$	
6,120		1st overtone $\nu(\text{CH}_2)$ cyclic	
6,985		1st overtone $\nu(\text{OH})$	
8,320		2nd overtone $\nu(\text{CH}_2)$	
Sandarac	4,063	3rd overtone $\delta(\text{CC})$	
	4,348	$\nu(\text{CH}_2)+\delta(\text{CH}_2)$	
	4,615	$\nu(\text{CO})+\nu(\text{CH}_2)$	
	4,720	$\nu(\text{CO})+\nu(\text{OH})$	
	5,176	$\nu(\text{OH})+\delta(\text{OH})$	
	5,800	1st overtone $\nu(\text{CH}_2)$	
	5,892	1st overtone $\nu_a(\text{CH}_2)$	
	6,113	1st overtone $\nu(\text{CH}_2)$ cyclic	

For calcium carbonate, the signal at 4,275 cm⁻¹ is likely due to the 2nd overtone mode of the strong asymmetric stretching of the carbonate anion ($3\nu_3$) [16]. On the burnt amber, the signal at 5,220 cm⁻¹ is attributable to OH

combination band and that at 4,516 cm⁻¹ is, most probably, due to the OH and Si–O combination band. Finally, the NIR spectrum of gypsum shows the typical features due to the combination and overtone modes of water [21].

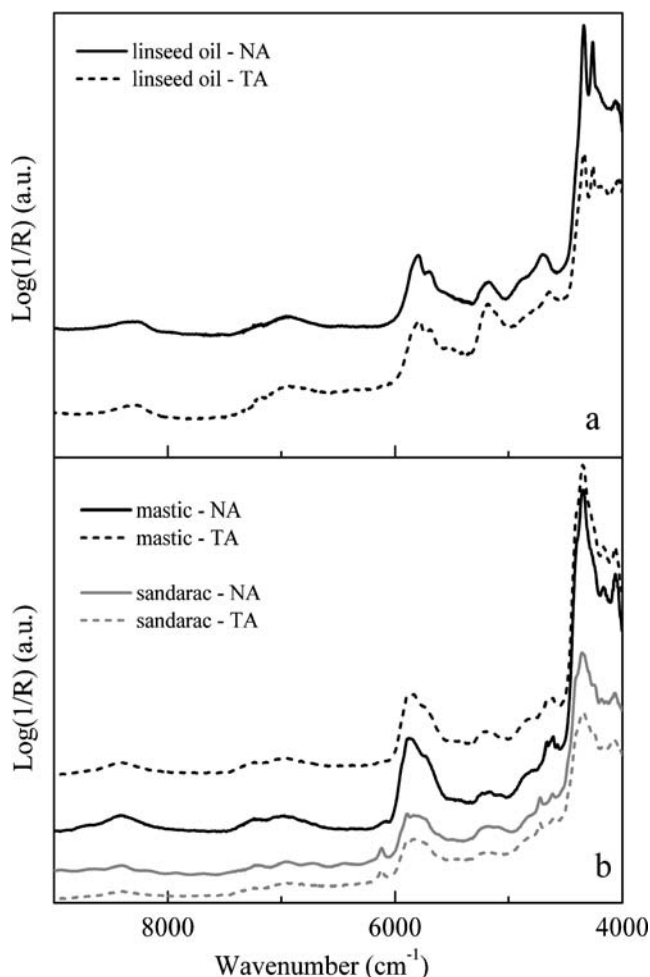


Fig. 4 Reflectance NIR spectra of pure standards of linseed oil, mastic and sandarac resins after natural (NA) and thermal (TA) aging

Paint models

Near-infrared spectra of the easel painting models prepared with cinnabar in whole egg and oil binders were recorded

and analyzed by first derivative (Figs. 5 and 6, respectively). The effect on the spectroscopic results relating to the presence of a varnish layer has also been evaluated in the case of oil (Fig. 6b). For all the painting models, gypsum and glue from the preparation layer have been easily detected irrespective of the used binder and of the presence of the varnish layer. On the other hand, due to the fact that signals of glue from the preparation layer were visible, the discrimination between oil and egg in the painting layer resulted to be more critical; thus, the subtle differences observed in the derivative transformation of the methylenic combination bands of the lipidic component have been used as distinctive markers for the two binders (see inset in Fig. 5). Finally, when a varnish layer made of turpentine resin was added to the oil painting, unambiguous new spectral features appeared in the ranges of 5,600–6,000 cm^{-1} and of 4,000–4,500 cm^{-1} (Fig. 6b).

It is worth to note that the recognition of paint ground, binder, and varnish in the laboratory models was possible only by applying the derivative transformation to NIR spectra. This clearly highlighted, as also recently evidenced in literature [9], how data treatment is crucial to fully exploit NIR spectroscopic information content for the specific investigation of painting materials.

Case study

A painting on canvas, dated back to circa mid-seventeenth century by an unknown artist and representing a high prelate, was utilized as a case study. The painting belongs to a private owner and no historic documentation is available. The analyses here presented have been performed in occasion of its restoration during which it has been found that the original portrait has been repainted to add physiognomic details and it has been coated with a finishing protective varnish. During the restoration, the

Table 4 Experimental NIR wavenumbers of pigments and tentative assignment

Pigments	Experimental wavenumber (cm^{-1})	Band assignment
Lead white	4,300	$\nu(\text{OH})$ +deformation modes (Pb–OH)
	4,980	$\nu(\text{OH})$ + $\nu(\text{CO})$
	6,905	1st overtone $\nu(\text{OH})$
	10,120	2nd overtone $\nu(\text{OH})$
Gypsum	4,510	$\nu_s(\text{OH})$ + $\delta(\text{OH})$
	5,145	$\nu_a(\text{OH})$ + $\delta(\text{OH})$
	6,495	1st overtone $\nu(\text{OH})$
	6,700	1st overtone $\nu(\text{OH})$
	6,910	1st overtone $\nu(\text{OH})$
Calcium carbonate	4,273	2nd overtone $\nu(\text{CO})$
Burnt umber	4,516	(OH)+ $\nu(\text{Si-O})$
	5,220	$\nu(\text{OH})$ + $\delta(\text{OH})$

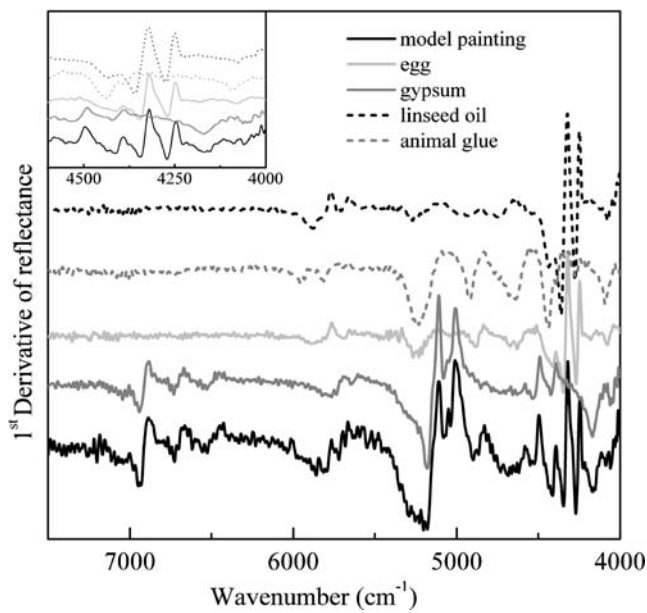


Fig. 5 Derivative NIR spectra of the pictorial model prepared with cinnabar in egg tempera compared with pure standards

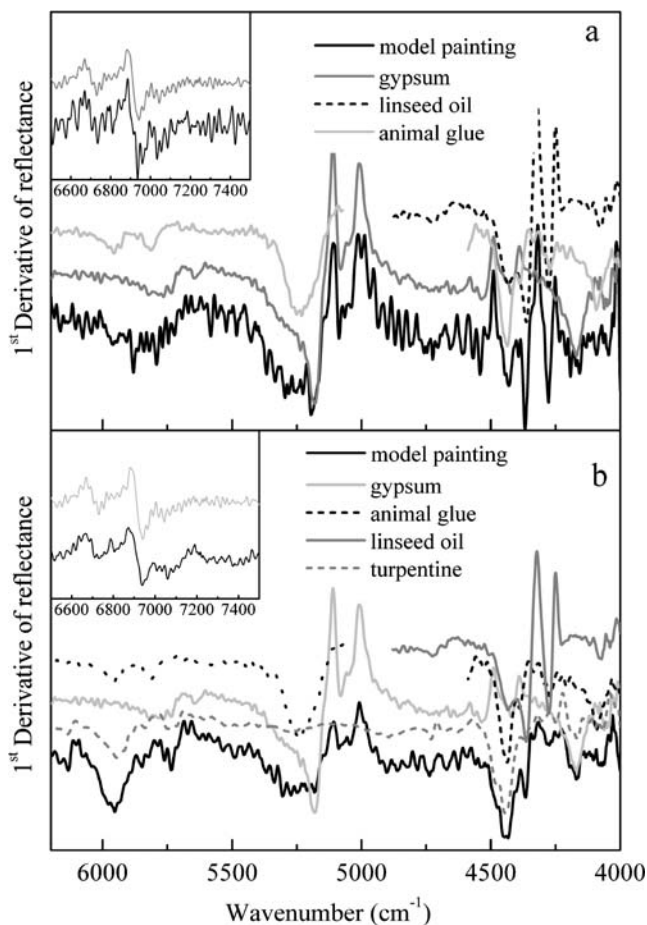


Fig. 6 Derivative NIR spectra of the pictorial model prepared with cinnabar in oil **a** without and **b** with varnish coating compared with pure standards

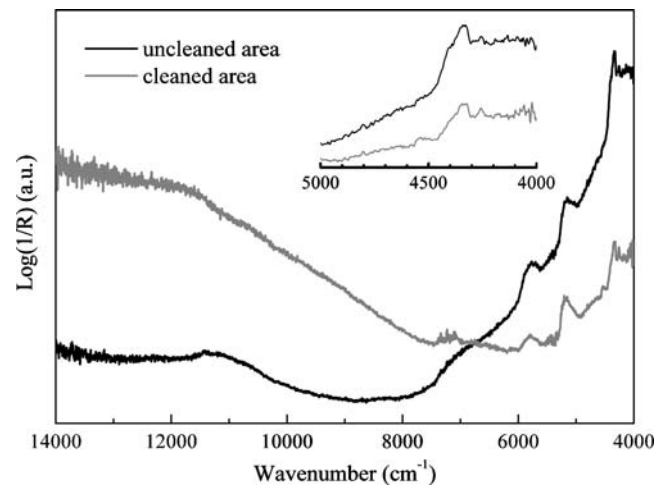


Fig. 7 NIR spectra of the clean and unclean areas of a seventeenth-century painting

face and part of the hair have been cleaned for a complete removal of the over-paint and of the varnish. Near-infrared spectra have been acquired in both the clean and unclean areas of the dark hair (Fig. 7) in order to obtain information

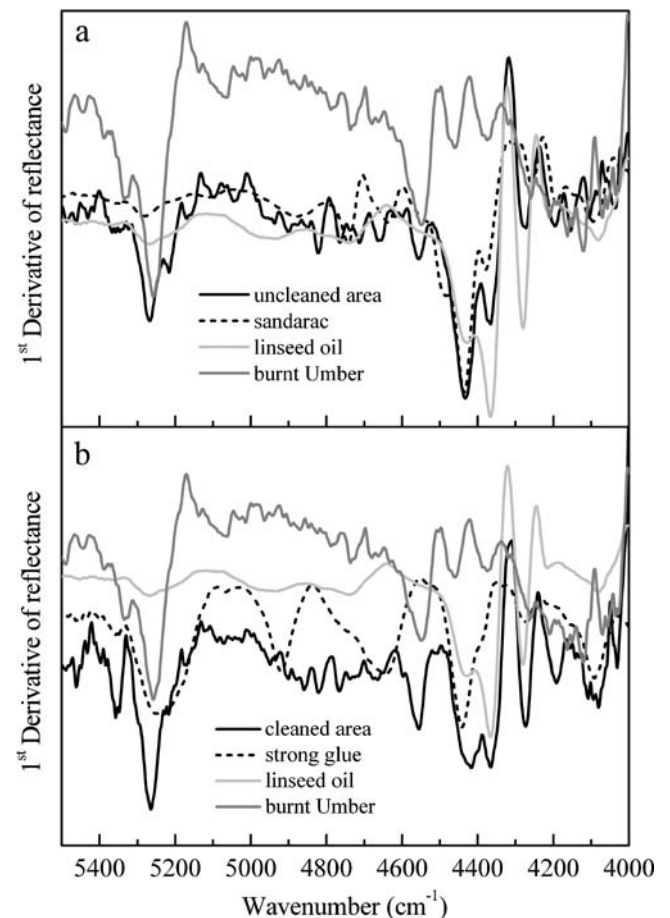


Fig. 8 Derivative NIR spectra of the unclean **(a)** and clean **(b)** areas of a seventeenth-century painting in comparison with some standards

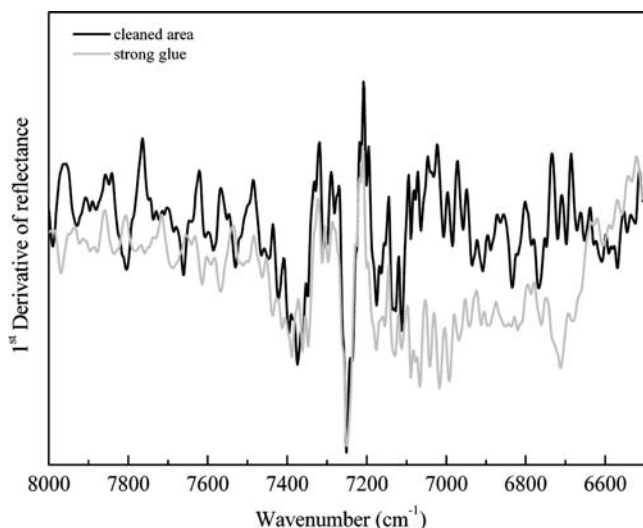


Fig. 9 NIR derivative spectrum of the clean area in the range 6,500–8,000 cm^{-1} in comparison with the strong glue standard

on the original and the overlaid materials. A micro-sample containing all the layers has been collected for comparative GC-MS analysis on the unclean area.

The observation in the NIR reflectance spectra of methylenic combination bands in the range of 4,400–4,200 cm^{-1} clearly indicates the occurrence of organic compounds, but the absence of other specific signals does not allow to clearly identify their nature (see inset). The weak band around 11,500 cm^{-1} is due to the electronic transition of an iron(III)-based pigment [21].

When considering the derivative spectra of both clean and unclean areas (Fig. 8a and b), the presence of siccativ oil is clearly shown by the characteristic minima and maxima sequence in the 4,400–4,200 cm^{-1} range and relate to the methylenic combination bands. Furthermore, burnt

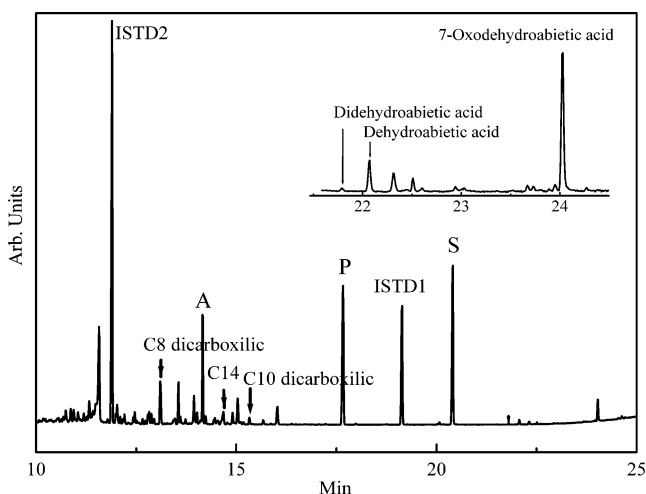


Fig. 10 Total ion chromatogram of the acidic fraction of the real sample (A azelaic acid, P palmitic acid, S stearic acid)

umber is recognized as the pigment originally used by the artist being more evident in the clean area (Fig. 8b). Here, it is also possible to distinguish the presence of animal glue which looks like strong glue as evidenced by the unambiguous features around 7,250 cm^{-1} (Fig. 9).

On the unclean area (Fig. 8a), the over-paint partially hinders the glue signals from the preparation, while the sharp signal around 4,435 cm^{-1} points towards the presence of a natural resin showing the spectral features of a diterpenic resin, very similar to sandarac.

Near-infrared results were further confirmed by GC-MS analyses. The acidic fraction of the organic extract evidenced the occurrence of palmitic and stearic fatty acids combined with the presence of dicarboxylic acids (Fig. 10), being azelaic the most abundant one. Quantitative analysis provided an A/P wt ratio of 1.0 and a total amount of dicarboxylic acids of 39 wt.%. These results clearly indicate the use of a siccativ oil as binder for both the original and the over-paint, most likely linseed oil as inferred by a P/S wt ratio of 1.0. The observation of oxidation products of abietanes in the acidic fraction (see inset of Fig. 10) also suggests the presence of a diterpenic resin, likely from the *Pinaceae* family. Neutral organic fraction did not show any significant signal.

GC-MS analysis of amino acids revealed the presence of a proteinaceous component. This has been identified as animal glue according the principal component analysis (PCA) of the aminoacidic weight percentage profile [24] carried out by using as reference data set about 30 standard samples containing the three main proteinaceous binders: egg, glue, and casein (see score plot in Fig. 11). The first two principal components account for 82% of the variance of the data.

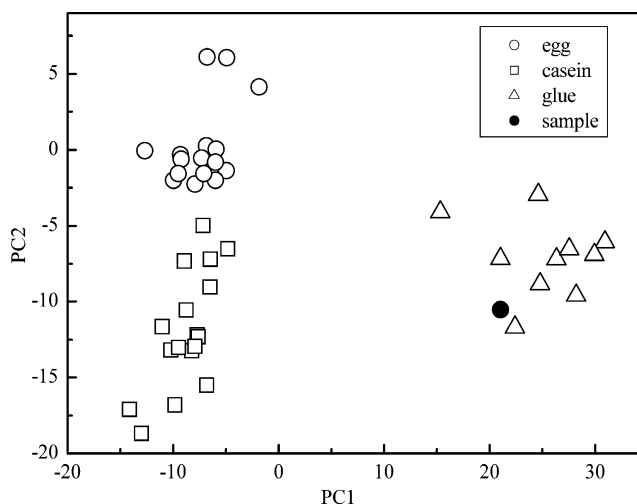


Fig. 11 Score plot from PCA analysis of the percentage weight aminoacidic profile of the real sample from GC-MS

Conclusions

The present study shows how near-infrared spectroscopy can be exploited to analyze easel paintings, providing useful information in a non-invasive manner on natural organic materials probing the whole stratigraphy. Until now, this kind of spectroscopy has been underemployed in the field of cultural heritage in consideration of the complexity and variety of the analyzed organic materials leading to convolutions of spectral signals of difficult interpretation.

Literature about the application of near-infrared spectroscopy in different scientific fields point to the use of the first-derivative transformation as an effective approach to spectra analysis. It demonstrated to be a simple and fast way to deduce much more information from spectroscopic data by the enhancement of the spectral differences.

In the present study, first-derivative transformation of reflectance NIR spectroscopic data has been used to non-invasively characterize painting materials with particular attention to organic components. Indeed, different compounds belonging to the classes of lipids, proteins, and resins are hardly distinguishable in the conventional spectroscopic approach. To this purpose, at first, a library of near-infrared data was built for a significant number of pure natural organic compounds. By comparison of the first-derivative transformation of the spectra, it was possible to identify spectral features that can help to distinguish the compounds here considered, included those belonging to the same organic class. This result is certainly achievable for pure standards, representing the most favorable situation; more difficult is the transition to the study of real cases where unknown complex mixtures of materials are present and arranged in multiple layers. To this purpose, our analytical approach was validated on pictorial models reproducing the typical stratigraphy of an easel painting. For non-absorbing or slightly absorbing pigments, the analysis of data derivative transformations provided successful recognition of paint ground, binder, and varnish. It is worth commenting that as this analytical approach is based on the collection of spectral data in reflectance mode, when dealing with real painting surface, the choice of the area to be investigated is of crucial importance. In fact, in order to collect spectra of good quality and to fully explore the paint depth, the main condition of low absorptivity of the inorganic pigments has to be fulfilled. To this aim, a careful selection of the probed surface can be carried out by exploiting the non-invasiveness of the technique.

The analytical value of the methodology was verified on a real painting used as case study. The investigation had the double aim of characterizing original and over-painted materials by measurements on clean and unclean painting

areas. Signals from siccative oil, from glue and from a terpenic natural resin, as well as from an earth pigment were clearly identified and results were cross-validated by GC-MS analysis.

The positive results obtained in this preliminary study revalue NIR spectroscopy as a useful analytical tool in cultural heritage when appropriate data treatments for spectra interpretation are used (see, for example, [9]). Our findings evidence how derivative transformation of NIR spectra is an easy and fast way to successfully increase the specificity of the method. We also propose this technique as a valuable approach to resolve spectral signal of original materials from those of over-paintings by surface monitoring before and after cleaning procedures.

Further research work is in progress following two main guidelines: expansion of the available database with other organic materials historically used in artworks (waxes, gums, etc.), and, secondly, actuation of aging protocols by exposure of laboratory standards to light and heat in order to deeper investigate the possible spectral modification induced by degradation of the materials.

Acknowledgments The financial support provided by Eu-ARTECH (contract RII3-CT-2004-506171), a project of the Sixth FP of the European Union within the program of Research Infrastructure, is acknowledged. The authors are also grateful to COO.BE.C (Cooperativa Beni Culturali) of Spoleto, Italy.

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