CHARACTERIZATION OF COLOURING COMPOUNDS IN ANNATTO (BIXA ORELLANA L.) USED IN HISTORIC TEXTILES BY MEANS OF UV-VIS SPECTROPHOTOMETRY AND FT-IR SPECTROSCOPY.

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ABSTRACT: *Valencian historic textiles were commonly woven with coloured silks and natural dyes in the 18th century. The optimisation of analytical methods to extract and classify the dyes used is required to understand not only their degradation process, but also the state of conservation of these textiles. This paper shows the results obtained in the optimisation of the preparation process of the two main colouring components in annatto (Bixa Orellana L.), namely, bixin and norbixin, prior to analysis by means of UV-Vis Spectrophotometry and FTIR spectroscopy. The results of the analysis were also satisfactor*

KEYWORDS: natural Dyes, carotenoids, annatto, Bixa Orellana L, silk, FTIR spectroscopy, UV-Vis spectrophotometry

1. INTRODUCTION

Natural dyes obtained from plants, animals and minerals have been historically combined according to ancient procedures and recipes and have resulted in a wide spectrum of tones and shades.

In the 18th century, dyes were classified into two groups according to their inherent characteristics and quality. The first group consisted of the colourants considered 'major or good dyes' ('*tinte mayor o bueno')*, whereas the second group comprised dyes known as 'minor or false dyes' ('*tinte menor o falso'*). Those of the latter group were used "as base-dyes to obtain specific shades and also more affordable mixtures. At other times, they were also used alone, but they were considered low-quality materials" (Roquero, 2006).

Annatto, the dye obtained from the *Bixa Orellana* L., was considered a "minor" dye. Joseph Macquer's treatise refers to annatto as a weak material that changed and deteriorated easily. He also stated that *"its colours are so beautiful, and this is the only reason to use them since beauty is preferred to duration when it comes to dyeing silks"* (Macquer, 1771: 107).

Given the beauty of the yellow and orange tones obtained with annatto, and despite being classified as a "minor" dye, it was widely used for silk dyeing in Spain. Indeed, eighteenth-century textiles are a good example of this practice.

Dye treatises from the 18th century included a wide range of tones that could be obtained from annatto pure dye or diluted at different concentrations. The combination of annatto with other colourants also produced all types of warm colours ranging from gold to maroon, which were reported in the treatises of that period (Fernández, 1995: 51).

Annatto is a natural dye obtained from a tropical plant growing in America. Its seeds are covered by a red resin which contains a number of carotenoid compounds that constitute the main colouring agents: bixin is the predominant colouring compound in liposoluble preparations, and its chemical structure is shown in Figure 1. Norbixin (see also Figure 1), which can be obtained by means of bixin saponification, is the main colouring agent in hydrosoluble preparations. Nevertheless, norbixin can also be found in both seeds and liposoluble solutions.

Annatto colouring compounds can be identified by UV-Vis Spectrophotometry and FTIR Spectrometry. Devia developed an extraction method to determine the maximum absorption wavelength of annatto dyes. It consisted of 1gr of annatto dye seeds in a 100 ml-calibrated flask containing a 5% KOH solution. Then, 1 ml aliquot of this solution was transferred to a second calibrated flask (100 ml), which also contained a 5% KOH solution. The absorption spectrum finally obtained was in the range of between 300nm and 600nm (Figure 2).

Absorption bands with a maximum value at 450nm for the dye extracted from annatto (bixin) can be obtained as referred to in the literature (Mosquera, 1989; Devia and Saldarriaga, 2003: 8 and Devia, 2005), and at 483 nm for norbixin (Natukolor, 2004). The UV-Vis spectrum of annatto was reported by Britton (Briton, 1995), which is dominated by an absorption band with three maximum values.

Additionally, Devia developed the IR analysis of annatto dye seeds in the following way: pressed KBr powder is prepared with the coloured powder and the infrared spectrum is obtained (Devia, 2005)). Devia indicates: *"a band close to 1600 cm⁻¹ can be observed that represents the stretching vibration of the -C=O groups (typical of bixin structures). Moreover, several consecutive bands can be observed in the area between 1700 and 1800 cm-1, that are found typically in double bonds which are usually present in the organic structures that characterise bixin"* (Devia and Saldarriaga, 2003: 8).
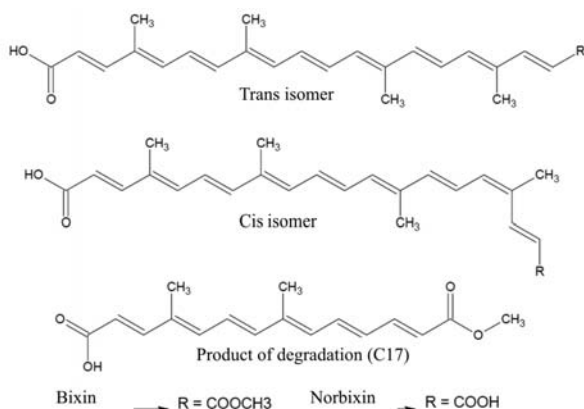
Figure 1. Chemical structure of bixin and norbixin carotenoids, cis and trans isomers and their main degradation products (Scotter, 1995)

Figure 2. UV-Vis spectrum of annatto extracts from solid dye seeds (Devia, 2005)

Extracting the colouring compounds from the fabric (silk in this case) prior to analysing by spectroscopic techniques is required to identify natural dyes in historic textiles. Thus, several methods have been proposed for this purpose. Among them, it is worth mentioning that of Orska-Gawrys *et al* (2003: 239), which uses a mixture of ethanol and hydrochloric acid for the hydrolysis stage. Chloro-solvents such as dichloromethane can be used for direct extraction, as suggested by Tocchini and Mercadante (2001: 310). Other authors, however, propose the use of dimethylformamide and acetic acid for the direct extraction of synthetic dyes from cotton fibre (Chao *et al*, 1998: 59).

Being a proteinaceous material, silk can be affected by acid or basic environments that deteriorate it as a result of the hydrolysis process. The typical functional groups contained in their amino acids, which are present in the coloured extracted solution, can be identified by FTIR.

As Becker states: *"Over 80% of the fibroin molecule consists of glycine, alanine and serine in a 3:2:1 ratio. The highly repetitive sequence of fibroin is described by: Gly-Ala-Gly-Ala-Gly-Ser-Gly-Ala-Ala-Gly- [Ser-Gly-(Ala-Gly)$_n$-]^8-Tyr, where n is usually 2".* (Becker *et al*, 1997:27). The chemical structure of these amino acids is characterised by the existence of primary amides.

Seok Ki *et al*. developed the structural characterisation of silk by means of several analytical techniques such as FTIR: *"Protein conformation is determined by identifying the peak positions of amide I, II, and III corresponding to C=O, N-H, and C-N stretching, respectively. All regenerated silk filaments present amide I and II peaks at around 1630 and 1520 cm^{-1}, as well as an amide III peak at 1260 cm^{-1} as a shoulder peak, thus indicating the β-sheet structure known as silk II type. On the other hand, SS powder presents amide I and II peaks at slightly different positions (1623 and 1516 cm^{-1}) with the absence of a shoulder peak at 1260 cm^{-1}. This is probably due to an aggregation of SS molecules, and not to β-sheet conformation."* (Seok Ki *et al*, 2007: 346)

This research shows the earliest results obtained in the optimisation of the extraction process of the two main colouring components of annatto, bixin and 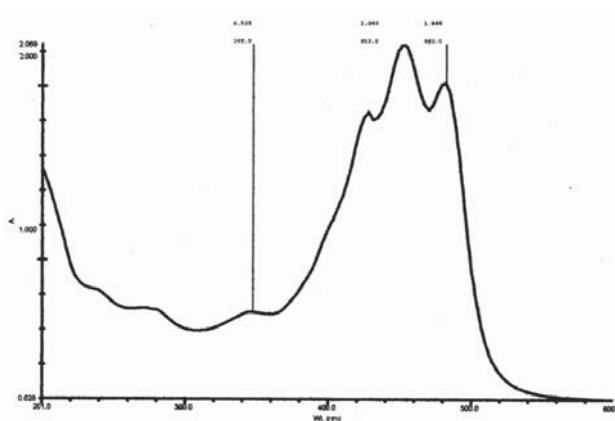norbixin, from silk textiles which was carried out prior to the spectroscopic characterisation of annatto colouring compounds. Two types of extraction processes have been evaluated. The first method was carried out in two stages: acid hydrolysis followed by extraction with organic solvents. The second method is simpler than the first, and consists of direct extraction with organic solvents without the need for a previous acid hydrolysis stage.

Secondly, the colouring compounds extracted from the dyed textiles were characterised by UV-Vis Spectrophotometry and FTIR spectrometry. The assignment of the main absorption bands in the UV-Vis-IR regions allowed us to not only differentiate the different colouring compounds present in the dyed textiles, but to also evaluate the efficiency of the extraction method tested.

2. EXPERIMENTAL

2.1. Reagents and Reference Materials

The chemical reagents and solutions used were ethanol, 96%, pa, Panreac; hydrochloric acid, 37%, pa, Carlo Erba; Dichloromethane (DCM), pa, Carlo Erba; Chloroform, 99+%, Acros; Ethanol: hydrochloric acid 3M (1:1) solution. Acetonitrile (ACN) and Methanol (MeOH), HPLC-gradient grade, Carlo Erba; Potassium bromide, 99+%, RS, Aldrich; Trifluoroacetic acid (TFA) 99% (Panreac); Acetic acid 100% (Prolabo), Dimethylsulfoxide (DMSO), RS, Carlo Erba, Deionised water, HPLC grade, Medica Elga (Eolia Water). HCl 3M:MeOH:H$_2$O (2:1:1) solution and MeOH:H$_2$O (1:1) solution

Annatto seeds (ref. 37350) were provided by Kremer pigmente (Figure 3).

The experiments were performed on 100% silk, whose commercial name was *ponge textile*, supplied by Soditex, S.L.. This textile was characterised (Vicente-Palomino *et al*, 2006: 139) as:

2.2. Instrumentation

UV-Vis Spectrophotometry. The spectra in the UV and visible region were measured with a HITACHI U2010 recording double-beam

	Name	Composition	Number of yarns		Density (taffeta)		Fabric grammage
			warp	weft	warp	weft	
	Pongee	100% Silk	7 tex	4 tex	60	60	0'00459g/m^2

Table 1 Characterization of textile.

Figure 3. Annatto seeds.

Figure 4. UV-Vis spectra of samples ACH-1C (-----) and T00 (▫) corresponding to the dye extracted from 50 µL of dyeing bath and 0.0023g of dyed silk, respectively, obtained by means of the acid hydrolysis-dichloromethane extraction procedure

Figure 5. UV-Vis spectrum of sample T00 corresponding to 0.0055g dyed silk without mordent obtained by means of the direct extraction procedure using 40µL DMF:acetic acid.

spectrophotometer. The work conditions were: a spectral range of 200-1000 nm, a scan speed of 800 nm.min^{-1}, a sampling interval of 1 nm, a slit width of 2 nm and a path length of 10 mm. Data were processed with UV Solutions software version 1.2. (HITACHI INSTRUMENTS, Inc.).

FTIR Spectroscopy. IR absorption spectra were performed in the attenuated total reflectance mode (ATR) with a Vertex 70 Fourier transform infrared spectrometer (BRUKER OPTIK GmbH) with an FR-DGTS (fast recovery deuterated triglycine sulphate) temperature-stabilised coated detector made by BRUKER OPTICS® with an MKII Golden Gate Attenuated Total Reflectance (ATR) accessory. Number of co-added scans: 32; resolution: 4 cm^{-1}. Data were processed with the OPUS software, version 5.0.

2.3. Procedures

2.3.1. Preparation of reference materials
Aqueous extracts of the colouring compounds from annatto seeds were used for the dyeing procedure. Non-mordanted silk was then immersed in the dye bath.

2.3.1.1. Preparation of the dyeing baths
Forty grams of annatto seeds were soaked in 200 mL of deionised water for 20 h at room temperature, and were then heated at 90°C for 20 minutes. The hot solution was filtered using a nylon filter, resulting in a concentrated dyeing bath (1C). This solution was then diluted to 600 mL in order to obtain that a dyeing bath (ACH-1C).

2.3.1.2. Dyeing of silk textiles
Wetted and unmordanted silk was immersed in the dyeing solution (ACH-1C) at 80°C for 2h. After cooling the dyeing solution, the dyed silk (T00) was rinsed in deionised water and left to dry in the dark.

2.3.2. Extraction Methods
Following a series of prior experiments, we propose three procedures to extract the colouring compounds from the textiles and the dyeing bath.

1) Acid hydrolysis and organic solvent extraction

a) 50µL of the dyeing solution (ACH-1C), or 1.5-2.5mg of the dyed silk textiles (T00), were hydrolysed by adding 40µL of (1:1) HCl:EtOH solution for 15 minutes at 100°C. Afterwards, 60µL dichloromethane were added and two phases were obtained. The yellow extract formed in the organic phase was then analysed.

b) A second extraction with 50µL H$_2$O was carried out on the former yellow extract, and 120µL of HCl:EtOH solution (1:1) and 100µL of dichloromethane were used in the first and second steps.
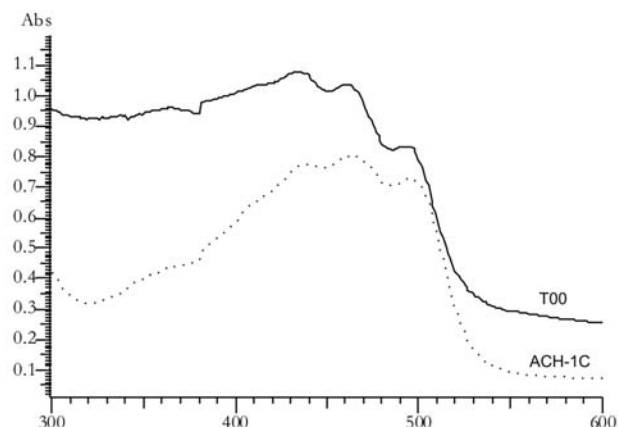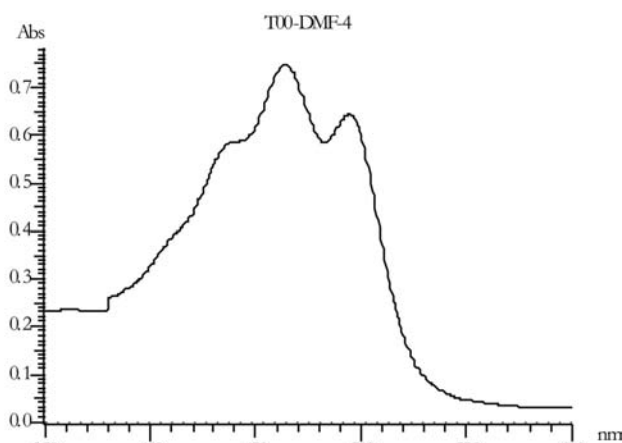
2) Direct solvent extraction

a) Extraction was performed by adding an organic solvent, 60µL chloroform, or

b) 60 µL DMF: acetic acid (9:1).

Experimental measurements
50 µL yellow extract were placed into a quartz microcell and a UV-Vis spectrum was obtained.
50 µL yellow extract were gently added into 0.1mg of finely powdered and dried (at 100°C for 2 hours) KBr. The IR spectra were obtained in the ATR mode from the measurements performed on the dyed KBr powder obtained after evaporating the solvent.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Spectrophotometry

The absorption spectrum obtained from the dyeing bath (ACH-1C) and the dyed silk (T00) by means of the above-mentioned Extraction Method 1-a (acid hydrolysis - solvent extraction) is shown in Figure 4, whereas the absorption spectrum obtained from the dyed silk (T00) by means of Method 2 using DMF: Acetic acid is shown in Figure 5.
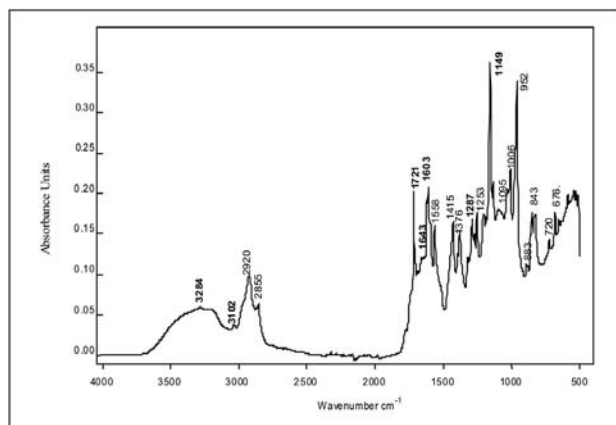
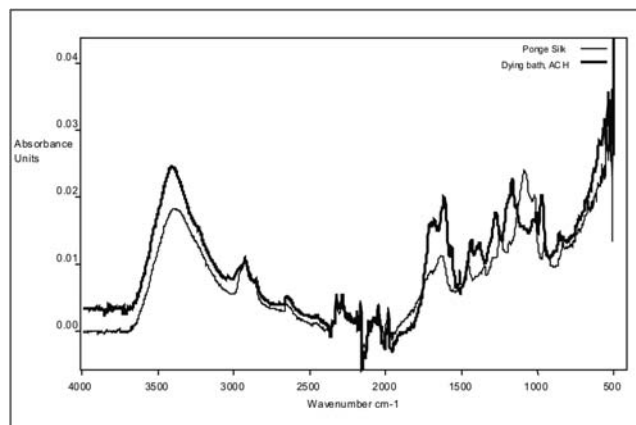Figure 6. IR spectrum of annatto extracts from solid dye seeds.

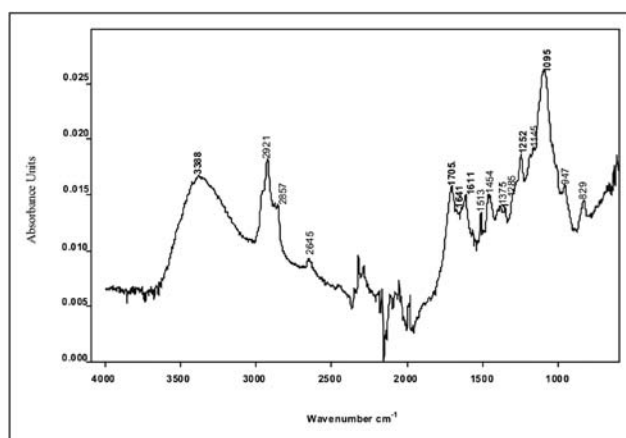Figure 7. FTIR spectra of ponge silk and annatto dyeing bath extract by Method 1a.

Figure 8. IR absorption spectrum of sample T00 corresponding to the dyed silk (0.0013g) obtained by 40μL EtOH:HCl - 60μL Dichloromethane (Method 1a).

The UV-Vis spectrum in Figure 5 corresponding to dyed silk using Extraction Method 2 displays absorption values at 495, 464 and 438 nm. This result matches that of Britton (1995), and shows that it is possible to discriminate among three maximum absorption values for annatto extracts unlike those obtained by Mosquera (1989) and Devia (2003 and 2005). The UV-Vis spectra depicted in Figure 4 correspond to the colouring compounds extracted from both the dyeing bath and the dyed silk samples using Method 1-a, which are similar to those in Figure 5 where the maximum absorption values at 493-6, 461-4 and 435-9 nm appear. Satisfactory results were obtained by both extraction methods.

3.2. FTIR Spectroscopy

The main frequencies of the absorption bands, relative intensities and tentative assignments are provided in Table 1 for the annatto extracts from solid dye seeds, silk, the dyeing bath and dyed silk.

The IR spectrum obtained from the solid finely powdered annatto seeds is shown in Figure 6. This spectrum displays a broad band at 3284 cm^{-1} which is ascribed to the stretching vibration of the OH groups and to 2920 and 2855 cm^{-1} associated with the stretching vibrations of the hydrocarbon skeleton, as well as with sharp bands at 1721, 1603, 1149 and 962 cm^{-1} whose assignment is summarised in Table 1. These absorption bands are typical of carotenoid compounds, in particular bixin.

The IR spectra of the *pongee silk* samples and the dyeing bath obtained by Method 1a are presented in Figure 7. We observe a significant decrease in the absorption values of the IR bands if we compare them with the spectrum obtained from the annatto seeds. On the other hand, the IR absorption ranges typically found in the amino acids contained in the chemical structure of silk are shown in Figure 1, as indicated by both Becker and Seok Ki (Becker *et al*, 1997: 27 and Seok Ki *et al*, 2007: 346)

The IR spectra of the dyed silk, T00, obtained by Methods 1a (Figure 8) and 1b, are shown. Typical annatto bands can be seen in both spectra. They exhibit a broad band at 3388 and 3465 cm^{-1} absorbed to the stretching vibration of the OH groups and to the sharp bands from C=O to 1705 and 1721 cm^{-1} absorbed to stretching, C-O to 1285-1145 cm^{-1} and 1288-1158cm^{-1} (stretching), C=C to 1641-1611cm^{-1} and 1631-1613cm^{-1} (stretching, alkenes), respectively. Despite the bands not being as sharp as those obtained from the dying solution, the confirmation of the dye concentration in the dyed silk is lower than its concentration in the dyeing solution. Furthermore the additional water extraction stage equally contributes to a noticeable dilution of the dye. Therefore, we suggest that Method b) is not a suitable extraction method.

The main IR absorption bands identified by Method *1a* in samples extracted from the silk specimens, the annatto solid seeds, the dyeing solution (ACH-1C) as well as from dyed silk (T00) are shown in Table 1. If we observe the sample corresponding to the T00 dyed silk, both IR bands are typically found in annatto dyes (3388 and 1611 cm^{-1}), while those corresponding to the silk (1705, 1513 and 1454 cm^{-1}) may also be identified. This is a direct result of the extracting method of the dyed silk specimen where the protein structure of silk is also hydrolysed by hydrochloric acid. Consequently, it can be said that this extraction method highlights the bands corresponding to the silk itself together with those corresponding to the coloured compounds.

The literature suggests a direct extraction method with chloroform (Souza, 2005). This led us to develop Method 'c'. The IR spectra obtained by a direct extraction with chloroform (Method 'c') from both the dyeing solution (ACH-1C) and dyed silk (T00) reveal a broad band at 3349 cm^{-1} absorbed to the stretching vibration of the OH groups, as well as a band at 1716 cm^{-1} absorbed to a stretching vibration of the C=O group, 1285-1159cm^{-1} to a stretching vibration of C-O, and a band at 1610 cm^{-1} to a stretching vibration of C=C group. As seen, direct extraction is more effective when performed in the dyeing solution than in the dyed silk sample since sharper bands are obtained.

At this point, it is interesting to show the comparison between the two organic solvents used in Method 'c'; chloroform and DMF:acetic acid; applied to the dyed silk T00 extraction. The IR spectra are

Silk	Annatto solid seeds	Dyeing bath ACH-1C	Dyed Silk T00	Assignment and comments
Wavelength (cm⁻¹)	Wavelength (cm⁻¹)	Wavelength (cm⁻¹)	Wavelength (cm⁻¹)	
3391	3284	3402	3388	Stretching vibration of OH group associated
--	3102	--	--	Stretching vibrations of carboxylic moieties and/or C-H
2957	--	--	--	Asymmetric stretching vibrations –CH$_3$
2918	2920	2918	2921	Asymmetric stretching vibration of –CH$_2$ groups from the hydrocarbon skeleton
2856	2855	2846	2857	Symmetric stretching vibration of –CH$_2$ from the hydrocarbon skeleton
2650	--	2642	2645	Stretching vibration of –O-H group associated to aliphatic carboxylic acid
2452	--	--	--	
--	1721	1711	--	Stretching vibration of -C=O group associated to aliphatic carboxylic acid
1701	--	--	1705	Stretching vibration of -C=O group
1627	1643	1673	1641	Alkenyl stretching vibration of C=C group
--	1603	1610	1611	Stretching vibration of conjugated C=C group
--	1558	1562	1562	
1509	--	--	1513	Stretching vibration of aromatic ring C=C-C group; Bending vibration of NH
1457	--	--	1454	Stretching vibration of aromatic ring C=C-C group; Symmetric stretching vibration of COO- group
1408	1415	--	--	Phenol or tertiary alcohol, bending vibration of O-H
--	--	1433	--	Asymmetric bending vibration of -CH$_3$ group
1367	1376	1380	1375	Symmetric bending vibration of -CH$_3$ group
1356	--	--	--	Bending vibration of methyne C-H bend
1287	1287	--	1285	In plane bending vibration of -OH group from alcohol
1241	1253	1265	1252	Stretching vibration of C-N
1182	1149	1155	1154	Stretching vibration of -CO group from carboxylic acid
1078	1095	--	1095	Bending vibrations of –C-(C=O)-C- group of ketone (probable overlapping with stretching vibration of -CO group from secondary alcohol and/or with stretching vibrations of –C-O groups from ether)
1019	--	--	--	C-N Primary amine, stretching vibration of C-N
--	1006	1007	--	Stretching vibration of -CO group from primary alcohol
953	952	964	947	OH deformation
824	883, 843	--	829	CH out-of-plane deformation
726	720	--	-	Methylene -(CH2)n- rocking, (n = 3)
--	676	--	--	Out-of-plane bending vibrations of aromatic C-H

Table 2. The main IR absorption bands identified in the samples of extracts from silk, solid annatto seeds, dyeing bath (ACH-1C) and dyed silk (T00) by Method 1a.

obtained by direct extraction with chloroform and DMF:acetic acid from the dyed silk T00. Both organic solvents can extract coloured compounds from dyed silk. However DMF:acetic acid extraction and absorption present sharper and larger bands which may be caused by the acid hydrolysis that took place during the procedure.

4. CONCLUSIONS

In the 18th century, Valencian historic textiles were commonly made out of coloured silks and used a variety of natural dyes. The annatto natural dye (*obtained from Bixa Orellana L*) was particularly popular at that time. Dye extractions, as well as UV-Vis Spectrophotometry and FTIR Spectroscopy analyses, have worked well according to their chemical characterisation.

A two-stage extraction process (firstly acid hydrolysis and then organic solvent extraction with dichloromethane) proved to be a suitable procedure to analyse natural dyes from historic silk textiles as evidence of early results. Annatto-dyed silk was also analysed by UV-Vis Spectrophotometry and FTIR Spectrometry. Satisfactory results were obtained since the analytical signal was obtained at a visible range spectrum. FTIR has helped identify the chemical structure of the extracted annatto corresponding to the carotenoid compounds.

Additionally, these results provide more in-depth knowledge of not only the behaviour of dyes and dyed textile fibres, but also of their detection and identification in textiles.

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Versión española

TITULO: *Caracterización de los componentes coloreados del achiote utilizado en los tejidos históricos valencianos mediante Espectrofotometría UV-Vis y Espectrometría FTIR.*

RESUMEN: *Los tejidos históricos valencianos del siglo XVIII fueron fabricados con seda teñida con colorantes naturales. Optimizar los métodos analíticos de extracción y caracterización de los colorantes naturales es necesario para comprender los procesos de degradación y por tanto, de conservación de estos tejidos. En este artículo se presentan los resultados obtenidos de la optimización de los procesos de extracción y caracterización mediante Espectrofotometría UV-Vis y Espectrometría FTIR de los dos componentes coloreados del achiote (Bixa Orellana L.), denominados bixina y norbixina. Los resultados obtenidos han sido satisfactorios.*

PALABRAS CLAVES: *tintes naturales, achiote, Bixa Orellana, Espectrometría FTIR, eEspectrofotometría UV-Vis, seda, carotenoides*