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Investigation of crimson-dyed fibres for a new approach on the characterization of cochineal and kermes dyes in historical textiles



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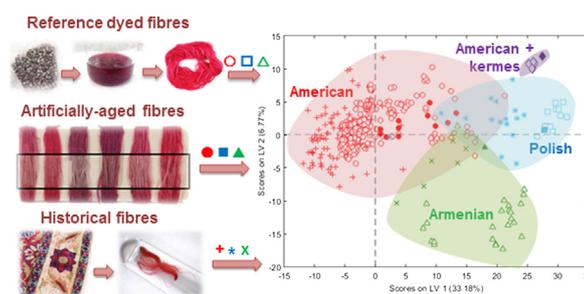
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HIGHLIGHTS

- Chromatographic composition is related to the type of fibre and dyeing parameters.
- Photo-degradation compounds are detected in artificially-aged and historical fibres.
- UHPLC, MS and EDX show similarities between dyed references and historical fibres.
- UHPLC along with PLS-DA provide precise classifications of close-related dyestuffs.
- PLS-DA of UHPLC data gives more conclusive results than compounds quantification.

GRAPHICAL ABSTRACT



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ABSTRACT

The colorant behaviour of cochineal and kermes insect dyes in 141 experimentally-dyed and 28 artificially-aged samples of silk and wool was investigated using ultra-high performance liquid chromatography coupled to photodiode array detector (UHPLC-PDA), liquid chromatography electrospray ionisation mass spectrometry (LC-ESI-MS) and image scanning electron microscopy – energy dispersive X-ray spectroscopy (SEM-EDX). Partial-least squares discriminant analysis (PLS-DA) was then used to model the acquired UHPLC-PDA data and assess the possibility of discriminating cochineal insect species, as well as their correspondent dyed and aged reference fibres. The resulting models helped to characterize a set of 117 red samples from 95 historical textiles, in which UHPLC-PDA analyses have reported the presence of cochineal and kermes insect dyes.

Analytical investigation of the experimentally-dyed and artificially-aged fibres has demonstrated that the ratio of compounds in the insects dye composition can change, depending on the dyeing conditions applied and the type of fibres used. Similarities were observed when comparing the UHPLC-MS and SEM-EDX results from the dyed and aged references with the historical samples. This was verified with PLS-DA models of the chromatographic data, facilitating the classification of the cochineal species present in the

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historical samples. The majority of these samples were identified to contain American cochineal, which is in agreement with historical and dye identification literature that describe the impact of this dyestuff into European and Asian dyeing practices, after the Iberian Expansion in the 16th century.

The analytical results emphasize the importance of using statistical data interpretation for the discrimination of cochineal dyes, besides qualitative and quantitative evaluation of chromatograms. Hence, the combination of UHPLC-PDA with a statistical classification method, such as PLS-DA, has been demonstrated to be an advisable approach in future investigations to assess closely related species of natural dyes in historical textile samples. This is particularly important when aiming to achieve more accurate interpretations about the history of works of art, or the application of natural dyes in old textile production.

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1. Introduction

In the last decades, a growing interest has been put onto the analytical characterization of natural organic dyes in cultural heritage objects, as a way of achieving more information about provenance, date and production of the objects, evaluating conservation and restoration treatments for those objects or to respond questions that historical documentation has not fully covered [1–4].

This characterization has been widely undertaken using high performance liquid chromatography (HPLC) [4–10]. Cultural heritage objects are often coloured with complex matrices of closely related species of certain families of plant and animal dye sources. These comprise similar compounds and minor compounds with variable ratios. Accurate HPLC characterization is essential to distinguish between each species, as the knowledge of their specific chromatographic profiles can help identifying them in the objects [10–16].

In this context, there has been an increasing awareness to improve resolution and detection in HPLC results, in order to obtain more detailed chromatographic information. For instance, an increasing number of dyestuff extraction methods has been developed [4,8,9,17–20]: as the type of dye extraction adopted can influence the quality and the quantity of the isolated compounds, a suitable extraction method can bring more detailed information about the dye source [6]. Also, a method using ultra-high performance liquid chromatography coupled to a photodiode array detector (UHPLC-PDA) has been recently optimized for the characterization of natural dyes in cultural heritage objects, which has been shown to deliver more efficient and sensitive results than an HPLC-PDA method previously used [10].

Chromatographic information can be moreover supported by additional techniques. Mass spectrometry (MS), for instance, has become an indispensable tool for the investigation of molecular structures of unknown dye compounds detected with liquid chromatography [3–6,8,21–24]. In addition, multivariate statistical analysis has recently become a powerful tool for the study of natural dyestuffs, if a large amount of data displaying similar chromatographic profiles is available [11,12,16,25]. For example, Serrano et al. used principal component analysis (PCA) to discriminate the HPLC results of (closely related) species of cochineal insect dyes and characterize them in historical textiles [11].

Cochineal and other insect dyes have always been among the most appreciated natural organic dyestuffs for colouring textiles. Their fame was especially owed to the brilliant and enduring shades of crimson they provided to animal fibres (silk and wool). Though, given their scarcity, laborious collection and complex dyeing process, they were inevitably expensive and, hence, principally used in the manufacturing of luxury fabrics [2,26–29].

Until the 16th century, Asian and European manufacturers obtained their crimson from insects of lac (*Kerria lacca*), kermes

(*Kermes vermilio*) and Polish and Armenian cochineal (*Porphyrophora polonica* and *Porphyrophora hamelii*, respectively). Growing in specific geographic regions, these dyestuffs were used locally or traded to the most important dyeing centres, through the main trade routes connecting both continents [2,27,29]. With the Iberian Expansion at the beginning of the 16th century, the Spanish started to bring from Mexico a new species of cochineal (*Dactylopius coccus*), which European dyers soon realized to be much richer in red colorant than the European and the Asian insect dyes (10–12 times more colorant than kermes, for instance). This allowed for a lesser number of American insects to be used for dyeing the fabrics, thus bringing a decrease to their final price [2,26–31]. This economical factor would inevitably raise the demand for this dyestuff in European and Asian markets, in such way that it would become one of the most profitable Spanish imports from the Americas [28,36,32].

Contemporary historical publications have been systematically affirming that, within few decades after the first shipments of American dyestuff started to arrive to Europe, this was swiftly adopted by textile manufacturers, replacing all other insect sources of red, by the end of the 16th century. Some further support that a similar process occurred when the American dyestuff was traded to Asian regions [26–28,30–36]. While the European case is reasonably well documented, historical sources confirming the use of American cochineal in Asia are rare [34]. Extensive chemical studies have attempted to identify the precise insect dye used in European and Asian historical textiles [31,33,34] and paintings [36], to provide important evidence for tracing the global impact of American cochineal.

The HPLC characterization of insect dyes in cultural heritage objects was firstly suggested by Wouters and Verhecken [17,37,38] and it is generally based on the visual examination and quantification of representative compounds detected with a UV–vis detector, Table 1.

Cochineal and lac dye insects comprise different compounds and minor compounds that make them easily discernible in colorant mixtures. While lac dye comprises laccaic acids, cochineal is currently well characterized by the major compound carminic acid (ca, 2-C-glucopyranoside of kermesic acid) and other minor compounds, such as dcII (2-C-glucopyranoside of flavokermesic acid), dcIV (2-C- α -glucofuranoside of kermesic acid) or dcVII (2-C- β -glucofuranoside of kermesic acid) [22]. On the other hand, kermes alone can be distinguishable from cochineal or lac, but not in mixtures with them, since they share similar compounds (flavokermesic acid (fk) and kermesic acid (ka)). As for cochineal insect species, they also share analogous compounds and, hence, their differentiation depends on the ratio of minor compounds dcII, fk and ka. Based on this ratio, Wouters & Verhecken [17] were able to determine the cochineal species present in wool and cotton historical fibres.

Table 1
Representative compounds in insect dye extracts and respective relative abundances, obtained with the integration of chromatograms at 275 nm [17,38].

Insect dyes	Representative compounds	Relative abundances (%)
Lac dye	Laccaic acids (la's), flavokermesic acid (fk), kermesic acid (ka)	La's (71–96), fk + ka (3.6–9.0)
Kermes	Fk, ka	Fk (0–25), ka (75–100)
Polish cochineal (<i>P. polonica</i>)	ppl, carminic acid (ca), dclV, dcVII, fk, ka	Ca (62–88), fk + ka (12–38)
American cochineal (<i>D. coccus</i>)	dclI, ca, dclV, dcVII, fk, ka	dclI (1.4–3.8), ca (94–98), fk + ka (0.4–2.2)
Armenian cochineal (<i>P. hamelli</i>)	dclI, ca, dclV, dcVII, fk, ka	dclI (0.1–1.2), ca (95–99), fk + ka (1.0–4.2)

Recently, the same methodology was used to determine the cochineal species present in silk historical fibres. Though, a significant variation of the minor compounds was observed in the insects dye extracts, thus compromising the characterization of the precise species [11,38]. Indeed, similar chromatographic compositions have frequently brought inconclusive results when ascribing cochineal species to cultural heritage objects [7,8,11,31,38–43]. For this reason, results are often supported by historical interpretations, and hence, studies claim to identify American cochineal in European [31,33] and Asian textiles [34,35,44], dating to and after the 16th century.

Furthermore, cochineal species identification in historical samples is generally made by comparing the unknown chromatographic results with that from reference insect dyes. However, it should be considered that different dye extraction methods [11,18], dyeing treatments [45,46], or the natural ageing of both colorant [47–49] and fibres [49,50], can potentially influence the chromatographic composition of the historical cochineal-dyed fibres. In fact, it has been recently reported that the amount of dclI present in silk fibres dyed with American cochineal is much smaller than that detected in wool dyed fibres or in insect dye extracts [42].

In this study, an analytical investigation was developed to fully assess the colorant behaviour of cochineal insect species, before establishing comparative interpretations with historical fibres. UHPLC-PDA analyses were undertaken on 66 samples of kermes, American, Armenian and Polish cochineal insects, 141 wool and silk fibres dyed at different dyeing conditions using these insects, and 28 artificially-aged fibres (from the total of 141). After qualitative and quantitative examination of the chromatographic results, and given the large amount of data obtained, partial-least squares discriminant analysis (PLS-DA) was applied to model the data and assess the possibility of discriminating cochineal insect dyes and their correspondent dyed and aged reference fibres. In addition, given the possibility of detecting a mixture of cochineal with kermes in historical fibres, this type of chromatographic composition was assessed here as well. A set of 117 fibres from 95 historical textiles was then analysed with UHPLC-PDA and, afterwards, the results identified with cochineal species were projected on the models constructed with the dyed and aged reference samples. Cochineal representative compounds and unknown compounds detected in artificially-aged and historical fibres were investigated with electrospray ionisation mass spectrometry (ESI-MS) coupled to the UHPLC system. Additionally, the 28 samples selected for artificial-ageing and the historical fibres were compared with image scanning electron microscopy – energy dispersive X-ray spectroscopy (SEM-EDX), in order to compare the dyeing conditions used for both experimentally-dyed and historical samples.

2. Experimental

2.1. Materials and solvents

Methanol (99.9%, HPLC gradient grade) and formic acid 98% from Sigma–Aldrich (Steinheim, Germany), and deionised water (Millipore Simplicity™ Simpapak® 2, R = 18.2 MΩ cm, U.S.A.) were

used for the UHPLC gradient grade and UHPLC-MS analyses. For sample preparation, besides methanol and deionised water, dimethyl sulfoxide (DMSO) from Merck (Munich, Germany) and hydrochloric acid 37% (HCl) from Acros Organics (Geel, Belgium) were used.

About 129 experimentally-dyed samples were prepared on tussah silk and fleece wool from P&M wool craft (Hanslope, England). Given that reproducibility was an important factor when comparing experimentally-dyed samples, it was opted to use, when possible, laboratory grade materials: distilled water (pH ~5), tap water (pH ~8.2), lake water (pH ~8.5), rain water (pH ~6.2), bottled water (pH ~8), Marseilles soap, alum (potassium aluminium sulfate 99.5%) and starch from Lamers en Indemans/Interpharm b.v. ('S-Hertogenbosch, Netherlands'), tin chloride and tannic acid from Riedel-de Häen (Seelze, Germany), cream of tartar (potassium hydrogen tartrate) and potash (potassium carbonate) from Merck (Darmstadt, Germany), calcium nitrate from Interchema b.v. (Oosterzee, Netherlands), calcium carbonate from Acros Organics (Geel, Belgium), sodium nitrate from Sigma–Aldrich (Steinheim, Germany), oxalic acid from Sigma–Aldrich (Steinheim, Germany), oak galls and gum Arabic from Kremer-Pigmente (Aichstetten, Germany), turmeric, copper sheets and sea salt from local stores and ashes from burnt oak wood. The 129 samples, along with other 12 experimentally-dyed samples donated for this study, were submitted to UHPLC-PDA analyses (6 replicates per sample).

A total of 66 types of insect samples of kermes and American, Armenian and Polish cochineal insect species were analysed with UHPLC-PDA (3 replicates per type of insect sample) for further comparison with the experimentally-dyed samples. American cochineal supplied by Kremer-Pigmente (Germany) was used as a standard for UHPLC-PDA and UHPLC-MS analytical conditions and for carrying out most dyeing experiments, since it is more accessible and economic. Experiments were carried out as well using kermes, Armenian and Polish cochineal.

A total of 117 red samples of silk and wool were obtained from 95 historical textiles belonging to diverse cultural heritage institutions. The majority of these samples had been previously analysed in other papers [11,33,38,51] and, hence, the presence of insect dyes was expected. In addition, new samples (of unknown dye source) were obtained as well. Sample selection was principally based on the crimson shade exhibited by the respective historical textiles, but also on their date (mostly between 15th to 17th centuries), provenance (European and Asian regions), and type of fibres (silk or wool).

Additional information on the materials used for carrying out the dyeing experiments, as well as details about the provenance of the insects, the experimentally-dyed samples and the historical samples are given in Electronic Supplementary Material, [ESM 1](#).

2.2. Dyeing experiments

Based on the revision of several contemporary studies [17,45,46,52] and historical recipes dated between the 15th and 18th centuries [2,53–57], about 129 dyeing experiments on silk and on wool were undertaken following a three-step procedure of soap,

mordant and dye baths, while varying different parameters: types of mordant (alum and tin), additives (cream of tartar, tannic acid, oak galls, calcium nitrate, calcium carbonate, sodium nitrate, turmeric, gum Arabic, starch, copper sheets, wood ashes and sea salt), insect dyes (kermes and American, Armenian and Polish cochineal), water (distilled, tap, lake, rain and bottled – used in all baths, as well as for washing and rinsing the fibres), temperatures (100 °C, 80 °C, 40 °C and room temperature), pH (4, 7 and 10 – controlled by potash or oxalic acid) and time of exposure to both mordant or dye baths (30 min, 1 h and overnight). These parameters were tested to evaluate the quality of the dyed results, as well as possible influences on the chromatographic ratio of compounds in the insects' colorant. Ultimately, the aim was to achieve a relatively close shade to that observed in crimson historical fibres, as well as comparable chromatographic results. Details on the general procedure and parameters used to carry out dyeing experiments are given in [ESM 2](#). Colour coordinates (CIE L* a* b*) measurements were performed in all 129 samples (three times per sample), using a Minolta spectrophotometer CM-2600D (Konica Minolta Sensing, inc., Japan).

2.3. Artificial ageing

A selection of 28 experimentally-dyed samples was made, based on the most representative dyeing parameters applied. These were submitted to artificial light ageing conditions, using a Xenotest, Alpha High Energy (Atlas[®]) equipment, with a filtered Xenon-Arc lamp (Xenochrome type 320 (nm)). Samples were exposed for a total period of 160 h, with a test chamber temperature of 50 ± 5 °C, relative humidity (RH) of $40 \pm 5\%$ and an illuminance of 105 Klux. Colour coordinates measurements and UHPLC-PDA and UHPLC-MS analyses were performed on the aged samples to investigate the photo-oxidation damage, depending on the quality of the fibres (silk or wool) and the dyeing conditions.

2.4. UHPLC-PDA and UHPLC-MS

2.4.1. Sample preparation

In order to accurately characterize both insect dyes and textile samples in this study, it was important that acquired chromatograms displayed high reproducibility and resolution.

Insect samples were grinded and accurately weighted (circa 0.2–0.3 mg). They were then dissolved in 100 μ L of DMSO, subjected to mechanical agitation and heated up to 80 °C in a water bath for 10 min. To American cochineal samples which were often too concentrated, 100 μ L of DMSO was added. Samples were centrifuged for 10 min at 2000 rpm and part of the resulting supernatants were transferred to new vials. These vials were centrifuged once more prior analyses.

Because the colorant in cochineal-dyed fibres is mainly in mordant form, an acidic extraction solution of HCl 37%: MeOH: H₂O (2:1:1, v/v/v) [17] was used. This provided efficient and reproducible chromatograms, essential to compare quantitatively both insect dyes and textile samples. Then, a two-step extraction method was adapted [58], using DMSO prior to hydrochloric acid. In this way, information on the potential presence of additional dyestuffs (especially in unknown historical samples) can still be obtained, as DMSO is able to extract vat and direct dyes: 1) DMSO was added to the fibres and heated up to 80 °C in a water bath for 10 min, after which, the extract was transferred to another vial; 2) acidic extraction solution was added to the fibres and heated up to 100 °C in a water bath for 10 min. After the extraction, the dye extracts were evaporated to dryness under gentle nitrogen flow, and the resulting dry residues were reconstituted with the DMSO extracts, thus combining the two steps. These were then subjected to

mechanical agitation and centrifugation twice, as performed for the insect samples, before analyses. For the dyed and aged reference samples, a relatively large sample size (1 mg), along with 100 μ L DMSO and 100 μ L acidic solution, was adopted, in order to determine the chromatographic behaviour of the colorant. For historical samples, however, a smaller sample size was used (0.1–0.3 mg), along with 50 μ L DMSO and 50 μ L acidic solution.

2.4.2. Apparatus

UHPLC-PDA analyses were performed using a Waters Acquity[™] H-class UHPLC system (Waters Corporation, Milford, MA, U.S.A.) equipped with a quaternary solvent delivery system, a column oven, an autosampler and a PDA detector. PDA data was recorded from 200 to 800 nm with a resolution of 1.2 nm (2 scan/s), and the analyses monitoring was settled at a detection wavelength of 254 nm. The equipment was controlled by Empower 2.0 Chromatography Data Software from Waters Corporation.

Separation was performed using a method published earlier by Serrano et al. [10], which has demonstrated to deliver suitable chromatographic detection and resolution for the analysis of mixtures of insect dyes. Thereby, analytical conditions were carried out using in a Waters Acquity[®] UHPLC BEH Shield RP18 1.7 μ m of 2.1×150 mm column, protected by a filter unit (0.2 μ m), with 2 μ L injection volume, a flow rate of 0.2 ml min⁻¹ and a constant temperature of 40 °C. The mobile phase comprised 10% aqueous methanol (v/v) (solvent A), pure methanol (solvent B) and 1% aqueous formic acid (v/v) (solvent C) in a gradient elution program scheduled for a 40 min run: 0–1.33 min, isocratic gradient of 80A:10B:10C (v/v/v); 1.33–2.33, linear gradient to 74A:16B:10C (v/v/v); 2.33–5.33, linear gradient to 55A:35B:10C (v/v/v), kept in isocratic gradient until 9 min; 9–14 min, linear gradient to 30A:60B:10C (v/v/v); 14–25 min, linear gradient to 5A:85B:10C (v/v/v); 25–26 min, linear gradient to 100B, kept for 4 min; and 30–32 min, linear gradient to 80A:10B:10C (v/v/v), kept for 8 min.

ESI-MS Micromass Q-tof-2 was coupled to UHPLC to assess the minor compounds in the cochineal standard and dyed fibres (silk and wool), as well as photo-oxidation products present in one aged extract from the cochineal standard and in extracts of artificially-aged and historical samples (silk and wool). A flow split of 1/5 (v/v) was set between the MS and the PDA, respectively. MS detection was undertaken in the negative ionisation mode, which gives higher sensitivity for anthraquinones [11,38], a collision energy of about 8–10 eV for single MS mode, a capillary voltage of 3.2 kV, a cone voltage of 40 V and a source temperature of 80 °C. The nitrogen gas flow rate had a desolvation temperature at 150 °C, and was set for 120 L h⁻¹ for cone gas, 90 L/h for nebulizer gas and 120 L h⁻¹ for desolvation gas. The scan range for *m/z* was set for 0–800.

2.5. Data treatment

2.5.1. Compounds evaluation

For the qualitative interpretation of the chromatographic results, and further evaluation of the integrated peak areas [11,17,38], chromatograms were always examined at 275 nm, as the major and minor compounds from the insect dyes, as well as the photo-degradation products, could be detected at this wavelength. Hence, characterization of the compounds and minor compounds in cochineal insects, dyed-fibres and historical samples was undertaken based on the integrated peak areas at which the respective PDA spectra could still be recognized. This step was essential to recognize whether minor compounds should be considered noise, something that is particularly helpful when characterizing species of cochineal in unknown historical samples. Given the relatively high amount of ca compound in cochineal extracts, it was expected

that this would be always detected in “real” samples, along with the minor compounds. Indeed, if the response of ca would be too small, the minor compounds should not be detected and, for this reason, such chromatographic results could not be considered.

2.5.2. Multivariate statistical analyses

Given the considerable number and the variation of data acquired with UHPLC-PDA, a supervised classification method, partial-least squares discriminant analysis (PLS-DA), was used to model and, furthermore, assess the possibility of discriminating the chromatographic profiles between cochineal species, as well as their corresponding experimentally-dyed and artificially-aged samples. The chromatographic data region considered (absorbance at 275 nm) was between 14.5–17.5 and 20.5–24 min (retention time), because this includes relevant dye compounds (dcIV, dcVII and/or fk and ka). Models were built with samples of known identity (insect dyes and artificially aged and non-aged silk and wool fibres experimentally-dyed with them), grouped into classes of insect dye species (American cochineal, Armenian cochineal, Polish cochineal and a mixture of American cochineal and kermes). Historical samples characterized by UHPLC-PDA with the presence of cochineal or, possibly, a mixture with kermes, were projected onto these models to reveal their identity. For this, the PLS Toolbox 7.9.3 (Eigenvector Research, Manson, WA, USA) in Matlab R2014a (Mathworks, Natick, MA, USA) was used. Details on the models construction are given in [ESM 3](#).

2.6. SEM-EDX

Few fibres, from the 28 selected experimentally-dyed samples (prior to ageing) and from the historical samples, were fixed on carbon adhesive over aluminium stubs for SEM-EDX analyses. Samples were observed with a SEM, JSM5910LV from JEOL, to assess possible contaminating particles on the textile fibres, using 20 kV accelerating voltage, 11 mm working distance, 48 spots size, and backscattered signal (BES) at 40 Pa. EDX analyses were undertaken using a SDD detector from Thermo Fisher scientific and a NSS software. Analyses were undertaken on regions free of contaminating particles to evaluate the presence of mordants and additives, using 80 s live time and pulse processor with projected maximum throughput of 71400 cps. Owing to the non-uniform surface and variable thickness of the fibres, EDX analyses were performed three times for each sample, to obtain a qualitative assessment of the elements present.

3. Results and discussion

3.1. Experimentally-dyed samples

The characteristic compounds of cochineal and kermes dyes could be properly characterized in the insects and textile samples analysed with UHPLC-PDA and UHPLC-MS, as displayed in [ESM 4](#). An additional compound, DCOFK (3-O-glucoside of flavokermesic acid, with $475 [M-H]^-$) recently characterized by Stathopolou et al. [22], was detected as well in the insect extracts and in the dyed wool samples. Other compounds identified in this publication were not found, which can be explained by the fact that different extraction and analytical conditions were used.

The best crimson shades are obtained when cochineal and kermes dyes are applied to animal fibres. UHPLC-PDA results for silk and wool cochineal or kermes-dyed fibres obtained in this study can be explained by three types of reactions that can occur depending on the dyeing parameters used: complex reactions that produce stable and insoluble chelate complexes of dye–aluminium cation–fibre; direct reactions between polar groups of both

colorant and fibres; and acid reactions, through the ionic linkage of carboxylic groups from the colorant and the fibres amino groups [45,59,60].

In general, skipping the soap bath, using other water than distilled or rain, or using calcium carbonate or sodium nitrate has brought light, uneven dyed silk fibres. Indeed, these can trigger side reactions creating insoluble compounds that attach to the fibres or precipitate the colorant, and thus, the yield of colorant interacting with the fibres diminishes [45,46]. For this reason, historical recipes recommend to use water or alum as much pure as possible [46,55,56].

To avoid side reactions, historical recipes often suggest the use of additives, such as cream of tartar and oak galls [46,53–57]. These contain acidic compounds that produce complex reactions with contaminants, freeing the mordant and the colorant to interact with the silk fibres [46,55]. Cream of tartar, applied in both mordant and dye baths, has revealed to have a greater influence than tannic acid (or oak galls), as lower concentrations are required [46].

The above-mentioned parameters had a small effect on wool. This generally has a higher dye adsorption than silk, and this is clearly observed when comparing their chromatographic profiles, [Fig. 1](#). Indeed, it was observed that the ratio and response of dye compounds in dyed wool are similar to those of insect extracts, and they are not prone to alterations as much as silk. This might be related to the fibres structural composition: wool principally comprises amorphously arranged amino acid chains with functional side groups, connected by disulphide cross-linked bonds, and

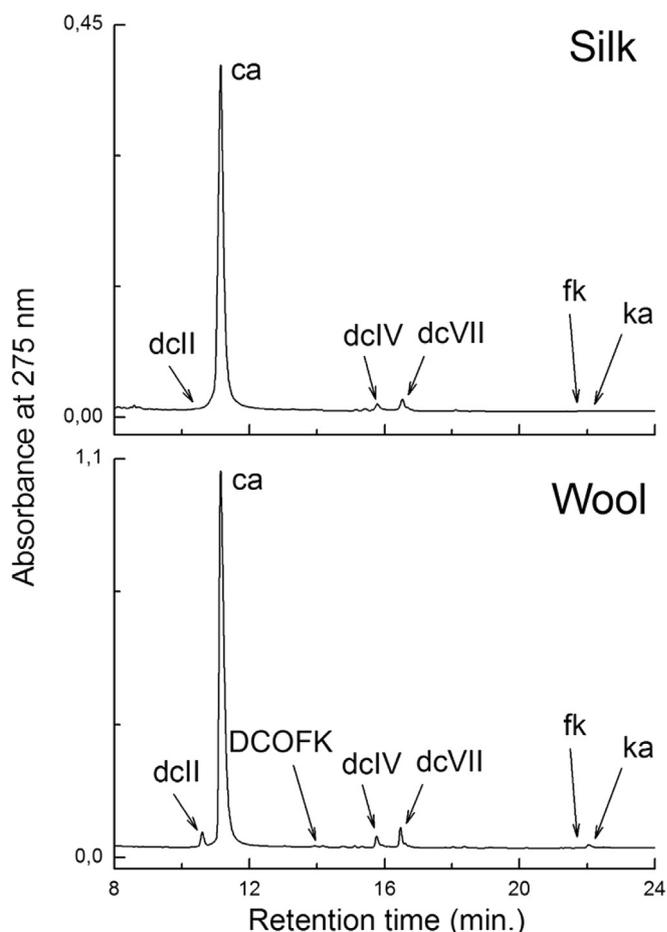


Fig. 1. Chromatograms obtained for silk and wool samples dyed with American cochineal at optimal conditions.

readily available for interactions with alum and cochineal; silk, on the other hand, mainly comprises very crystalline regions with chains of small organized amino acids with side hydroxyl groups that make silk very difficult to interact with outside compounds [61].

On the other hand, it is possible that dye compounds react differently to both mordant and fibres because of their molecular structures (ESM 4), as previously reported for alizarin and purpurin, when dyeing with madder [62]. At optimal dyeing conditions, the neighbouring carbonyl and hydroxyl groups in cochineal compounds create stable complex reactions with aluminium cations, which are partly connected to the hydroxyl groups of silk amino acids [59,60]. Since complex reactions occur on both sides of the ca, dcIV, dcVII and ka molecules (double-sided), stable cross-linking chains should form with alum and silk, besides less stable direct and acid reactions. On the other hand, dcII, DCOFK and fk compounds have one less hydroxyl group (single-sided) and, hence, they can only engage in complex reactions with one side of the molecule. Therefore, stable cross-linked chains cannot be formed, which might explain why silk samples dyed at optimal conditions have shown a decreased response/absence of dcII and fk (and no DCOFK at all). On the contrary, if dyeing conditions are not propitious, the rate of mordant reactions decreases and, consequently, the amount of double-sided compounds. In this case, it is likely that direct and acid reactions play a more important role in the binding mechanism, thus increasing the rate of single-sided compounds. This was furthermore verified when the colorant was easily extracted from the fibres, using DMSO.

Given the amorphous composition of wool, it is expected that a balance of mordant, direct and acid reactions would occur with the dye compounds. The same could be applied for tin chloride-mordanted silk fibres (mordant and dye baths at low pH and no additives), since tin seems to interact stronger than alum on silk, thus creating a stable ground to receive the dyestuff.

Optimal conditions to dye crimson with alum were obtained using cream of tartar in the mordant and dye baths (pH 7); mordant for 1 h at 80 °C, followed by 24 h at room temperature; dyestuff extraction for 1 h at 100 °C; and dye bath for 1 h, kept at 80 °C [45,46]. At these conditions, UHPLC-PDA results were highly reproducible, indicating high yields of colorant and with silk fibres displaying a lower response of dcII and fk. When altering the dyeing parameters, opposite results were obtained.

In order to achieve shades closer to crimson historical samples, calcium nitrate was added to the mordant bath. In old dyeing practices, calcium would have been added through wood ashes, alum rocks or diluted in water [45]. Indeed, EDX analyses performed in this study on historical fibres have systematically displayed the presence of this cation, as also reported elsewhere [63]. In addition, by following a historical recipe [52], results on wool were similar to those achieved before but, on silk, very similar results to those of historical textiles were obtained. Although further research is required to assess the individual role of the ingredients added in the dyeing process (copper sheets, sea salt, turmeric or gum Arabic), it is evident that their presence is fundamental, not only to enhance the final colour, but to protect silk from abrasion as well [2,56,61].

When testing the colorant behaviour for other insect dyes (kermes, Polish and Armenian cochineal), dyeing conditions applied were similar to those optimized for American cochineal, although it was soon observed that lighter shades were obtained with these insects on silk. Further research would be required to assess the best approach for obtaining crimson with these insects, although their availability is quite limited [29]. Nevertheless, deeper shades were achieved on wool, with kermes displaying orange red shades, rather than crimson. This is probably related

with the insects' colorant composition: kermes-dyed fibres comprise a mixture of (red) ka and (orange yellow) fk, whereas the pinkish red cochineal-dyed fibres, mainly contain (more bluish red) ca [60]. This might explain why turmeric (yellow colorant) is mentioned in post-16th-century historical recipes to achieve orange red shades with American cochineal, while recipes with kermes are not.

UHPLC-PDA results for silk dyed with Armenian cochineal have demonstrated colorant behaviour similar to that reported for American cochineal-dyed silk, with a decrease/absence of dcII and fk response. The same was observed for a mixture of American cochineal and kermes, which chromatographic profile becomes similar to that of silk dyed with Polish cochineal (fk and ka compounds from kermes amount for c. 20% relative abundance) [17,37].

Due to this alteration, compounds quantification [17,37] can be compromised. As previously demonstrated by Serrano et al. [11], Fig. 2 shows that this method is not sufficient for dyed fibres, especially when dcII and fk are too small. Indeed, scores for American and Armenian insects and dyed samples show dispersion; fall outside the zones of highest probability; and overlap each other zones.

PLS-DA models were built to discriminate cochineal species, using chromatographic regions which had relevant common compounds - dcIV, dcVII, fk and ka. Regions including ca or dcII compounds were not considered, because they substantially vary between samples of the same class. Given the dissimilar chromatographic profiles exhibited by dyed-wool and silk, more accurate discriminations were obtained when considering the two types of fibres in separate models, Figs. 3 and 4.

In both figures, the first two LV's depict a higher degree of separation between cochineal species (American, Armenian, Polish and mixture of American with kermes), in comparison with Fig. 2. For instance, American cochineal insects and respective dyed fibres are well distinguished from other dyestuffs, generally displaying negative scores in LV1 (higher amount of dcIV and dcVII). The majority of these scores show high congregation, indicating a low variability in the examined chromatographic region. American cochineal samples displaying positive LV1 mostly correspond to fibres dyed at unsatisfactory conditions (lower dcIV and dcVII). On

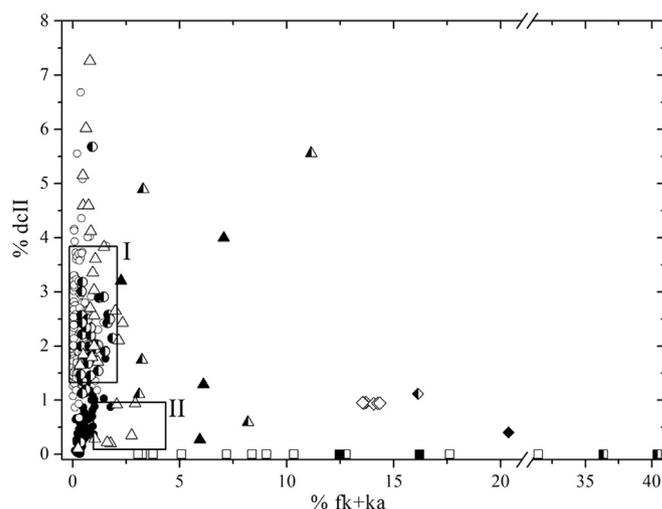


Fig. 2. Graphical system based on the relative percentages of minor compounds dcII and fk + ka, with rectangular areas representing the zones of higher probability (I. American cochineal, and II. Armenian cochineal), according to [17,38]; American cochineal (circles); Armenian cochineal (triangles); Polish cochineal (squares); mixture American cochineal and kermes (diamonds)/insects (white shapes), silk fibres (black shapes), wool fibres (left black shapes).

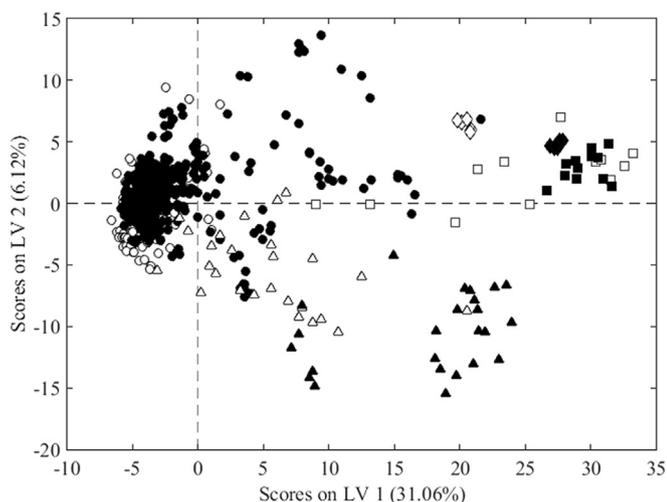


Fig. 3. Silk: Scores on the first 2 LV's of a PLS-DA model of insect dyes and their respective dyed fibres: American cochineal (circles); Armenian cochineal (triangles); Polish cochineal (squares); mixture American cochineal and kermes (diamonds)/insects (white shapes), silk or wool fibres (black shapes).

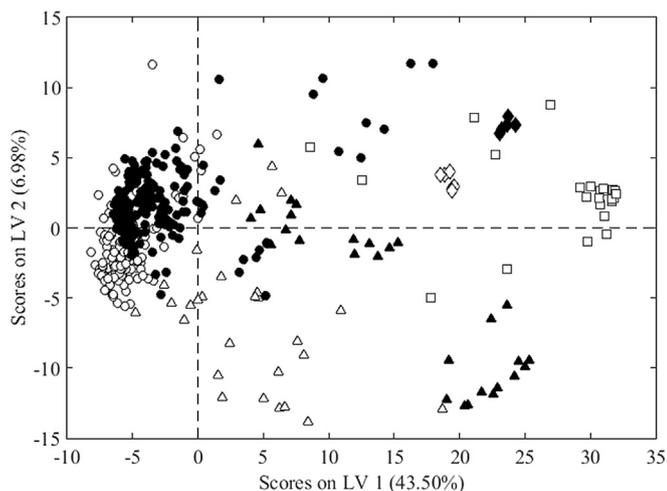


Fig. 4. Wool: Scores on the first 2 LV's of a PLS-DA model of insect dyes and their respective dyed fibres: American cochineal (circles); Armenian cochineal (triangles); Polish cochineal (squares); mixture American cochineal and kermes (diamonds)/insects (white shapes), silk or wool fibres (black shapes).

the other hand, Armenian and Polish cochineal samples show higher within-class variation. Although they show some separation in relation to other classes, there is some overlap with the region of American cochineal samples (on the first 2 LV's).

However, cross-validation has demonstrated that a higher number of LV's is optimal to classify both sets of samples. When using 5 LV's for the silk model and 9 LV's for the wool model, approximately 5.2% (silk) and 4.1% (wool) of samples were wrongly predicted (confusion matrices in [ESM 3](#)).

3.2. Artificially-aged samples

As historical textiles are expected to have been through photo-degradation during their lifetime, induced ageing of the experimentally-dyed samples was carried out. This was important to ascertain possible changes in the chromatographic ratio of the colorant, which could influence the insect dyestuffs discrimination

and possibly give comparable results to those of historical samples.

After submitted to artificial ageing, samples were compared with their non-aged correspondents, using colour measurements. In [Table 2](#) it is noticeable that the rate of fading can vary between samples, depending on the quality of the dyeing and the type of fibre. In all of them, ΔL^* values are always positive, which means that all samples have suffered fading (became lighter). Wool dyed with American cochineal became slightly redder (positive Δa^*), while most wool samples dyed with other insects and all silk samples tended to become less red. Additionally, more than half of the samples tended to become more yellow (positive Δb^*).

"Yellowing" of samples is expected to occur during photo-oxidation of the fibres, as UV radiation interacts with the keratin of wool and fibroin of silk. These interactions occur with their side groups, principally tyrosine in silk, and cysteine, tryptophan and tyrosine in wool. By absorbing UV radiation, they are reduced into new groups, such as aspartic, glutamic and cysteine acids in wool. These new groups are responsible for the yellow shade exhibited by fibres [\[48–50,61\]](#), especially those prepared with tannic acid.

Although no significant photo-degradation had been previously observed in aged cochineal-dyed fibres [\[49\]](#), here UHPLC-PDA results have generally shown lower amounts of chromophore compounds in relation to the non-aged ones. Absolute amounts of colorant registered with UHPLC-PDA before and after ageing are given in [Table 2](#). Here, it is observable that the percentage of colorant loss in wool is reported to be smaller than in silk [\[50\]](#). Moreover, fibres most severely faded are those prepared at unsatisfactory dyeing conditions. This demonstrates that the occurrence of stable complex reactions (fibre-alum-colorant) at optimal conditions brings more resistant and light-fast colours, preserving the fibres protein matrix [\[45,49,50,59\]](#).

Chromatograms for artificially-aged and historical samples also revealed the occurrence of similar photo-degradation compounds, in agreement with Degano et al. [\[49\]](#). In general, these compounds have shown a higher response in aged silk, than in aged wool samples. These were found as well in the extract of an old cochineal standard, prepared about one year before and kept at room temperature and UV light exposure. Non-aged fibres or fresh insect extracts either did not contain these compounds, or their response was very small.

The first two compounds eluted at an early retention time and were colourless according to their UV–vis spectra ([ESM 5](#)). This loss of colour and early retention time can be related to a strong increase in polarity, due to degradation mechanisms. MS analyses indicated that the first compound ($\lambda_{\max} = 255$ nm) has a molecular mass of 452 ($[M-H]^-$ 451; very small response). This can probably be a by-product of splitting bonds in the conjugated system of anthraquinone molecules or a ring opening reaction, possibly followed by a reduction process of adjacent molecules. Due to its small response in the MS detector, the molecular mass of the second compound ($\lambda_{\max} = 280$ nm) could not be characterized. This could perhaps correspond to hydroxybenzoic acid, which has been suggested to be a by-product of the photo-degradation of tyrosine and tryptophan in protein fibres [\[47,50\]](#), as well as flavonoids present in aged textile samples [\[47,48\]](#).

Two more photo-degradation compounds were detected as well, displaying spectra in the visible area. The third compound ($\lambda_{\max} = 498$) was found in aged samples (experimentally-aged and historical fibres and insect extract), but its molecular mass could not be ascertained because of its small response in the MS detector. Though, owing to its UV spectrum, close to that of red anthraquinones ([ESM 5](#)), it could be a photo-degradation by-product of small induced changes occurring in these molecules. The fourth compound ($\lambda_{\max} = 340$) was reported to be an isomer of dcII ($[M-H]^-$ 475), and it only occurred in the cochineal insect extract. Further

Table 2

Differences in colour registered with colour measurements and UHPLC-PDA analyses, between the aged and non-aged experimentally-dyed samples.

	Fibre	ΔL^*	Δa^*	Δb^*	$\Delta \text{Colorant } (\%)^a$
No additives	Silk	16.82	-26.61	18.27	86.33
Dye extract 100 °C + low pH (dye bath)	Silk	15.96	-21.85	9.08	65.09
Cream of tartar + low pH (dye bath)	Silk	10.80	-20.24	2.54	79.14
	Wool	1.59	2.00	2.73	30.63
Tannic acid + low pH (dye bath)	Silk	20.41	-28.01	14.65	44.96
	Wool	3.74	3.28	6.53	54.14
Cream of tartar + Ca(NO ₃) ₂)	Silk	11.93	-21.86	1.32	64.58
	Wool	10.21	10.01	17.61	55.91
Tannic acid + Ca(NO ₃) ₂)	Silk	7.87	-16.74	11.39	66.84
	Wool	9.09	7.87	15.67	62.04
Tap water	Silk	10.87	-18.74	6.59	60.17
	Wool	12.86	0.48	-9.06	53.26
Lake water	Silk	15.90	-23.41	5.69	74.93
	Wool	9.70	6.26	-5.16	49.94
Rain water	Silk	11.99	-16.05	-1.52	58.77
	Wool	3.61	4.17	6.22	42.31
Historical recipe adapted	Silk	5.03	-9.07	-1.10	24.27
Tin chloride	Silk	8.62	-17.02	-10.06	62.04
	Wool	5.86	1.04	10.06	53.35
Kermes (no additives)	Wool	6.48	4.47	11.18	7.15
Kermes (cream of tartar + Ca(NO ₃) ₂)	Silk	9.50	-13.76	5.97	45.14
	Wool	13.56	-11.89	-0.26	60.73
Armenian cochineal (low pH)	Silk	7.58	-14.28	7.85	71.44
	Wool	6.95	-3.50	-7.64	54.50
Polish cochineal (no additives)	Silk	12.07	-14.67	10.32	73.34
	Wool	7.86	-7.38	-8.46	32.35
American cochineal + kermes	Silk	5.22	-14.04	-1.71	42.67
	Wool	3.18	2.69	5.44	45.81

^a Difference calculated with the sum of absolute amounts (integrated peak areas) for the representative dye compounds detected with UHPLC-PDA (dclI, ca, dclV, dcVII, fk and ka) in the aged and non-aged (100%) experimentally-dyed samples.

research on the characterization of these compounds would be necessary to understand their photo-degradation path, although this falls outside the scope of the current study.

3.3. Historical samples

The red samples from a large group of 95 European and Asian historical textiles were analysed with UHPLC-PDA to determine the cochineal species present. This not only confirmed the methodology developed in this study, but also the impact of American cochineal into local dyeing practices. About 50% of these textiles are of Italian origin, but other exemplars are from Spanish, Dutch, Turkish, Iranian, Indian and other regions. They are dated before and after the documented arrival of this dyestuff in Spain in 1521, Italy (and the Ottoman Empire) in 1543, Iran before 1618, and India in 1612 [28–30]. For details about these textiles and their chromatographic results, see ESM 6.

In a first phase, UHPLC-PDA results were visually examined and the presence of cochineal and/or kermes dyes was detected. In some samples, compounds from other dyestuffs were reported as well, such as indigotin, type C component, alizarin or luteolin. These compounds were often recognized as cross-contaminations from adjacent fibres in the textiles weaving structure, although, in specific cases, they were considered part of the colorant mixture as well. Gallic acid and/or tannic acid compounds were detected in about 65% of the samples, which indicates the importance of tannins, such as gallnuts, in crimson dyeing recipes. This ingredient would be added to improve the strength of the fibres [41], while protecting them from contaminations [46]. Also, it would make them advantageously heavier, as silk was sold by weight [2,40,60,61].

From the total of 117 samples, 13 samples from Italian and Spanish textiles, and dated before the 16th century, were characterized with the presence of kermes dyes. From these, one sample

(MT33357, pile) was characterized with a mixture of kermes and Polish cochineal; and two samples (MT22864), with a small amount of ca compound, along with kermes compounds. Compounds quantification of UHPLC-PDA chromatograms was often undertaken to ascribe the cochineal species used but, in this case, the small amount of ca compound and the absence of cochineal minor markers made this task impossible. Indeed, when minor compounds dclI, fk and ka were not detected, quantification was not achievable and, for this reason, species characterization for 21 of the samples was based on the chromatograms visual examination.

If at least one of the compounds was detected, quantified results were plotted in Fig. 5. Given the alteration of minor compounds in dyed silk fibres reported in section “3.2. Experimentally-dyed samples”, it was opted to not include the results for the insect dyes. Hence, for more accurate results interpretation, the unknown historical samples were compared with the reference experimentally-dyed and aged samples. Though, it is worthwhile noticing that, despite their low response, minor compounds in the reference samples were still considered, because the respective cochineal species were known *a priori*. However, and as previously reported in Fig. 2, the overlap of American and Armenian cochineal reference samples compromises the proper characterization of these species and, therefore, their identification in historical textiles. As for unknown samples distanced from this region and showing an absence of dclI, this graphical system can be more clearer about the presence of Polish cochineal, or a mixture with kermes.

PLS-DA models for silk and for wool were made with the experimentally-dyed and the aged samples of cochineal species and the mixture of American cochineal and kermes. Since insects have shown variation in relation to the respective dyed fibres in Figs. 3 and 4, it was opted to not consider them. In this way, a more accurate classification of the insect sources in unknown historical

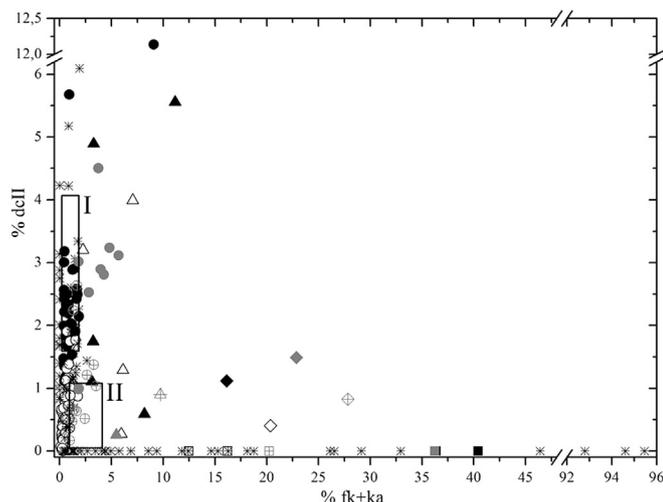


Fig. 5. Graphical system based on the markers relative percentages as described in Fig. 2, with rectangular areas representing the zones of higher probability (I. American cochineal, and II. Armenian cochineal): American cochineal (circles); Armenian cochineal (triangles); Polish cochineal (squares); mixture American cochineal and kermes (diamonds)/silk fibres (white shapes); wool fibres (black shapes); aged silk fibres (grey crossed shapes); aged wool fibres (grey shapes); historical fibres (asterisks).

samples could be obtained. Furthermore, outliers were removed, based on the 95% confidence intervals of the Hotelling T^2 and the Q -statistic: it was found that 3.9% silk and 4.3% wool experimentally-dyed samples were above both limits, and they correspond to very unsatisfactory dyeing conditions. As a result, cross-validation has shown a decrease of optimal number of LV's (3 LV's), as well as fewer misclassifications, with 0.4% of the silk reference samples and 3.3% of the wool reference samples predicted wrongly (confusion matrices in *ESM 3*). This means that almost 100% of the reference samples were classified correctly.

Cochineal-dyed historical samples (97 silk and 8 wool fibres) were projected on these models. These samples did not include the two samples characterized with a small amount of ca compound (MT22864), neither a sample (AS4921), in which a complex mixture of dyestuffs was detected along with cochineal compounds. In these cases, the chromatographic regions analysed are too different from those in the reference samples and, therefore, results may not be accurate.

Classification of cochineal species in historical samples was based on strict predictions (a sample being classified in precisely one class), and when this was not available, classification was based on most probable predictions, and on visual inspection of *Figs. 6 and 7* (2 LV's). These results were then verified with those already obtained with the qualitative and quantitative interpretation of the chromatographic results, as well as the textiles date and provenance. For instance, for sample SGL1907/114, the most probable prediction was American cochineal (99% of highest probability), and the respective plot corresponded to that region in *Fig. 6*. This was furthermore in agreement with the chromatogram's qualitative and quantitative interpretations, and the textile's date and provenance. On the other hand, for sample MNAA1616, American cochineal was the most probable prediction (though, only 30% of highest probability) and the respective plot was relatively close to the region of Polish cochineal in *Fig. 6*. When comparing with the qualitative and quantitative results and the textile's date and provenance, Polish cochineal was a more reasonable attribution.

From the total of 105 historical samples projected onto the PLS-DA models, almost 80% of the samples were classified based on

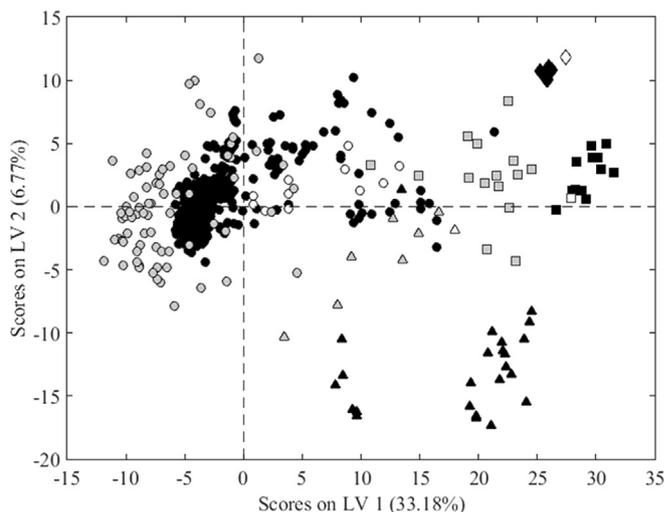


Fig. 6. Silk: Projection of historical samples over PLS-DA models (2 LV's) of aged and non-aged dyed fibres: American cochineal (circles); Armenian cochineal (triangles); Polish cochineal (squares); mixture American cochineal and kermes (diamonds)/non-aged silk and wool fibres (black shapes); aged silk and wool fibres (white shapes); historical fibres (grey shapes).

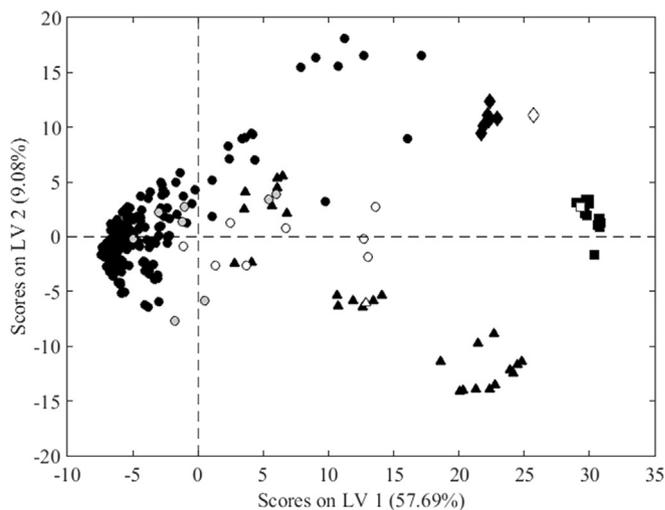


Fig. 7. Wool: Projection of historical samples over PLS-DA models (2 LV's) of aged and non-aged dyed fibres: American cochineal (circles); Armenian cochineal (triangles); Polish cochineal (squares); mixture American cochineal and kermes (diamonds)/non-aged silk and wool fibres (black shapes); aged silk and wool fibres (white shapes); historical fibres (grey shapes).

strict predictions. The results achieved have successfully shown agreement with the textiles date and the provenance (*ESM 6*), except for six samples that were strictly predicted with American cochineal, although they belong to 15th-century textiles. In these cases, the date of the textiles should probably be reconsidered.

It is possible that a higher percentage of strict predictions would be obtained if the number of reference samples for Polish and Armenian would be increased. Moreover, it is important to emphasize that reference samples can never be complete reproductions of the historical samples and, for this reason, PLS-DA classifications are not as accurate as the cross-validation results on the experimentally dyed and aged samples. *Figs. 6 and 7* show that historical samples have a lower score on LV1 (higher amount of dcIV and dcVII) than experimentally-dyed and the aged samples.

These results indicate that historical samples are well conserved, and this is supported by the very small response of

photo-degradation compounds reported in the respective UHPLC-MS results. Also, these results point out that historical fibres possess more colorant than the experimentally-dyed ones, probably due to the efficiency of the dyeing recipe, but also due to the type of fibres. Indeed, SEM analyses revealed that historical fibres are somewhat thicker, which could perhaps provide a wider surface for more reactions to occur with the colorant, thus increasing the colorant yield.

Furthermore, some historical fibres presented many dirt particles under SEM observation, and this was translated in high EDX peaks of aluminium, magnesium, potassium, silicon, calcium, sodium or chloride. Smaller peaks of these elements in historical fibres that were apparently clean, could be an indication of diluted cations in water used for dyeing, or due to additives. The presence of potassium could be related to the use of wood ashes or from the alum, while peaks of sodium and chloride might indicate the use of salt, by contamination or additives. On the other hand, the presence of phosphorus could indicate possible use of phosphate-containing detergents used in conservation treatments [61].

From a historical point of view, the majority of the samples (70%), dating to and after the 16th century and produced in both European and Asian regions, were characterized with the presence of American cochineal. Textiles dated before the 16th century were classified as containing Armenian and Polish cochineal. Interestingly, only one sample was reported with a mixture of kermes and Polish cochineal, while none of the samples was characterized with a mixture of kermes and American cochineal, as suggested by Pearson [56]. These are very important interpretations, as they bring more accurate knowledge of the use of cochineal insects in historical textiles, while establishing invaluable connections with historical and dye identification literature, concerning the impact of American cochineal in local dyeing practices [26–28,30–34].

On the other hand, it is worthwhile noticing that these results contradict those obtained by Serrano et al. [11]. In the previous study, not only insects were mainly used as references, but also a non-classification method, PCA, was adopted. In this case, unknown historical samples were predicted according to the dominant class in the region of the principal components space, where the samples were projected on. While PCA has often been used for discrimination, this is not a particularly reliable approach and more suitable methods exist, such as PLS-DA [64]. PLS-DA is a classification method that performs discriminant analysis on scores on latent variables, which are designed to capture information in the data that is most useful for predicting the class [65]. Thus separation between classes is improved, and the use of discriminant analysis allows estimation of the accuracy of the models (ESM 3). For this reason, PLS-DA analyses, along with the qualitative and quantitative interpretation of chromatographic results, constitutes a recommendable approach for future studies about cochineal and kermes dyes identification in historical textiles.

However, in such studies, researchers would need to prepare their own reference dyed-fibres, although Armenian and Polish cochineal are very hard to obtain nowadays, since they are near extinction [29]. In addition, researchers in the field of dye identification in cultural heritage do not often have the knowledge to carry out multivariate statistical analyses. As a future perspective, a possible solution would encompass the creation of an international online database that could include the PLS-DA models developed here. Researchers analysing unknown textile samples at similar analytical conditions, would be able to plot their results onto these models and, hence, ascertain the identity of their samples. This could be developed in such way that researchers could even add their own reference samples and the results for their historical samples, thus expanding the database for future studies. The potential success of such platform could be even prolonged to other

applications, such as the identification of cochineal dyes in historical paintings.

4. Conclusions

A combination of UHPLC-PDA, UHPLC-MS, SEM and multivariate statistical analysis for the study of cochineal dyes in experimentally-dyed and aged samples has brought fruitful insights for the characterization of historical textiles. The methodology developed demonstrated that it is possible to obtain consistent information about dyed samples and their composition, depending on the experimental parameters and the textile substrate used. Furthermore, it has been demonstrated that compounds ratio in the colorant composition are directly related to the type of fibres (especially silk) used and the dyeing parameters applied to them, because of different types of reactions occurring between the fibres and the insects' colorants. For this reason, minor compounds quantification alone cannot be considered for characterizing cochineal species in silk historical textiles. On the other hand, comparison between experimentally-dyed and aged samples has revealed, for the first time, the occurrence of photo-degradation compounds in cochineal and kermes dyestuff composition and the fibres matrix, especially in silk fibres. This was reported in historical fibres as well. Research on the chemical nature of these photo-degradation compounds is advisable in future studies.

Considering the experimentally-dyed and aged samples as references for the identification of the cochineal dye source in historical textiles, it has been possible to obtain more conclusive classifications, rather than with direct comparison with reference insect dye extracts. Hence, by combining PLS-DA analyses, with the qualitative and quantitative interpretation of chromatographic results, it became possible to obtain accurate classifications of cochineal species present in historical textiles. Further historical interpretation of these results has come to support historical and dye identification literature, which defend a strong impact of American cochineal into European and Asian dyeing traditions, from the 16th century onwards. These successful results are proof of the powerful combination of UHPLC-PDA with a statistical classification method, to accomplish more accurate interpretations about closely related dyestuffs. Therefore, it is highly recommended for future studies on natural dyes identification in historical textile objects.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.aca.2015.09.046>.

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