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The Impact of Enzymes on the Physical and Chemical Properties of Paint Layers in Historical Oil Paintings: An Experimental Study

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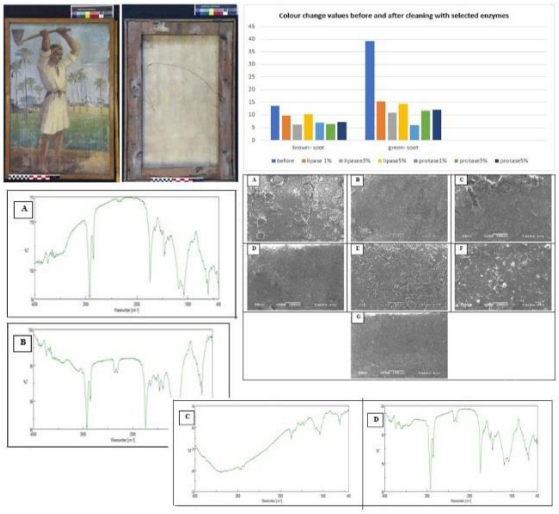
HIGHLIGHTS

- A 5% concentration of enzymes effectively removes surface contaminants without altering paint layers.
- FTIR analysis shows minimal chemical interaction at optimal enzyme concentration levels.
- Enzyme-based cleaning presents a safer alternative to traditional mechanical cleaning methods.
- The proposed enzyme concentrations can be adapted for conservation practices across diverse painting media.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims to investigate the effectiveness of lipase and protease enzymes in removing soot stains from the paint layers of historical oil paintings while minimizing changes to color and chemical composition. Using experimental mock-ups based on a historical oil painting from Fatima Ismail Palace, varying concentrations (1%, 3%, and 5%) of each enzyme was applied. The results were evaluated using scanning electron microscope (SEM-EDX), colorimetric analysis, and Fourier transform infrared (FTIR) spectroscopy.

The SEM analysis revealed that higher concentrations of both enzymes achieved greater stain removal. Colorimetric analysis indicated that 3% concentrations of both lipase and protease provided optimal cleaning with minimal color alteration, while higher concentrations led to increased color changes. FTIR analysis showed that the enzymes, particularly at 3% and 5%, altered specific chemical bonds associated with the soot stains. Overall, the study demonstrates the potential of lipase and protease enzymes in controlled cleaning of oil paintings, suggesting that 5% concentrations are most effective for removing stains while preserving the original paint layer's integrity.

1. Introduction

Oil paintings suffer from various deterioration phenomena, particularly the accumulation of surface dirt such as dust, grime, grease, molds, and fungi over time [1]. This accumulation can slowly alter the appearance of the oil paintings, making the colors duller and attracting further deposits. Furthermore, surface dirt can gradually bond more readily with the underlying original materials, partly due to its tendency to retain atmospheric moisture, leading to further decay and deterioration. Recognizing the significance of addressing these issues, the study aims to emphasize the importance of removing these stains from oil painting surfaces [2].

While organic solvent-based methods have proven successful, the study acknowledges the reluctance of many expert restorers to employ them due to their invasive nature. Confronting this challenge, the study aims to explore alternate solutions, considering the need to regulate the penetration and retention of solvents in the original paint layer and the limited understanding regarding the long-term effects of such treatments. Mechanical cleaning methods, though effective, have the potential to over clean and abrade the original surface layers [3]. In this context, the study turns to enzymatic cleaning methods, which have gained wider acceptance and application in the conservation of various artifacts. Some commercially available enzymes were used in the cleaning of cultural heritage [4], whether organic materials such as paper [5-7], oil painting [8], textiles [9], and wood [10], or inorganic materials such as stones [11,12], and wall paintings [13].

Furthermore, numerous researches have been conducted to evaluate the cleaning efficacy of enzymes and the surface characteristics of various archaeological artefacts before and after enzymatic treatment [5,10,14-16].

The fundamental nature of proteins was explored as a foundation for understanding enzymes in conservation practices. A general classification of enzymes was provided, focusing on the characteristics of proteases, lipases, and glycosides, with discussions on various enzyme sources relevant to conservation treatments. Specific enzymes were highlighted, and their applications in conservation processes were examined, offering insight into how enzymes can be selected based on specific treatment requirements [17].

Enzymes are increasingly employed in the preservation of oil paintings, particularly for the removal of surface dirt. Their use has expanded to cleaning various types of artifacts, often incorporated into cellulose compresses or combined with aqueous and organic solvent mixtures. Among the most used enzymes in conservation are hydrolases, such as protease, lipase, and amylase. Lipase, in particular, targets lipids (fats) and has demonstrated success in removing resinous layers, including acrylic coatings like Paraloid B72, without negatively affecting the artifact. This method has proven to be both safe and effective for cleaning purposes [18].

In addition, a hydrolytic enzyme was employed to remove layers of aged acrylic resin (Paraloid B-72) from two artworks: a 15th-century tempera painting and a 19th-century oil painting on canvas. The study proposed a plausible mechanism for the enzyme's action, contributing to the understanding of enzyme-based cleaning in artwork conservation [19]. The effectiveness of lipase enzymes in reducing a discolored, hard, and fragile preservative layer applied during the 19th-century restoration of Salisbury Cathedral was evaluated. The testing indicated that the enzyme poultice achieved a highly satisfactory level of cleaning, addressing both aesthetic and structural concerns [20].

A yeast-based enzymatic mixture was used to remove oil-based overpainting from the fragile surfaces of valuable historical paintings, demonstrating the method's applicability to sensitive, historical materials [3]. The present study aims to use lipase and protease enzymes due to their successful applications in the cleaning of various archaeological materials such as textiles, wall paintings, and wood. Notably, the use of lipase enzyme in removing varnish layers from the surface of oil paintings has shown efficacy in dealing with oil-based materials. This study seeks to extend their application to the removal of stains on oil paintings' surfaces, aiming to observe and analyze the changes in stains, pigments, and the oil medium before and after the enzyme treatment.

To assess the effect of the enzymes on stain removal, surface topography, color changes, and chemical alterations in the oil medium following the cleaning process, various analytical tools were employed; including scanning electron microscope (SEM), portable digital microscope, colorimeter, and Fourier transform infrared spectroscopy (FTIR). Samples required for the experimental study were prepared based on the results of the examination and analysis of several samples taken from a historical painting at Fatima Ismail palace (the Agricultural Museum in Cairo). The case study painting is an unsigned, undated oil painting on canvas, representing the Egyptian farmer in his work (Fig.1.).

The artist succeeded in capturing the sharp facial features to illustrate the intensity of the man's dedication to work and the expression of movement in his active state. He executed the drawing of a man standing in a three-quarters position, holding an axe with all his strength, ready to strike the land and prepare it for cultivation. As for the man's clothing, the artist rendered it in a realistic manner, mimicking what Egyptian farmers typically wear while working in the field. Additionally, the artist effectively depicted a background that simulates the agricultural landscape of the Egyptian countryside, using green colors and palm motifs that reflect rural life. He also employed light brown tones to illustrate some houses, representing the architecture of rural village buildings. The artist considered dimension and depth in the painting by including figures passing near the edge of the agricultural land. Furthermore, he successfully depicted the sky in its natural heavenly color, suggesting a rural environment free from pollution, which makes the sky appear clear.

Fig.1. The front and verso of the case study painting.

2. Materials and methods

2.1. Analytical methods for the case study painting

XRD model EMM 0143 GBC, at the Central Laboratory, South Valley University, Qena, Egypt, was used to identify the filler in the ground layer and the pigments in the paint layer. FTIR spectroscopy model JASCO FTIR-4100, at the Central Lab, Chemistry Department, Faculty of Science, South Valley University, Qena, Egypt, was utilized to identify the binding material in the ground layer. GC-MS spectroscopy model Thermo ISQ 7000, at the Research and Conservation Center for Antiquities, Ministry of Tourism and Antiquities, Cairo, Egypt, was employed to identify the oil medium in the paint layer.

2.2. Experimental setup

2.2.1. Samples preparation

Based on the results of the analytical methods applied to the case study painting, six mock-ups measuring 5 cm by 5 cm were constructed. These mock-ups consisted of cotton canvas, a ground layer composed of zinc oxide, calcium carbonate, and animal glue, and a paint layer made of chromium oxide and raw umber mixed with linseed oil as a medium.

2.2.2. Accelerated thermal ageing

The ageing process was done in a drying oven model JSON-150, in the central laboratory, Animal Department, College of Science, South Valley University. The process was carried out at a temperature of 105°C for 180 hours [21]. This is thought to be equivalent to about 100 years of natural ageing. The oven was then turned off and allowed to cool to room temperature. The samples were removed and left to settle for 24 hours under ambient conditions.

2.2.3. Preparation and application of soot stain

After the initial accelerated aging of the samples, soot stains were applied using a 5% carbon black solution with a soft brush [22]. Soot stains are tiny particles of carbon that are spread on the surfaces of oil paintings due to the presence of carbon atoms, resulting in industrial pollution, and as a result, they blacken the image [23]. Since most museums housing oil paintings are located in the capitals of countries or large cities, which are known for high levels of air

pollution due to factories and car exhaust, especially in Egypt, these pollutants can easily enter the museums and affect the appearance of the oil paintings.

The samples then underwent a second round of accelerated aging to further integrate the stains with the sample surfaces, making them more difficult to remove [24]. This aging process was carried out at a temperature of 105°C for 18 hours, which is estimated to be equivalent to approximately 10 years of natural aging [21]. The oven was then turned off and allowed to cool to room temperature. The samples were removed and left to settle for 24 hours under ambient conditions.

2.2.4. Preparation and application of enzymes

A lipase enzyme from ADVENT® and a protease enzyme from Solarbio Life Science® were selected for the experimental study. Lipase enzyme was chosen for this study due to their specific ability to hydrolyze ester bonds in lipid-based materials, such as those found in aged oil paintings [25]. Historical oil paintings typically use drying oils like linseed oil, which undergo oxidation and cross-linking over time. Lipase effectively targets and breaks down fatty acid residues and organic contaminants present on the surface, without affecting the underlying paint layers. By degrading lipid-based contaminants, lipase helps remove accumulated grime, varnish residues, or environmental pollutants while preserving the integrity of the cross-linked oil matrix. This enzymatic approach offers a controlled and selective cleaning method, minimizing the risk of damage compared to mechanical or solvent-based techniques.

Protease was included in the study to address potential proteinaceous contaminants often present on historical oil paintings. These contaminants may originate from environmental pollutants, aged varnishes, adhesives, or handling residues, which can include protein-based materials. While protease primarily hydrolyze peptide bonds [26], their application can assist in breaking down these proteinaceous layers [25], facilitating the removal of surface deposits without directly affecting the cross-linked linseed oil substrate. Additionally, protease may interact with protein-lipid complexes or other organic residues, weakening their adhesion to the painted surface. This could explain the observed cleaning efficacy, similar to that of lipases. Therefore, the inclusion of protease aimed to target mixed organic contaminants, contributing to an overall cleaning effect suitable for delicate oil painting surfaces.

2.2.4.1. Preparation of buffer solution

For enzyme preparation, a stable pH suitable for enzyme activity was maintained using a buffer solution, which stabilizes the pH during the cleaning process (enzyme action). Reaction products formed by the enzyme during dirt breakdown may be acidic or alkaline, potentially affecting enzyme activity. A carbonate buffer solution (0.1 M, pH 8.7) containing ammonium carbonate (0.1 M) was used [27].

2.2.4.2. Preparation of enzymes

To prepare enzyme solutions, varying concentrations of lipase and protease were dissolved in 12.5 ml of buffer solution. For each enzyme, three concentrations were prepared: 0.125 g for a 1% solution, 0.375 g for a 3% solution, and 0.6 g for a 5% solution. These concentrations were chosen to assess the effectiveness of each enzyme at different activity levels (Table 1.).

Table 1. Preparation of lipase and protease enzyme solutions at varying concentrations in 12.5 ml of buffer solution.

Enzyme	Weight (g)	Buffer solution (ml)	Concentration (%)
Lipase	0.125	12.5	1%
Lipase	0.375	12.5	3%
Lipase	0.6	12.5	5%
Protease	0.125	12.5	1%
Protease	0.375	12.5	3%
Protease	0.6	12.5	5%

2.2.4.3. Enzymes application

Enzymes were applied to the samples surface by brushing at room temperature [18]. The enzymes were left on the surface for 5 minutes, after which the results were observed. After cleaning, alcohol was applied with cotton to deactivate the lipase and protease enzymes [13].

2.2.5. Analytical methods for evaluating the efficiency of enzymes in cleaning the paint layer

2.2.5.1. Portable digital USB microscope

A portable digital USB microscope connected to the PC, with a magnification of up to 200×, was used to examine the samples before and after cleaning with enzymes in different concentrations.

2.2.5.2. Scanning electron microscope

To investigate surface morphology and assess any topographical changes caused by enzyme exposure, a JEOL JSM-5500 LV scanning electron microscope (JEOL, Japan) was used at the Central Laboratory, South Valley University, Qena, Egypt.

2.2.5.3. Color change

Color change was assessed using the Optomatch 3100® device from SDL Company at the National Institute of Standards (NIS) in Cairo, Egypt. The color change (ΔE) was calculated using the following equation:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

The (L) index was the color illustrating the black-to-white color, the (a) was the index signifying green-to-red color, and the (b) was the index characterizing blue-to-yellow color [28].

2.2.5.4. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was conducted to observe chemical changes in the oil medium of the aged, stained, and cleaned samples using a KBr disc on a JASCO FTIR-4100 (Japan) at the Central Lab, Chemistry Department, Faculty of Science, South Valley University, Qena, Egypt.

3. Results and Discussion

3.1. Painting materials composition

The XRD results (Figs.2, 3.) indicated that the artist used red ochre (Fe_2O_3) as a red pigment, chromic oxide II (Cr_2O_3) as a green pigment, and Manganese dioxide (MnO_2) – Pyrolusite- as a black pigment. Additionally, calcite ($CaCO_3$) and zinc oxide (ZnO) were identified as fillers in the ground layer. While FTIR analysis results (Fig.4.) showed that the binder is animal glue, as the functional groups of the historical sample matched those of a standard animal glue sample [29].

For the paint layer medium, the extracted samples were analyzed using GC-MS after derivatization with hexane to form fatty acid methyl esters (FAMEs), enhancing volatility and separation [30]. The chromatograms showed characteristic peaks corresponding to palmitic acid (C16) and stearic acid (C18), identified at retention times of 51.65, 53.75, and 58.03 minutes, respectively. Notably, the expected peaks for oleic, linoleic, and linolenic acids — typically found in fresh linseed oil—were either

absent or significantly reduced. This absence can be attributed to the advanced oxidation and polymerization processes in aged linseed oil films, which break down or cross-link unsaturated fatty acids, rendering them undetectable by GC-MS. Meanwhile, palmitic acid and stearic acid (P/S) — remain relatively stable during the drying process. Consequently, the P/S ratio has been used as one of the main criteria to differentiate between drying oils [31]. So the medium was identified by calculating the ratio between palmitic (P) and stearic (S) acids. GC-MS analysis (Fig.5.) of the sample revealed that the palmitic acid content was 63.25%, and the stearic acid content was 36.76%. The resulting palmitic-to-stearic acid ratio of 1.72 indicates that the oil medium used in the case study oil painting is linseed oil [32].

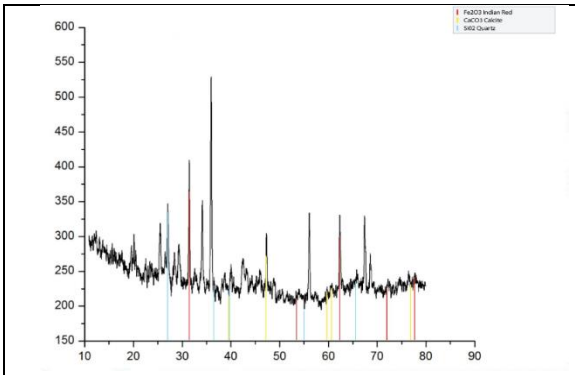

Fig.2. XRD spectra of paint layer sample.

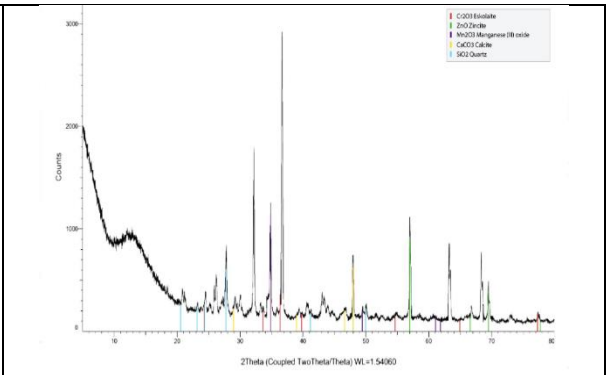

Fig.3. XRD spectra of paint layer sample.

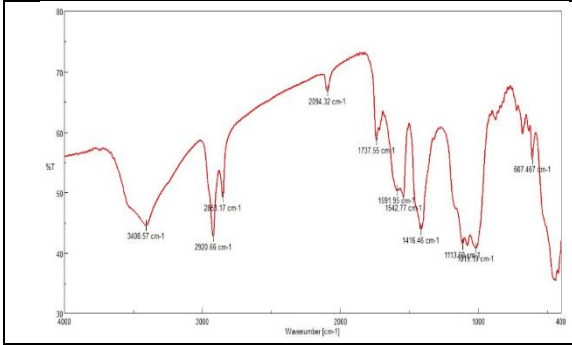

Fig.4. FTIR spectrum of the binding material in the ground layer.

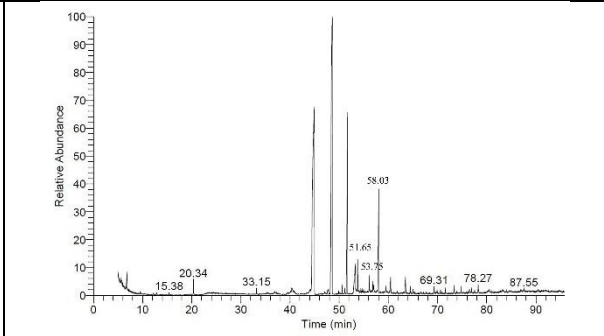

Fig.5. GC-MS chromatogram of FAMEs in a sample taken from the case study painting.

3.2. Evaluating the efficiency of enzymes in cleaning the paint layer

3.2.1. Portable digital USB microscope

It is clear that the soot stain covers the surface of the brown pigment sample (Fig.6A.). The results demonstrated that applying lipase at a concentration of 1% (Fig.6B.) resulted in the removal of only a small part of the soot stain. This indicates that at this concentration, the cleaning effectiveness was limited, with only a portion of the stain successfully eliminated. Cleaning with lipase at a concentration of 3% (Fig.6C.) showed greater efficacy, as a larger portion of the soot stain was removed compared to the 1% concentration. This suggests that increasing the concentration of lipase enhanced its cleaning performance, leading to more significant stain removal. The highest concentration, lipase at 5% (Fig.6D.), yielded the most substantial results. It successfully eliminated a significant portion of the stain, indicating that the 5% concentration of lipase was the most effective, resulting in a significant reduction or complete removal of the soot stain from the brown pigment samples.

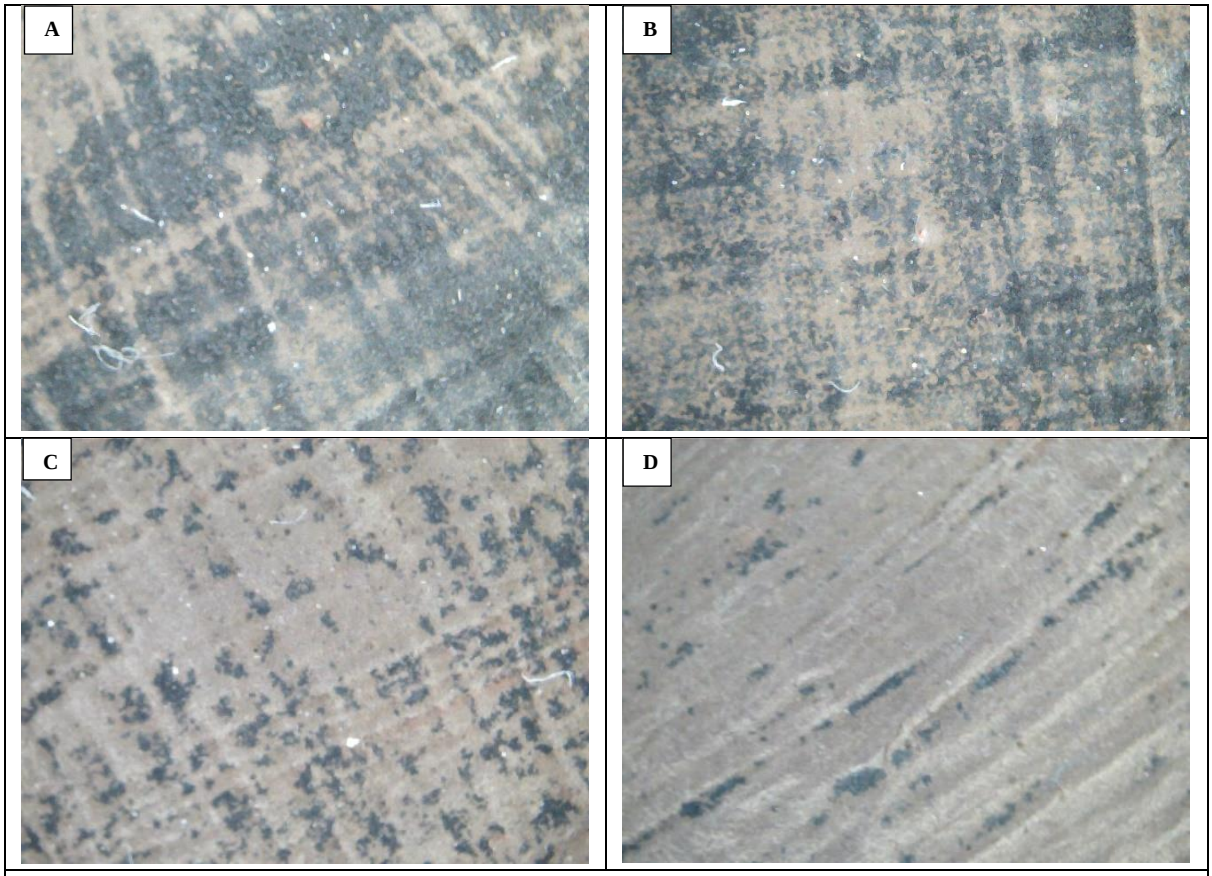

Fig.6. Portable digital USB microscope images of brown pigment samples: (A) Before cleaning soot stains; (B) After cleaning with 1% lipase enzyme; (C) After cleaning with 3% lipase enzyme; (D) After cleaning with 5% lipase enzyme.

It is clear that the soot stain covers the surface of the green pigment sample (Fig. 7A.). The results showed that the application of protease at a concentration of 1% (Fig.7B.) removed only a small portion of the soot stain. This indicates that, at this concentration, the cleaning effectiveness was limited, and only part of the stain was successfully eliminated. Cleaning with protease at a concentration of 3% (Fig.7C.) demonstrated increased efficacy. A larger portion of the soot stain was removed compared to the 1% concentration. This suggests that as the concentration of protease increased, so did its cleaning performance, resulting in more significant stain removal. The highest concentration, protease at 5% (Fig.7D.), produced the most substantial results. It successfully removed a large percentage of the stain, indicating that the highest concentration of protease was the most effective for cleaning, leading to a significant reduction or complete removal of the soot stain.

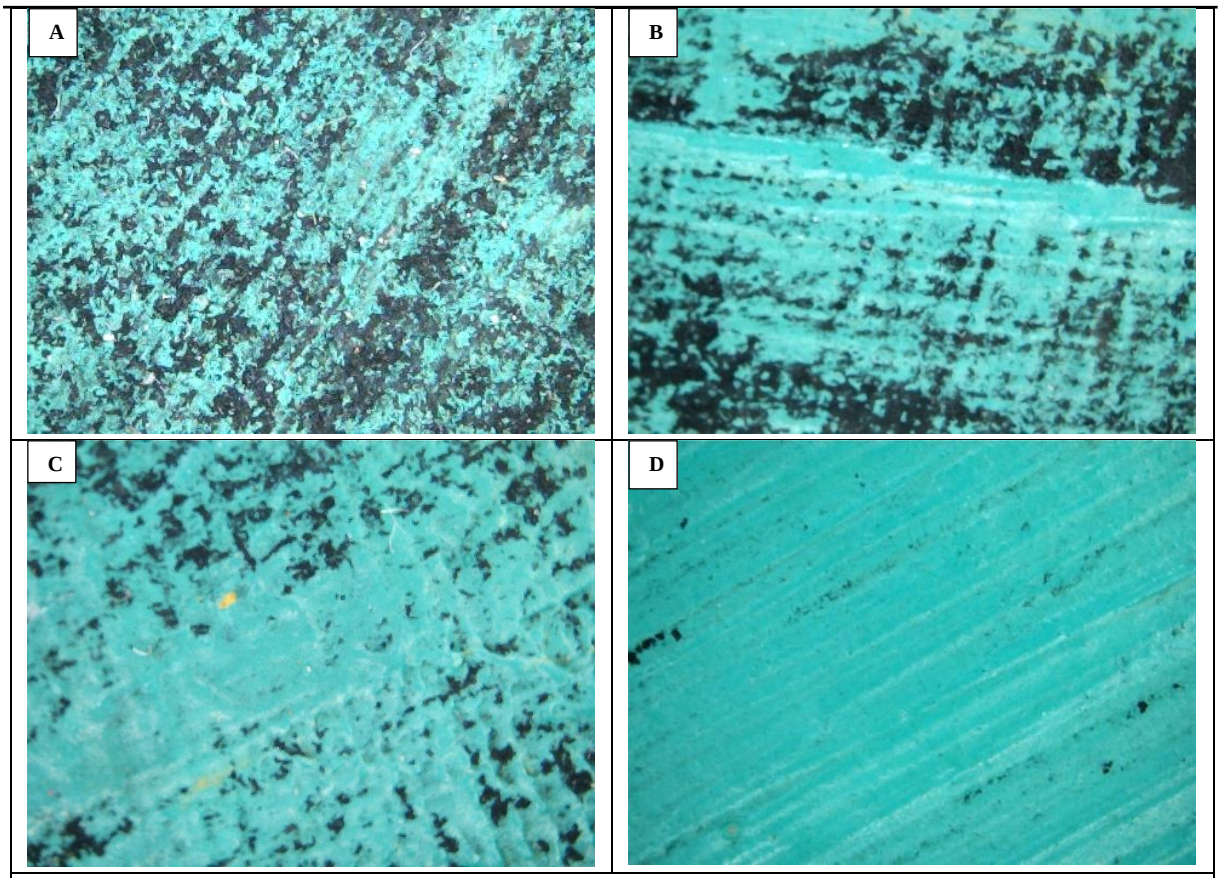

Fig.7. Portable digital USB microscope images of green pigment samples: (A) Before cleaning soot stains; (B) After cleaning with 1% lipase enzyme; (C) After cleaning with 3% lipase enzyme; (D) After cleaning with 5% lipase enzyme.

3.2.2. Scanning electron microscope

Scanning electron microscope (SEM) examination of the samples before cleaning indicated that soot stains fully covered the surface (Fig.8A.). Application of 1% lipase (Fig.8B.) removed a small portion of the soot stain, suggesting limited cleaning effectiveness at this concentration. Increasing the lipase concentration to 3% (Fig.8C.) improved cleaning efficacy, with a larger portion of the stain removed compared to 1%. The highest concentration, 5% lipase (Fig.8D.), achieved the most significant stain removal, indicating that higher enzyme concentrations correlate with improved cleaning effectiveness.

Similarly, protease at 1% (Fig.8E.) only partially removed the soot stain, reflecting limited cleaning ability. At 3% protease (Fig.8F.), a more substantial stain removal was observed, showing increased efficacy with concentration.

The highest concentration, 5% protease (Fig.8G.), led to a near-complete stain removal, suggesting optimal cleaning performance. Overall, the results demonstrate that the effectiveness of both enzymes increases with concentration, with 5% solutions achieving the most substantial soot stain removal.

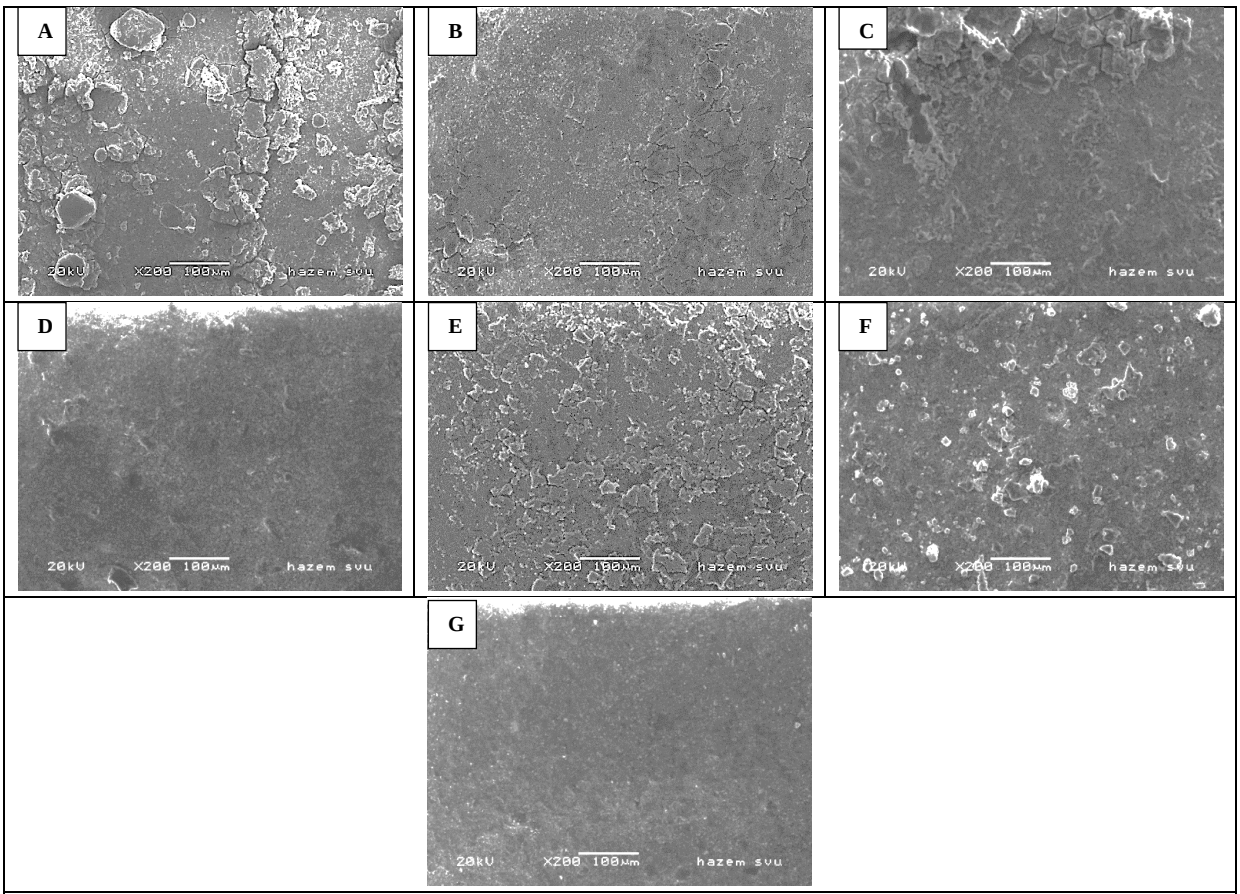

Fig.8. SEM examination results of samples showing soot stains before and after enzyme cleaning: (A) Before cleaning; (B) After cleaning with 1% lipase; (C) After cleaning with 3% lipase; (D) After cleaning with 5% lipase; (E) After cleaning with 1% protease; (F) After cleaning with 3% protease; (G) After cleaning with 5% protease.

3.2.3. Color Change

Results of color change examination by spectrophotometer (Table 2. & Fig.9.) show the CIE Lab color changes in the experimental samples before and after cleaning with the selected enzymes.

3.2.3.1. Brown pigment

Before cleaning, the initial ΔE value of 13.6 indicates a notable color change in the brown pigment integrated with the soot stain, suggesting visible alterations to the surface color characteristics. Cleaning with 1% lipase reduced the ΔE value to 9.7, indicating a moderate restoration of color with this concentration. Lipase at 3% resulted in a further ΔE reduction to 6.1, implying a more controlled cleaning effect with minimal impact on the original color. However, cleaning with 5% lipase increased the ΔE to 10.3, suggesting a more pronounced color change compared to the 3% concentration.

Protease at 1% produced a ΔE of 7.0, indicating controlled color restoration. This concentration achieved a reduction in color change relative to the initial state. Protease at 3% showed a similar effect to 3% lipase, with a ΔE of 6.4, suggesting effective color restoration and minimal color alteration. The 5% protease concentration resulted in a ΔE of 7.2, maintaining controlled restoration with minimal color change, similar to the effect seen with 1% protease. This outcome suggests that higher protease concentrations achieve effective stain removal with limited impact on the original color.

3.2.3.2. Green pigment

Before cleaning, the initial ΔE value of 39.3 indicates a significant color change in the green pigment integrated with the soot

stain, suggesting a substantial alteration in surface color characteristics. Cleaning with 1% lipase reduced the ΔE to 15.3, reflecting a moderate color restoration at this concentration. At 3% lipase, the ΔE further decreased to 10.9, suggesting a more controlled restoration with minimal impact on the original color. However, cleaning with 5% lipase increased the ΔE to 14.4, indicating a slightly higher color change compared to the 3% concentration, suggesting that higher concentrations may lead to a more pronounced restoration effect.

For protease, similar to observations with brown soot, a 1% concentration produced a ΔE of 6.0, signifying a substantial color restoration effect on the green soot stain. Protease at 3% yielded a ΔE of 11.7, providing controlled restoration similar to the effect observed with 3% lipase and indicating moderate impact on the original color. The 5% protease concentration resulted in a ΔE of 12.0, achieving a similar effect to the 3% concentration, indicating effective stain removal with only moderate alteration to the original color.

Table 2. ΔE values indicating color change in experimental samples before and after cleaning with selected enzyme concentrations.

	ΔE	
Stain / Enzyme	Brown Pigment	Green Pigment
	Soot	Soot
Before cleaning	13.6	39.3
Lipase 1%	9.7	15.3
Lipase 3%	6.1	10.9
Lipase 5%	10.3	14.4
Protease 1%	7	6
Protease 3%	6.4	11.7
Protease 5%	7.2	12

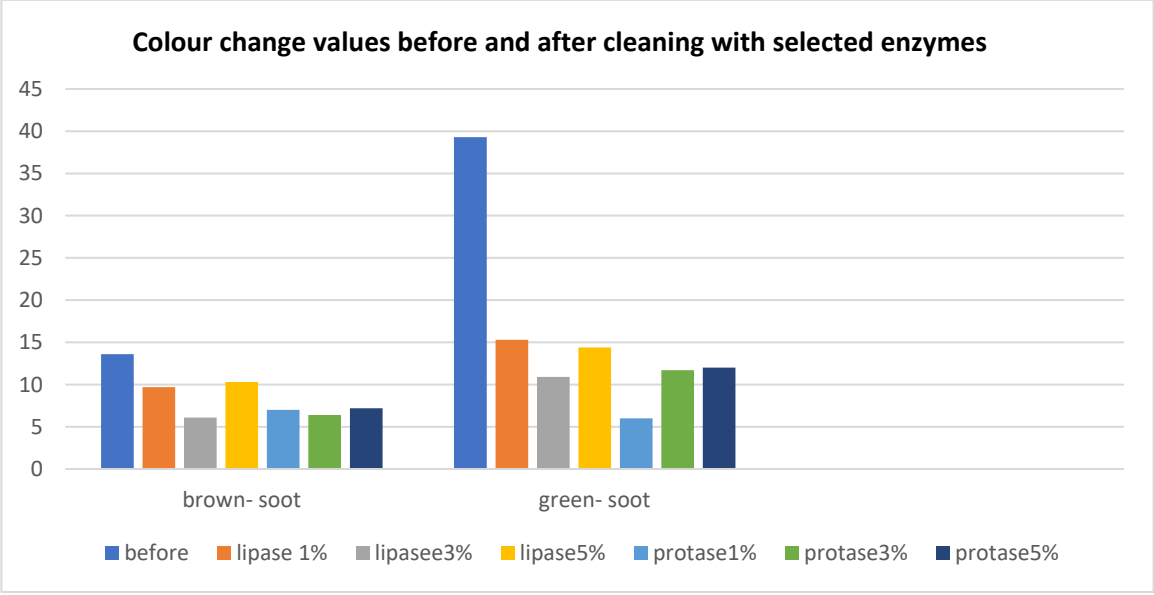

Fig.9. ΔE values showing color change in experimental samples before and after cleaning with selected enzyme concentrations.

3.2.4.Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were used to identify functional groups and assess chemical changes qualitatively. The results of FTIR analysis of the experimental samples (Fig.10.) showed that the band at 2926 cm^{-1} in the standard sample before the application of soot stain assigned to broadband represents the (C-H) stretching band. For the standard sample, the intensity of this band was 94.272. After applying the soot stain, the intensity of this band reduced to a lower value of 86.181. The intensity of this band in the cleaned sample with lipase 3% was 85.432. This band appeared at 2925 cm^{-1} for the cleaned sample with lipase 5% and the intensity was 85.925. It was obvious that this band disappeared in the cleaned sample with lipase 1%.

The peak at 1743 cm^{-1} is related with the C=O stretching band in the standard sample. The intensity of this band was 97.833. The intensity of this band was reduced to a lower value of 85.445 in the cleaned sample with lipase 5%. This band appeared at 1742 cm^{-} in the stained sample, cleaned sample with lipase 1%, and lipase 3%. The intensities of this band were 86.361, 75.130, and 88.442 for the stained sample, cleaned sample with lipase 1%, and 3% respectively.

The peak observed at 1460 cm^{-1} is assigned to C-H bending band in the standard sample. This band appeared at 1462 cm^{-1} with the stained sample and cleaned sample with lipase 3%. For the cleaned sample with lipase 5%, this band appeared at 1463 cm^{-1}. It disappeared in the cleaned sample with lipase 1%. The intensities of this band were 103.163, 91.515, 92.386, and 89.598 for the standard sample, the stained sample, cleaned sample with lipase 3%, and lipase 5% respectively.

The bands at 1082 cm^{-1}, 1080, and 1079 are assigned to (C-O) bending band in the standard sample, stained sample, and cleaned sample with lipase 1% respectively. This band disappeared after cleaning samples with lipase 3% and 5%. The intensities of this band were 94.802, 86.922, and 76.969 for the standard sample, the stained sample, cleaned sample with lipase 1%, respectively.

The intensity of the (C-H) stretching band in the cleaned sample with protease 3% was 81.019. This band appeared at 2925 cm^{-1} in the cleaned sample with protease 1%, but it appeared at 2924 in the cleaned samples with protease 5%. The intensity of this band in the cleaned sample with protease 1% was 54.968, while with protease 5% was 84.358.

The peak at 1743 cm^{-1} is related with the C=O stretching band in the standard sample. the intensity of this band was 97.833. This band appeared at 1742 cm^{-1} in the stained sample, cleaned sample with protease 5%. The intensities were 86.361 and 78.915 respectively. It also appeared at 1745 cm^{-1} in the cleaned sample with protease 1% and protease 5%. The intensities were 69.707 and 90.617 respectively.

The peak observed at 1460 cm^{-1} is assigned to C-H bending band in the standard sample, cleaned sample with protease 3% and protease 5%. The intensity of the standard sample was 103.163, but it decreased in the cleaned sample with protease 3% and protease 5%. The intensities were 97.864 and 84.556 respectively. This band appeared at 1462, and 1459 with the stained sample, cleaned sample with protease 1% respectively. The intensities of this band were 91.515 and 82.833 respectively.

The bands at 1082 cm^{-1}, 1080, and 1079 are assigned to (C-O) bending band in the standard sample, stained sample, and cleaned sample with protease 5% respectively. The intensities were 94.802, 86.922, and 68.147 respectively. This band disappeared after cleaning samples with protease 1% and protease 3% [29].

Lipase enzyme at different concentrations effectively alters the functional groups or bands associated with soot stains. Lipase 1% shows a significant impact on hydrocarbons, indicated by the disappearance of the C-H stretching band. Variations in carbonyl

group vibrations (C=O stretching and C-O effectiveness in different concentrations. Lipase 3% and 5% demonstrate effective cleaning, particularly in altering carbonyl groups, as evidenced by functional band shifts and intensity changes.

Protease enzyme at different concentrations induces varying changes in functional groups or bands associated with grime stains. Protease 3% and 5% show a complex impact on hydrocarbons bending) highlight the enzyme's varying and carbonyl groups, with Protease 5% indicating more profound changes. The disappearance and reappearance of bands at different concentrations suggest a nuanced interaction between the protease enzyme and grime components. Further detailed analysis may be required to understand the specific enzymatic actions involved.

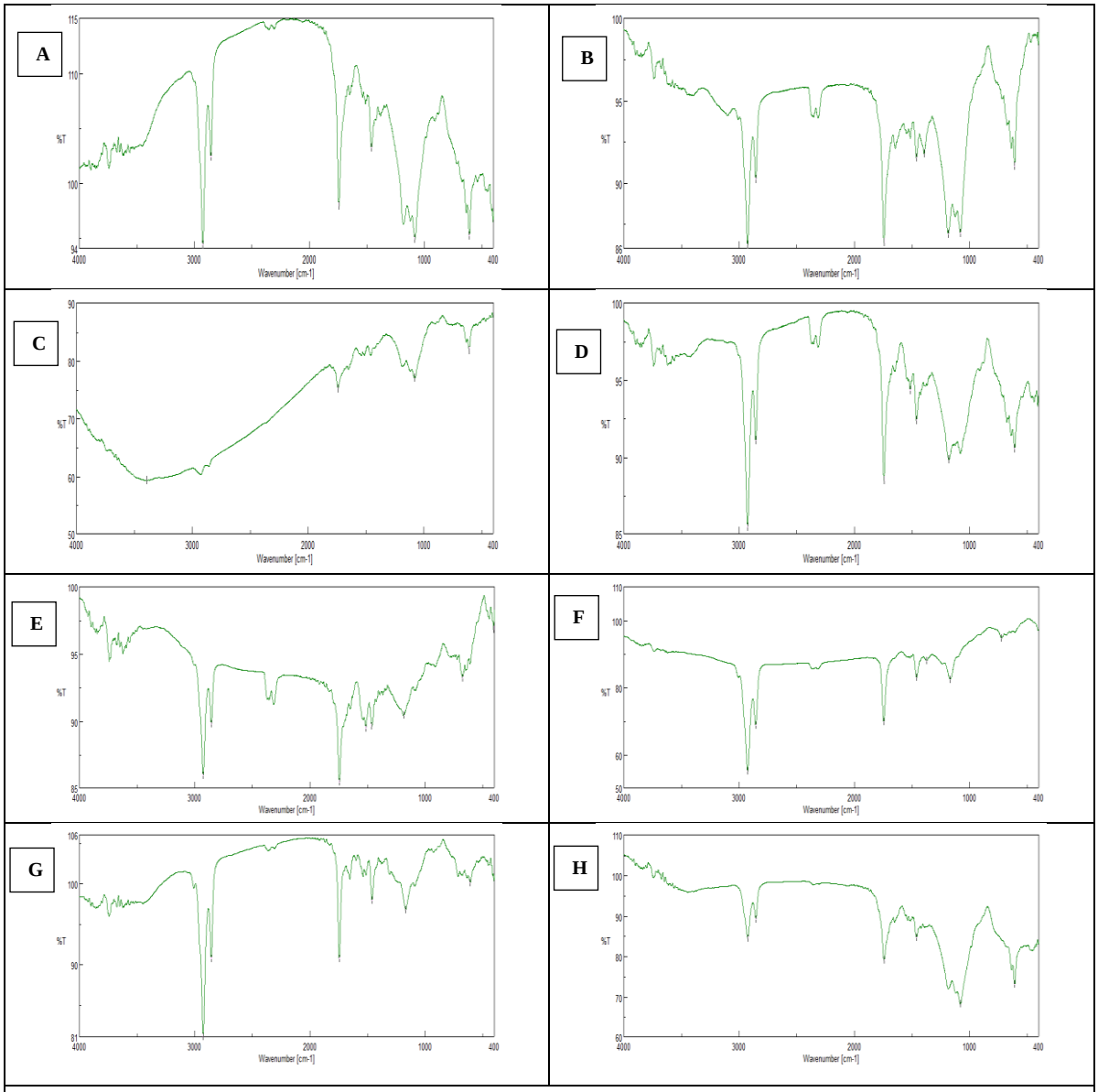

Fig.10. FTIR spectra of experimental samples: (A) Standard sample; (B) Before cleaning, with soot stains; (C) After cleaning with 1% lipase; (D) After cleaning with 3% lipase; (E) After cleaning with 5% lipase; (F) After cleaning with 1% protease; (G) After cleaning with 3% protease; (H) After cleaning with 5% protease.

4. Conclusion

This study highlights the potential of lipase and protease enzymes as effective, non-invasive cleaning agents for cleaning historical oil paintings. Both enzymes, applied at a 3% concentration, effectively removed soot stains while preserving the integrity of the original paint layers. SEM analysis demonstrated significant stain reduction, particularly at higher concentrations, while colorimetric data confirmed that a 3% enzyme concentration provided an optimal balance, effectively reducing stains with minimal color alteration. FTIR analysis further revealed that higher enzyme concentrations interact with specific functional groups in the soot, indicating that enzyme concentration directly influences both cleaning efficacy and chemical stability.

SEM analysis of mock-up samples showed that both lipase and protease, especially at 5% concentrations, effectively removed soot stains. However, the 3% concentration provided the best balance of cleaning efficacy and preservation of surface features, consistent with findings on the selective removal of contaminants using enzymes [18]. The CIE Lab color change (ΔE) values indicated that 3% concentrations minimized color alteration while achieving effective cleaning. Higher concentrations, such as 5%, produced more pronounced color changes, aligning with previous studies [3], supporting the recommendation to use concentrations at or below 3% to maintain aesthetic integrity. FTIR analysis indicated that enzyme treatments at higher concentrations affected functional groups, especially in carbonyl and hydrocarbon bonds. At a 3% concentration, however, enzyme activity was sufficient to clean the surface without significantly altering the chemical composition, a desirable outcome for preserving the stability of the painting materials.

This study demonstrates that enzyme-based cleaning, particularly with 5% lipase and protease, is a promising method for oil painting conservation. This approach aligns with conservation principles of minimal intervention and preservation of both aesthetic and chemical integrity, positioning it as a viable alternative to traditional cleaning techniques. The findings also highlight enzyme-based cleaning as a gentler method than mechanical or solvent-based treatments, reducing the risk of damage. Future research could further refine enzyme applications for various paint media and explore long-term effects, ultimately broadening enzyme-based cleaning techniques for use across diverse conservation contexts.

Conflict of Interest:

No potential conflict of interest was reported by the authors.

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