

Stone sustainable protection and preservation using a zein-based hydrophobic coating

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ABSTRACT

In the present work, the efficiency of an ecofriendly 5% (w/v) solution of zein in DMSO was evaluated as protective material for stone. The solution was sprayed on $5 \times 5 \times 1$ cm slabs of Serena stone, a sandstone widely used in Florentine architecture, in order to create a hydrophobic coating. The obtained coating turned out to be hydrophobic, showing WCA of around 120° , while a reduction of 8.4% in water uptake was observed during 15 days. SEM and profilometer analyses stated that the zein coating application resulted in the formation of a compact film on the stone's surface with a continuous structure characterized by micro-scale roughness. XPS analysis confirmed the presence of the thin conformal layer over the stone. A mechanism of hydrophobic surface formation, in which both the roughness and the solvent's evaporation style are believed to play a significant role, was finally proposed. Accelerated aging tests indicated a good resistance to aging in terms of hydrophobicity and color variations, where the overall color parameter of $\Delta E < 2.0$ was maintained, indicating invisible color changes to the naked eye. This sustainable approach can be easily scaled and may represent a valuable alternative to the most common synthetic treatments used nowadays in stone protection.

1. Introduction

Synthetic polymers, such as poly acrylates, siloxanes and fluorinated polymers, have been extensively applied as protective coatings for stone surfaces in cultural heritage mainly because of their excellent water repellent and optical clarity properties [1–9]. Nevertheless, degradation due to prolonged UV-light exposure, humidity, extreme temperature variations, etc., can induce unwanted cross-linking and/or chain scission reactions resulting in reduced protection, yellowing or detachment of the polymeric layers [10–16]. The addition of inorganic silica (SiO₂) and titanium dioxide (TiO₂) nanoparticles to silicon-based polymers was also tested in order to enhance the coating properties and durability [3,17–20]. Nevertheless, essential issues are still pending regarding the effectiveness, long-term stability and impact of nanoparticles on human health and environment [21].

Recently polymers from natural sources have attracted interest as

protective materials for stones, as sustainable alternatives to the synthetic coatings, but also due to the possibility of easier removal from the stones' surface. Up to now, the protection properties of zein, chitosan, poly(lactic acid) (PLA), poly(3-hydroxybutyrate) (PHB) [22,23], poly(hydroxyalkanoate)s (PHAs) [24], and fungal hydrophobins [25] have been investigated. However, research in this field is still in a preliminary stage.

Zein is an amphiphilic prolamine extracted from corn endosperm and represents about 80% of proteins in corn. It has an amphiphilic character due to its unusual amino acid sequence, which contains over 50% hydrophobic residues, including high percentages of leucine (20%), proline (10%) and alanine (10%). The hydrophilic component is due to the relatively high content (21%–26%) of glutamine [26]. So far, zein has been mainly applied as renewable and biodegradable material for food coating, pharmaceutical and biomedical applications [27,28]. Zein is insoluble in water but soluble in ethanol/water and acetic acid/water

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mixtures [29,30]. Recently dimethyl sulfoxide (DMSO) was also used to dissolve zein, and a robust zein film was obtained [31]. DMSO is able to solubilize a wide range of polymers and is miscible with all common solvents, including water. Furthermore, this solvent is recyclable and readily biodegradable [32–34]. Therefore, zein can be easily processable with environmental-friendly, nonhazardous chemicals, an increasing requirement for the materials used in the field of cultural heritage and not only [35].

In the present work, the effectiveness of a protective zein coating applied on sandstone slabs by spray coating of a 5% (w/v) solution of zein in DMSO was evaluated. Zein was tested as protective layer on Serena stone, a sandstone very commonly used as ornamental stone in historical buildings. Like limestone, sandstone can undergo several degradation processes regulated by intrinsic (such as mineralogical-petrographic characteristics and physical properties of the stone itself) and external factors (such as the conservation environment and pollution) [36–38]. Protective strategies are especially required to preserve sandstone from the action of water, ensuring at the same time the need to maintain compatibility with the support (avoiding significant water vapor permeability changes and color alterations), and to guarantee durability and possibility of retreatment of the stone surface [24,39]. Considering that, the type of the used solvent, the application method, the evaporation rate and the concentration of zein in the initial solution strongly affect the wettability of the resulting protective layer, specific efforts were put in obtaining a hydrophobic coating with careful tuning of all the above parameters [29,40,41]. Finally, a specific mechanism for the formation of the hydrophobic protective zein surface is proposed.

2. Materials and methods

2.1. Preparation of the stone samples

In this study, Serena stone, a fine-medium grain sandstone characterized by a grey-bluish color, was chosen to investigate the protective performances of a zein-based protective coating. It is mainly made of quartz feldspars and phyllosilicate grains and white micaceous lamellae (muscovite) with minor amounts of siliciclastic rock fragments, calcite and dolomite [3,42]. Porosity of Serena stone was measured by helium pycnometry and mercury intrusion porosimetry (MIP), performed with Pascal 140 Evo and Pascal 240 Evo mercury porosimeters (Thermo Fisher Scientific) [43,44]. Porosity was found 3.11%, and a bimodal pore size distribution was measured, with median pore size in the ranges 0.03–0.02 μm and 6.30–3.98 μm .

The stone was quarried from the district of Fiorenzuola; 5 cm \times 5 cm \times 1 cm slabs were cut and polished with 280-grit silicon carbide paper. Samples were then washed in deionized water, dried in oven at 60 °C and kept in a desiccator to bring them to a constant weight prior to use.

2.2. Preparation and application of the coating

Zein powder (Z3625, Sigma-Aldrich, Inc. USA) was washed in ethanol (99.7 Sigma-Aldrich, Inc. USA) following the procedure illustrated by De Boer et al. [45] with the aim to remove the colored impurities. Zein (10 g) was stirred in ethanol (1.5 L) overnight and the suspension was left to sediment by gravity. The liquid phase, containing most of the impurities, was discarded, while the collected sediment was dried and used to prepare a 5% (w/v) solution of zein in DMSO. The mixture was maintained under stirring for 30 min at room temperature and any insoluble aggregates were removed by filtration using a 0.45 mm filter (PVDF, Millipore). UV-visible absorption spectra of purified and unpurified solutions were collected using a Cary JEOL UV-spectrophotometer in order to verify the purification process.

Particle size distributions of both purified and unpurified solutions were also analyzed by Dynamic Light Scattering (DLS) with a Zetasizer Nano S Malvern Panalytical Instrument.

Serena stone slabs were coated by zein 5% (w/v) solution using a

spray coater (Paasche Airbrush VL with 73 mm head and 1.06 mm tip). The spray coating application method should allow a thin, conformal coating formation that does not change the physical characteristics of the stone. A total of 3 mL of solution was sprayed on each slab surface (5 cm \times 5 cm). The quantity of sprayed coatings was kept constant by controlling both spray pressure (2 bar), application time (3 s) and spraying distance from the sample (40 cm). Samples were dried at room temperature under fume hood for 48 h and subsequently placed in oven at 60 °C for other 48 h, in order to remove any solvent from the coating.

2.3. Chemical characterization of zein coating

A Fourier Transform Infrared (FTIR) spectrometer (VERTEX 70v, Bruker) equipped with an ATR (attenuated total reflection) accessory (MIRacle ATR, PIKE Technologies) with a diamond crystal was used for the infrared spectra acquisition from the zein coatings. ATR-FTIR was applied to study the secondary structure of zein. All spectra were recorded in the range from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} , accumulating 64 scans. Three analyses were performed for each sample to ensure the reproducibility of obtained spectra.

ATR-FTIR spectrum of zein coating obtained by spray coating technique was compared with spectra of zein films obtained by solvent casting. The amide I region, between 1600 and 1800 cm^{-1} , was selected and a linear baseline was applied to the spectrum. Deconvolution was carried out as previously reported by Bicudo et al. [46] and Forato et al. [47]. Spectra were normalized with respect to the peak of amide I and second-order derivative analysis was performed on each spectrum using PeakFit 4.11 software [48] to confirm that each fitting peak position represented a real spectral signal from the samples.

X-ray photoelectron spectra were acquired before and after coating application by a SPECS XPS spectrometer, using a monochromatic Mg K α source operating at 12 kV and 7 mA and a pass energy of 90 eV for the survey and 30 eV for the high resolution. Sample charging was compensated by a flood of low-energy electrons, and energy scale calibration was performed setting the main carbon C 1s peak at 284.8 eV. The spectra were analyzed using CasaXPS software [49,50].

2.4. Morphological characterization of zein films on stone

SEM was used to analyze the micro-morphology of the samples before and after the zein application. SEM images were acquired using a JEOL JSM-6490LA (Japan), operating at 10 kV acceleration voltage. Prior to imaging, the samples' surfaces were sputtered with a 10 nm thick film of gold (Cressington 208HR sputter coater, UK). SEM images were collected at different magnifications [51]. Surface roughness of the stone substrates before and after the application of the coating was investigated by 3D Optical Profilometry. Different magnifications, ranging from 2.5 \times to 20 \times were used corresponding to a Z profile resolution ranging from 25.0 μm to 0.5 μm respectively [52]. Moreover, the adherence of the coating to the stone surface was evaluated by performing a peeling test using Scotch® Magic™ tape (3 M). The changes in stone surface morphology were observed by SEM. The test was carried out according to previously reported methods [53,54,55].

2.5. Effectiveness of zein as protective layer of stone: wettability, hygroscopicity and water vapor permeability

A contact angle instrument (OCAH-200 DataPhysics, Germany) was used to measure the wettability of the treated stone surface. Static water contact angle (WCA) was measured at room temperature (\sim 23 °C). A gas-tight 500 mL Hamilton precision syringe with blunt needle of 0.52 mm internal diameter was used to deposit milli-Q water droplets of 5 μL on the stone sample placed on a sample holder, with the testing surface placed in a horizontal position; 10 measurements were acquired on each sample surface [56]. Obtained values were averaged and standard deviation was calculated. The measurements were performed both before

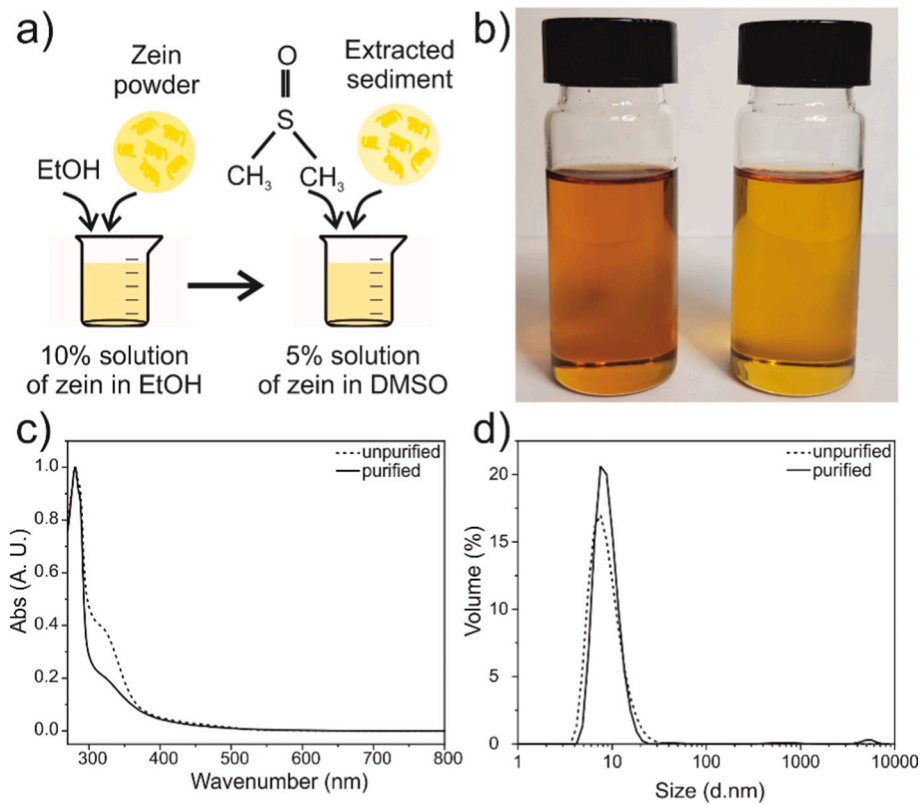


Fig. 1. a) Schematic representations of zein purification and preparation of the solution used for the spray coating. b) Photograph of DMSO solutions made with unpurified (left) and purified (right) zein. c) UV-Vis absorption spectra of unpurified (dot line) and purified (solid line) solutions of zein, d) DLS (dynamic light scattering) analyses performed on solutions of unpurified (dot line) and purified (solid line) zein powder.

and after the application of the treatments. WCAs were acquired also in continuous mode for 5 min (1 acquisition/s) to track the droplet behavior in time.

Water vapor uptake measurements were carried out both on untreated and treated stone. Dry samples weighed with the electronic balance (0.0001 g accuracy) were placed in the humidity chamber at 100% R.H. for 14 days, until stabilization of weight (daily measures); the amount of adsorbed water vapor was calculated based on the initial dry weight, according to the following formula:

$$\text{Water vapor adsorption (\%)} = \frac{m_f - m_0}{m_0} \cdot 100 \quad (1)$$

where m_f is the sample weight at 100% RH condition and m_0 is the sample weight at 0% RH.

Water vapor permeability (WVP) of the untreated and treated stone was determined at 25 °C and 100% RH according to the ASTM E96 standard method. [57] 100% humidity gradient was reached by placing 400 μ L of deionized water in the permeation chambers of 7 mm inner diameter and 10 mm height. In order to be able to place the samples in the chambers, 6 slices of stone 5.0 mm \times 5.0 mm \times 0.8 mm were cut, 3 of them were treated with the zein solution, while the other 3 were used as reference of the untreated stone. The samples were placed on the top of the permeation chambers and sealed. The chambers were placed in a desiccator, maintained at 0% RH by anhydrous silica gel desiccant at room temperature (20 °C and \sim 40% RH). The mass loss over time was registered by weighting samples every hour for 8 consecutive hours, with an electronic balance (0.0001 g accuracy). The water mass loss of permeation chambers was plotted as a function of time. The slope of each line was calculated by linear regression. The water vapor transmission rate (WVTR) was determined as below:

$$\text{WVTR} \left(\text{g}(\text{m}^2 \text{d})^{-1} \right) = \frac{\text{slope}}{\text{area of the sample}} \quad (2)$$

The water vapor permeability (WVP) of the samples was calculated as follows:

$$\text{WVP} \left(\text{g}(\text{mdPa})^{-1} \right) = \frac{\text{WVTR} \cdot L \cdot 100}{p_s \cdot \Delta RH} \quad (3)$$

where: L(m) is the thickness of the sample, measured with a micrometer with 0.001 mm accuracy; ΔRH (%) is the percentage relative humidity gradient; and p_s (Pa) is the saturation water vapor pressure at 25 °C.

2.6. Stability of zein coating by artificial aging

Treated and untreated stones were placed in a Memmert Climate chamber with three cold light fluorescent lamps (D65, 6500 K) and two UV lamps (320–400 nm), at 25 °C and 60% R.H., simulating an accelerated aging of 7.5 Mlxh (million lux hours) [12,24,56]. A four-week aging was carried out. After aging, samples were subjected to static contact angle measurement and to colorimetric analysis in order to evaluate both protective effectiveness of treatment and color changes [58,59].

According to NORMAL 43/93 [60], total chromatic variation upon application of the product was evaluated using a Konica Minolta CM 2600d spectrophotometer with a small area view (SAV) of 3 mm diameter, the 10° detector and the D65 primary source in the CIELAB system. L^* , a^* , b^* chromatic parameters were obtained, where L^* is the brightness, a^* is the red/green color component (positive for red and negative for green), and b^* is the yellow/blue component (positive for yellow and negative for blue), respectively [61,62].

The total color change ΔE^* was calculated for each sample, according

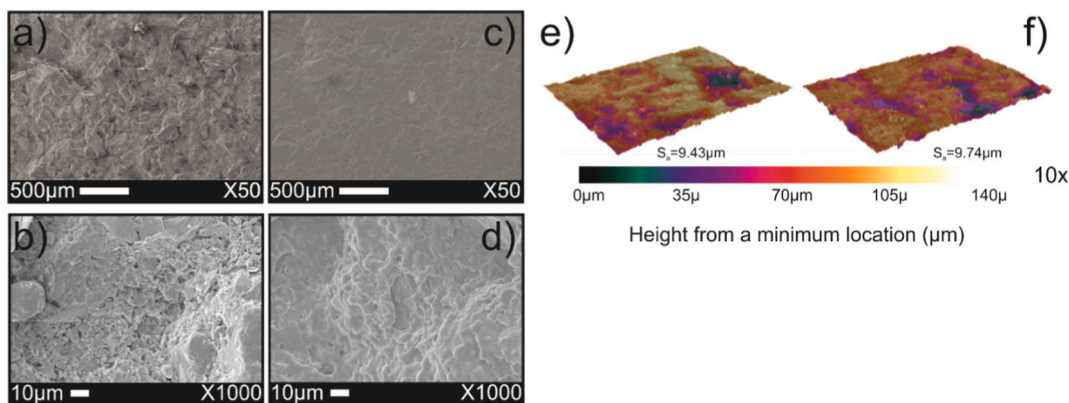


Fig. 2. SEM images of (a,b) uncoated and (c,d) zein-coated Serena stone and optical profilometer topography: 3D surface images of Serena stone before (e) and after (f) the application of the zein 5% coating.

to the formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

The measured ΔE^* represents the deviation from the original value due to the presence of the coating. According to Delgado-Rodrigues and Grossi [63] a treatment maintaining the color unchanged, or at least under the threshold value of 3, is considered suitable to cultural heritage application.

The measurement points were localized by a reference spatial grid to ensure precise repeated measurements at the same points before and after the treatment as well as before and after the artificial aging.

2.6.1. Microbial colonization test

Considering the bio-based origin of the coating, we wanted to exclude any potential pro-vegetative effect on the treated stones. The effect of zein on microbial colonization was assessed by a semi-quantitative method observing the growth of either *Agrocybe aegerita* (*Aae*) mycelium or naturally occurring microorganisms on the stone surface. Biological growth was classified as follows: – = no colonization observed on the stone; + = mild colonization on the stone; ++ to +++ = stone partially covered by colonies; ++++ = high colonization observed on the stone.

The mycelium from the fungal strain *Agrocybe aegerita* (*Aae*), was chosen as a test microorganism, considering its ability to grow even on inert substrates thanks to the outstanding oxidizing properties of its secretome (Patent No. IT102020000016798) [64]. Its growth in presence of the nutrient medium PDB was monitored for 2 weeks on both treated and untreated Serena stone, previously sterilized with UV radiation.

Square samples (5 cm side) of either treated or untreated Serena stone were placed in a glass jar (diameter 10 cm) filled with 30 mL of either Potato Dextrose Broth (PDB) or water. Jars and media were sterilized by autoclave, while UV radiation was used to sterilize the stone not to alter the zein film through the thermal process. Briefly, samples were exposed for 20 min to the radiation of a UV lamp with emission peak at 253,7 nm (U.V.C.). This wavelength is optimal to ensure sterilization by damaging microorganisms' nucleic acids (absorbance maximum at 260 nm), while minimizing the potential effects on zein (the UV absorbance spectrum of photosensitive aminoacids tryptophan and tyrosine shows a minimum around 250–260 nm and maximum at 280 nm) [65].

Samples were inoculated with a disk (8 mm diameter) of mycelium on the top surface of the stone and incubated for 15 days in climatic chamber at 78% RH and 27 °C. In parallel, control samples without the mycelium underwent the same process in order to monitor potential growth of naturally occurring microorganisms on stone. In this second

Table 1

Average roughness (S_a) and root mean square roughness (S_q) measurements obtained with 3D optical profilometer.

Sample	S_a ($\mu\text{m} \pm \mu\text{m}$)	S_q ($\mu\text{m} \pm \mu\text{m}$)
Treated	9.747 ± 0.7858	12.25 ± 0.9711
Untreated	9.434 ± 0.9748	12.36 ± 0.9346

set of experiments two samples (G and H) did not undergo the sterilization procedure to ensure that UV treatment was not modifying the zein coating. Two replicates were prepared for each sample.

For a semi-quantitative evaluation of the fungal colonization, samples were monitored on a daily basis [58,59,66].

3. Results and discussion

3.1. Preparation and purification procedure of zein

Zein powder was washed in ethanol (99.7%) as illustrated by De Boer et al. [45] with the aim to remove the colored impurities. Fig. 1a illustrates a schematic representation of the procedure followed for the preparation of the zein 5% (w/v) in DMSO formulation before spray coating of the stones. Fig. 1b shows the color differences between unpurified (left) and purified (right) zein solutions. In order to verify the effective purification of zein, the absorption spectra of solutions of unpurified and purified zein solutions were compared by UV–Vis spectroscopy (Fig. 1c). After zein purification, a lower absorption was observed in the range 300 to 500 nm, which indicates removal of impurities that absorb in that region of the electromagnetic (EM) spectrum. DLS (dynamic light scattering) analyses performed on solutions of unpurified and purified zein powder (Fig. 1d), showed that no significant changes in particle size in solution were detected.

3.2. Morphological and topographical evaluation of treated stone

SEM (scanning electron microscopy) observations of untreated stone showed the characteristic aspect of Serena stone, with many irregularities due to the presence of different minerals with a specific morphology (Fig. 2 a-b). In the treated samples, the presence of a layer of zein is detectable (Fig. 2 c- d). The application of the zein coating resulted in the formation of a compact film on the stone's surface with a continuous structure showing micro-scale roughness.

The roughness observed with SEM analysis was measured by optical profilometry. The topography of non-treated and treated samples was compared using 3D surface images, as shown in Fig. 2e, f (e not treated, f treated). The calculated average roughness (S_a) and root mean square roughness (S_q) parameters are reported in Table 1. No statistically

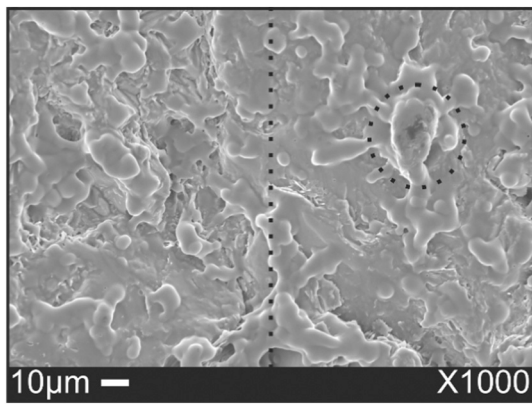


Fig. 3. SEM images of Serena stone after the peeling test. On the left side of the image, the non-tested surface is presented, on the right side, the corresponding tested surface is presented.

significant roughness differences were detected between the untreated sample and the treated one. This suggested that a conformal layer of zein was formed onto the stone's surface that did not mask the stone's intrinsic roughness.

Fig. 3 shows the SEM image acquired after peeling test. The removal of a very small amount of material from the sample surface was observed after peeling, indicating a satisfactory adhesion of the coating to the stone. This is confirmed by the slight loss of mass observed (1.8 ± 0.4 mg) after the tape removal.

3.3. Effectiveness of zein as protective coating for stone: wettability, hygroscopicity and water vapor permeability

Fig. 4a shows water contact angle (WCA) measurements over time

for untreated and zein-treated stone. A total absorption of the water droplets was observed for untreated stone within the first minute of the droplet deposition. When zein coating was applied on the stone, the just deposited water contact angles increased, with values varying between 100° and 120° , most likely depending on the roughness of the stone's surface underneath. Both Cassie-Baxter [67] and Wenzel [68] models explain how roughness can affect the wettability of a surface, and especially they describe how hydrophobicity increases with the surface roughness when the respective flat surface is hydrophobic [69]. The contact angle behavior shown in Fig. 4a is referred to the most hydrophobic areas where 120° were reached for the just deposited water drops.

In Fig. 4b the water drop volume reduction over time is also reported: the drop volume was reduced by 90% after 5 min from its deposition on untreated stone (absorption). In the case of treated surface the volume only decreased by 10% after 5 min (evaporation).

Fig. 4c shows the water vapor uptake at 100% RH (relative humidity) for untreated and treated stone. Due to intrinsic characteristic of Serena stone, the amount of water vapor absorbed by the untreated stone was not high (0.24% after 24 h); still, with the application of the coating a reduction of 8.4% in water vapor uptake was observed during 15 days.

The presence of zein led to a slight decrease ($18 \pm 4\%$) in water vapor transmission rate (WVTR), as shown in Fig. 4d, indicating that the natural vapor transmission process, necessary for the stone, is still allowed. Otherwise, moisture can be trapped, causing chemical or physical decay of the stone. The small decrease in the water vapor transmission can be ascribed to the low gas permeability of zein [70,71].

Thus, zein applied by spray coating seems to confer a good degree of water repellency to the stone. Water contact angle is comparable to the one obtained with other natural-based coating, such as poly(hydroxyalkanoate)s [24] or fungal hydrophobins [25]. Results obtained in terms of water repellency are even improved with respect to commercially available polysiloxanes coatings [72], which gave a water contact

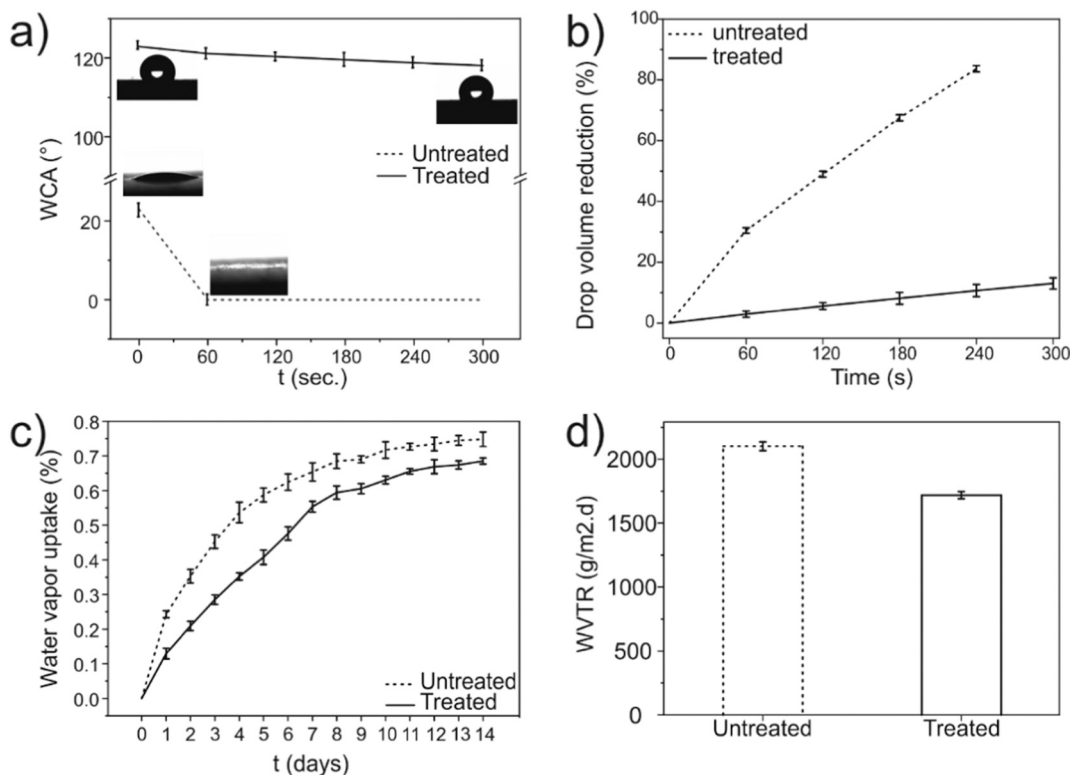


Fig. 4. Water-related properties of Serena stone before (dot line) and after (solid line) the treatment with zein solution. a) Water contact angle over time. Static WCAs are reported at 0 s, 60 s, 120 s, 180 s, 240 s and 300 s. b) Drop volume reduction over time (%). c) Water vapor uptake of untreated and zein-treated stone at 100% RH over time. d) Water vapor permeability of untreated and zein-treated stone.

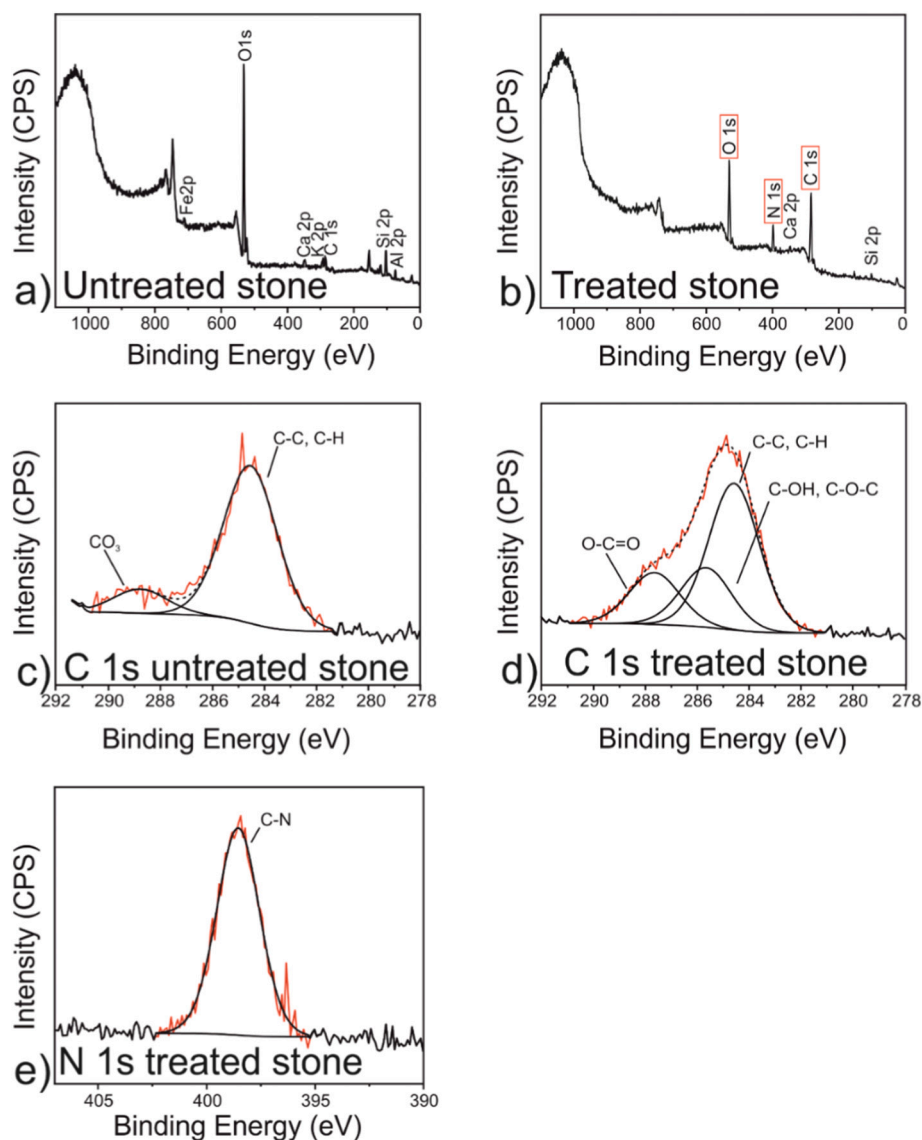


Fig. 5. Full XPS scanning spectrum of a) Serena stone untreated and b) Serena stone treated with zein coating. XPS high-resolution spectra of C 1s for c) untreated Serena stone and d) Serena stone treated with zein coating. e) XPS high-resolution spectra.

angle of 90–100°.

3.4. Chemical characterization of zein

3.4.1. Chemical characterization of coated stone

XPS (X-ray photoelectron spectroscopy) experiments were performed on treated and untreated stone in order to investigate the surface chemistry and the possible zein-stone chemical interactions. Fig. 5a and b show the full spectra of untreated Serena stone and Serena stone with zein coating respectively.

In the spectrum of Serena stone (Fig. 5 a) the characteristic elements of the stone were detected. Once the zein coating was applied, the peaks attributed to the stone disappeared, except for Si 2p and Ca 2p, whose intensity however decreased considerably. The treated Serena stone was found to be rich in C 1s (284.8 eV), O 1s (530.9 eV) and N 1s (398.5 eV) peaks. N 1s peak, in particular, is ascribed to the presence of a zein coating thick at least 10 nm.

The differences in elemental compositions (%) between treated and untreated stone obtained from the survey spectra are reported in Table 2. A higher percentage of C 1s was observed in treated stone, however, in order to better understand the origin of the peaks, the high-

Table 2

Surface chemical composition obtained through XPS analysis for treated and untreated Serena stone.

	Elemental composition (At.%)							
	C 1s	O 1s	N 1s	Si 2p	Ca 2p	Al 2p	Fe 2p	K 2p
Serena Stone	7.9	57.4	–	19.1	1.6	11.2	0.6	1.7
Serena Stone + coating	59.5	24.0	11.5	4.2	0.6	–	–	–

resolution XPS spectra of C 1s peak was acquired for untreated and zein-treated stone.

Fig. 5c and d show the high-resolution XPS survey spectra of untreated and treated stone respectively: the difference in the shape of the peaks is evident. In the high resolution spectra of C 1s of untreated Serena stone only two peaks were observed: the peak at 284.5 eV, related to the C–C and/or C–H (aliphatic carbons), due to the adventitious carbon, and a minor peak at 289.2 eV due to the CO₃ of the small amount of calcite present in the stone. C 1s peak of zein-treated stone, instead, was deconvoluted in three fitted peaks: the component located

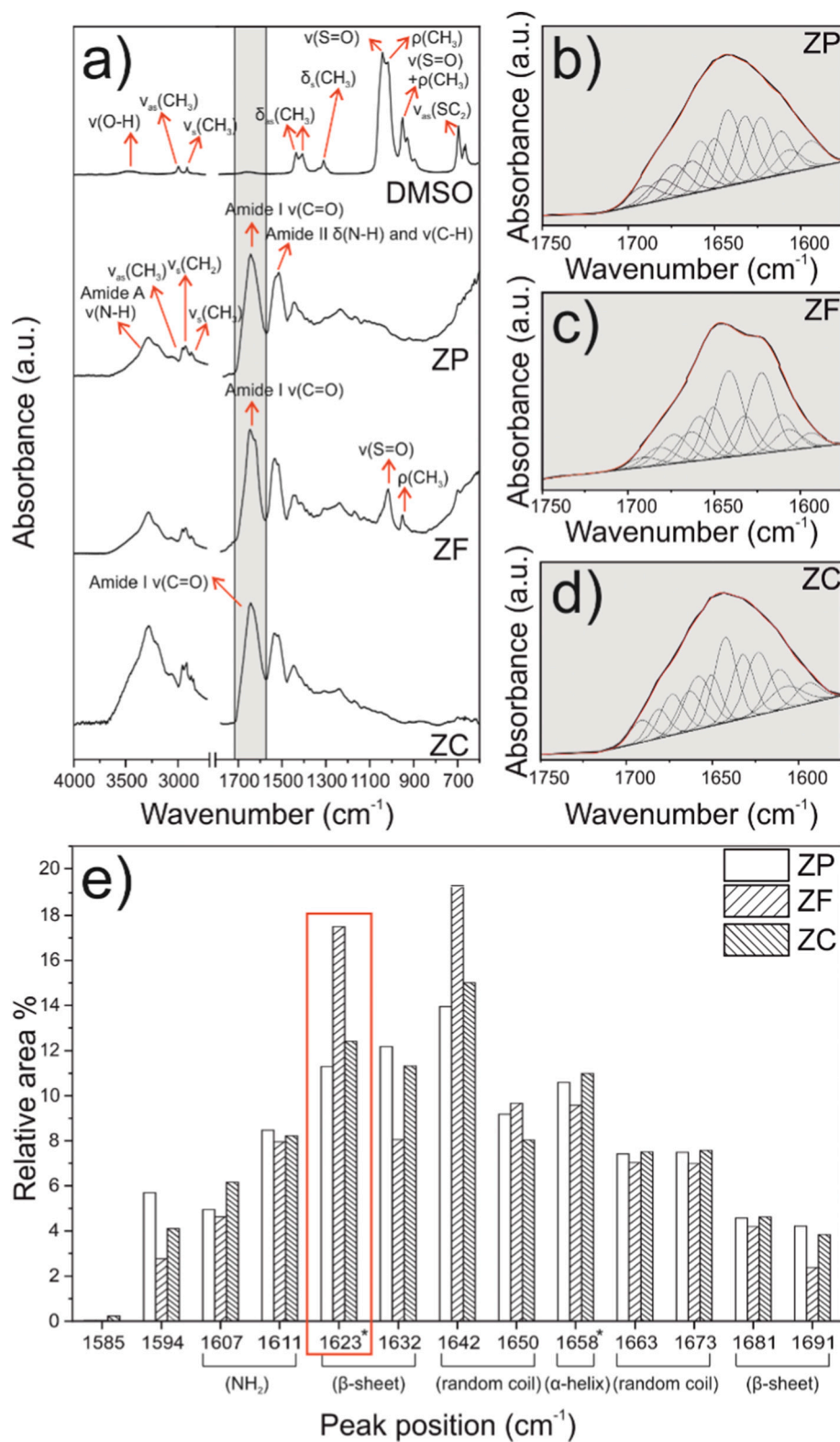


Fig. 6. a) ATR-FTIR spectra of DMSO, zein powder, zein film obtained by casting and zein coating obtained by spray coating in the 4000–600 cm⁻¹ region. Deconvolution of amide I peak (1750–1570 cm⁻¹) of b) zein powder c) zein film and d) zein coating. e) Changes in zein secondary structure content (%).

at 284.5 eV corresponded to C–C and/or C–H (aliphatic carbons), the component at 285.6 corresponded to C–O–C and/or C–OH, while the component at 287.7 eV corresponded to C–C=O [28,29,73,74].

3.4.2. Zein secondary structure and hypothesis of mechanism of hydrophobic surface formation

The secondary structure of zein protein in the developed films was studied by attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectroscopy. The spectrum of the zein coating (ZC) formed by spray coating and used for the stones' treatment was compared with the

ATR-FTIR spectra of a zein hydrophilic film (ZF) formed by drop casting. Briefly, 3 mL of zein 5% w/v solution was poured into a teflon Petri dish (diameter of 3 cm) and dried at room temperature under fume hood for 48 h and subsequently placed in oven at 60 °C for other 24 h. The collected ATR-FTIR spectra (4000–600 cm^{-1}) of DMSO solvent, zein powder (ZP), zein film (ZF) and zein coating (ZC) are reported in Fig. 6a.

DMSO exhibited its typical peaks reported in literature [75–78], whereas ZP showed the typical zein protein absorption bands. The amide A band associated to the N–H and O–H bonds of the amino acids of zein appears from 3600 to 3100 cm^{-1} . The methyl group vibration (ν_{CH_3} asymmetric and symmetric) produces the small bands at 2957 and 2872 cm^{-1} , while the peak at 2932 cm^{-1} is due to the methylene group asymmetric stretching vibration ($\nu_{\text{as}}\text{CH}_2$). Another band, due to the stretching of the carbonyl ($\nu_{\text{C=O}}$) of the peptide groups (amide I) appears at 1640 cm^{-1} . The band at 1530 cm^{-1} (amide II) corresponds to the deformation of the N–H bond (δ_{NH}), and the band at 1235 cm^{-1} corresponds to the deformation of the C–N bond (δ_{CN}) [27,73,79].

The characteristic peaks of zein in powder [27,73,79] were observed both in the ZC and in the ZF spectra. In the ZF spectrum, the characteristic peaks of DMSO were still evident. Recently, Wey et al. [31] attributed the amount of DMSO remained within zein film after the drying process to the high boiling point of DMSO solvent (189 °C). Apart from the presence of DMSO peaks, ZF showed also a different shape in the amide I signal (1760–1670 cm^{-1}), with respect to ZP and ZC. The amide I signal contains most of the information of the protein secondary structure [79], therefore the C=O peak deconvolution of ZP, ZF and ZC, was carried out in order to understand the relationship between the secondary structure of the protein and the hydrophobic and hydrophilic nature of the coating and the film respectively. The spectra are presented in Fig. 6b, c, and d respectively. Amide I band of ZP is symmetric, the deconvoluted spectra show a strong signal at 1640 cm^{-1} (Fig. 6b) related to the high α -helix content of zein powder as reported in literature [40,47]. In ZF spectra (Fig. 6c), the presence of a shoulder at 1623 cm^{-1} is clearly evident, which is attributed to the β -sheet structure by several authors [46,47,79]. For ZC (Fig. 6d), no obvious peak or shoulder at 1623 cm^{-1} can be observed from the spectra, revealing a secondary structure similar to the one of zein in powder.

In Fig. 6e the proportion of secondary structures (%) for ZP, ZC and ZF are reported. In ZF a higher proportion of β -sheet structure at 1623 cm^{-1} is observed (17.5%) with respect to the ZC, where β -sheet structure only reached 10.9%, which is almost the same percentage observed for zein in powder (10.5%). Several authors [40,80] illustrated that the increase in β -sheet structure at 1623 cm^{-1} is associated to the α -helix to β -sheet transformation that occurs during solvent evaporation in ethanol-water mixed solutions of zein. This α -helix to β -sheet transformation involves a process of hydrogen bonds rearrangement, which needs the solvent as a medium to take place. DMSO is a hygroscopic solvent with strong affinity for water and high boiling point [81].

DMSO, when left drying under environmental conditions for the zein film formation, might behave like water, promoting the formation of hydrogen bonds with zein. This could also explain the presence of DMSO in ZF after the drying process, as observed with ATR-FTIR analysis. During spray coating, the solvent evaporates in a short time, so no occurrence of the α -helix to β -sheet transformation is observed and, thus, in the coating, the structure is dominated by α -helix structure [40,73]. Spray coating, thus, seems to be the right technique to form a surface film without any solvent entrapped.

The application method plays a central role also in the wettability of the zein surface. When the zein 5% w/v in DMSO solution was allowed to dry slowly after casting on the stone, a smooth hydrophilic film was formed. Using spray coating instead, the solution was ejected as small droplets in the air, forcing the solvent to evaporate from the droplets' surface at a fast rate. In a relative short period of time, before complete solvent evaporation, a radial concentration gradient of zein is expected to form within each droplet [40,82]. In particular, zein starts solidifying from outside, at air-liquid interface, towards inside the droplet. Due to

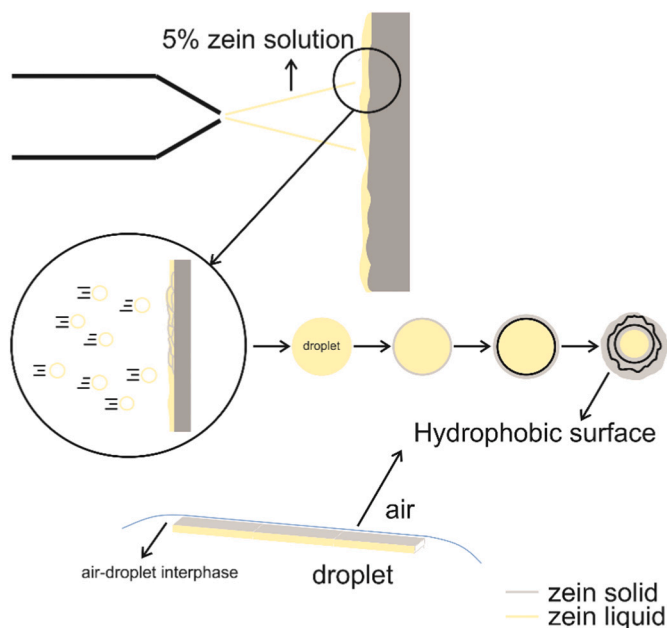


Fig. 7. Schematic illustration of zein hydrophobic surface formation during spray coating.

the DMSO still present inside the droplet, the non polar hydrophobic side of zein is forced to face the outer part of the droplets, while the hydrophilic polar side remains in contact with the solvent. As the drying process proceeds, the solvent evaporates completely also from the inner part of the droplets, while the atmospheric pressure exerted onto the semi-solidified zein particles and the force applied to the droplets due to their impact on the stone surface, make the droplets to collapse one over the other, giving rise to the hydrophobic coating. A similar mechanism was proposed by Dong et al. [40] for the formation of a super-hydrophobic/hydrophobic surface during electrospinning of zein in ethanol-water mixture, in which the solvent evaporation contributed significantly to the orientation of zein molecule. We have adapted this mechanism to the coating obtained by spraying 5% w/v zein in DMSO solution on Serena stone and is demonstrated graphically in Fig. 7.

3.5. Stability of zein coating by artificial aging

Fig. 8 shows the results of static contact angle measurement and color variation before and after one month of artificial aging in the climatic chamber. After aging of the zein-treated stone a decrease in water contact angle was observed. Nevertheless, a water contact angle around 100° was maintained, showing the same trend observed for unaged sample (Fig. 8a).

The color variation was measured for zein-treated samples before and after aging with respect to the non-treated samples. Fig. 8b shows the measured colorimetric variations ΔE^* due to the aging. A ΔE^* of 1.21 ± 0.06 and 1.29 ± 0.08 was observed for unaged and aged stone, respectively. Hence, the measured values are much below the limit value of 3 even after accelerate aging. This means that the color changes are always maintained lower than the threshold value beneath which the variations are accepted by conservators [63].

Overall, the zein coating showed a good resistance to aging, a satisfactory water repellency was maintained and no significant color variations were observed.

3.5.1. Microbial colonization test

Fig. 9 shows the growth of microorganisms on treated and untreated stone after 15 days for each experimental condition, while results of the daily observation are summarized in Fig. 9b. The mycelium colonized

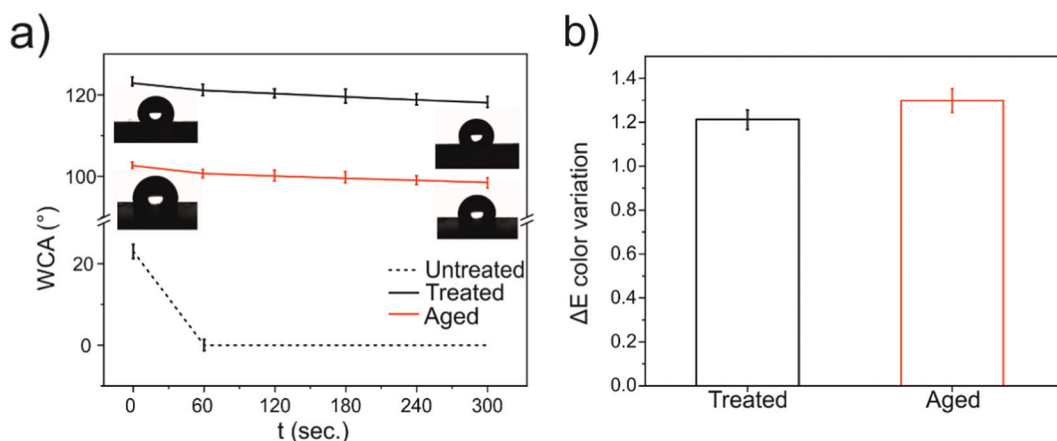


Fig. 8. a) Water contact angle over time of untreated (dot line), treated (black solid line) and aged (red solid line) samples. Static WCA are reported at 0 s, 60 s, 120 s, 180 s, 240 s and 300 s; b) ΔE^* color variation for unaged zein-treated (black) and aged zein-treated (red) samples with respect to untreated stones. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

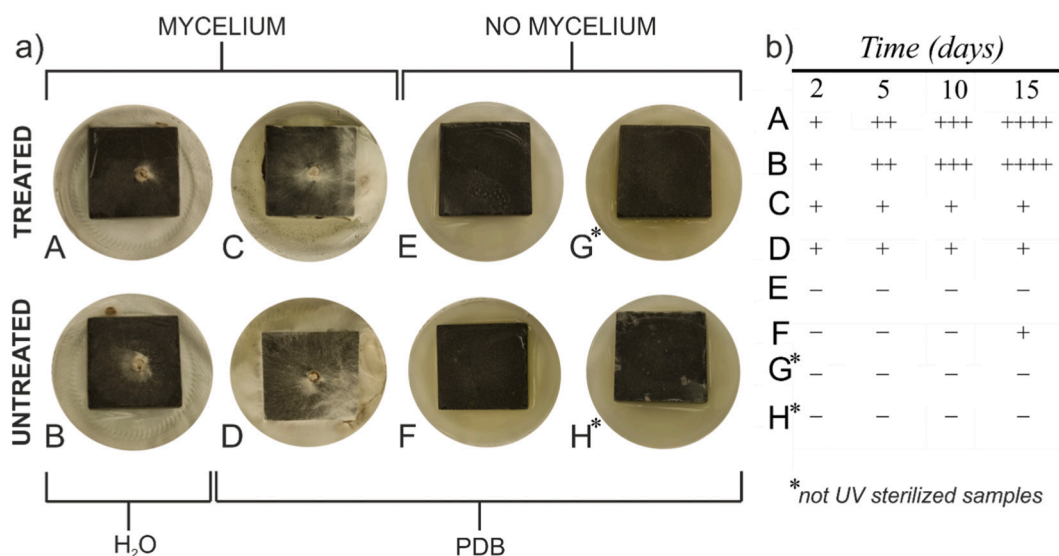


Fig. 9. a) Fungal and microbial degradation test on stone samples after 15 days. Treated (A) and untreated (B) Serena stone, UV treatment, mycelium in H₂O; treated (C) and untreated (D) Serena stone, UV treatment, mycelium in PDB; treated (E) and untreated (F) Serena stone, UV treatment, no mycelium in PDB; treated (G*) and untreated (H*) Serena stone no UV treatment, no mycelium. b) Temporal evolution of the growth of Aae or naturally occurring microorganisms on stone samples.

the top surface of both treated and untreated samples (Fig. 9a C and D respectively) and the surrounding medium, irrespective of the zein coating. On the contrary, when the test was repeated using pure MilliQ water as growth medium, negligible colonization was detected (Fig. 9a A and B). These results proved that zein does not seem to serve as nourishment for the fungal mycelium. During a second set of experiments, no mycelium was added while treated and untreated stone samples were incubated in PDB. In absence of the competing mycelium, some microorganisms resident on stone grew in PDB but did not colonize the stone samples (Fig. 9 E and F). The growth was more intense in case the samples did not undergo UV sterilization before incubation (Fig. 9 G* and H*). These results confirm that zein does neither limit nor promote the growth of common microorganisms naturally occurring in the environment.

4. Conclusions

An investigation of the characteristics of a zein film and its potential application as a protective coating for stone in cultural heritage was carried out. A thin and conformal coating, which did not affect the

underneath roughness, was obtained by spray coating a 5% w/v solution of zein in DMSO. A good water repellency was obtained after the application of the zein coating, reaching WCAs around 120°. The slight decrease in water vapor permeability observed is likely due to the high barrier properties of zein, but the stone retained its ability to permeate natural vapors. Our analysis demonstrated that both the use of the spray technique for the application of the zein coating and the underlying surface roughness contributed to the formation of a hydrophobic coating on the stone surface. Moreover, the amide peak deconvolution performed with ATR-FTIR analysis suggested that it might be a correlation between the drying process which drives the orientation of zein molecules and the different proportion of a helix and β sheet observed in the amide peak. Based on the results obtained, zein could represent a suitable alternative to the commercially available synthetic polymers considering that, even after the aging test, WCAs higher than 90° were maintained and no significant color changes were observed. Even though biodegradation was not seen to be related to the presence of zein, the addition of a proper anti-fouling system to the developed material will be tested in order to address the problems related to the biological growth observed on the stone.

CRedit authorship contribution statement

M. Z., E.Z. and A.A. conceived the study and designed the experiments. G. M. contributed for FTIR analyses. L. B. and M.Z. conceived the microbial degradation test. R. C. performed XPS analyses and analyzed the related data. M. Z. and L.B. wrote the main manuscript text. All authors reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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