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Original article

From the bottom of the sea to the display case: A study into the long-term preservation of archaeological maritime silk textiles in controlled atmosphere



Ana Serrano^{a,b,*}, Agnes Brokerhof^a, Bart Ankersmit^a, Maarten van Bommel^{b,c}

^a Cultural Heritage Agency of the Netherlands (RCE), Cultural Heritage Laboratory, Hobbemastraat 22, 1071 ZC Amsterdam, The Netherlands

^b Conservation and Restoration of Cultural Heritage, University of Amsterdam, PO Box 94552, 1090 GN Amsterdam, The Netherlands

^c Van't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, PO Box 94157, 1090 GD Amsterdam, The Netherlands

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ABSTRACT

An exceptional group of silk fragments was unearthed in 2014 from a shipwreck, which sank in the mid-17th century, in the Wadden Sea, The Netherlands. A unique example of 17th-century fashion, it comprises about 300 textile fragments from garments, parts of garments and furnishing fabrics, almost entirely made of silk and embroidered or woven with metal thread. These are in remarkably good condition, which may be related to the archaeological environment, the quality of the silk yarns in the fragments, and the presence of metal threads and other metallic objects from the shipwreck. Surviving archaeological maritime silk textiles are extremely rare, which makes this a distinctive find posing challenges for selecting the most suitable conditions for exhibition and storage.

Scientific research was carried out with the aim to evaluate the response of modern and archaeological silks to temperature, relative humidity, light and oxygen, in order to define the most suitable parameters for the long-term storage and exhibition of the collection. Artificially aged samples taken from one of the archaeological fragments were analysed at the visual, structural and molecular level by means of colour measurements, Fourier transform infrared spectroscopy (FTIR) and ultra-high performance liquid chromatography coupled to a fluorescence detector (UHPLC-FLD). The results showed that light exposure, in combination with high temperatures, oxygen and moisture strongly affected the silk's structure and molecular composition. Limiting exposure to light and removing oxygen reduced this effect and increased life expectancy significantly. Therefore, the analytical results obtained were essential to defining a preliminary preservation strategy for the collection: while on display, anoxic conditions slow down degradation of the silk by a factor of 4–5, whereas in a dark storage, a low RH is the most important factor, with anoxic conditions providing additional reduction of decay.

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1. Introduction

A large collection of archaeological silk fragments was found in 2014 among the debris of a shipwreck (registered as BZN17) that sank in the mid-17th century, in the Wadden Sea, The Netherlands. Buried in a time capsule for centuries, this collection represents a unique example of undisturbed 17th-century fashion. It comprises some 300 textile fragments, almost entirely made of silk velvets, damasks and brocades, with many dyed reds with coccid insects

or madder, and embroidered or woven with silver and silver gilt thread. It includes entire ensembles, garments and furnishing fabrics. Previous assessment of their condition (published elsewhere), supported by scanning electron microscopy-energy-dispersive X-ray (SEM-EDX), revealed that most fragments are well preserved, as fibres appeared generally clean, smooth, round and flexible [1,2]. This may be related to the quality of the silk yarns in the fragments, the presence of metal threads and other metallic objects from the shipwreck, and the archaeological maritime conditions that promoted their preservation – low temperatures and the absence of oxygen and light beneath the sea sediment [3–5]. These conditions are similar to those of other archaeological textiles that have survived in extremely dry or wet and frozen conditions, secluded from light, and subjected to low oxygen or extreme pH levels [5,6]. On the other hand, some more fragile fragments contained variable

* Corresponding author at: Cultural Heritage Agency of the Netherlands (RCE), Cultural Heritage Laboratory, Hobbemastraat 22, 1071 ZC Amsterdam, The Netherlands.

E-mail address: afaserrano13@gmail.com (A. Serrano).

amounts of surface deposits generated in the archaeological environment, such as sand, clay, salts, silver sulphide from corroded metal threads and iron sulphide. Fibres of fragile fragments also showed heavy pitting from biodegradation, longitudinal and transverse cracking, surface peeling and fibre collapse from prolonged wetness [1]. Although these features provide information about the physical condition of the fragments, they do not reveal anything about the chemical stability of the fibres.

Studies of surviving archaeological maritime textiles are rare [7–13], and they often focus on the characterisation and/or conservation of surviving woollen and plant fibres in small, fragmentary textile fragments. So far, only the excavation of the shipwreck of the 19th century *S.S. Central America* has reported findings of whole, well-preserved silk garments, along with other woollen, cotton and flax textiles [12,13]. Therefore, the BZN17 collection, with its almost complete silk garments, is a substantial find that receives much attention from textile experts, art historians, material culture historians, conservators, curators, and the general public. Given its significance, it is of the utmost importance to preserve it for generations to come. However, because little is known about the deterioration of maritime archaeological silk textiles, a detailed understanding of the degradation mechanisms would be useful for conservators to select the most suitable conditions for exhibition and storage.

Silk has always been a valuable material. Mainly obtained from the cultivated *Bombyx mori* silkworms, it is produced when the caterpillar extrudes a proteinaceous filament to prepare its cocoon. This filament comprises two fibroin strands, bound together by sericin. The latter makes fabrics stiff and hinders their homogeneous dyeing, which is why it is often removed with a degumming mild alkaline solution. The BZN17 fragments and many historical silks are degummed, essentially leaving the fibroin [14–16]. The chemical composition of fibroin mainly consists of the amino acids glycine, alanine and serine. These are connected through intra-chain bonds, in a highly ordered sequence of six peptide (or amide) groups, known as the primary structure or polypeptide backbone (-Gly-Ala-Gly-Ala-Gly-Ser-). Adjacent repeats of this sequence stack on one another, connecting through inter-chain hydrogen bonds and forming parallel and antiparallel β -pleated sheets, which is the secondary structure. This well-ordered, three-dimensional conformation forms the very stable crystalline regions of the fibroin (~60%). These regions are alternated with amorphous regions (~40%), arranged as α -helix and/or random coils, and generally consisting of amino acids with bulky and polar side groups, namely tyrosine and smaller percentages of valine, aspartic acid, leucine, isoleucine, glutamic acid, threonine, arginine, methionine, proline, phenylalanine, lysine, cysteine, histidine and tryptophan [13,16–20].

Silk is one of the most sensitive natural fibres to be affected by common deterioration agents in a museum environment, such as radiation (UV-visible light), oxygen, inappropriate temperatures (T) and relative humidity (RH). Prolonged exposure to one or a combination of these environmental agents can trigger (photo-)oxidation and hydrolysis of silk, and cause irreversible chemical and physical changes that lead to discoloration and/or yellowing, embrittlement and friability [17,19,21,22]. Research into the effect of these agents is usually carried out by artificial ageing of modern silk. Tests frequently involve high temperatures, extreme levels of humidity, oxygen and/or exposure to high irradiation levels of UV and light [16,19,22–30]. Relatively little attention has been given to the influence of removal of oxygen (anoxia) [26,27,31]. Once artificially aged, silk is assessed with several analytical techniques that identify and characterise chemical and physical degradation markers. These are often compared with those from historical silk objects, in order to evaluate the extent of their degradation. Determination of tensile strength and viscosity, pH and colour

measurements, and morphology assessments have been used to study macroscopic alterations [16,19,22]. However, some of these techniques are destructive and require large samples, which is highly undesirable when analysing historical textiles. More sensitive techniques, that are non-invasive or require very small samples, can provide more detailed information on the structural and molecular levels. For example, Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR) has provided insights into structural changes [16,26,29,32], whereas chromatographic techniques have helped determining modifications in the amino acid composition [15,16,29,33].

2. Research aims

The aim was to investigate the effect of temperature, RH, irradiation and oxygen on the stability of the BZN17 silks, to define the most suitable parameters for their long-term storage and exhibition. However, common artificial ageing techniques are not proficient enough to recreate maritime archaeological silk from modern material, as they lack characteristics such as soil contamination, microorganism attack, and waterlogging [7]. Hence, it was impossible to predict the response of the BZN17 collection to a museum environment by relying on data obtained from artificially-aged modern silk only. To tackle this problem, small silk strips from one BZN17 fragment were exposed to combinations of light, oxygen, temperature and humidity. For comparison, undyed and cochineal-dyed modern silks were exposed as well. The aged specimens, along with the correspondent non-aged ones, were analysed at the macroscopic, structural and molecular level, with colour measurements, FTIR-ATR, and ultra-high performance liquid chromatography coupled to fluorescence detector (UHPLC-FLD). This has been used for amino acid analysis in food science and biomedical research [34]. A bespoke protocol was developed to investigate modifications in the chemical composition of silk upon exposure to different environments. Principal component analysis (PCA) explored statistical correlations in the data obtained with FTIR-ATR and UHPLC-FLD.

3. Material and methods

3.1. Sample preparation

A small maritime archaeological silk fragment (BZN17-f26) of cinnamon brown colour was used for the exposure experiments. This fragment had been used as a conservation test, being rinsed with demineralised water by conservators. Chromatographic analyses indicated that this fragment was originally dyed with a small amount of cochineal, while observation with SEM-EDX showed that its fibres were brittle and evidenced pitting from biodegradation; although they were relatively clean from soil contamination [1].

Additionally, two pieces of modern degummed silk fabric were washed with demineralised water and Marseille soap, and one of them was further mordanted and dyed with cochineal (*Dactylopus coccus* COSTA), following an adapted historical recipe described elsewhere [32]. Cochineal was chosen because of its stability against photo-oxidation, and because it was reported to be present in many BZN17 fragments [1].

Strips of 5 × 1 cm were cut from the undyed, the cochineal-dyed and the archaeological f26 silk fabrics, and mounted on a support. A strip of ISO blue wool standard nr 4 (BWS4) was included as a reference for light exposure. The support was placed in a bespoke open container that created a 75 mL air volume and protected the strips from direct contact with other materials. Both support and box were made of aluminium sheets, and aluminium tape was used

to fix the strips onto the support and to construct the container. Aluminium was chosen because it is inert and does not react with silk. The containers were sealed inside low-gas-permeability pouches, as depicted in Supplemental Online Material 1 (SOM 1). Detailed information about the materials used and suppliers is available in SOM 2.

To create a low RH (Table 1), 2 g of dried silica gel was added to the pouches; 100% RH was created with small pieces of sponge soaked in demineralised water. Near anoxic conditions were obtained using two bags of oxygen absorbers inside each pouch. RH and oxygen levels were verified before and after exposure with humidity indicator strips and oxygen indicators Oxy-eye placed inside the pouches during the first exposure (A).

RH/T data loggers and oxygen sensors were placed inside some of the pouches during the second exposure (B). RH and temperature were recorded with self-contained mini temperature and humidity data loggers tempmate[®]-B series (suitable for extreme T and RH), and evaluated with TempIT software. Oxygen was monitored with fibre optic oxygen transmitter Fibox 4 trace, from PreSens Precision Sensing GmbH (Regensburg, Germany), with non-invasive mini oxygen sensors and sensor probes, and using PreSens Data-manager software.

3.2. Artificial exposure of samples

To assess the effect of environmental conditions, comparable to those in a museum storage, on silk in general, pouches with only modern undyed and cochineal-dyed silk strips were exposed for 912 h (38 days) in a Thermo Scientific Heraeus[®] oven, in the dark, to $85 \pm 5^\circ\text{C}$ with four combinations of high and low RH with ambient and low oxygen concentrations (exposure A). Thermal ageing was conducted in duplicate (a and b) (Table 1).

To assess the effect of environmental conditions, comparable to those in a museum exhibition, on silk, modern undyed and cochineal-dyed silk, together with archaeological silk and BWS4 strips, were exposed to high light intensities in a Xenotest, Alpha High Energy (Atlas[®]), containing a filtered Xenon-Arc-lamp (Xenochrome type 320 (nm)) (exposure B). Exposure was carried out for 600 h (25 days), at a test chamber temperature of $50 \pm 5^\circ\text{C}$ and an illuminance of 105 klx. The polyester of the pouches absorbs radiation with wavelengths smaller than 320 nm (SOM 2), providing protection against the most powerful radiation in the spectrum. Light exposure was conducted twice for combinations of ambient and low RH, with ambient and low oxygen concentration. In the first experiment, samples were exposed in duplicate (a and b), in the repeat experiment, singly (c).

3.3. Analytical methods

3.3.1. Colour measurements

CIELab* colour coordinates for the aged silk and BWS4 strips were compared with the corresponding non-aged ones. Measurements were made with a Konica-Minolta 2600D Spectrometer and the software SpectraMagic[™] NX Lite, using primary illuminant D65 and 2° observer angle. Three replicate measurements were made at random locations on each strip (SOM 3). The colour difference, between the aged and the non-aged specimens (ΔE^*), was calculated from the mean of the replicates, and according to CIE76 (SOM 4) [14,31,36].

3.3.2. FTIR-ATR

Spectral data was collected using a Perkin Elmer Spectrum 1000 FTIR spectrometer combined with a Graseby Specac Golden Gate Single Reflection Diamond ATR. The silk strips were placed over the ATR accessory, and three replicate measurements were carried out at random locations on each silk strip. Spectra were obtained

using a range of $4000\text{--}450\text{ cm}^{-1}$, 32 scans, 4 cm^{-1} resolution and scan speed of 0.2 cm/s . The acquired spectra were interpreted with PerkinElmer Spectrum software. Each spectrum was subjected to baseline correction at 1800 cm^{-1} , where no absorbance band was observed for any of the samples; and normalised to the peak of highest absorbance in relation to all spectra [16]. To quantify structural changes occurring in the artificially-aged silk, the crystallinity index was calculated with the theoretical absorbance heights corresponding to Amide I's β -sheets conformations at 1615 cm^{-1} and α -helix/random coils at 1655 cm^{-1} (SOM 5) [26,37].

3.3.3. UHPLC-FLD

Sample preparation. A standard solution was prepared by combining the amino acids present in silk: aspartic acid, glutamic acid, serine, histidine, glycine, arginine, alanine, tyrosine, lysine, methionine, proline, valine, tryptophan, threonine, cysteine, phenylalanine, isoleucine and leucine. Each 1.5 mg amino acid was first dissolved in 4 mL 0.1 N hydrochloric acid (HCl), and then diluted ten times with the same solution (1:9, v/v) [38]. Silk samples (0.2–0.3 mg) were hydrolysed in sealed glass vials with $100\ \mu\text{l}$ 6 N HCl, kept in a pre-heated oven at 110°C for 20 h. These were evaporated to dryness under nitrogen flow and the resulting dry residues reconstituted with $100\ \mu\text{l}$ 0.1 N HCl, vortexed and centrifuged. $10\ \mu\text{l}$ were transferred to new inserts and diluted ten times with the same solution [23,24].

The standard solution and the hydrolysed silk samples were always pre-column derivatised, using a programmable automatic addition, with a total injection volume of $2\ \mu\text{l}$ derivatisation agent: 0.1 μl sample (20:1, v/v) [38,39]. The derivatisation agent was prepared by diluting 2.55 mg o-phthalaldehyde (OPA) in 0.5 mL methanol (MeOH), $5\ \mu\text{l}$ 3-mercaptopropionic acid (MPA) and 2 mL borate buffer 0.1 M pH 9.2 (17 g borax and 3 g boric acid in 0.5 L H_2O), and left at 5°C for about 90 min to allow full reaction. This solution was prepared every two days and kept in the UHPLC autosampler in the dark at 7°C [40,41].

Apparatus. Analyses were performed using a Waters Acquity[™] H-class UHPLC system (Waters Corporation, Milford, MA, U.S.A.), equipped with quaternary solvent delivery system, column oven, autosampler and fluorescence detector, using 240 nm band-pass excitation (λ_{ex}) and 450 nm long-pass emission (λ_{em}) filters, which give optimal response and baseline stability for the study of amino acids derivatised with OPA [40]. The equipment was controlled by Empower 3.0 Chromatography Data Software from Waters Corporation. Analytical conditions were carried out using a Waters Acquity[®] UHPLC BEH Shield RP18 $1.7\ \mu\text{m}$ of $2.1 \times 150\text{ mm}$ column, protected by a filter unit ($0.2\ \mu\text{m}$), with 0.2 mL min^{-1} flow rate and 40°C constant temperature.

Separation was resolved by adapting a high-performance liquid chromatography (HPLC) gradient for amino acid analyses [40]. Solvent A was 10% aqueous MeOH (v/v), solvent B pure MeOH, and solvent C sodium acetate 0.1 M pH 7 (20.6 g sodium acetate and $50\ \mu\text{l}$ acetic acid in 0.5 L H_2O). The developed 33 min-gradient elution program was: 0–3 min, isocratic 75A:15B:10C (v/v/v); 3–10, linear to 54A:36B:10C (v/v/v); 10–13, linear to 39A:51B:10C (v/v/v), isocratic for 3 min; 16–18, linear to 36A:54B:10C (v/v/v); 18–19, linear to 10A:80B:10C (v/v/v), isocratic for 3 min; 21–23 min, linear to 75A:15B:10C (v/v/v), isocratic for 10 min. Validation of the analytical method is discussed in SOM 6.

Amino acids in the silk samples were identified by their retention times in relation to the standard solution, which was injected twice a day. To assess alterations in the artificially-aged silk, the content (mole %) of serine, alanine, glycine and tyrosine amino acids was determined (SOM 7).

Table 1
Types of exposure and RH/T and oxygen (ambient (A), low (L) and high (H)) registered inside the pouches – unregistered data notated as not available (n.a.).

		Exposure		T (°C)	RH (%)	O ₂ (%)
Thermal ageing	A1 – H _{RH} A _{O2}	High RH	a) ^a	85	~100	n.a.
		Ambient O ₂	b) ^a		~100	n.a.
	A2 – L _{RH} A _{O2}	Low RH	a) ^a		~10	n.a.
		Ambient O ₂	b) ^a		~10	n.a.
	A3 – H _{RH} L _{O2}	High RH	a) ^a		~100	<1
		Low O ₂	b) ^a		~100	<1
	A4 – L _{RH} L _{O2}	Low RH	a) ^a		~10	<1
		Low O ₂	b) ^a		~10	<1
Light ageing	B1 – A _{RH} A _{O2}	Ambient RH	a) ^b	54	35–45	19–4
		Ambient O ₂	b) ^a		n.a.	~50
	B2 – L _{RH} A _{O2}	Low RH	a) ^b	59–76	55–35	16.6–0.7
			b) ^a	58	1–7	n.a.
		Ambient O ₂	b) ^a	n.a.	~10	n.a.
			c) ^b	53–65	50–21	19.2–17.3
	B3 – A _{RH} L _{O2}	Ambient RH	a) ^b	52	60	0.2
			b) ^a	n.a.	~50	<1
		Low O ₂	c) ^b	53–76	29–59	0.2
			a) ^b	53	20	0.5
	B4 – L _{RH} L _{O2}	Low RH	b) ^b	55–63	20–45	0.7
			c) ^b	50–63	45–55	0.2

^a Registered before and after exposure with RH and O₂ estimate indicators.

^b Registered throughout exposure with O₂ sensors and RH/T data loggers.

3.3.4. Statistical analyses

PCA was applied to model acquired data, using R software, with ChemSpec package. A total of 147 FTIR-ATR spectra (aligned and normalised with PerkinElmer Spectrum software) were modelled considering the region between 1900–800 cm⁻¹, where the most relevant peaks are found. The region between 2.4 and 9 min of a total of 117 UHPLC-FLD chromatograms was analysed, excluding the glycine peak (3.3–4.4 min), as it did not help differentiate the samples. Chromatograms were aligned and normalised to the peak of highest intensity. Chromatograms from exposure A were not considered because they showed a high correlation (0.997–0.998) that hindered the differentiation of exposure B chromatograms.

4. Results and discussion

Since silk is a natural material with an heterogeneous structure and the conditions inside the duplicate and triplicate pouches were not exactly the same, reproducibility was not achievable and the analytical results showed considerable variation. The colour measurements of modern undyed silk and cochineal-dyed silk, for example, showed large differences between the duplicates under thermal ageing at high RH and ambient oxygen concentration (exposure A1 in Fig. 1). Nevertheless, it is possible to determine trends and draw conclusions.

4.1. Thermal ageing

Colour measurement of the samples after thermal ageing showed the largest differences compared to the non-aged samples for silk aged in an oxygen-rich and humid environment (A1 H_{RH}A_{O2}, Fig. 1). This was particularly noticeable for undyed silk, which showed a large positive shift in b* values towards yellow (SOM 3 and 4). In cochineal-dyed silk, the colour changes observed were the result of those in the colourant and the silk support. If yellowing of the silk occurred, it was obscured by the greater colour loss of the dye, which showed an increase in L* and a negative shift in a* and b*. At a low RH and ambient oxygen levels (A2 L_{RH}A_{O2}), the shift in b* for undyed silk is significantly smaller, while for cochineal-dyed silk the changes in both a* and b* lessened. This confirms that hydrolysis reactions cause yellowing of

undyed silk, while they also play a role in the colour change of the dye [22,27].

In a low-oxygen atmosphere, the yellowing of undyed silk in a humid environment (A3 H_{RH}L_{O2}), as well as in a dry environment (A4 L_{RH}L_{O2}), decreased significantly. The colour changes in cochineal-dyed silk were also much lower than in humid, oxygen-rich conditions. However, for undyed silk, the benefit of a low-oxygen atmosphere seems less than that of dry conditions. For the dyed silk, the effect of low-oxygen or low humidity environment seems comparable. For both undyed and dyed silk, the combination of a low-oxygen environment with low humidity does not seem to add anything to the effect of either one. The colour measurements suggested that in dark situations, such as in storage, ageing of silk, whether undyed or dyed, is a combination of hydrolysis and oxidation reactions, where absence of moisture or oxygen can slow down deterioration. In museum practice, colour changes are significantly curtailed when silk is stored in a dark environment with low RH [22,25]. However, colour measurements alone do not provide enough information to determine whether this is sufficient to preserve silk on a structural and molecular level.

In Fig. 2, FTIR spectra depict characteristic absorption bands that correspond to specific vibrational modes of the molecules and their conformation in the silk's polypeptide backbone – assignments in Table 2. The sharp and distinct bands in non-aged silk (NA) reflect ordered crystalline and amorphous regions. As degradation occurs, these regions become disordered, the molecules' vibrational modes change, and the respective FTIR bands lose definition [18,26]. This could be observed for cochineal-dyed silk kept in an oxygen-rich environment, either at high or low RH (A1 H_{RH}A_{O2} and A2 L_{RH}A_{O2}). Moreover, the crystallinity index of Amide I in these spectra confirmed a slight increase of the relative proportion of β-sheets in relation to α-helix/random coils conformations (Fig. 3).

In contrast, undyed silk aged under the same conditions, as well as undyed and cochineal-dyed silk kept in low-oxygen atmosphere (A3 H_{RH}L_{O2} and A4 L_{RH}L_{O2}), maintained a closer profile to that of non-aged silk. In addition, the crystallinity indexes did not alter significantly. When plotting the spectral data with PCA (Fig. 4), these corresponded to scores with deeply negative PC2 and close-to-zero PC1. On the other hand, scores for cochineal-dyed silk kept in an oxygen-rich humid environment showed positive PC2 and negative PC1. Hence, it is possible that cochineal-dyed silk is affected

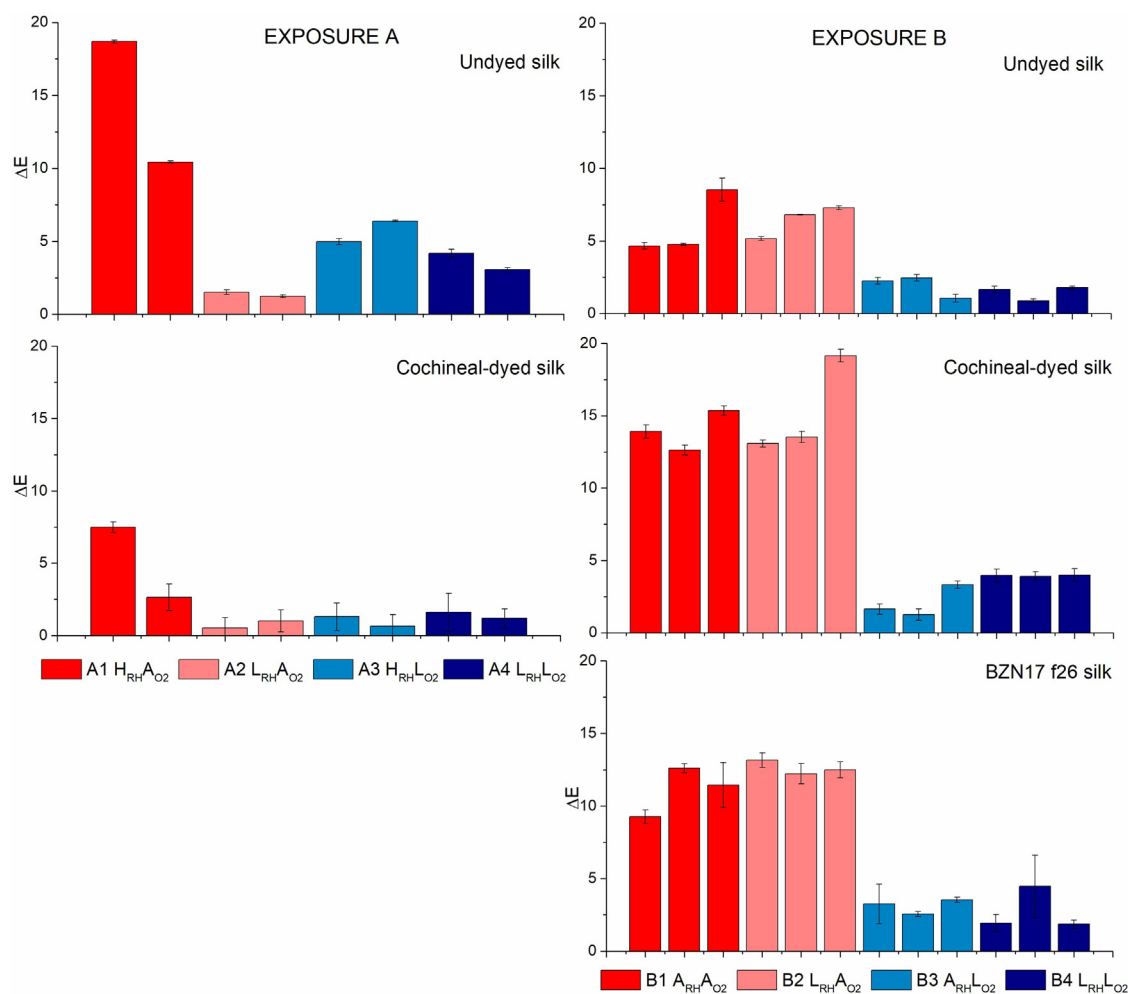


Fig. 1. Colour differences (ΔE^*) of silk strips after thermal (exposure A) and light (exposure B) ageing with ambient (A), low (L) and high (H) RH/T and oxygen.

Table 2
Representative absorption bands in silk spectra, with respective assignments [16,26,32,46].

Wavenumber (cm^{-1})	Assignment
1615 (parallel β -sheets)	Amide I, carbonyl stretching vibrations and, in a lower percentage, C–N stretching and N–H bending vibrations
1655 (random coils and α -helices)	
1698 (antiparallel β -sheets)	
1515	Amide II, N–H and C–N bending vibrations
1260 (β -sheets)	
1230 (random coils)	Amide III, N–H bending and C–N stretching vibrations and side-chains in the polypeptide backbone
1460–1350	
1164	
1083	
997	
977	
977	

by mordant and dyeing treatments, making it more susceptible to deterioration agents than undyed silk [42]. There is no correlation between the observed colour changes and the structural changes, as revealed by the FTIR spectra.

When considering alterations in amino acid content obtained with UHPLC-FL (Fig. 5), the differences are not so obvious between thermal-aged and non-aged modern silk, for both undyed and dyed

silk. All thermal-aged silk samples have relatively high, if somewhat variable, contents of tyrosine and glycine, comparable with those of non-aged silk. Hence, these results, in combination with those from FTIR, indicate that, despite the presence of oxygen or humidity, only small alterations occurred in the structure and molecular content of thermal-aged silk. The only observable change is a colour change upon exposure to high humidity in the presence of oxygen. It is to be expected that a longer period of exposure and/or higher temperatures will cause more obvious structural and chemical alterations [16]. Therefore, the results of these thermal exposures are only indicative for the long-term preservation of silk.

4.2. Light exposure

Light exposure was expected to be much more damaging than thermal ageing [16], which is why the limited number of archaeological BZN17 f26 silk samples was only included in the light exposure experiments.

Colour changes were directly observable after light exposure of undyed, cochineal-dyed, BZN17 f26 and BWS4 samples kept in an oxygen-rich atmosphere (SOM 3 and 4). In contrast, samples kept in low-oxygen atmospheres showed much smaller colour changes. The B1 $A_{RH}A_{O_2}$ and B2 $L_{RH}A_{O_2}$ samples all displayed colour differences that were at least two to three times larger than the B3 $A_{RH}L_{O_2}$ and B4 $L_{RH}L_{O_2}$ (Fig. 1). This difference was particularly significant in both cochineal-dyed and BZN17 f26 samples; although the standard deviation for the latter was somewhat higher, which

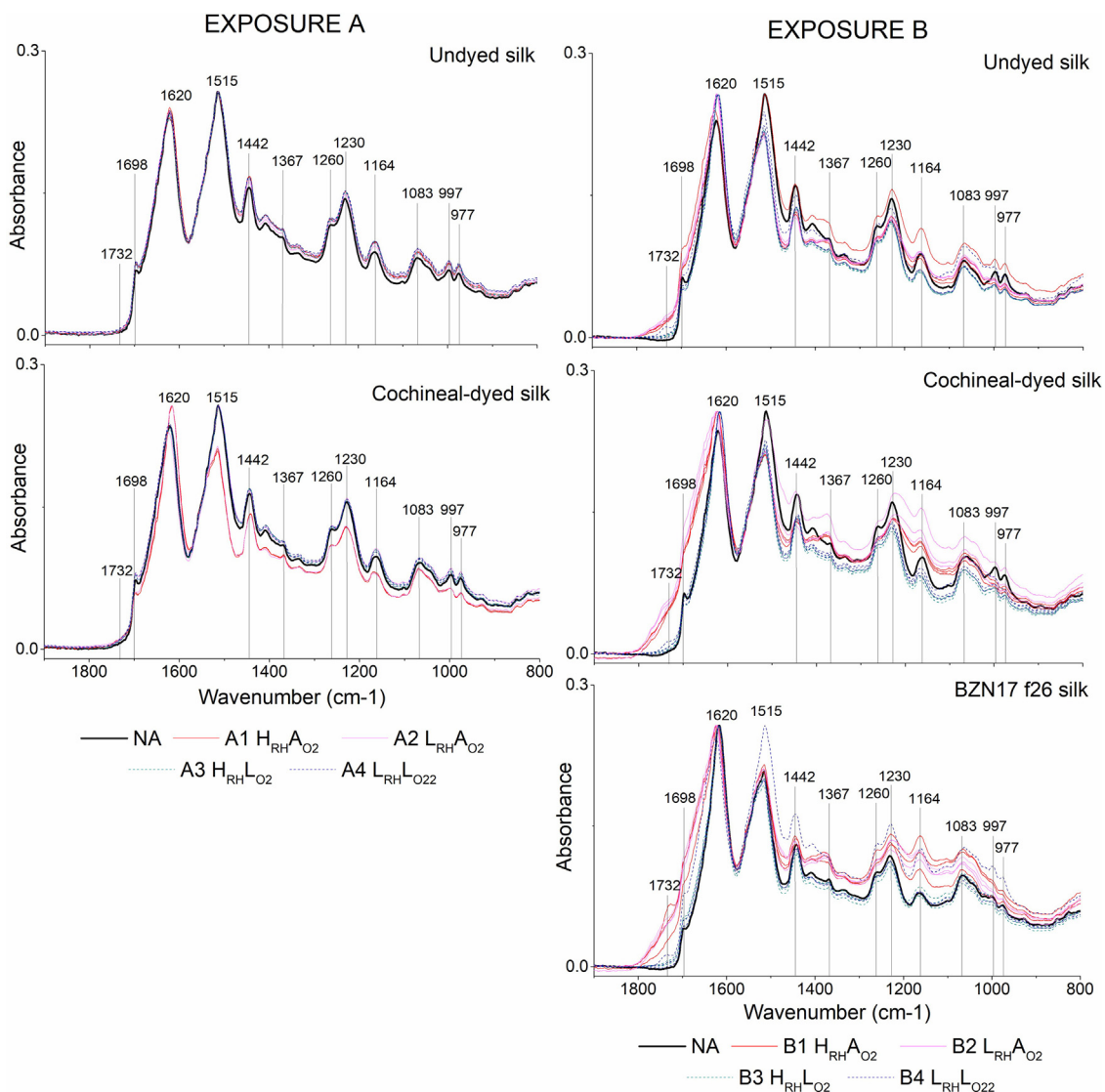


Fig. 2. FTIR spectra of non-aged (NA), thermal-aged (exposure A) and light-exposed (exposure B) silk samples, submitted to ambient (A), low (L) and high (H) RH/T and oxygen.

can be explained by their less homogeneous colour after centuries in the bottom of the sea. These results confirm that a low-oxygen atmosphere reduces the rate of discolouration of dyed fabrics due to light considerably [31,36]. Contrary to thermal ageing, reducing the RH in oxygen-rich atmospheres had no significant effect on the discolouration of the silk, nor the dyestuff. This suggests that photo-oxidation reactions are more powerful than hydrolysis, even though this still plays an important role in the whole process [22].

Colour changes in undyed silk do not describe the effects of light ageing on the fibres to its full extent. The colour difference compared to the non-aged silk was not as prominent as that in the thermal exposure, but in oxygen-rich atmospheres, yellowing was still measured as a positive shift in b^* values (SOM 3). As in the thermal exposure, during light ageing, water and oxygen that are present in the pouches reacted with the polar amino acids in the amorphous regions of the silk, breaking the molecular chains and forming conjugated systems which result in yellowing [19,25]. The presence of UV-vis radiation during Xenon light ageing will accelerate the rate and intensity of photo-oxidative reactions; though the effect is reduced, due to the UV filtering properties of the polyester of the pouches.

At the structural level, FTIR results also showed that RH did not have a significant impact on the silk structure. Yet, a striking difference could be observed between silk samples kept in oxygen-rich and low-oxygen atmospheres; the latter maintaining profiles closer to that of non-aged ones. Fig. 2 presents alterations in FTIR spectra of silk samples exposed to light under conditions B1 $H_{RH}A_{O_2}$ and B2 $L_{RH}A_{O_2}$ conditions. Particularly, cochineal-dyed and BZN17 f26 silk show spectral changes that may be due to their increased susceptibility after mordant and dyeing treatments. Significant structural transformations can be observed at the 1698 cm^{-1} and 1732 cm^{-1} bands, which have been attributed to an increase of antiparallel β -sheet contribution, and to the formation of new carboxylic groups, respectively. The broadening of the wider amide III band at 1230 cm^{-1} corresponds to a distortion in the random coil (amorphous), rather than in the stable (crystalline) β -sheet conformation. In addition, the decrease of the amide II at 1515 cm^{-1} can be related to a decrease of N-H groups in the polypeptide backbone [16,20,32,43,44].

Plotting the FTIR data with PCA (Fig. 4) reveals non-aged silk to have deeply negative scores in PC2 and close-to-zero in PC1, whereas silk kept in a low-oxygen atmosphere, a positive PC2 and a negative PC1, as well as a high scores congregation, which indicates

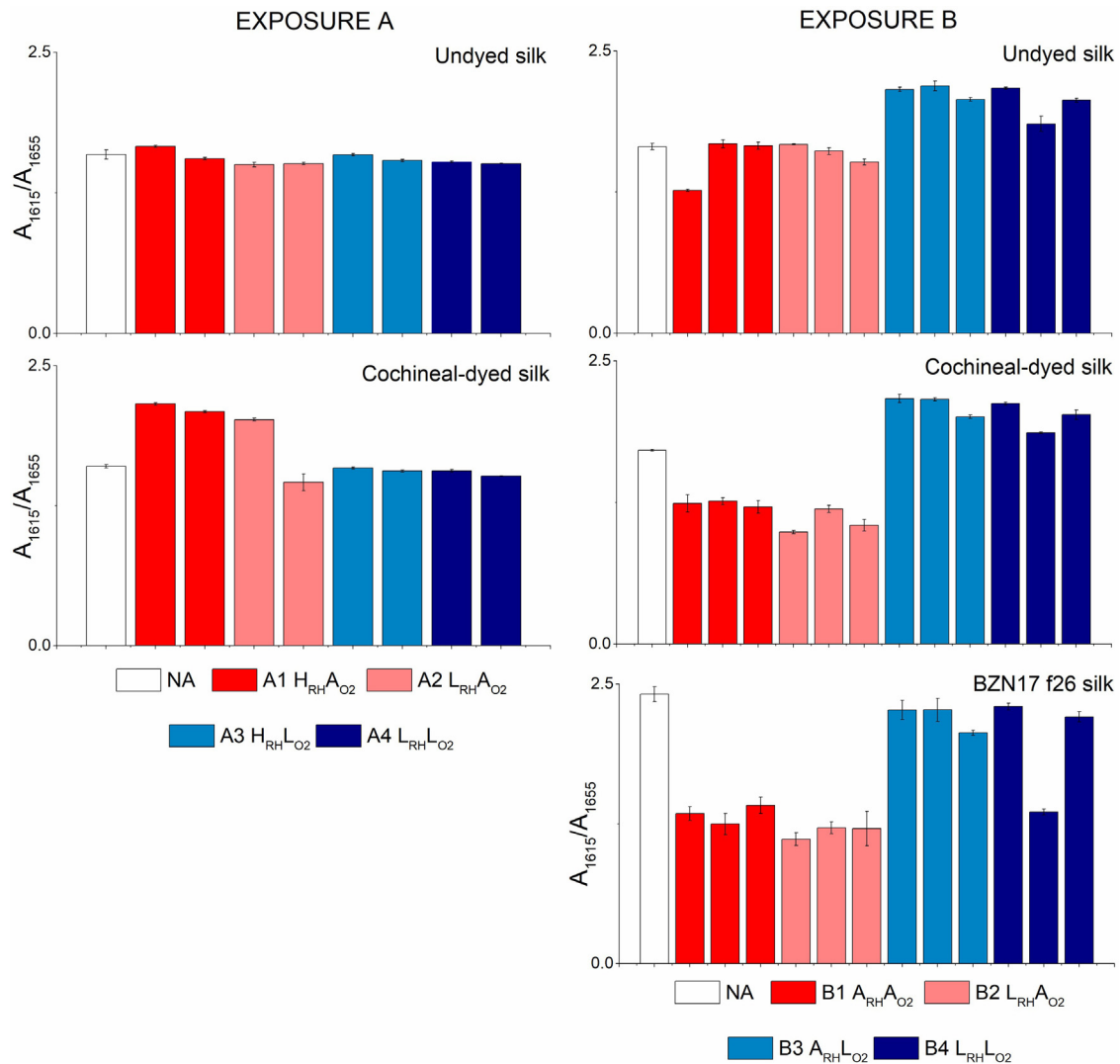


Fig. 3. Crystallinity index (A_{1615}/A_{1655}) for the non-aged (NA), thermal-aged (Exposure A) and light-exposed (exposure B) silk samples, submitted to ambient (A), low (L) and high (H) RH/T and oxygen.

small spectral variability. In an oxygen-rich atmosphere, undyed silk exposed to light has a PC1 closer to zero, while the scores for the cochineal-dyed and the BZN17 f26 samples present more positive PC1 scores, as well as higher scattering, indicating greater structural changes and spectral variability.

When considering Amide I's crystallinity index, and, as demonstrated by previous research on time-based artificial light ageing, photo-oxidation reactions and hydrolysis of the polar groups occurring in the amorphous regions (random coils) lead to the molecules' chain scission and to their re-organisation into crystalline groups (β -sheets), increasing the crystallinity index. As photo-oxidation progresses, the crystalline regions will be affected as well, leading to a decrease in the crystallinity index [26,37,43]. In agreement to this, Fig. 3 shows an increase of the crystallinity index in silk kept in a low-oxygen environment, corresponding to a higher relative proportion of β -sheets in relation to α -helix/random coils conformations, and thus, to an initial phase of silk's photo-oxidation. In contrast, the decrease of the crystallinity index in silk kept in an oxygen-rich atmosphere translates into a lower relative proportion of β -sheets conformations and to an advanced state of photo-oxidation.

Significant alterations in the amino acid content of the silk samples were observed upon light exposure in oxygen-rich atmospheres. The RH appeared to have no influence. As shown in Fig. 5,

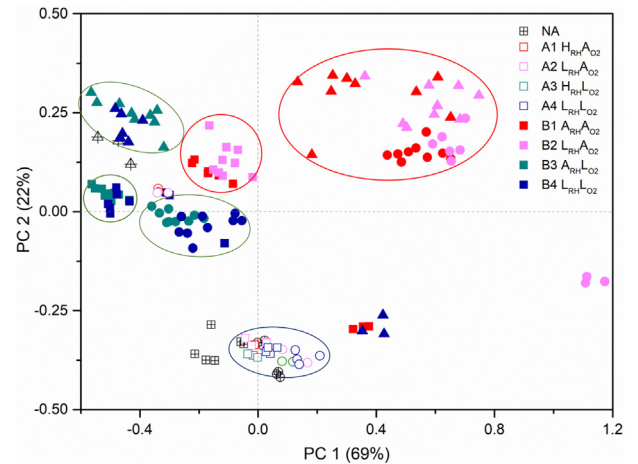


Fig. 4. PCA scores from FTIR spectra, representing 91% of total data variance: non-aged (NA), thermal-aged (A) and light-exposed (B) samples of undyed (squares), cochineal-dyed (circles) and BZN f26 (triangles) silk, submitted to ambient (A), low (L) and high (H) RH/T and oxygen.

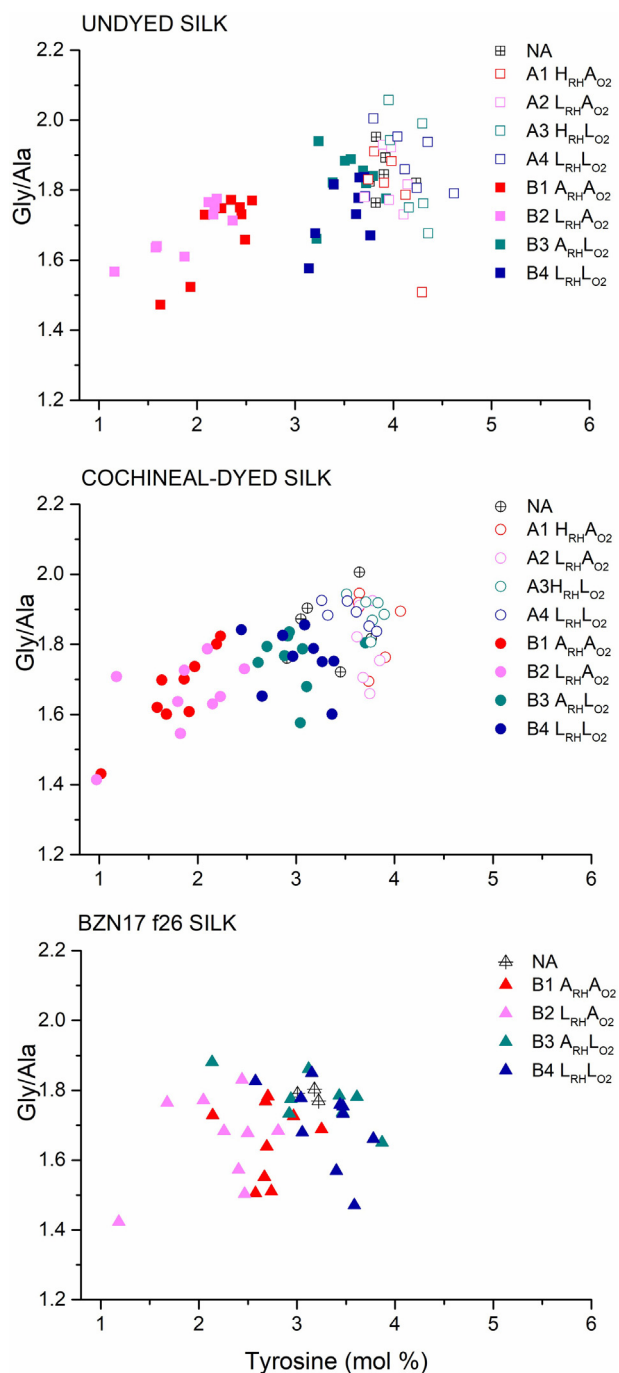


Fig. 5. Tyrosine plotted against Gly/Ala ratio: non-aged (NA), thermal-aged (exposure A) and light-exposed (exposure B) samples of undyed (squares), cochineal-dyed (circles) and BZN f26 (triangles) silk, submitted to ambient (A), low (L) and high (H) RH/T and oxygen.

a considerable decrease of tyrosine and a slight increase of alanine in relation to glycine occurred in oxygen-rich conditions (B1 A_{RH}A_{O2} and B2 L_{RH}A_{O2}). This was likely caused by radical reactions occurring in the amorphous regions, transforming tyrosine into alanine through the loss of phenol groups, and the formation of side products, such as o-quinones [13,20,21]. Silk kept in a low-oxygen atmosphere presented less modifications in tyrosine content, and were somewhat comparable with that of non-aged silk. Similar interpretations could be attained with the PCA plot of the chromatograms (Fig. 6), where most non-aged silk and silk exposed in a low-oxygen atmosphere displayed negative scores in PC1, whereas

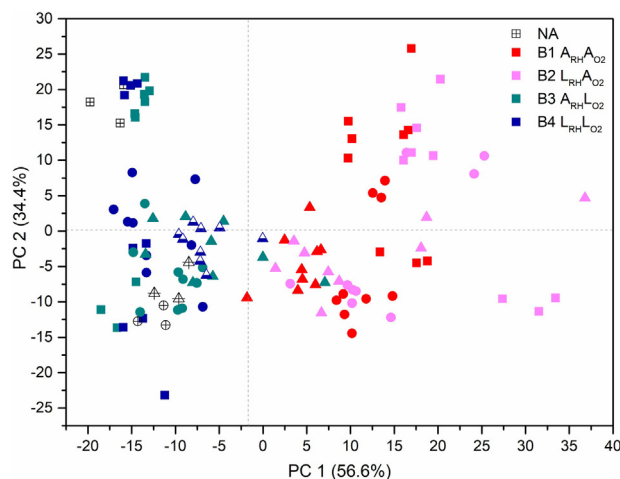


Fig. 6. (single-column fitting image) PCA scores from UHPLC-FL chromatograms, representing 91% of total data variance: non-aged (NA) and light exposed (exposure B) samples of undyed (squares), cochineal-dyed (circles) and BZN f26 (triangles) silk, submitted to ambient (A) and low (L) RH/T and oxygen.

in oxygen-rich conditions the samples exhibited positive scores in PC1, as well as wider chromatographic variability.

The tyrosine content in non-aged cochineal-dyed and BZN17 f26 samples was lower than in non-aged undyed silk. This corresponds with the FTIR data, which indicate that the structure of both cochineal-dyed and BZN17 f26 silk had been altered already, probably due to mordant and dyeing. This contradicts studies that have suggested that these treatments have a preserving effect on silk [33,35].

5. Conclusions

The experiments described in this paper were performed with a limited number of samples and under not entirely repeatable conditions. Thermal and light exposure periods were too short to allow extrapolation to long-term preservation. Nevertheless, trends in the results could be observed and provisional conclusions could be drawn. Silk is a natural and complex product, which has a heterogeneous composition at the structural and molecular levels. Its degradation will never be completely reproducible, as it occurs unevenly within the same silk strip and will always provide analytical results with significant standard deviation [23,33]. Testing a larger number of silk samples with longer exposures could possibly bring more conclusive interpretations.

Nonetheless, it was still possible to investigate the degradation of silk with the data acquired from colour measurements, FTIR-ATR and UHPLC-FL analyses, supported by a multivariate statistical method. The FTIR results showed that, after more than 300 years buried in the bottom of the sea, the structure of the BZN17 f26 archaeological silk was not very different than that of modern dyed silk, with the UHPLC profiles showing a similar amino acid composition as non-aged modern cochineal-dyed silk. It is likely that comparable results would be obtained with silks unearthed from other archaeological contexts.

High intensity light exposure in oxygen-rich atmospheres at a slightly elevated temperature caused severe damage to the silk, irrespective of humidity. This was evident in FTIR spectra, where alterations in the amide bands and the formation of new carboxylic groups were observed, as well as significant changes in amide I's crystallinity index. This was principally noted for cochineal-dyed and BZN17 f26 silk, which appear to be more susceptible than undyed silk, probably due to mordant and dyeing treatments. Moreover, UHPLC analyses showed a significant decrease of the

tyrosine content for all samples, possibly as a result of transformation into alanine. In contrast, only small alterations in colour, structure and amino acid composition were observed after light exposure in low-oxygen environments. Results from thermal ageing were insufficient to draw meaningful conclusions about the degradation process of undyed and cochineal-dyed silk. Colour changes could be observed, but changes occurring at the structural and molecular levels were too small to understand the processes causing them. Results from the colour measurements showed that reducing RH or oxygen concentration in otherwise dark conditions decreased the extent of discolouration, which suggests that a combination of hydrolysis and oxidation may take place. Compared to light exposure, the rate of colour change in the dark is considerably slower.

The implications of this study for a preliminary preservation strategy for the maritime archaeological silk objects from the BZN17 shipwreck are that, in storage, light should be excluded and humidity levels kept low. RH values below 50% are often advised in textile conservation literature, but for this collection, the RH should be kept constant at 30%, to preserve the metal threads in the fragments and to avoid the hydrolysis of iron sulphide particles present on the fibres of many fragments [17,45]. For the display of the collection, a low-oxygen atmosphere slows down photo-oxidation reactions by a factor of 4 to 5 compared to ambient oxygen levels, according to the colour measurements and FTIR analyses. Humidity levels are of minor importance in a low-oxygen environment; yet it is advisable that they should be kept below 50% nevertheless. When objects are stored in the dark, the absence of oxygen appears to be less relevant. However, this does not imply that dark storage ensures long-term preservation of archaeological silk. There is anecdotal evidence of completely disintegrated archaeological silks that were retrieved after many years of storage in the dark. Unfortunately, there is no information about their condition and previous treatment before going into storage. Internal factors can play a role as well. Therefore, low-oxygen storage of the collection could be considered as a cautious strategy. Whether the costs of high-tech, air-tight low-oxygen display cases outweigh the benefit of increased exhibition time of the silk objects is ultimately a decision for the museum. Given that many objects will, most of the time, reside in store, low-oxygen storage in sealed bags with oxygen absorbers is an affordable option, and it is relatively accessible to be handled by the museum staff. This is also advisable for other silks originating from other archaeological contexts; although their long-term response to museum deterioration agents should be always assessed beforehand.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.culher.2020.04.004>.

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