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Infrared and Raman Spectroscopy for the Analysis of Pictorial Materials: Leonardo da Vinci's *Last Supper* in the Convent of Santa Maria delle Grazie, Milan †

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† This article is dedicated to Prof. Giuseppe Zerbi in recognition of his outstanding scientific contributions to Spectroscopy.

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Abstract: This study presents the application of infrared (IR) and Raman vibrational spectroscopy to the analysis of the pictorial materials used in Leonardo da Vinci's Last Supper, located in the Convent of Santa Maria delle Grazie in Milan. The research was conducted during the extensive restoration campaign led by Giuseppina Brambilla Barcilon between 1977 and 1999, in collaboration with Professor Giuseppe Zerbi's research group at the Polytechnic University of Milan. A total of fourteen microsamples were taken from different areas of the mural to identify both inorganic and organic components through molecular-level spectroscopic analysis. Infrared and Raman spectra revealed the predominant presence of calcium carbonate as an inorganic support, along with organic materials such as shellac and beeswax, while synthetic polymers like polyvinyl-acetate and Paraloid B-72 appeared to be absent The results demonstrate that shellac, applied during Pelliccioli's 1946 restoration, is the most ubiquitous material across all samples, and that organic binders have penetrated deeply through the painting's stratigraphy, reaching the plaster layer. The study confirms the effectiveness of vibrational spectroscopy as a non-destructive diagnostic tool for documenting the chemical composition and restoration history of complex mural paintings.

Keywords: Cenacolo; diagnostics; infrared and Raman spectroscopy

1. Introduction

The conservation and study of cultural heritage require non-destructive or minimally invasive analytical techniques to identify materials, pigments, binders, and degradation products. Among these, infrared (IR) spectroscopy and Raman spectroscopy represent essential tools, capable of providing detailed chemical and structural information without damaging the artifact.

In the field of cultural heritage conservation, infrared spectroscopy is generally employed to:

- Identify pigments, binders, and restoration materials
- Analyse oils, resins, and waxes used in paintings and sculptures
- Investigate degradation processes, such as oxidation or hydrolysis of materials

Analyses are typically conducted using FTIR (Fourier Transform Infrared Spectroscopy), allowing rapid and sensitive measurements even on micro-samples, or in Attenuated Total Reflectance (ATR) mode, which is more suitable for surface analyses.

Raman spectroscopy, complementary to infrared techniques, offers several advantages in this context:

- Almost always non-destructive, requiring no sampling
- Ability to distinguish between very similar pigments, including metallic oxides and sulfides
- Capability to study micro-areas of color or degraded regions without removing material

Raman spectroscopy is particularly valuable for the analysis of mineral pigments, lacquers, and inorganic materials, supporting studies on authenticity, provenance, and conservation status.

As noted, the two techniques are complementary: infrared spectroscopy is highly sensitive to polar bonds and organic materials, while Raman spectroscopy excels with inorganic and crystalline substances. Their combined use allows for a comprehensive characterization of an artwork's constituent materials, from the chemical structure of pigments to the presence of degradation products or prior restoration interventions.

The application of IR and Raman spectroscopy in cultural heritage has become an indispensable tool for conservators, restorers, and researchers. Their ability to provide detailed information in a non-invasive manner helps protect and enhance cultural heritage, contributing to scientific studies, restoration, and authentication of artworks.

2. The Restoration of Leonardo's Last Supper

The Cenacolo, also known as The Last Supper*,* is Leonardo da Vinci's masterpiece, painted between 1494 and 1498 on the wall of the refectory of the Convent of Santa Maria delle Grazie in Milan. As is well known, Leonardo did not employ the conventional fresco technique on wet plaster but experimented with a "dry" method, applying pigments onto a preparatory layer on the wall. This choice allowed for greater control over details and chiaroscuro effects, but rendered the work extremely fragile and susceptible to deterioration over time.

The painting began to deteriorate only a few years after its execution, with 16th-century sources already reporting significant damage by 1517. Over the subsequent centuries, numerous restorations were carried out (at least nine since the 18th century), many of which were essentially retouches of deteriorated areas rather than true conservation interventions. While these early efforts were well-intentioned, they nonetheless contributed to altering the original appearance of the work.

Solvents, resins, waxes, and animal glue were periodically used in attempts to preserve the painting, ranging from detergents applied to clean the pictorial layer, to varnishes and oils intended to restore opacity and vibrancy, including the use of shellac dissolved in alcohol in 1953 for consolidation purposes.

In the three documented restorations of the 20th century [1] the Cavenaghi (1903–1908), Silvestri (1924), and Pelliccioli (1947–1954) interventions, the main objective was the conservation of the existing material, although documentation provides limited information on the specific materials used for consolidation and cleaning. Prior to the pictorial integrations executed through watercolors by Cavenaghi and tempera retouches by Silvestri and Pelliccioli, it is known that Silvestri consolidated the pigment with injections of a fixative composed of "mastic resin dissolved in petroleum essence and mixed with wax." The procedure concluded with the application of heated iron rollers and, finally, a rubber roller, which appeared to restore consistency and vibrancy to the color.

The most significant and enduring restoration in the history of the Cenacolo was directed by Pinin Brambilla Barcilon, one of the most important restorers of the 20th century. This intervention spanned over twenty years, from 1977 to 1999, and was characterized by a rigorous and scientific approach, focusing not on mere repainting but on meticulous recovery of the original pictorial layers.

Barcilon's restoration stood out for the use of scientific methods for material analysis—chemical, spectroscopic, and physical—aimed at understanding the nature of pigments, binders, and degradation phenomena. This pioneering approach enabled targeted and conservative interventions, protecting what remained of Leonardo's original work without invasive overpainting.

The diagnostic approach adopted was integrated and multidisciplinary, combining optical, chemical, and radiographic techniques. This strategy allowed for precise differentiation between original materials and later retouches, a better understanding of Leonardo's painting technique, and informed selection of consolidation materials and conservation strategies while minimizing invasive procedures.

Chemical analyses formed the core of the diagnostics, with particular attention to pigments and organic binders:

- Raman spectroscopy: enabling identification of mineral and organic pigments
- FTIR (Fourier Transform Infrared Spectroscopy)**:** used to determine the nature of organic binders and degradation products
- XRF (X-ray fluorescence): allowing the determination of the elemental composition of pigments, detecting elements such as lead, iron, copper, and mercury
- XRD (X-ray diffraction) employed to determine the mineralogical composition of inorganic pigments in the samples

Within this context, the long-unresolved issue of identifying the organic binders used over the centuries was revisited with great care.

For this purpose, the research group led by Professor Giuseppe Zerbi at the "G. Natta" Department of the Polytechnic University of Milan was tasked with documenting, through physicochemical measurements at the molecular level, the organic and/or inorganic components involved in the restoration process.

In previous years, scientific studies aimed at identifying organic components had been conducted by E. Fedeli [2] using mass spectrometry and gas chromatography, by H. Kühn [3] using colorimetric reactions for characterizing individual chemical classes of components (proteins, acids, etc.), and by V. Furlan [4] through gas chromatography. The data from Fedeli, Kühn, and Furlan were among the first documented chemical analyses (1981) of samples taken from the painting and helped demonstrate that the preparatory and pictorial layers did not have a simple or uniform composition, complicating identification of the original materials versus substances applied in past restorations.

Kühn's work was particularly useful in highlighting the complexity of stratifications and material associations (pigments, binders, consolidants, coatings present in the painting, including those left by previous conservation campaigns or interventions).

These analyses ultimately demonstrated that the surface layers were "contaminated" by more recent restoration materials, and the primary task of the analysts was to distinguish what was original from what was not.

Overall, protein-based organic binders compatible with tempera (glue-tempera) were identified, primarily used in the pictorial and preparatory layers, along with an oily component suggesting the use of a fat tempera or a protein-oil mixture rather than a purely fresco binder. Therefore, the characteristics of true fresco painting pigments fixed solely by lime carbonation were not observed; the presence of organic binders confirmed that the pigment was not chemically fixed exclusively to the plaster [5–7].

Professor Zerbi's contribution was integrated into this campaign as a proposal for the use of optical diagnostic techniques to identify inorganic and, especially, organic components in the samples taken from *The Last Supper* [8–10].

The data discussed in the present study therefore derive from the application of vibrational infrared and Raman spectroscopy to Leonardo's Last Supper.

3. Experimental Section

For this study, fourteen samples were taken from different areas of the painting. It was not possible to directly intervene in the selection of the sampling areas or in the sampling method, as this procedure had been carried out independently by the restorer.

The description of the samples and their respective areas of origin are reported in Table 1.

The average size of the samples was approximately 0.5 mm.

Table 1. Samples taken from The Last Supper.

No.	Sample Description	Sampling Area
1	3 layers	Saint Simon's cloak
2	White + preparatory layer	Tablecloth
3	3 layers before cleaning	Saint Philip
4	3 layers after cleaning	Saint Philip
5	Wax	Tablecloth
6	Plaster + dark material	Saint James
7	Dark material after cleaning	Saint James
8	Snake background	Lunette
9	Blue snake	Lunette
10	Green leaves (single layer)	Lunette
11	Green leaves (single layer)	Lunette
12	Green leaves (two layers)	Lunette
13	Red background	Lunette
14	Colored sample	Lunette

Most of the collected samples consisted of a series of superimposed layers, theoretically corresponding to the different stages of the mural's execution (Figure 1). In general, these include the plaster layer applied to the masonry substrate, a preparatory layer, an intermediate coating, and finally the pictorial film [11].

All infrared spectra were recorded on samples embedded in a KBr matrix and/or on solvent extractions subsequently deposited onto suitable glass slides. The material used for the analyses was collected from the samples through small scraping procedures. It is important to note that, since the pictorial materials used in the

painting constitute a heterogeneous mixture with a variable composition of organic and/or inorganic components, the presented infrared spectra provide only an average representation of the overall chemical composition of the samples.

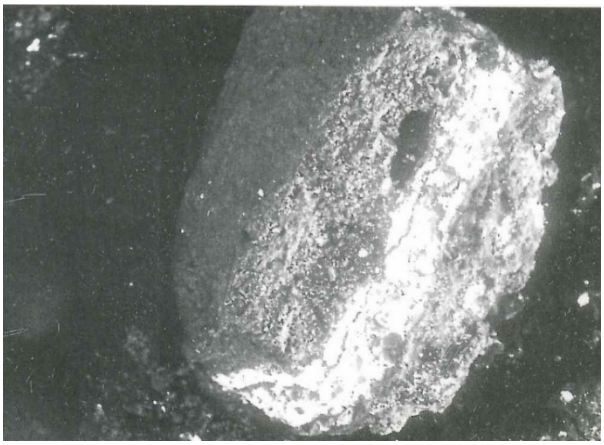

Figure 1. SEM image showing the layered structure of sample 1.

FTIR spectra were acquired using a Magna 550 spectrometer (Thermo Fisher Scientific, Nicolet brand, Madison, WI, USA). All spectra were recorded at a resolution of 4 cm^{-1} with 128 scans. No spectral corrections were applied.

Raman spectra were recorded using a Dilor Modular XY spectrometer with He–Ne (λ = 633 nm) and Ar^{+} (λ = 514 nm) lasers, as well as a Nicolet 910 FT-Raman spectrometer with an Nd:YAG laser (λ = 1064 nm). No fluorescence or baseline corrections were applied.

To facilitate spectroscopic analysis and enable the rapid identification of substances present in the samples, a reference spectra database was also compiled, containing materials analogous to those most commonly employed in the painting techniques of the period.

4. Spectroscopic Analysis: Identification of Components via Infrared and Raman Spectra

To identify the organic and inorganic fractions generally present in all analysed samples, the infrared spectrum corresponding to the Mantle of Saint Simon (sample 1) was taken as a reference. By comparing this spectrum (Figure 2) with those of calcium carbonate (peaks E, G, L) and magnesium hydroxide (peak A), it was possible to assign peaks A, E, G, and L to the inorganic fraction of the substrate.

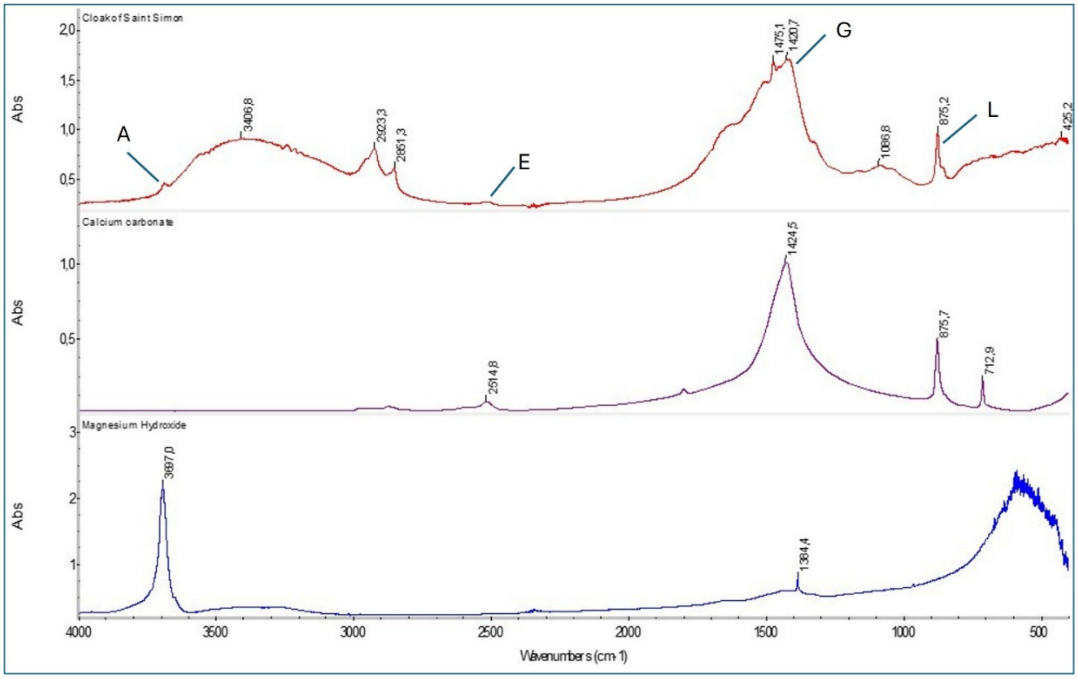

Figure 2. Comparison of the infrared spectrum of sample 1 with the infrared spectra of calcium carbonate and magnesium hydroxide.

The significant presence of calcium carbonate was also confirmed by the FT-Raman spectrum (λ = 1064 nm) of the same sample (Figure 3), which shows characteristic bands at 1085 cm^{-1} and 710 cm^{-1}.

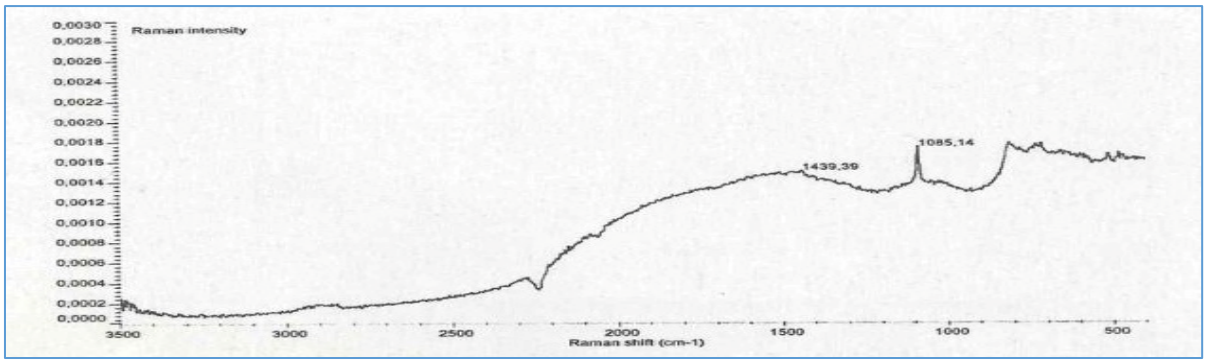

Figure 3. Cloak of Saint Simon: FT-Raman spectrum.

Previous stratigraphic studies had revealed the existence of a thin intermediate layer (approximately 20 μm), identified by Matteini and Moles [12] as lead white, and by Fedeli [2] as hydrated calcium sulfate. However, the IR and Raman spectra of these substances show absorption bands that were not observed in the samples we analysed. This absence does not necessarily exclude their presence but suggests that, if present, their concentration is minimal compared to other organic and inorganic components detected.

To identify the organic fraction, characteristic bands corresponding to vibrations of specific functional groups were recognized (Figure 4), allowing the classification of chemical compound families, further clarified through reference spectra. At high frequencies, a broad and intense band B around 3400 cm^{-1} indicates the presence of hydroxyl (OH) and/or phenolic groups, variably involved in hydrogen bonding, while the triplet C*, C, D corresponds to C–H stretching vibrations in methyl (CH_3) and methylene (CH_2) groups, typical of both natural oils (Figure 5) and waxes (Figure 6).

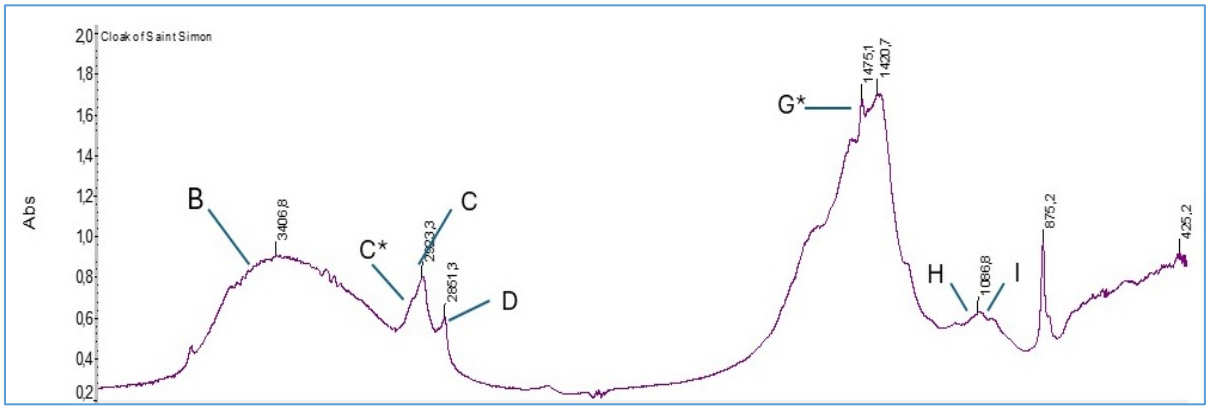

Figure 4. Saint Simon's cloak: assignment of bands to the organic fraction.

Deformation vibrations of CH_3 groups give rise to a band around 1470 cm^{-1} (G*), and another at 720 cm^{-1} (associated with CH_2 rocking modes of long polymethylene chains); the latter is too weak to be observed in The Last Supper samples but is clearly visible in reference spectra of linseed oil and beeswax.

In the 1050–1100 cm^{-1} region, a broad and complex structure was observed, where absorptions due to vibrations of alcohols, ethers, and esters are normally found. In particular, peaks H (1086 cm^{-1}) and I (1050 cm^{-1}) were analysed in detail. By comparing the spectra of sample 1 (Saint Simon's cloak) and sample 2 (Saint Philip before cleaning), the B peak (OH), the C*, C, D peaks (CH_2 and CH_3), and peaks H and I can be clearly observed.

The spectrum of shellac (Figure 7), an organic resin used during restorations after 1946 under the direction of M. Pelliccioli, corresponds almost exactly to the organic fraction identified in samples 1 and 2.

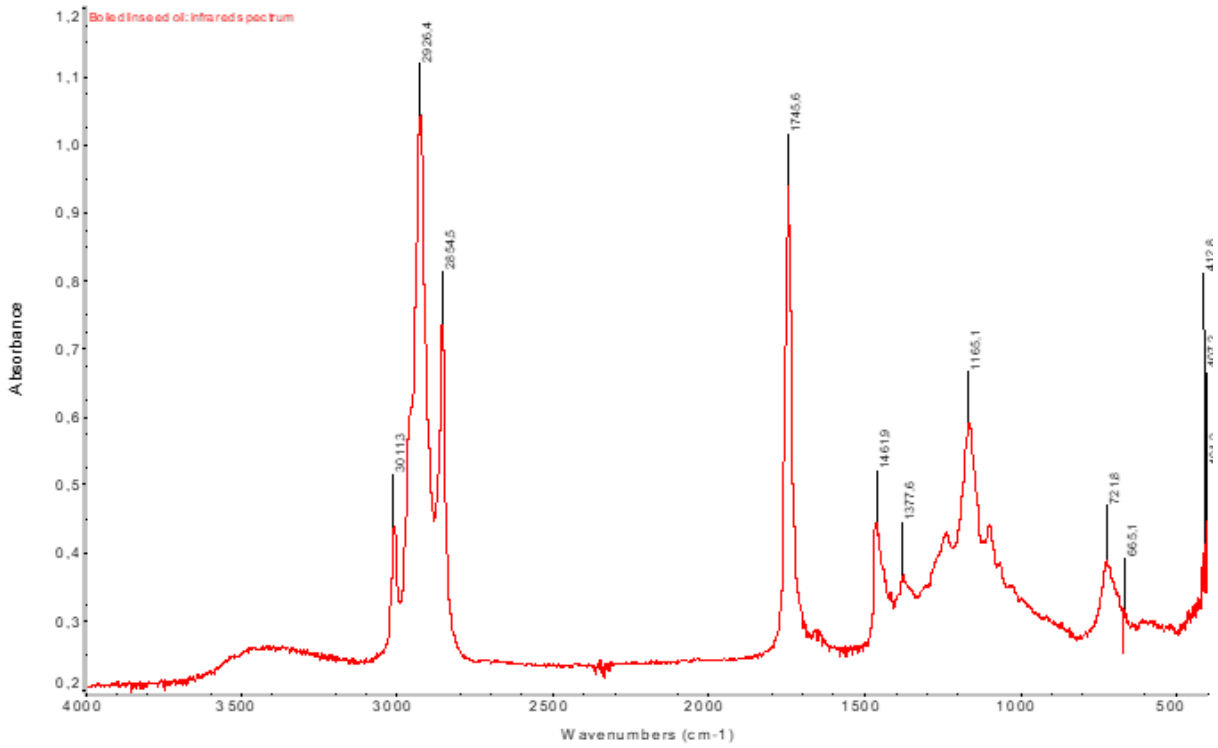

Figure 5. Boiled linseed oil: infrared spectrum.

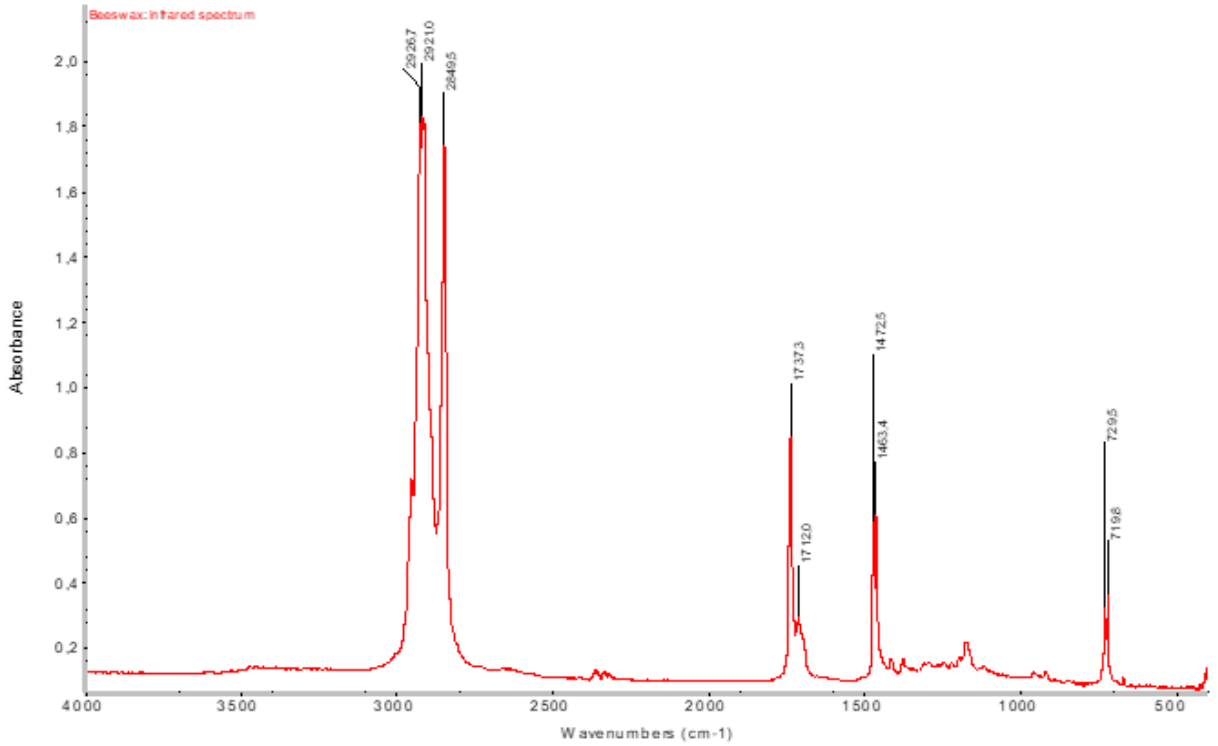

Figure 6. Beeswax: infrared spectrum.

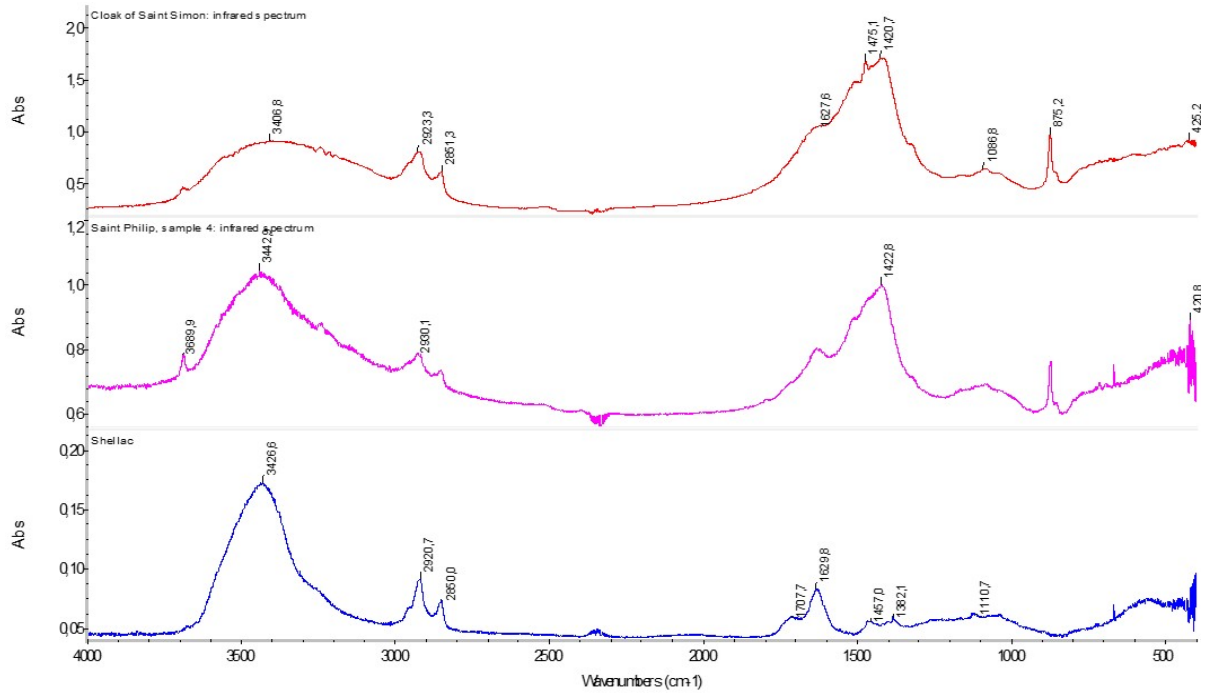

Figure 7. Comparison of the infrared spectra of Saint Philip and Saint Simon with the infrared spectrum of shellac.

Analyses conducted by Kühn [2] revealed that traces of polyvinyl-alcohol and polyvinyl-acetate were present in the examined samples. These synthetic organic materials, including Paraloid B-72 (copolymer of methyl methacrylate and ethyl methacrylate), belonging to the polyester class, have been used as binders or protective coatings in many paintings. Reference spectra of these polymers consistently show a characteristic, very intense C=O stretching band at 1740 cm^{-1} (Figure 8).

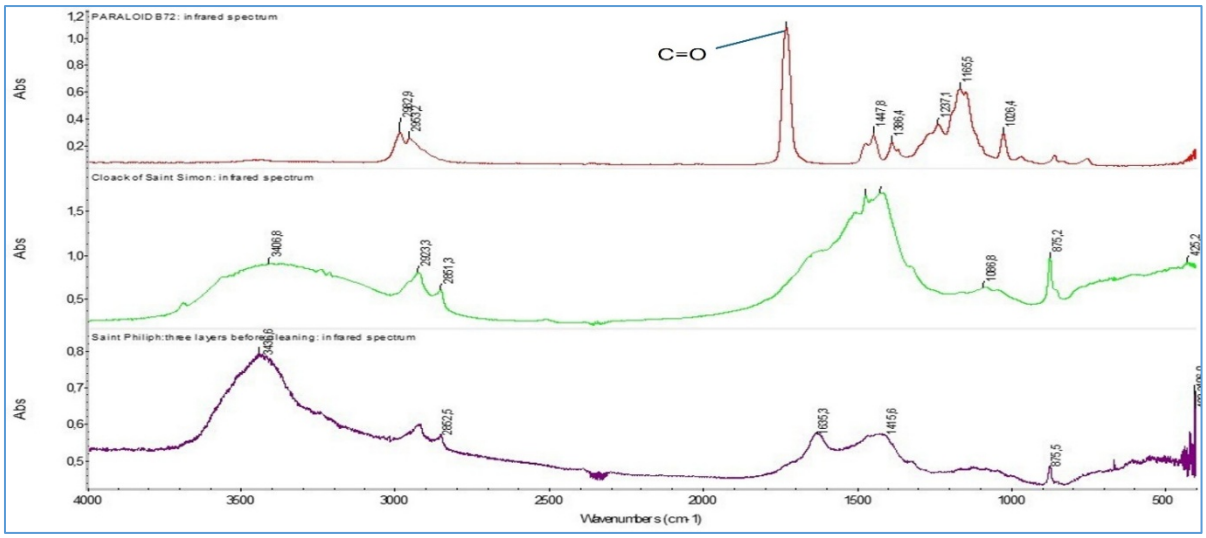

Figure 8. Comparison of the infrared spectra of Saint Philip and Saint Simon with the infrared spectrum of Paraloid B-72.

Since no distinct absorption at 1740 cm^{-1} was observed in the infrared spectra of The Last Supper that we examined except for a slight, poorly resolved shoulder the presence of a significant fraction of such synthetic polymers was considered unlikely in the samples we analysed, in contrast with the findings reported by Kühn.

During the restorations of The Last Supper, synthetic adhesives and consolidants were certainly employed, selected according to criteria of stability and reversibility. Although the contemporary restoration literature occasionally cites Paraloid B-72 as a reference acrylic since the 1970s, official publications on the restoration of the Cenacolo do not explicitly document the direct application of this resin on the artwork. At the conclusion of the investigations, Pinin Brambilla Barcilon generally refers to “synthetic adhesives” used in some micro-applications as consolidants for retouches and stuccoes [1].

We therefore consider the presence of synthetic polymers reported by Kühn to be plausible, but, as described in the literature, only in certain micro-areas that were evidently not included in our analysis samples. We can further confirm that such synthetic materials were almost certainly not applied indiscriminately across the entire surface of the painting; otherwise, a very clear and intense infrared signal would have been observed, which was not the case.

Analyses of the lunette samples (samples 8 and 13) revealed the same B, C*, C, D, H, and I peaks discussed for sample 1, indicating a dominant organic component identifiable as shellac The minimal presence of gum arabic cannot be ruled out (Figure 9), although it is difficult to isolate due to band overlap.

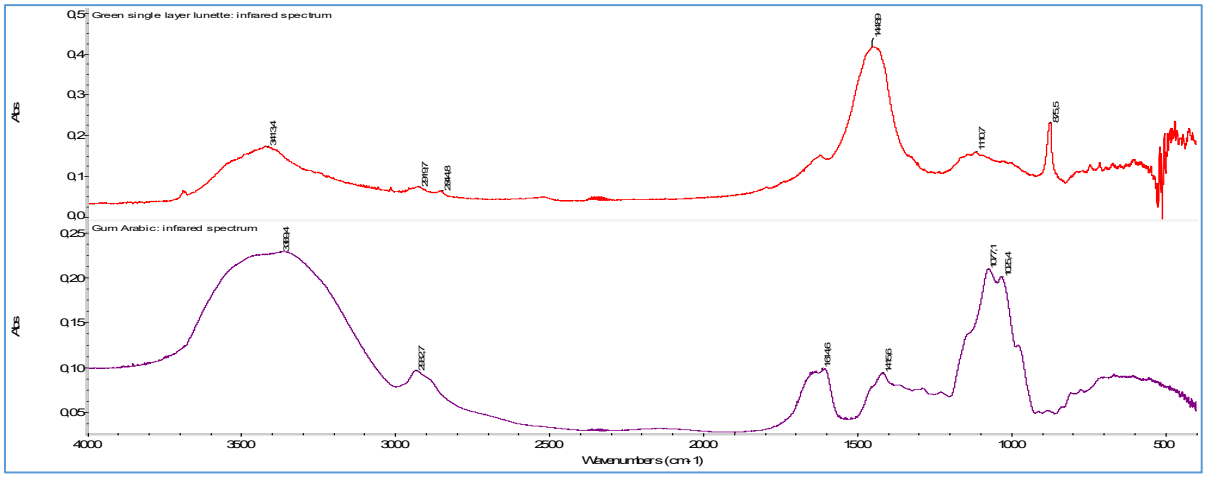

Figure 9. Comparison of the infrared spectrum of the green lunette with the infrared spectrum of gum arabic.

In samples 6 and 7, taken from Saint James, the spectra before and after cleaning (Figure 10) show features similar to those of samples 1 and 2: the presence of calcium carbonate and shellac is clearly recognizable. After cleaning, the absorption centered around 1500 cm^{-1} disappears, leaving well defined peaks at 1576 cm^{-1} and 1420 cm^{-1}. The additional peak at 1570 cm^{-1} is attributed to an organic material resistant to the solvents used during restoration, while the presence of alkyl chains or organic residues (bands at 2923 and 2850 cm^{-1}) was also observed.

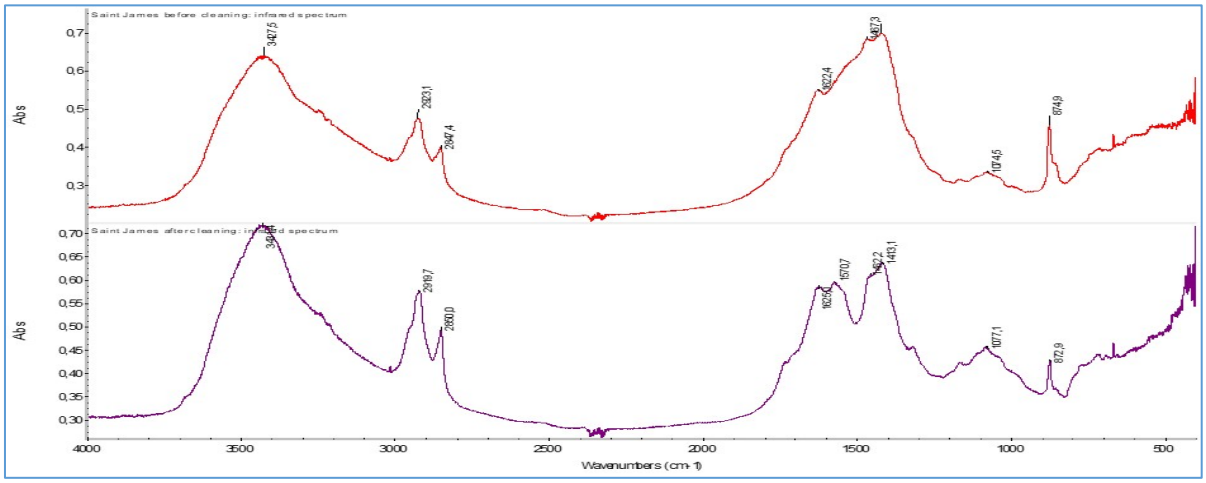

Figure 10. Infrared spectra of the Saint James samples before and after cleaning.

Similarly, in the Saint Philip samples (samples 3 and 4—Figure 11), cleaning leads to a significant increase of the band at 1420 cm^{-1} (calcium carbonate) and a reduction of the residual organic fraction. Extraction with chloroform confirms an enrichment of the inorganic fraction, while the organic peaks remain similar, indicating that the solvent or washing technique does not significantly remove the organic materials.

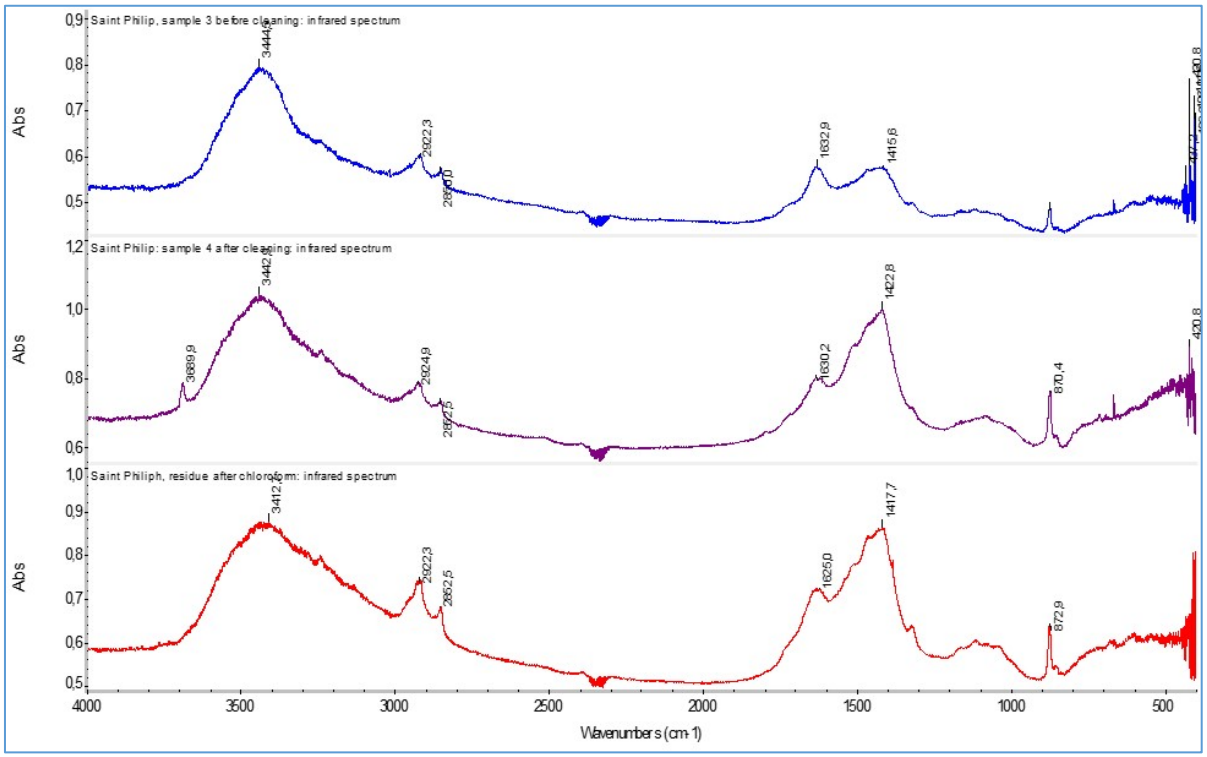

Figure 11. Cleaning and solvent extraction of the Saint Philip samples: infrared spectra.

The analyses also confirm the presence of beeswax, observed in sample 5 and detected by the FT-Raman spectrum (λ = 1064 nm—Figure 12).

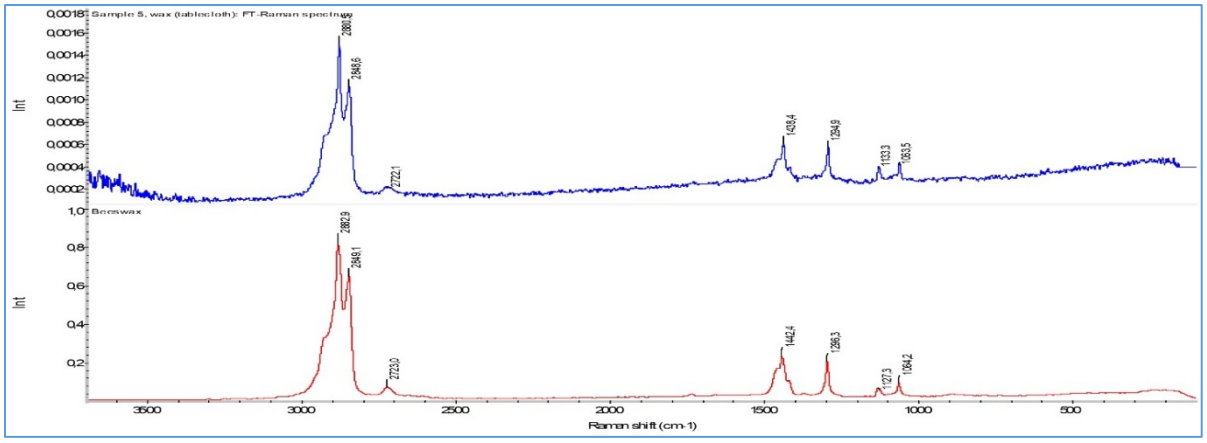

Figure 12. FT-Raman spectrum of sample 5 (tablecloth): comparison with beeswax.

5. Spectroscopic Analysis: The Contribution of Raman Spectroscopy

Using the instruments available in Professor Zerbi's laboratory, several Raman spectra were recorded on the considered samples, some already discussed, others corresponding to preparatory or plaster layers. It is important to note that at the time these analyses were carried out, Raman spectroscopy was a highly advanced diagnostic technique in the field of cultural heritage and was available in only a few university laboratories. Moreover, unlike today, there was no possibility of using this technique directly in situ with portable Raman spectrometers.

The challenges of applying Raman spectroscopy in this field were also little known. One of the most technically difficult aspects of using this technique on artistic materials, including the Cenacolo, is the strong fluorescence caused by organic materials or degradation products. This can make the spectra complex or obscure the Raman signals, as fluorescence interference often masks the weak Raman signal.

Intense fluorescence was observed in almost all examined samples. Useful results were obtained only using a source at λ = 1064 nm; it was not possible to record usable spectra in the visible range. Microscopic analyses revealed that the samples exhibited strong fluorescence in both the preparatory layers and the plaster, indicating

that the original organic binders or protective/consolidation materials applied during subsequent restorations had penetrated to the deeper layers.

Examining the FT-Raman spectrum recorded on sample 6, or the FT-Raman spectrum recorded on sample 1 (Figure 13), a consistent broad band around 1089 cm^{-1} can be observed, associated with a peak at 537 cm^{-1}. These bands are also observable in the spectrum of calcium carbonate, confirming that Raman spectroscopy is particularly suitable for the study of inorganic components or samples of higher purity.

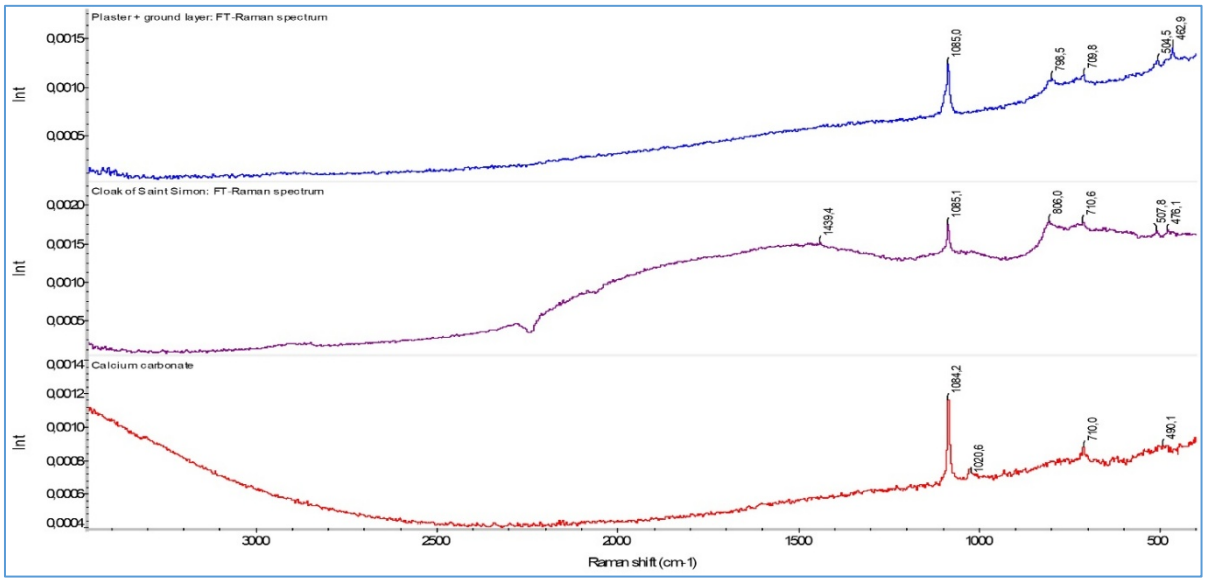

Figure 13. FT-Raman spectra recorded on samples 1 and 6: comparison with the FT-Raman spectrum of calcium carbonate.

In the case of the Cenacolo, Raman spectroscopy presented significant limitations at the time of Barcilon's restoration due to:

- the strong heterogeneity of the layers;
- interferences caused by degradation products.

For this reason, Raman results were always historically contextualized, compared with data from other techniques, and interpreted with extreme caution, particularly regarding the attribution of materials to Leonardo's original work.

Analyses of red pigments, conducted in subsequent years using micro-Raman spectroscopy in specific studies [13], enabled the identification of carmine lake (extracted from *Dactylopius coccus*), madder lake (extracted from *Rubia tinctorum*), and other red pigments derived from natural anthocyanins or anthraquinones. These studies employed advanced fluorescence subtraction techniques to obtain interpretable spectra.

6. Conclusions

The results obtained show both consistencies and divergences compared to other studies conducted using different diagnostic techniques. Any discrepancies are likely attributable to the heterogeneity of the samples, taken from different areas of an extremely complex and non-uniform artwork.

Table 2 summarizes the inorganic and organic components detected in the samples considered:

Table 2. Inorganic and Organic Components Detected.

No.	Sampling Area	Inorganic Components	Organic Components
1	Saint Simon's cloak	Calcium carbonate, Magnesium hydroxide	Shellac, Beeswax, Linseed oil
2	Tablecloth	Calcium carbonate	Shellac, Beeswax
3	Saint Philip	Calcium carbonate, Magnesium hydroxide	Shellac, Beeswax, Linseed oil
4	Saint Philip	Calcium carbonate, Magnesium hydroxide	Shellac, Beeswax, Linseed oil
5	Tablecloth	–	Beeswax
6	Saint James	Calcium carbonate, Magnesium hydroxide	Shellac, Beeswax, Linseed oil
7	Saint James	Calcium carbonate, Magnesium hydroxide	Shellac, Beeswax, Linseed oil
8–14	Lunettes	Calcium carbonate, Magnesium hydroxide	Shellac, Beeswax, Linseed oil, Gum arabic

The search for the original binders used by Leonardo was heavily complicated by the numerous materials applied during subsequent restorations. Shellac, applied during M. Pelliccioli's restoration after 1946, was found in almost all recorded spectra.

In the lunette samples, shellac was the predominant component, with a probable minimal presence of gum arabic, likely associated with later retouching interventions.

Although the four-layer structure was clearly observed through SEM analysis, Raman measurements demonstrated that the organic binder had penetrated and diffused throughout all layers, reaching even the plaster.

The spectroscopic techniques employed provided direct scientific documentation of the chemical nature of the pictorial materials and made it possible to identify substances deposited or removed during the different phases of restoration.

The combined use of IR and Raman spectroscopy provided a comprehensive understanding of the materials present in the Cenacolo*:* while infrared is more sensitive to organic materials and binders, Raman is better suited for the identification of pigments, inorganic compounds, and crystalline substances. Thanks to this complementarity, restorers were able to:

- Preserve and consolidate the original materials.
- Precisely remove non-original retouches.
- Plan targeted preventive conservation interventions.

Infrared and Raman spectroscopies proved to be decisive tools in the restoration of Leonardo da Vinci's Last Supper, allowing the artwork to be analysed scientifically and non-invasively. These techniques contributed to preserving the originality of the work, understanding degradation phenomena, and guiding restorations accurately, ensuring that the public can admire the masterpiece in optimal conditions.

Author Contributions

E.M. and G.Z.: conceptualization, methodology, software; E.M.: data curation, writing—original draft preparation; E.M.: visualization, investigation; G.Z.: supervision; E.M.: software, validation; E.M. and G.Z.: writing—reviewing and editing. All authors have read and approved the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

No new data were generated, or data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

Artificial intelligence was employed to assist with certain literature reviews.

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