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L'OPUNTIA COME LEGANTE PITTORICO PER LA CONSERVAZIONE DEI BENI CULTURALI



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In questo 8° numero presentiamo una ricerca condotta all'università di Palermo che esplora l'uso della mucillagine estratta da due diverse specie di Opuntia, concentrandosi in particolare su Opuntia ficus-indica.

La mucillagine è stata valutata come potenziale legante per applicazioni artistiche e di conservazione del patrimonio culturale.

I risultati hanno rivelato che la mucillagine di Opuntia ficus-indica presenta caratteristiche leganti notevoli, consentendo una distribuzione uniforme dei pigmenti e promuovendo la stabilità dei colori su diverse superfici. Inoltre, l'analisi della composizione monosaccaridica ha evidenziato un equilibrio tra diversi zuccheri, il che suggerisce una composizione ottimale per le applicazioni artistiche.

Queste caratteristiche la rendono un'opzione attraente per l'uso nei settori della pittura, della decorazione e della conservazione artistica. Inoltre, i test di invecchiamento UV hanno dimostrato la capacità della mucillagine di Opuntia ficus-indica di resistere efficacemente alla degradazione causata dalla luce ultravioletta, garantendo una maggiore durata nel tempo dei materiali artistici trattati.

TITOLO:

Opuntia ficus-indica e Opuntia stricta

Leganti pittorici a base di mucillagini per la conservazione del patrimonio artistico

INTRODUZIONE:

L'industria agroalimentare genera, ogni anno, elevate quantità di rifiuti, molto spesso stoccati in azienda, creando gravi problemi ambientali e richiedendo lo studio necessario per il loro riutilizzo. Si stima che nell'Unione Europea i rifiuti derivanti dalla filiera alimentare raggiungano punte di 130-150 tonnellate l'anno, e circa il 50% sia interamente prodotto all'interno della filiera agroalimentare aziendale. Al giorno d'oggi, molte industrie hanno cambiato il loro modo di pensare e i loro mezzi operativi. La maggior parte dei rifiuti, infatti, possono diventare e diventano nuove risorse per nuove materie prime come preziose sostanze chimiche, trovando applicazioni in settori come quello alimentare, nutraceutico, biodiesel, ecc.

Il genere *Opuntia* spp. comprende numerose specie appartenenti alla famiglia delle Cactaceae note per avere specifiche proprietà nutrizionali. La capacità di queste specie di vivere in condizioni di ridotta disponibilità idrica appare oggi sempre più rilevante in un contesto globale in cui la siccità colpisce vasti territori. Per questo motivo le Nazioni Unite hanno sollecitato a considerare l'importanza di queste specie che, in alcune aree del mondo, sono state spesso considerate invasive o prive di valore intrinseco. Nell'attuale crisi climatica, tuttavia, il genere *Opuntia* spp. deve essere considerata una risorsa importante, soprattutto dal punto di vista alimentare, e questo approccio si interseca con molti degli obiettivi di sviluppo sostenibile, sia in termini di mitigazione del cambiamento climatico che in termini di conservazione della biodiversità e di consumo e produzione responsabili. L'interesse dietetico per il genere *Opuntia*, pur in presenza di una vasta biodiversità, è quasi concentrato sui frutti di *O. ficus-indica* (OF). Altre specie, invece, rivestono interesse a scopo alimentare, legate ai frutti o ai cladodi privati delle spine. Tra questi *O. stricta* (OS) (Haw.) Haw. (sin. *Opuntia dillenii* (Ker-Gawl) Haw.) è apparsa da tempo di interesse strategico anche perché presenta abbondanza di frutti e straordinaria plasticità di adattamento.

Più recentemente, lo studio dei derivati ottenuti dalla lavorazione di molte parti di piante di cactacea ha evidenziato che queste specie rivestono grande rilevanza anche per l'elevata quantità di sostanze bioattive, oltre che di prodotti naturali, sempre con l'obiettivo di costruire un modello per estrazione a base biologica e applicazione di sottoprodotti di origine naturale. Questi composti bioattivi sono influenzati da specie e cultivar oltre che, ovviamente, dall'area climatica, e hanno molteplici effetti, dalle azioni antimicrobiche a quelle antiossidanti, fino all'estrazione di biopolimeri per attività industriali a basso impatto ambientale. Le mucillagini, tra queste, sono particolarmente presenti nei cladodi. Dal punto di vista biologico rappresenta la base di un sistema di adattamento naturale a condizioni di limitata disponibilità idrica che molto spesso caratterizza le aree di origine e diffusione di queste specie.

In un'ottica di economia circolare, la produzione di mucillagini derivate dai cladodi e la sua applicazione in molteplici direzioni rappresentano un'importante risorsa economica alternativa a quella dei frutti, poiché consente ai residui della potatura delle piante, effettuata annualmente in tutte le condizioni di coltivazione, di ricevere un nuovo valore.

I frutti, le infusioni di fiori secchi e i cladodi raccolti da OF e OS, presentano diverse attività biologiche confermate in letteratura. Sono ampiamente riconosciuti in etnomedicina, considerati interamente commestibili, sia bolliti che fritti, e non tossici per il consumo umano. Le mucillagini ottenute da diverse specie di Opuntia hanno ampia applicabilità, sia nell'industria alimentare che farmaceutica, come leganti, addensanti, gelificanti e stabilizzanti. Diverse ricerche hanno evidenziato come le diverse mucillagini dimostrino eccellenti proprietà salutistiche quali attività antiulcera, antinfiammatoria, citoprotettiva, ipolipemizzante, antiossidante, antimicrobica e ipocolesterolemizzante, e recentemente sono state riviste le loro proprietà biologiche e tecnologiche nella preparazione dei cibi. Nello studio di Naeji viene riportato l'uso della mucillagine per creare pellicole commestibili con proprietà antimicrobiche. Inoltre, la mucillagine può preservare gli alimenti dagli attacchi fungini e avere diversi effetti benefici, principalmente sul deterioramento microbico e sulle proprietà fisico-chimiche delle fragole fresche durante la conservazione refrigerata.

Per le loro proprietà nutritive le piante di questo genere sono sempre state sottovalutate ma vengono utilizzate principalmente come scarti agricoli. Negli ultimi decenni, infatti, diverse ricerche hanno sfruttato la capacità antiossidante delle mucillagini di diverse cultivar di cactus per la produzione di imballaggi alimentari biodegradabili con capacità antiossidante. Questo aspetto è, in ogni caso, agevolata dall'enorme quantità di rifiuti (cladodi) che annualmente vengono prodotti, in particolare da coltivazioni estese di OF.

Attualmente, ci sono pochi esempi in letteratura che descrivono la preparazione di biofilm utilizzando varie mucillagini, aggiungendo pectina, chitosano, ecc. Aparicio-Fernández et al. hanno dimostrato come l'unione delle mucillagini OF con la carbossimetilcellulosa abbia migliorato la capacità antiossidante e ridotto il potere dei biofilm; inoltre, gli autori hanno scoperto che l'utilizzo di perle di alginato evitava la formulazione di ceppi batterici sugli hamburger utilizzati come mezzo di controllo. Dai dati ricercati in letteratura, non vi è evidenza dell'utilizzo della mucillagine come legante per scopi pittorici da applicare nel campo della conservazione dei beni culturali. Lo studio di Karami et al. suggeriscono che le nanofibre di mucillagine preparate utilizzando il metodo dell'elettrofilatura hanno potenziali applicazioni nel campo dell'imballaggio alimentare per l'incapsulamento di questa sostanza con buone attività antiossidanti e antimicrobiche e anche per il loro rilascio prolungato.

L'OF si conferma una coltura di grande importanza su scala globale perché fornisce cibo per l'uomo e gli animali ma, allo stesso tempo, consente la produzione di sostanze bioattive dalle molteplici proprietà, anche farmacologiche.

Viene spesso riportato che il potenziale del frutto in campo antinfiammatorio, ipoglicemizzante e antimicrobico, così come il suo potere antiossidante, rendono questo frutto particolarmente importante nella dieta funzionale in molte comunità in tutto il mondo. Alcuni autori hanno inoltre verificato la possibilità di utilizzare estratti di OF come coagulante per il trattamento delle acque inquinate, rivelandosi quindi un processo rispettoso dell'ambiente e sostenibile. La creazione artistica ha accompagnato lo sviluppo umano fin dalle origini dell'umanità. In passato, i processi di produzione del colore erano spesso caratterizzati da tecniche e materiali molto complessi, richiedendo talvolta all'utente una conoscenza approfondita delle interrelazioni. Infatti, per la tempera, ad esempio, la realizzazione di queste tecniche prevedeva e prevede tuttora l'utilizzo di colori in polvere (pigmenti) e colle animali (caseina, latte, tuorlo d'uovo di gallina, colla animale, ecc.), solubili in acqua. , utilizzati come leganti e impiegati prevalentemente su supporti in gesso, dal XII al XV secolo, fino all'uso diffuso della pittura ad olio. Uno studio scientifico sui frammenti delle pitture murali giulio-claudie ha evidenziato come i romani utilizzassero principalmente coloranti provenienti dal mondo vegetale (ad esempio, *Basella rubra*, *Sesamum radiatum*, *Lamium purpureum*), cere vegetali, oli (*Brassicaceae*) e resine (*Pinaceae*). come leganti, sostituendoli con colle animali (es. collagene, uovo).

I popoli nordici e alcuni pittori italiani del Rinascimento (Gentile da Fabriano; Sandro Botticelli) usavano aggiungere miele all'impasto per ritardare l'essiccazione del colore; i popoli del sud aggiungevano lattice di fico e vino. In numerosi oggetti d'arte i pigmenti, sia naturali che sintetici, sono mescolati con vari leganti, alcuni di origine vegetale (gomma arabica; amido), altri di origine animale (gelatina, albume e tuorlo d'uovo). Il mezzo legante, attraverso deboli interazioni, può influenzare significativamente la tonalità finale e la stabilità del colorante.

La conoscenza esistente è stata tramandata nel corso dei secoli e utilizzata ripetutamente; ma inoltre le materie prime sono state sviluppate e opportunamente adattate ai più svariati ambiti di applicazione ed esigenze, a seconda dei tempi. Nell'ambito della nostra continua ricerca, queste due mucillagini sono state utilizzate, per la prima volta, in modo del tutto innovativo, come possibili nuovi leganti pittorici, testandone le proprietà e studiandone gli aspetti colorimetrici, evidenziandone le differenze in termini di composizione chimica e capacità raccoglitrice.

CONCLUSIONI:

L'utilizzo di sottoprodotti naturali richiede particolare attenzione sia per i concetti legati all'economia circolare che per le possibili interferenze fisico-chimiche legate alla loro applicazione. Per questo motivo in questo lavoro, con il supporto delle relative analisi chimico-fisiche, si è cercato di individuare nuovi possibili leganti da sfruttare nella tecnica pittorica della tempera. In particolare, la mucillagine estratta dall'*Opuntia ficus-indica* (L.) Mill. e *Opuntia stricta* (Haw.) Haw. furono usati come nuovi leganti. Mediante la tecnica spettroscopica ¹³CNMR sono state identificate le unità monosaccariche delle due mucillagini, evidenziandone le differenze compositive: una caratterizzata principalmente da un'elevata quantità di arabinosio (36,48%) e galattosio (32,31%), mentre l'altra diversificata quasi in egual misura presenza di arabinosio (23,65%), galattosio (20,87%), e isomeri del glucosio (23,89%).

I diversi pigmenti erano dispersi in entrambe le mucillagini (OF e OS), evidenziando, dopo analisi colorimetrica, enormi differenze estetiche: i pigmenti dispersi in OS, infatti, testimoniavano un cambiamento estetico maggiore, ben visibile ad occhio nudo, rispetto a quelli in DI. In generale, i pigmenti inorganici hanno funzionato meglio nei confronti della mucillagine, registrando cambiamenti trascurabili nella DE prima e dopo l'invecchiamento. Nessuna differenza significativa è stata riscontrata nei dati tra i campioni posati su supporti cartacei o gessosi; ciò significa che il supporto materico non ha influenzato in modo significativo l'aspetto estetico dei campioni colore. Si può concludere che OF è adatto come legante per pigmenti, producendo colori con tonalità profonda, piena luminosità, consistenza corposa e resistente all'invecchiamento UV.

**A SEGUIRE, LA RICERCA
SCIENTIFICA COMPLETA**

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Anno: 2019

Le proteine trasportatrici del colesterolo nelle cellule HepG2 possono essere modulate da composti fenolici presenti nelle soluzioni acquose di *Opuntia ficus-indica*

Article

Opuntia ficus-indica (L.) Mill. and *Opuntia stricta* (Haw.) Haw. Mucilage-Based Painting Binders for Conservation of Cultural Heritage

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Abstract: The possibility of using materials from the waste of agricultural products for the conservation of cultural and artistic heritage has led to important technological developments on mortars, plasters, colors, and other applications. In this experimental work, we investigated the binding properties of mucilage obtained from two different species of the genus *Opuntia*, both collected in Sicily, Italy: *Opuntia ficus-indica* (L.) Mill. and *Opuntia stricta* (Haw.) Haw. Through chemical acid hydrolysis, and subsequent spectroscopic analysis conducted at ¹³C-NMR, the main monosaccharide composition of both mucilage was studied, identifying considerable compositional differences. In fact, the mucilage of *O. ficus indica* had similar total amounts of arabinose (23.65%), galactose (20.87%), and glucose isomers (23.89%), while that of *O. stricta* was characterized by significant amounts of arabinose (36.48%) and galactose (32.31%) units. The samples were obtained by dispersing pigments on the mucilage and applying the obtained tempera by a brush onto both paper and chalk supports, in order to observe if the colors changed with different substrates. Colorimetric analysis, measuring ΔE , showed how the same pigment modifies its aspect depending on the binder used. After a two-week UV ageing process, pigments that had dispersed in *O. stricta* changed their aspect more than those dispersed in *O. ficus-indica*. Overall, it is also evident how ΔE data for organic pigments are higher than those for inorganic ones.

Keywords: mucilage; NMR; by-products; pigments; historical artistic artifacts; tempera



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1. Introduction

The agri-food industry generates, every year, high quantities of waste, very often stored on the farm, creating serious environmental problems and requiring the necessary study for its reuse [1]. It is estimated that in the European Union, waste resulting from the food supply chain reaches peaks of 130–150 tons per year [2], and around 50% is entirely produced within the company's agri-food chain. Nowadays, many industries have changed their way of thinking and means of operation. Most waste, in fact, can and does become new resources for new raw materials such as precious chemical substances, finding applications in sectors such as food, nutraceuticals, biodiesel, etc.

The genus *Opuntia* spp. includes many species that belong to the Cactaceae family known to have specific nutritional properties [3,4]. The ability of these species to live under conditions of reduced water availability appears increasingly relevant today in a global context in which droughts affect large territories [5,6]. For this reason, the United Nations has urged the consideration of the importance of these species, which, in some areas of the world, have often been considered invasive or lacking in intrinsic value [7]. In the current climate crisis, however, the genus *Opuntia* spp. must be considered an important resource, especially from a food perspective, and this approach intersects with many of the sustainable development goals, both in terms of mitigating climate change as well as in terms of biodiversity conservation and responsible consumption and production.

Dietary interest in the genus *Opuntia*, albeit in the presence of vast biodiversity, is almost concentrated on the fruits of *O. ficus-indica* (OF) [8]. Other species, however, are of interest for food purposes, related to the fruit or cladodes deprived of their spines. Among these, *O. stricta* (OS) (Haw.) Haw. (syn. *Opuntia dillenii* (Ker-Gawl) Haw.) has long appeared to be of strategic interest partly because it has an abundance of fruit and extraordinary plasticity of adaptation [9].

More recently, the study of derivatives obtained from the processing of many cacti plant parts [10–12] has highlighted that these species are also of great relevance because of the high amount of bioactive substances, as well as natural products, again with the aim of building a model for bio-based extraction and application of by-products of natural origin [13]. These bioactive compounds are influenced by species and cultivar as well as, of course, climatic area, and have multiple effects, from antimicrobial to antioxidant actions, to the extraction of biopolymers for industrial activities with low environmental impact [14].

Mucilage, among these, is particularly present in cladodes. From a biological point of view, it represents the basis of a system of natural adaptation to conditions of limited water availability that very often characterizes the areas of origin and spread of these species [15]. From a circular economy perspective, the production of mucilage derived from cladodes and its application in many directions represent an important alternative economic resource to that of fruits, as it allows the residues of plant pruning, which is carried out annually under all growing conditions, to be given new value [16].

The fruits, infusions of dried flowers, and cladodes collected from OF and OS, present different biological activities confirmed in the literature. They are widely recognized in ethnomedicine, considered entirely edible, whether boiled or fried, and are non-toxic for human consumption [17–19].

The mucilage obtained from different *Opuntia* species have a wide applicability, both in the food and in the pharmaceutical industry, as binders, thickeners, gelling agents, and stabilizers [20]. Various research has highlighted how the different mucilage demonstrate excellent health properties such as anti-ulcer [21], anti-inflammatory [22], cytoprotective [23], hypolipidemic [19], antioxidant [24], antimicrobial [25], and cholesterol lowering activities [26], and their biological and technological properties in food preparation have been recently reviewed [27]. The use of mucilage to create edible films with antimicrobial properties is reported in Naejeji's study [28]. Furthermore, the mucilage can preserve foods from fungal attacks and have several beneficial impacts, mainly on microbial spoilage and physicochemical properties of fresh strawberries during refrigerated storage [29].

Due to their nutritional properties, plants of this genus have always been underestimated but are mainly used as agricultural waste. In fact, in recent decades, various research has exploited the antioxidant capacity of the mucilage of various cactus cultivars for the production of biodegradable food packaging with antioxidant capacity. This aspect is, in any way, facilitated by the enormous quantity of waste (cladodes) that are produced annually, in particular from OF extension cultivations.

Currently, there are few examples in the literature describing the preparation of biofilms using various mucilage, adding pectin, chitosan, etc. Aparicio-Fernández et al. [30] demonstrated how the union between OF mucilage with carboxymethylcellulose improved the antioxidant capacity and reduced the power of biofilms; also, the authors found that

using alginate pearls avoided the formulation of bacterial strains on hamburgers used as a medium control [31]. From the data researched in the literature, there is no evidence of the use of mucilage as a binder for pictorial purposes to be applied in the field of conservation of cultural heritage. The study by Karami et al. [32] suggest that mucilage nanofibers prepared using the electrospinning method have potential applications in the food packaging field for the encapsulation of this substance with good antioxidant and antimicrobial activities and also for their sustained release.

OF confirms to be a crop of great importance on a global scale because it provides food for humans and animals but, at the same time, it allows for the production of bioactive substances with multiple properties, including pharmacological ones [33,34]. It is often reported that the fruit's potential in anti-inflammatory, hypoglycemic, and antimicrobial fields, as well as its antioxidant power, make this fruit particularly important in the functional diet in many communities around the world [35]. Some authors have also verified the possibility of using OF extracts as a coagulant for the treatment of polluted water, thus proving to be an environmentally friendly and sustainable process [36].

Artistic creation has accompanied human development since the origin of mankind. In the past, the manufacturing processes of color were often characterized by very complex techniques and materials, sometimes requiring the user to have expansive knowledge of interrelationships. In fact, for tempera, for example, the realization of these techniques involved and still involves the use of powdered colors (pigments) and animal glues (casein, milk, hen's egg yolk, animal glue, etc.), which are soluble in water, used as binders and mainly used on chalk supports, from the 12th to the 15th century, until the widespread use of oil painting [37]. A scientific study on the fragments of Julio-Claudian wall paintings has highlighted how the Romans mainly used dyes from the plant world (e.g., *Basella rubra*, *Sesamum radiatum*, *Lamium purpureum*), vegetable waxes, oils (Brassicaceae), and resins (Pinaceae) as binders, replacing them with animal glues (e.g., collagen, egg) [38]. The Nordic peoples and some Italian Renaissance painters (Gentile da Fabriano; Sandro Botticelli) used to add honey to the dough to delay the drying of the color; the southern peoples added fig latex and wine. In numerous art objects, pigments, both natural and synthetic, are mixed with various binders, some of vegetal origin (gum Arabic; starch), others of animal source (gelatin, egg white, and egg yolk). The binding medium, through weak interactions, can significantly influence the final shade and stability of the dye [39].

The existing knowledge has been passed down through the ages and used repeatedly; but furthermore, the primary materials have been developed and sensibly adapted to several areas of application and needs, depending on the times [40]. As part of our ongoing research, these two mucilage have been used, for the first time, in an utterly innovative way, as possible new pictorial binders, testing their properties and studying their colorimetric aspects, highlighting the differences in terms of chemical composition and capacity binder.

2. Materials and Methods

2.1. Chemicals and Pigments

Inorganic pigments, such as terra di Siena naturale, yellow ochre, yellow cadmium, red cadmium, red Indian, land of natural shade (raw umber), scorched shadow earth (burnt umber), black ivory, ultramarine, cerulean, chromium oxide (III), and emerald, were purchased from F.lli Maimeri S.A. (Bettolino di Mediglia, 20076, Mediglia, Italy) and used without further purification; organic pigments, such as safranin, scarlet red R, carmine, phloxine B, carmine Naccarat, vesuvine, Dahlia, cresyl fast violet, galloxyanine, cochineal, and crocein scarlet were purchased from Carlo Erba (Milan, 20010, Italy). White papers (Fabriano, 20135, Milan, Italy), chalk, rabbit glue, and paint brush (Antichità Belsito, 00136, Rome, Italy) were purchased without further modification; all chemical reagents were acquired from Sigma-Aldrich (Via Monte Rosa, 93, 20149 Milan, Italy) and were used without further modifications.

2.2. Plant Material

Fresh cladodes of **OS** were harvested in Isola delle Femmine, Sicily, (38°12'07" N, 13°14'40" E, 9 m.s.l.) Italy, in April 2023. Cladodes of **OF** were harvested in Alimena, Sicily, (37°42'03" N 14°07'12" E, 750 m.s.l.) Italy, in March 2023. Authentication was conducted by Prof. Francesco Sottile, and vouchers have been deposited in the STEBICEF Department, University of Palermo (PAL113518 and PAL113560).

2.3. Extraction of Mucilage from OF and OS

Several cladodes of **OS** (1000 g) and **OF** (1000 g) were used for the extraction of mucilage. The cladodes were washed and, after the removing of spines, were cut into small pieces and infused in water (2 L) at room temperature for 24 h in order to facilitate the release of the mucilage in dispersion. The mucilage formed was removed using a cotton cloth to remove plant residues. The extraction was repeated three times. The viscous filtrates were pooled together (about 6 L). A total volume of 50 mL of both dispersions were freeze-dried to present a yield of 2.55 and 1.04%, respectively, for **OS** and **OF**. A total volume of 1 L of single mucilage dispersions were concentrated under reduced pressure at 40–50 °C using rotavapor in order to obtain the same concentration (9%) for both solutions.

2.3.1. Chemical Hydrolysis of Mucilage

In order to characterize the mucilage, it was necessary to identify the monosaccharides that compose it. An aliquot of both mucilage (25 mg) was hydrolyzed by adding 2.5 mL of 2 M H₂SO₄ and maintained at a temperature of 80 °C for four hours. After hydrolysis, the solution was neutralized by adding 14 M NH₄OH. The solution was freeze-dried and then methanol was added to extract the simple sugars obtained via hydrolysis. The methanolic solution was filtered through silica gel, evaporated, and the remaining solid was solubilized in D₂O for NMR analysis.

2.3.2. NMR Analysis

The NMR spectra of the two hydrolyzed mucilage were acquired in heavy water (D₂O) using the Bruker Avance II spectrometer, present at the CGA (Centro Grandi Apparecchiature) of the University of Palermo, operating at a frequency of 100 MHz for the acquisition of ¹³C-NMR. The chemical shifts are reported relative to the carbonyl of the acetone residual peak, added as internal standard (215.94 ppm for ¹³C-NMR).

2.4. Preparation of Samples

The study carried out included the use of **OF** and **OS** mucilage solutions, used at the same concentration (9%) as a binder of organic and inorganic pigments for the creation of a new prototype of painting colors. Every single pigment, accurately weighed (0.15 g), was finely ground in a glass mortar with 1 mL of each mucilage solution. Finally, the obtained tempera were manually applied with the Winsor & Newton brush on square chromatic fields (2 × 2 cm), and left to dry for 48 h, on paper and chalk supports.

2.5. Colorimetric and Statistical Analyses

Color parameters of samples were measured using a colorimeter (NH300 Colorimeter, 3NH Shanghai Co., Ltd.) and elaborated with CQCS3 software for data acquisition. L* (lightness), a* (red-green), and b* (yellow-blue) parameters were measured and compared for the same pigment dispersed in the two different binders. In order to compare them, the total color differences (ΔE) was calculated [41]. In order to make an average estimation on the sample surface, we carried out three replicated color measurements. The average values are provided in the discussions section and the relative standard deviation was constantly below 5% of the obtained values.

3. Results and Discussion

3.1. Chemical Composition of OF and OS Mucilage

Numerous extraction techniques have been developed and largely improved over the years to facilitate the extraction of mucilage, trying to increase its yield by increasing its use and application at an industrial level. Classic extraction mainly involves methods that exploit the action of different solvents more or less similar to the chemical–physical characteristics of mucilage, but innovative techniques, such as ultrasound-assisted (UAE) and/or microware (MAE) extraction have proven to be widely useful and advantageous for isolating mucilage in good quantities [27]. Some studies have shown that conventional extraction techniques can provide a good yield of extracted mucilage [42] and that in some cases it can be superior to the results obtained with non-conventional methods [43].

In this scientific work, a conventional mucilage extraction method was used. This method was chosen mainly because it is simple and easy to implement and is economical; since it does not require robust equipment, it does not require the use of solvents that are harmful and toxic to humans and the environment.

With the aim to determine the chemical composition of the mucilage, an aliquot of both mucilage dispersions was subjected to acidic hydrolyses [44] to provide the single hydrolyzed monosaccharides mixture. The solid residual was dissolved in D₂O and analyzed via ¹³C-NMR spectroscopy. In particular, the range of chemical shifts between 90 and 100 ppm, typical of the anomeric carbons of monosaccharides, was observed (Figures 1 and 2).

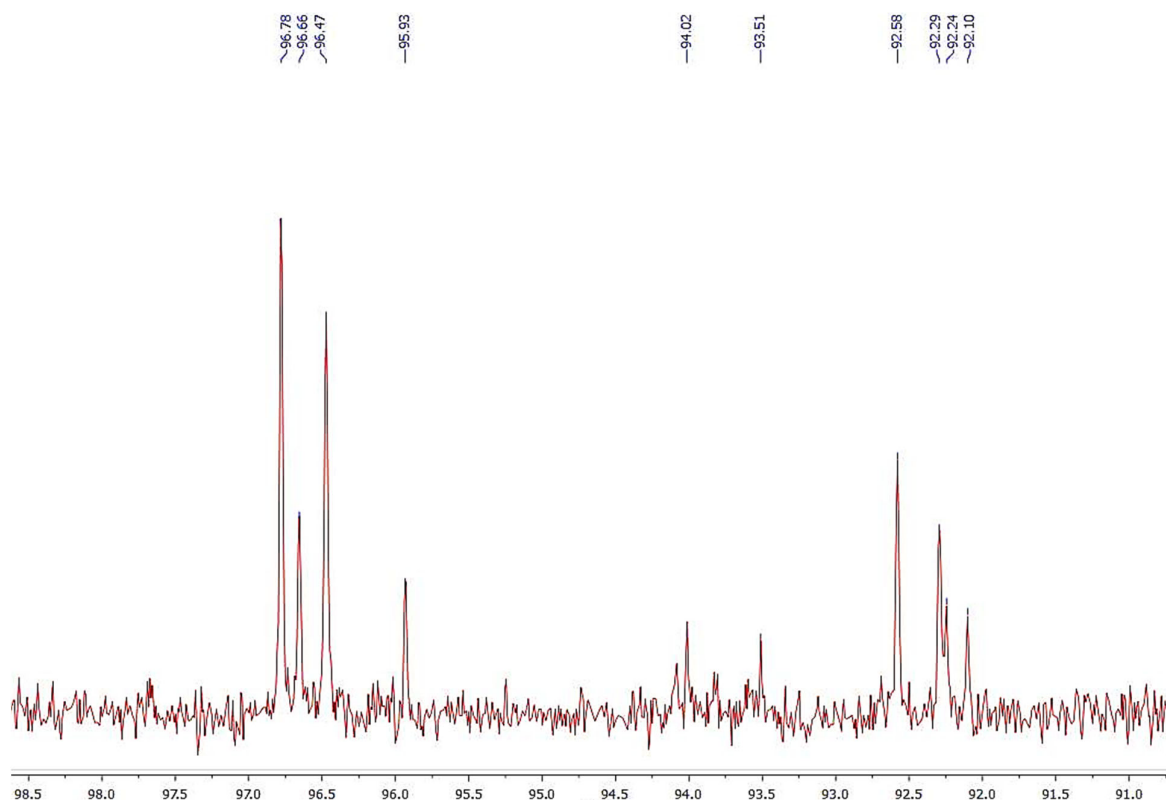


Figure 1. ¹³C-NMR spectrum (D₂O) of the anomeric carbons of the sugar composing the *O. stricta* (OS) mucilage.

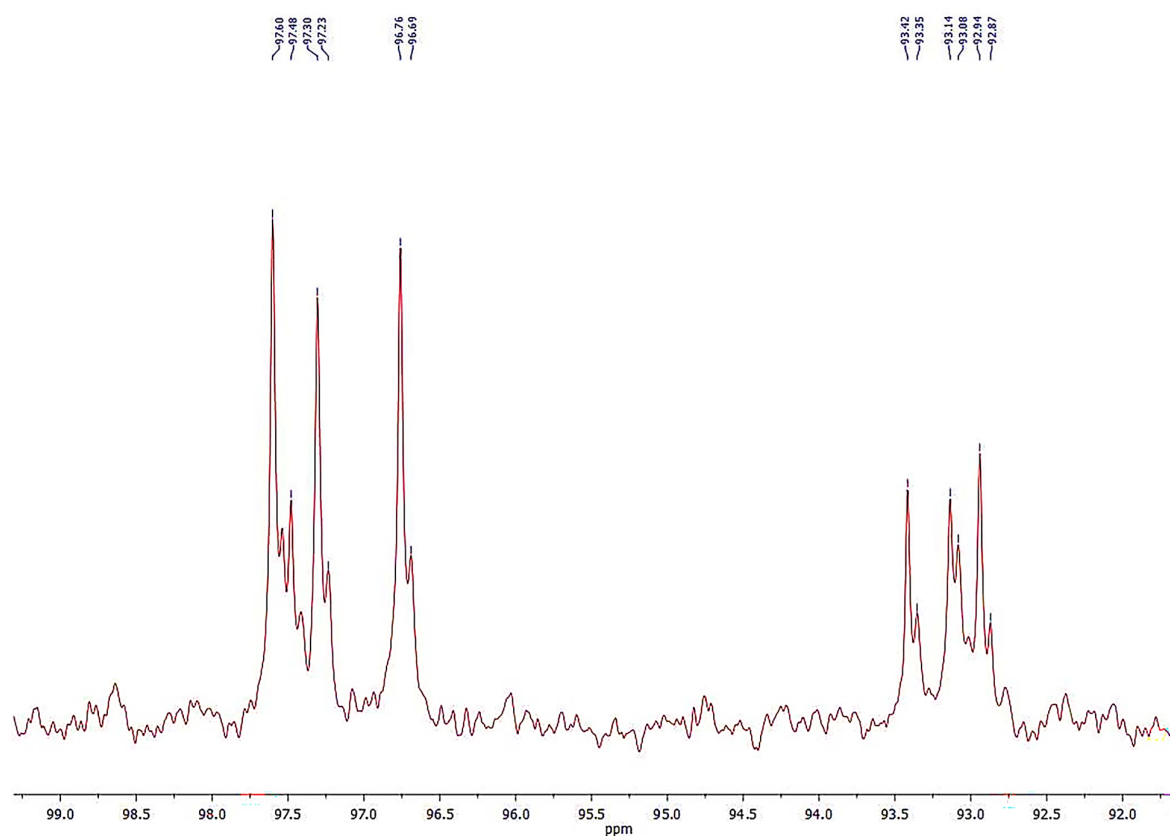


Figure 2. ^{13}C -NMR spectrum (D_2O) of the anomeric carbons of the sugar composing the *O. ficus indica* (OF) mucilage.

The comparison with the data reported in the literature [45] allowed for the identification of the monosaccharides present in the mixture. The signals observed within the spectra indicate both anomers of the single sugars detected in the hydrolyzed mucilage. By comparing the area of the peaks, it was possible to quantify the percentages of sugars present in both mucilage (Table 1). The main sugars occurring in the OS mucilage were arabinose (36.48%) and galactose (32.31%), followed by minor amounts of xylose (15.36%), glucose (10.45%), and rhamnose (5.40%). The only other paper reporting the composition of OS mucilage concerned an Indian accession of this plant [19]. The results of this analysis, carried out via gas–liquid chromatography of the alditol acetates, were similar but not identical with respect to the results of the Sicilian accession. In fact, although the main sugars identified were arabinose (38.80%) and galactose (33.00%), the other three sugars occurred in different amounts (rhamnose (15.70%), xylose (5.10%), and glucose (5.10%)). Similar quantities of arabinose, galactose, and glucose were instead identified in the mucilage of OF.

Table 1. Sugars composing the *O. stricta* (OS) and *O. ficus-indica* (OF) mucilage and ^{13}C -NMR value of the anomeric carbons.

Sugar	ppm	Sugar	ppm	Area (%) OS	Area (%) OF
α -arabinose	97.6	β -arabinose	93.4	36.48	23.65
β -fucose	97.2	α -fucose	93.1	-	11.74
β -galactose	97.3	α -galactose	93.2	32.31	20.87
β -glucose	96.8	α -glucose	93.0	10.45	23.89
β -glucuronic acid	96.7	α -glucuronic acid	92.9	-	8.88
α -rhamnose	94.9	β -rhamnose	94.4	5.40	-
β -xylose	97.5	α -xylose	93.1	15.33	10.96

A scientific study has evaluated the impact of seasonality and age on the sugar content of eight specimens of **OF**. From the data collected through HPLC-MS analysis, the presence of sugars similar to that reported in Table 1 has been verified, confirming, also in this case, the absence of neutral rhamnose [46]. However, it should be underlined that other authors report the massive presence of D-galactose and L-arabinose residues in **OF** mucilage [47], while other authors confirmed the presence of the latter two sugars accompanied by the chemical detection of glucuronic acids and rhamnose residues [48].

Unlike **OS**, **OF** mucilage also exhibited characteristic peaks of the two α - and β -isomers of fucose and glucuronic acids (11.74 and 8.88%, respectively). Finally, a minor presence of xylose (10.96% in **OF** than 15.33% in the **OS** mucilage) and the absence of rhamnose were detected in **OF**.

3.2. Colorimetric Analysis

Upon observing the samples made using pigments dispersed in both **OF** and **OS** binders, it was noticed how the same pigment changed its appearance, notably affected by binder changing. For this reason, a colorimetric analysis was conducted in order to obtain data to measure the change in appearance depending on the binders used, but also on the material when the color is laid. The parameters were measured using a portable colorimeter (NH300 Colorimeter, 3NH Shanghai Co., Ltd., Shanghai, China) and the CQCS3 software for data acquisition. L^* (lightness), a^* (red-green), and b^* (yellow-blue) were the variables measured and compared between the different binders and material supports. The total color differences (ΔE) were calculated using the following formula [41,49]:

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

From the data reported in Tables 2–5, it can be observed that, for most cases, pigments show high values of ΔE , changing the binder and material of the support in which it is laid. Since a ΔE value of 2.3 up to 4.0 corresponds to a noticeable difference according to human eyes, it is clear that these data match, albeit with a huge difference, in the appearance (Figures 3A and 4B), reaching a peak of $\Delta E = 31.0$ for cresyl fast violet pigment dispersed in **OF** and **OS**, laid on chalk support. These results emphasize the reported differences between pigments, which were immediately visible when laid in the supports (Figures 5A and 6B): changing binders, colors reveal distinct shades, brightness, and texture. Regardless of the support used, pigments exhibit a deeper color when dispersed in **OF**, with a full-bodied texture unlike the ones in **OS**, sometimes tending to transparency (Figure 4B).

Table 2. ΔE of inorganic pigments stretched upon a paper support.

Pigment	ΔE of OF and OS Samples *	ΔE of OF Samples Before and After Ageing *	ΔE of OS Samples Before and After Ageing *
Terra di Siena	5.1	0.5	3.6
Yellow ochre	4.4	0.1	6.9
Cadmium yellow	2.5	2.5	4.1
Cadmium red	16.8	0.7	2.7
Indian red	1.4	0.9	5.8
Raw umber	10.8	7.2	9.5
Burnt umber	15.2	0.5	15.8
Ivory black	3.9	1.9	10.8
Ultramarine blue	5.4	2.8	6.4
Cerulean blue	1.8	0.4	12.3
Chromium oxide	18.4	4.4	5.8
Emerald green	2.7	0.8	6.7

* Error was estimated for each sample by calculating a standard deviation no more higher than 5%.

Table 3. ΔE of organic pigments stretched upon a paper support.

Pigment	ΔE of OF and OS Samples *	ΔE of OF Samples Before and After Ageing *	ΔE of OS Samples Before and After Ageing *
Safranin	23.3	2.5	9.6
Scarlet R red	12.2	1.7	13.1
Carmine	4.1	3.4	9.5
Phloxine B	13.4	4.7	12.6
Carmine Naccarat	10.7	3.9	15.3
Vesuvine	12.1	3.9	8.7
Dahlia	21.0	4.5	16.6
Cresyl fast violet	27.0	3.9	15.2
Gallocyanine	3.9	2.2	15.5
Cochineal	4.2	7.9	8.3
Crocein scarlet	8.1	2.3	10.4

* Error was estimated for each sample by calculating a standard deviation no more higher than 5%.

Table 4. ΔE of inorganic pigments stretched upon a chalk support.

Pigment	ΔE of OF and OS Samples *	ΔE of OF Samples Before and After Ageing *	ΔE of OS Samples Before and After Ageing *
Terra di Siena	4.2	0.7	2.5
Yellow ochre	4.6	0.2	5.6
Cadmium yellow	2.2	3.1	3.6
Cadmium red	17.3	1.7	6.8
Indian red	1.1	2.9	3.4
Raw umber	11.2	5.4	8.1
Burnt umber	16.1	2.9	14.4
Ivory black	5.6	1.9	11.4
Ultramarine blue	4.7	3.1	5.3
Cerulean blue	2.8	0.5	13.8
Chromium oxide	17.6	3.7	5.1
Emerald green	3.8	0.6	6.4

* Error was estimated for each sample by calculating a standard deviation no more higher than 5%.

Table 5. ΔE of organic pigments stretched upon a chalk support.

Pigment	ΔE of OF and OS Samples *	ΔE of OF samples Before and After Ageing *	ΔE of OS Samples Before and After Ageing *
Safranin	20.0	1.4	9.8
Scarlet R red	10.6	0.9	13.5
Carmine	4.2	4.5	8.4
Phloxine B	12.9	3.9	13.1
Carmine Naccarat	8.9	4.1	15.6
Vesuvine	12.9	5.0	9.0
Dahlia	20.0	3.0	17.9
Cresyl fast violet	31.0	4.0	12.1
Gallocyanine	3.6	2.0	15.4
Cochineal	2.9	5.4	9.6
Biebrich scarlet	7.4	1.9	11.1

* Error was estimated for each sample by calculating a standard deviation no more higher than 5%.

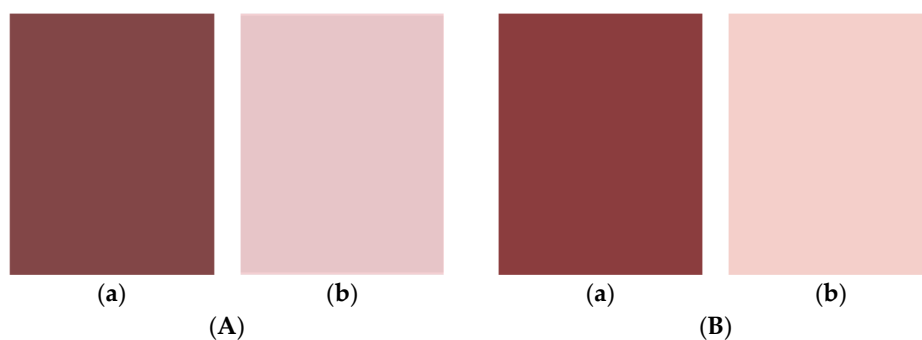


Figure 3. (A) Safranin pigment dispersed in OF (a) and in OS (b) laid in a paper support, produced using CQCS3 software. (B) Safranin pigment dispersed in OF (a) and in OS (b) laid in a chalk support, produced using CQCS3 software.

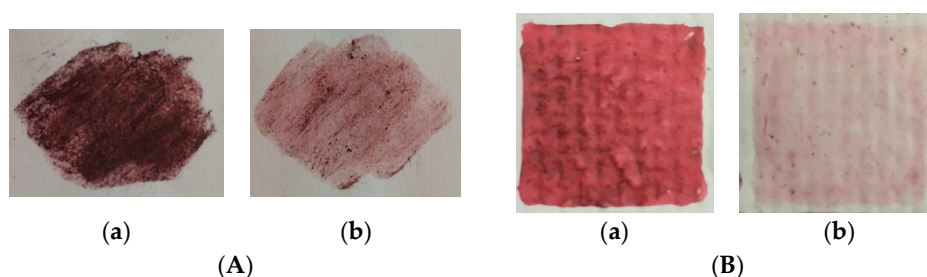


Figure 4. (A) Safranin pigment dispersed in OF (a) and in OS (b) laid in a real paper support. (B) Safranin pigment dispersed in OF (a) and in OS (b) laid in a real chalk support.

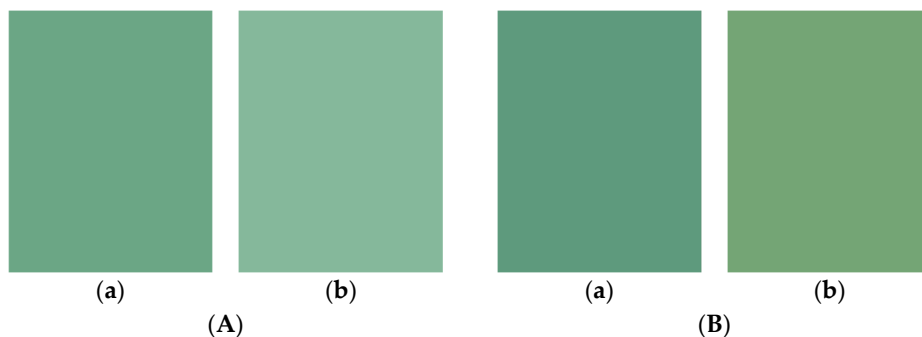


Figure 5. (A) Emerald green pigment dispersed in OF (a) and in OS (b) laid in a paper support, produced using CQCS3 software. (B) Emerald green pigment dispersed in OF (a) and in OS (b) laid in a chalk support, produced using CQCS3 software.

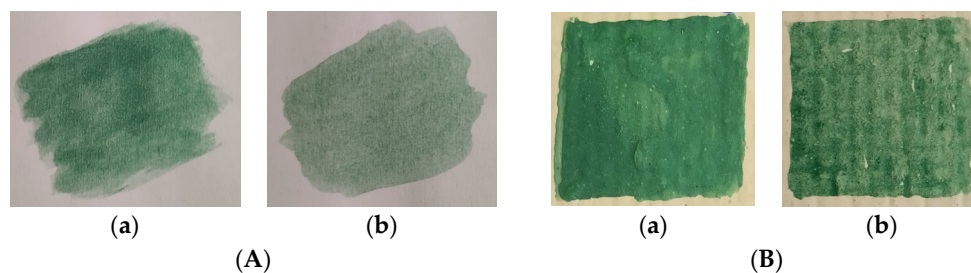


Figure 6. (A) Emerald green pigment dispersed in OF (a) and in OS (b), laid in a real paper support. (B) Emerald green pigment dispersed in OF (a) and in OS (b), laid in a real chalk support.

Comparing the two binders, it is also evident how ΔE data for organic pigments are higher than those for inorganic ones, equally on paper or chalk support. In this regard, it

has come to our attention that the cresyl fast violet ΔE values (27.0 and 31.0, respectively, laid on paper and chalk supports) were the highest data for the organic category, while for inorganic pigments, the highest ΔE values were for chromium oxide (III) pigments (18.4 and 17.6, respectively, on paper and chalk supports).

With regard to ageing, after 3 weeks of UV exposure, each sample showed aesthetic changes with a ΔE in no cases lower than 2, unlike the **OF** samples, which did not show aesthetic changes perceivable by the human eye (Figure 7A,B). In general, the **OS** samples had higher ΔE values than **OF** ones, no matter the support in which they were laid. Moreover, for most cases, ΔE indices after ageing for organic pigments were mainly higher than inorganic ones. Between paper and chalk samples before and after ageing, for inorganic pigments, the highest ΔE value is 15.8 (burnt umber laid on paper), while taking into account organic pigments, it registered a maximum ΔE of 17.9 (Dahlia laid on wood); in both cases, the worst ageing is reached with **OS** as the binder. These data are reflected in a visible change in color, evident for burnt umber, but more marked for Dahlia (Figures 8A and 9B).

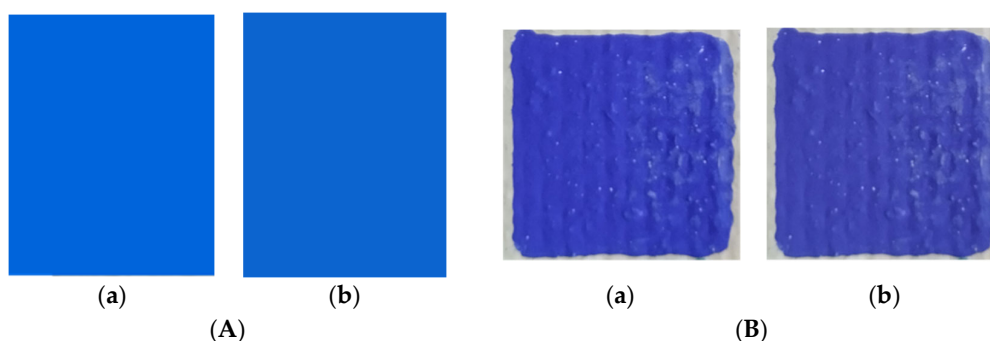


Figure 7. (A) Ultramarine blue pigment dispersed in **OF**, laid in a chalk support before (a) and after (b) ageing, produced using CQCS3 software. (B) Ultramarine blue pigment dispersed in **OF**, laid in a chalk support before (a) and after (b) ageing.

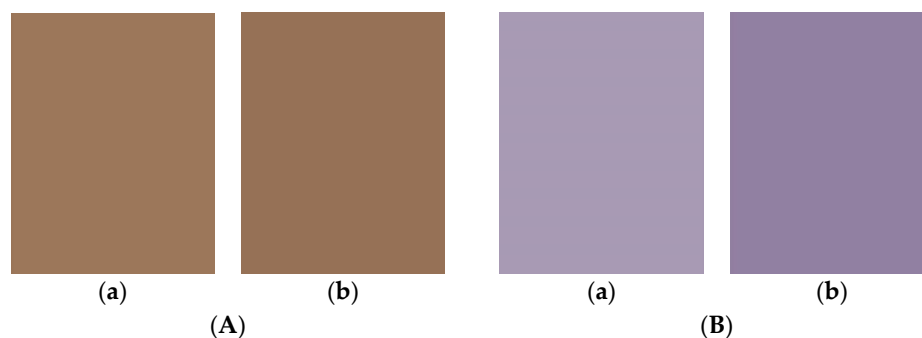


Figure 8. (A) Burnt umber pigment dispersed in **OS**, laid in a paper support before (a) and after (b) ageing, produced using CQCS3 software. (B) Dahlia pigment dispersed in **OS**, laid in a chalk support before (a) and after (b) ageing, produced using CQCS3 software.

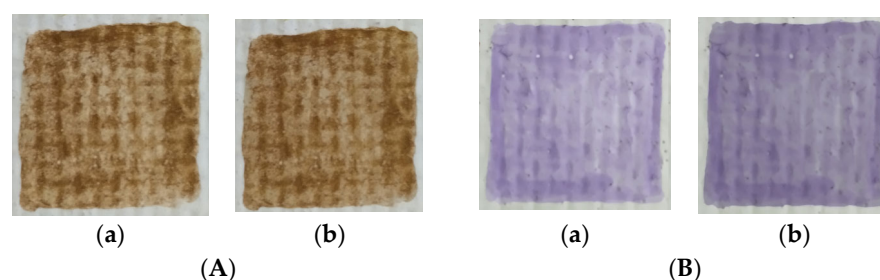


Figure 9. (A) Burnt umber pigment dispersed in OS, laid in a real paper support before (a) and after (b) ageing. (B) Dahlia pigment dispersed in OS laid in a real chalk support before (a) and after (b) ageing.

In artistic objects, dyes, natural or synthetic pigments, and polyxaridic (gum Arabic, starch) and/or protein (gelatin, egg white, etc.) binders are mixed together [39]. The scented binder significantly influences the final shade as well as stability over time. Vermeulen and colleagues [39] highlighted how the stability of arsenic sulfide varies depending on the binder chosen; gum Arabic, for example, allowed maximum temporal stability of the final dye, compared to the use of the egg in its different parts. Effects on stability were also confirmed by a study using carminic dyes. Haberova et al., in effect, have deduced, through the aid of parallel techniques (UV-Vis, FT-IR, GC-MS, and HPLC-MS techniques), the influence of albumin and gum Arabic on the thermal stability of final tempering, but not on the colorimetric parameters [50].

In the case reported here, the OF mucilage tends to make the dyes brighter when used. This can be interpreted as a greater presence of free methyl groups than the OS mucilage. The fucose units contain a $-CH_3$ group linked to position 5, which probably allows for a greater solubilization of the pigments used, compared to the more polar OS mucilage, also due to the presence of glucuronic acid units. However, in general, colorimetric analysis did not present significant differences in data between samples laid on paper or chalk support; this means that the material support did not considerably affect the aesthetic appearance of the color samples.

4. Conclusions

The use of natural by-products requires particular attention both for the concepts related to the circular economy and for the possible physical–chemical interferences related to their application. For this reason, in this work, with the support of the relative chemical and physical analyses, an attempt has been made to identify new possible binders to be exploited in the pictorial technique of tempera. In particular, the mucilage extracted from *Opuntia ficus-indica* (L.) Mill. and *Opuntia stricta* (Haw.) Haw. were used as new binders. The monosaccharic units of the two mucilage were identified by means of the ^{13}C -NMR spectroscopic technique, highlighting their compositional differences—one mainly characterized by a high amount of arabinose (36.48%) and galactose (32.31%), while the other diversified by almost the same presence of arabinose (23.65%), galactose (20.87%), and glucose isomers (23.89%). The different pigments were dispersed in both mucilage (OF and OS), highlighting, after colorimetric analysis, enormous aesthetic differences: the pigments dispersed in OS, in fact, witnessed a greater aesthetic change, clearly visible to the naked eye, compared to those in OF. In general, inorganic pigments performed better toward mucilage, recording negligible changes in ΔE before and after aging. No significant difference was found in the data between the samples laid on paper or chalk supports; this means that the material support did not significantly influence the aesthetic appearance of the color samples. It can be concluded that OF is suitable as a binder for pigments, producing colors with a deep shade, full brightness, full-bodied texture, and is resistant to UV ageing.

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