

A COMPARATIVE EVALUATION OF CHEMICAL TEST METHODS AND ATR-FTIR FOR THE CHARACTERISATION OF VEGETABLE TANNINS

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Abstract

Tannin characterisation of vegetable tanned leather has been an important part of leather conservation for the past 35 years. Previous research has demonstrated that leathers tanned with condensed tannins are more prone to deterioration through hydrolysis and oxidation than leathers tanned with hydrolysable tannins. Consequently, by characterising the tannins in historic leather objects, the degradation susceptibility of an object can be ascertained. This paper presents a comparative evaluation of several chemical test methods developed for the characterisation of vegetable tannins in leather fibres, including Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) analysis. The tests were carried out on both modern and historic leather specimens, with results suggesting ATR-FTIR was the most reliable and practical method for the characterisation of vegetable tannins in historic leather.

Keywords:

Leather conservation, vegetable tanning, ATR-FTIR, chemical test methods, tannins.

Introduction

The characterisation of tannins in historic leather objects and modern leather is of great value in leather conservation, making it possible to obtain information about the degradation, condition and leather technology of both modern and historic vegetable-tanned leather. Extensive research undertaken over the past 30 years has established that leather tanned with condensed tannins, such as mimosa, birch or oak bark, experience a higher degree of deterioration, compared to leathers tanned with hydrolysable tannins (Larsen 1994; 1996; 2008; Dirksen 1997; Florian 2006). This means that special care has to be taken when dealing with objects featuring leather tanned with condensed tannins, as they are prone to deterioration from hydrolysis and oxidation, mainly through the uptake of sulfur dioxide in the atmosphere. Depending on the general condition of the leather object, this may over time lead to a weakening of the physical stability of the leather and a loss of mechanical strength, which may result in leather that is brittle and prone to cracking, in addition to the development of so-called red rot. Therefore, an initial assessment in the conservation process of vegetable-tanned leather objects, verifying the tanning method, is beneficial for assessing the measures needed in the preventive conservation of the object and is also a recommended first step in the conservation process of historic leather objects (Kite et. al. 2006: 124). An assessment of the tannins in new leather considered for use in conservation treatment is also useful, as this makes it possible to establish the degradation susceptibility of the repair. It is essential that the tests available for

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tannin characterisation are of such a nature that they are easily conducted on a small sample quantity, in addition to being reliable and practical. This project aims to evaluate different qualitative tests developed for the characterisation of tannins in leather. This includes the ferric test, vanillin test, rhodanine test, acid butanol test and nitrous acid test. These are supplemented with tannin characterisation using ATR-FTIR.

Vegetable tanning

It has been proposed that the development of vegetable tanning - tanning using vegetable materials such as wood, bark and leaves - should be attributed to the Romans (van Driel-Murray 2008). However, with the development of various analytical techniques for the detection of vegetable tannins in leather during the last decade, new light is being shed on the history of vegetable tanning. In 2017, Egyptian leather objects from the Metropolitan Museum of Art, dating back to 2000 BC, were analysed for vegetable tannins using High Pressure Liquid Chromatography (HPLC), with positive results (Elnaggar et. al. 2017). This indicates that vegetable tanning existed in pre-Roman times. Since then, vegetable tanning has been a common and widespread tanning method in Europe, until the end of the 19th century. Drastic socio-economic changes in Western society led to an increased demand for leather, which led to the development of the chrome tanning process (Thomson 2006). This gave good quality leather in considerably less time than vegetable-tanned leathers, and this method is the most commonly-used in modern leather production.

The main principle behind vegetable tanning is the use of plant materials that contain polyphenols, which are large molecules with a molecular weight between 500 – 20000 Da, to replace the natural water content of the leather and to prevent the fibres from collapsing and becoming stiff (Bickley 1991: 16; Larsen and Rahme 1999: 45). It has been stated that for the phenols to be effective in the tanning process, they need to have a molecular weight between 500 and 3000 Da – larger molecules are insoluble and classified as gums (Covington 2006: 23; Larsen and Rahme 1999: 46). Although the theory behind how vegetable tannins react with collagen protein in skin is not yet fully understood, the consensus seems to be that the tannins interact with the collagen through both hydrogen and covalent bonding, causing crosslinking of the protein's chains, increasing the hydrothermal stability of the

skin (Khanbabaee and van Ree 2001; Mavlyanov et. al. 2001; Covington 2006; Schröpfer and Meyer 2016; Covington and Wise 2020).

Vegetable tannins can be divided into three main categories, according to their chemical structure: hydrolysable tannins, condensed tannins and complex tannins (Khanbabaee and van Ree 2001: 642-643). This project will be limited to the use of hydrolysable and condensed tannins in the tanning process of leather and hides, so complex tannins will only briefly be addressed in the discussion. For further details of the different sources of tannins, see Table 1 on the right.

The hydrolysable tannins are derived from gallic acid esters with carbohydrates and are also known as pyrogallol tannins (Covington 2006: 23; Schröpfer and Meyer 2016: 59; Falcaõ and Araújo 2018: 3). See Figure 1 for the chemical structure of hydrolysable tannins. Hydrolysable tannins can be divided into two subgroups: gallotannins and ellagitannins. Gallotannins occur in sumac, tara and aleppo oak, and are characterized as glucose esterified with gallic acid. Ellagitannins are esterified by ellagic acid and chebulic acid in addition to gallic acid, and they can derive from valonea, chestnut and myrtle (Larsen and Rahme 1999: 47; Covington 2006: 23). A common feature of hydrolysable tannins is that they create pale-coloured leathers that are resistant to darkening upon exposure to light (Covington and Wise 2020: 344).

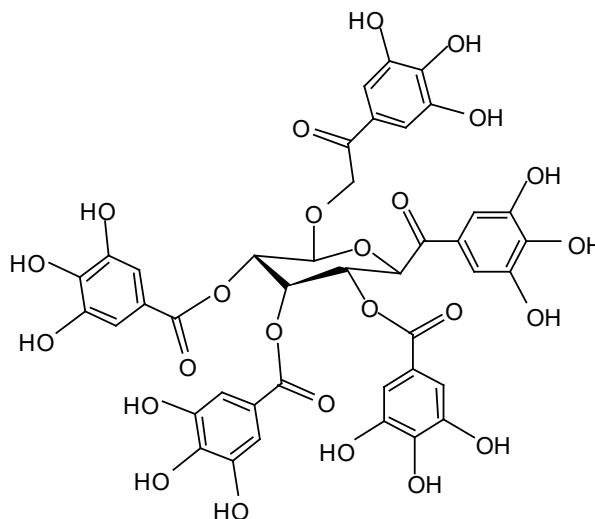


Figure 1: Chemical structure of a hydrolysable tannin, more specific gallic acid esterified to glucose, as illustrated in Schröpfer and Meyer 2016.

Table 1: Common vegetable tanning materials and their tannins. Based on Larsen and Rahme (1999) and Falcão and Araújo (2018).

Common vegetable tanning materials		
	Common name	Latin name
Ellagitannins	Myrtle	<i>Mirtus communis</i>
	Oak (wood)	<i>Quercus ilex/robur/petraea/pyrenaica/suber</i>
	Valonea	<i>Quercus vallonea/aegilops</i>
	Chestnut	<i>Castanea sativa</i>
	Myrobalan	<i>Terminalia chebula</i>
Gallotannins	Sumac	<i>Rhus coriaria</i>
	Tara	<i>Caesalpinia spinosa/tinctoria</i>
	Aleppo oak	<i>Quercus infectoria</i>
	Smoke tree	<i>Cotinus coggygria</i>
Condensed tannins	Mimosa	<i>Acacia decurrens/mearnsii</i>
	Pine	<i>Pinus cembra</i>
	Willow	<i>Salix fragilis</i>
	Quebracho	<i>Quebrachia lorentzii, Schinopsis balansae/lorentzii</i>
	Aleppo pine	<i>Pinus halepensis</i>
	Norway spruce	<i>Picea abies</i>
	Larch	<i>Larix</i>
Complex tannins	Birch	<i>Betula</i>
	Oak (bark)	<i>Quercus ilex/robur/petraea/pyrenaica/suber</i>

This might be explained by the natural antioxidant ability of hydrolysable tannins, which inhibits oxidative deterioration through light, although this feature may be reduced by long-term exposure to oxidizing agents such as UV, ozone and hydrogen peroxide (Greenhow and Shafi 1975; Florian 2006: 47). Hydrolysable tannins tend to break down by hydrolysis, a chemical reaction where water ruptures one compound into two compounds, creating acidic products that deposit in the leather (Covington 2006: 24).

Condensed tannins can be derived from mimosa, birch and salix, and are also known as catechol tannins (Covington 2006: 24; Larsen and Rahme 1999: 47). The chemical classification of condensed tannins has been termed proanthocyanidins, a group of polymers that contain catechin residues in a flavonoid ring structure (Bickley 1991: 17; Schröpfer and Meyer 2016: 59). See Figure 2 for the chemical structure of condensed tannins. Condensed tannins give a reddish-brown leather which darkens when exposed to light, due to crosslinking from oxidation (Covington 2006: 25-26; Florian 2006: 38; Larsen 2008: 900; Falcão and Araújo 2011: 150). Research suggests that condensed tannins are prone to both oxidative and hydrolytic breakdown, creating insoluble compounds and depositing acid products and monomers in the leather (Wouters 1994; Wouters and Claeys 1997; Wouters et. al. 1997; Florian 2006). In the conservation of vegetable-tanned leather, a common occurrence is the formation of so-called red rot – a condition where the leather fibres partly or totally disintegrate into a fine red

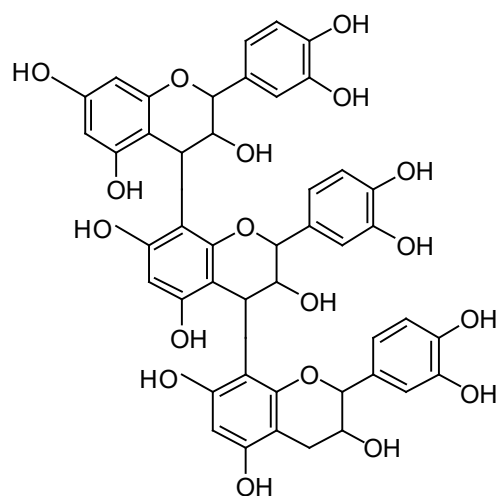


Figure 2: Chemical structure of condensed tannins as illustrated in Schröpfer and Meyer 2016.

powder. This process is irreversible and will eventually lead to the total decomposition of the leather. Although there are not many sources available regarding red rot dating from after the 2000s, there seems to be a consensus that this type of degradation is most commonly seen in leather tanned with condensed tannins, due to the fact that they more easily absorb sulfur dioxide and undergo hydrolysis – the suggested culprit of red rot (Haugsted 1981: 13; Dirksen 1997; Falcão and Araújo 2018: 3).

Materials and methods

A comparative evaluation of five chemical test methods and one analytical method was made based on the testing of five samples from each of ten different leather specimens, including both modern and historic leather, with both known and unknown tannage. The tannin products of catechin, gallic acid and ellagic acid were not included in this study, but would be a useful aspect to research in the future.

The chemical test methods are qualitative colorimetric tests, meaning that leather fibres are subjected to one or more reagents that will yield a change in colour in the presence of the respective classification of tannins. The tests are invasive and destructive in that they require removal of fibre samples from the specimen, and that the sample is ruined in the process of testing.

Leather specimens

Five samples from each specimen were collected from five different areas, to ensure that the chemical tests were consistent and to see if the colour formation varied, depending on the location of the sample. The modern leather samples are of a known tannage, the historic leather samples are of an unknown tannage. Different institutions across Europe kindly donated the samples – see Table 2 for an overview of the specimen characteristics and suppliers.

Table 2: Overview of leather specimen characteristics and suppliers.

Leather specimens	Additional information	Supplier
ConA	Modern leather tanned with mimosa/condensed tannins	School of Conservation, Det Kongelige Danske Kunstakademi, Denmark. Tanned at Forschungsinstitut Leder und Kunststoffbahnen, Germany.
ConB	Modern leather tanned with oak bark/condensed tannins	Sturge Conservation Studio, England. Tanned at J & JF Baker and Co Ltd.
GalA	Modern leather tanned with sumac/gallotannins	School of Conservation, Det Kongelige Danske Kunstakademi, Denmark. Tanned at Forschungsinstitut Leder und Kunststoffbahnen, Germany.
GalB	Modern leather synthetic tanned, re-tanned with sumac and tara/gallotannins	J Hewit & Sons Ltd. England
ElIA	Modern leather aldehyde pre-tanned, post-tanned with chestnut/ellagitannins	Forschungsinstitut Leder und Kunststoffbahnen, Germany.
ElIB	Modern leather aldehyde pre-tanned, post-tanned with valonea/ellagitannins	Forschungsinstitut Leder und Kunststoffbahnen, Germany.
HisA	Historic leather, lining from a Gladstone bag, probably from the 1930s	Sturge Conservation Studio, England.
HisB	Historic leather, carriage exterior, probably 19 th century	Sturge Conservation Studio, England.
HisC	Historic leather, dining chair seat, probably from the 1930s	Sturge Conservation Studio, England.
HisD	Historic, waterlogged leather, from the Metta Catharina shipwreck in 1786. 18 th century Russia leather	Sturge Conservation Studio, England.

Test methods

Standard laboratory equipment of glassware, an analytical balance, hot plates and stirrers were used. The following chemicals were used: acetone, ferric ammonium sulfate, hydrochloric acid (HCl), iron(III) sulfate, methanol, n-butanol, potassium hydroxide (KOH), pyridine, rhodanine, sodium nitrite (NaNO_2) and vanillin. All tests were carried out under a fume hood, with the use of personal protective equipment (PPE) such as a lab coat, goggles and gloves. Additional protective gear is required for the nitrous acid test, such as antistatic protective clothing and respiratory protection.

Ferric test for vegetable tannins

The following procedure was described in Florian (1984), Larsen (1991) and (1996), and is based on the principle that ferric ions react with phenolic compounds to give dark-coloured, mainly blue, grey or green products, indicating the presence of vegetable tannins.

Ferric reagent was prepared by adding 2% w/v iron(III) sulfate ($\text{Fe}_2(\text{SO}_4)_3 \cdot 5\text{H}_2\text{O}$) in water. Approximately 1 mg of leather fibres were scraped off the flesh side and placed on a glass slide. Another 1 mg of fibres were placed on the opposite side of the glass slide, to be used as a control sample. Both samples were hydrated with one drop of demineralized water and excess water removed with filter paper. One drop of ferric reagent was added to only one of the samples – the test sample – and the colour formation noted and compared with the control sample. A dark blue or black colour formation indicated the presence of vegetable tannins.

Vanillin test for condensed tannins

This procedure was reported in Larsen (1991). The principle behind this test is that vanillin (4-hydroxy-3-methoxybenzaldehyde) reacts with condensed tannins to produce triphenylmethane dyes, which yields a bright red colour in acid solution. The reaction will give no colour formation or only a light-yellow colour in the reaction with hydrolysable tannins, and in the case of historic leather or oxidized leather the colour might range from dark brown to red-brown.

Vanillin reagent was prepared by adding 1% vanillin in 86% ethanol. Approximately 1 mg of leather fibres was scraped off the flesh side and placed on a glass slide.

Another 1 mg of fibres was placed on the opposite side of the glass slide, to be used as a control sample. One drop of vanillin reagent was added to the test sample. When the vanillin was absorbed, one drop of 37% HCl was added to both the test sample and the control sample, and the colour formation noted and compared with the control sample. A red or magenta colour formation indicates the presence of condensed tannins.

Rhodanine test for gallotannins/gallic acid

The following test was executed as described in Poulsen (2002), based on work by Inoue and Hagerman (1988). The principle behind this test is that under basic conditions, rhodanine (2-thio-4-ketothiazolidine) will react with the vicinal hydroxyl groups of gallic acid to yield a red complex, thereby indicating the presence of gallotannins. To identify gallotannins it will therefore be necessary to hydrolyse the gallotannins into gallic acid with sulfuric acid (H_2SO_4) prior to the reaction with rhodanine

Rhodanine (2-thio-4-ketothiazolidine) reagent was made by adding 0.667% w/v rhodanine in 99% ethanol. 0.5 N aqueous potassium hydroxide (KOH) and 2 N H_2SO_4 was prepared. Three small fibre samples of each leather sample were placed side by side on a glass slide; each fibre sample equalled approximately 1 mg of fibres and was scraped off the flesh side of the leather with a scalpel. One drop of 2 N H_2SO_4 was added to the first fibre sample and soaked for five minutes to hydrolyse. Excess acid was then removed with filter paper and one drop of rhodanine solution added to the first and second fibre sample. Excess rhodanine reagent was removed with filterpaper after five minutes. Lastly, one drop of 0.5 N KOH was added to the three samples. A red or magenta colour formation yielded a positive result for either gallotannins or gallic acid, depending on the fibre sample. The first sample was tested for gallotannins, the second sample was tested for free gallic acid and the third sample was the control sample.

Acid butanol test for condensed tannins

This test was carried out as described in Falcão and Araújo (2011). The acid butanol test promotes an oxidative depolymerisation of condensed tannins in heated acidic alcohol to yield correspondent red anthocyanidins.

Acid butanol reagent was prepared by mixing 95% of n-butanol with 5% of 37% HCl. Iron reagent was made by first preparing 2 N 37% HCl and then adding 2% w/v ferric ammonium sulfate ($\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) in 2 N 37% HCl. 5 mg of leather fibres was scraped off the flesh side with a scalpel and put in a small glass vial. 2.4 ml of acid butanol reagent and 40 μl of iron reagent were added to the vial and the mixture loosely capped and put in a boiling water bath for 50 min. The progressive colour formation was noted, where colours ranging from orange to brown indicated the presence of condensed tannins.

Nitrous acid test for ellagitannins/ellagic acid

The following procedure is reported in Falcão and Araújo (2011). The principle behind the nitrous acid test is that ellagitannins present in the leather will react with nitrous acid and yield a red or pink colour that may slowly change to purple or blue during incubation.

First, 1% w/v sodium nitrite (NaNO_2) in water was prepared. 2 ml pyridine ($\text{C}_5\text{H}_5\text{N}$) was added to 5 mg of leather fibres in a small glass vial. Then, 150 μl of 37% HCl was added, and the mixture heated to 30°C in a water bath. The temperature was monitored with a non-contact infrared thermometer. After five minutes, 150 μl of 1% w/v sodium nitrite in water was added, mixed and incubated for 20 minutes at 30°C and the colour formation noted. Blue colour formation after incubation indicates the presence of ellagitannins. Red colour formation after incubation indicates the presence of ellagic acid, a degradation product often present in aged leathers due to hydrolysis.

ATR-FTIR analysis

Due to time limitations, the analyses were conducted on only one sample from each leather specimen, which was prepared as described in Falcão and Araújo (2012), with slight modifications: the acetone was evaporated during the preparation to ensure that it did not affect the adhesive in the crystal of the instrument. Additionally, this step increased the concentration of the tannin extract, which in turn might have helped to yield better results.

The basis of this procedure relies on the different absorption patterns in the fingerprint region of the spectrum of hydrolysable and condensed tannins, where tannins from the different vegetable sources present characteristic common bands. These bands

are as follows: For condensed tannins: 1288-1282, 1162-1155, 1116-1110, 976 and 844-842 cm^{-1} . For hydrolysable tannins: 1731-1704 and 1325-1317 cm^{-1} and for gallotannins: 1088-1082, 872-870 and 763-758 cm^{-1} . See Table 3 for the assignment of bands to chemical bonds in the tannin structure. The identification of ellagitannins can only be assumed by the absence of gallotannins, as there are no particular bands that can be used as markers for this class of tannins.

For this analysis, a PerkinElmer Spectrum One Fourier-Transform Infrared Spectroscopy (FTIR) instrument with Universal Attenuated Total Reflectance (UATR) Polarization Accessory was used, and spectra were recorded from 4000 to 650 cm^{-1} by averaging 32 scans at a spectral resolution of 4 cm^{-1} .

Table 3: Assignment of bands to chemical bonds.

	MARKERS cm^{-1}	Bands
Tannins	1615-1606	C=C aromatic ring
	1518-1507	C=C skeletal ring
	1452-1446	C=C aromatic ring
	1211-1196	Aromatic C-OH
	1043-1030	C-H deformation
Condensed	1288-1282	C-O pyran ring
	1162-1155	Asymmetric C-O-C cyclic ether
	1116-1110	Asymmetric C-O-C cyclic ether
	976	
Hydrolysable	844-842	Tetrasubstitued aromatic C-H
	1731-1704	C=O phenolic esters
	1325-131	C-O lactones and O-H deformation
Gallotannins	1088-1082	Symmetric C-O-C aryl phenolic ester
	872-870	
	763-758	OH and aromatic C-H Symmetric skeletal

10 mg leather fibres were removed by scalpel from the flesh side of the leather and added to a vial with 1 ml aqueous-acetone (1/1 v/v) solution, which was then capped and kept under continuous magnetic stirring at room temperature for 48 hours. The extracts were then filtered through 0.45 µm PTFE syringe filters, before being left uncapped for 12 hours for the acetone to evaporate. A small drop of the extract was deposited on the surface of the ATR crystal and evaporated until dry, before spectra were recorded.

Results

Test methods

The results from the chemical test methods are summarised in Table 4.

Ferric test for vegetable tannins

The ferric test was conducted on the historic specimens only, as the modern leather specimens are tanned with vegetable tannins, according to the suppliers. The samples of specimen HisA, HisC and HisD darkened upon exposure to iron(III)sulfate, meaning that they yielded positive results and are vegetable tanned – see Figure 3 for results of sample HisD. Sample #1, #3 and #4 of specimen HisA were collected from dyed areas of the leather specimen, which resulted in a less visible colour formation in reaction to the iron(III)sulfate solution compared to the remaining samples, which were collected from areas without dye. The samples from specimen HisB proved to be hydrophobic and did not soak up the aqueous solution of iron(III)sulfate, and the results were therefore rendered inconclusive.

Table 4: Summary of results from chemical test methods. Determination of tanning method is based upon the discussion of the results from the chemical test methods and ATR-FTIR analysis in the discussion.

	Ferric test	Vanillin test	Rhodanine test	Acid butanol test	Nitrous acid test	Tanning method
ConA	N/D	+	-	+	N/D	Condensed
ConB	N/D	+	-	+	N/D	Condensed
GaIA	N/D	-	+	-	N/D	Gallotannins
GaIB	N/D	-	+	-	N/D	Gallotannins
ElIA	N/D	-	+	-	N/D	Gallotannins
ElIB	N/D	-	+	-	N/D	Ellagitannins
HisA	+	+	+	+	N/D	Gallotannins
HisB	N/D	N/D	N/D	+	N/D	Inconclusive
HisC	+	-	-	+	N/D	Condensed
HisD	+	-	-	+	N/D	Inconclusive, condensed in literature

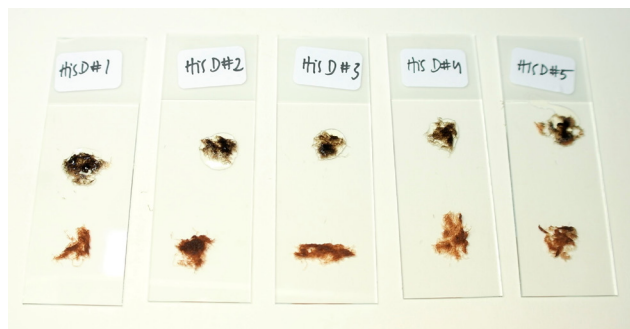


Figure 3. Results sample HisD for the ferric test. Photo: Ina Halvorsen.

Vanillin test for condensed tannins

ConA and ConB were the only specimens that reacted to the HCl and vanillin solution, yielding a bright red or magenta colour formation, indicating a positive result for condensed tannins – see Figure 4 for the results from sample ConA. HisA also yielded a weak red colour formation, but this might be due to dye present in the leather. The samples from specimen HisB did not soak up the vanillin solution, due to being hydrophobic, which renders the result inconclusive.

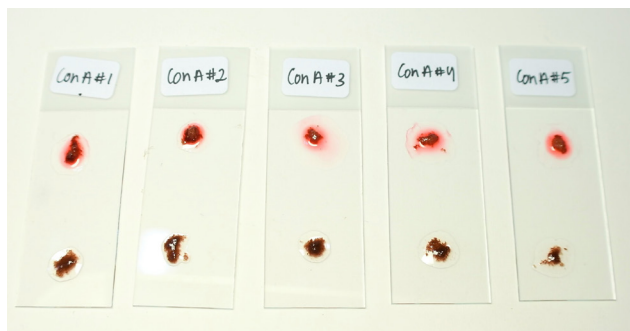


Figure 4: Results sample ConA for the vanillin test. Photo: Ina Halvorsen.

Rhodanine test for gallotannins/gallic acid

GalA and GalB yielded positive results, indicating the presence of gallotannins and gallic acid – see Figure 5 for results from sample GalB. A weak red colour formation was seen in EIIA and EIIB for both gallotannins and gallic acid, which might indicate a positive result. #1 and #3 of specimen HisA yielded a magenta colour formation, but this was most likely due to dye. However, a faint red colour formation in sample

#2, #4 and #5 of HisA might indicate the presence of gallotannins. The above-mentioned results must be viewed in light of the results from the other tests. The samples from specimen HisB were too hydrophobic to absorb the aqueous H_2SO_4 and KOH solutions, rendering the test inconclusive. The remaining specimens yielded negative results.

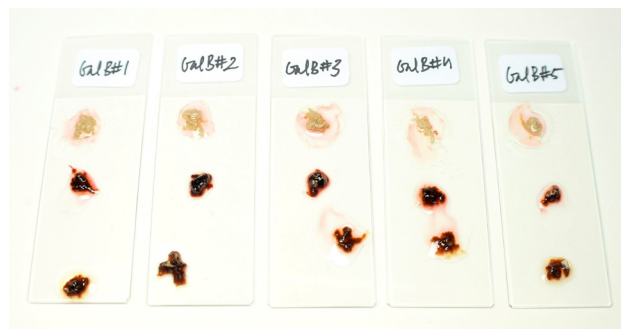


Figure 5: Results sample GalB for the rhodanine test. Photo: Ina Halvorsen.

Acid butanol test for condensed tannins

The colour formation indicating positive results ranged from pale orange to brown, depending on the specimen. ConA developed red and dark red colour, which indicates the presence of condensed tannins. The same accounts for specimen ConB. HisA yielded orange and red colour, which might indicate the presence of condensed tannins, although this specimen contained red dye that might have caused equivocal results – see Figure 6. HisB developed a brown colour on all samples, which might indicate the presence of condensed tannins. Both HisC and HisD yielded pale red and orange colours, which might indicate the presence of condensed tannins. The remaining specimens yielded negative results.

Nitrous acid test for ellagitannins/ellagic acid

Too little acid was added to sample #5 of HisA, which rendered the result inconclusive. The remaining specimens yielded colours ranging from green to black, which means the testing was unsuccessful. The colours should have ranged from pale yellow (as with the control sample), to blue or red, indicating a positive result and the presence of ellagitannins or ellagic acid, respectively. Instead, the solution appeared almost black in all samples, as seen in Figure 7.



Figure 6: Results sample HisC for the acid butanol test. Photo: Ina Halvorsen.

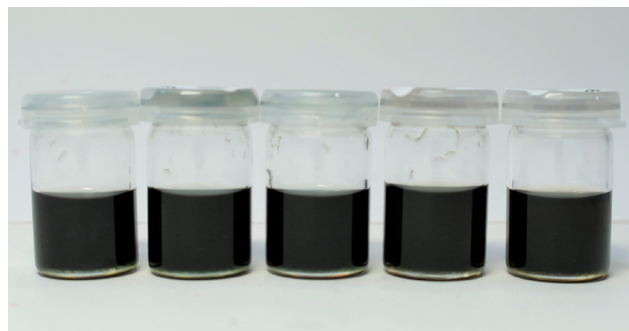


Figure 7: Results sample EIBB for the nitrous acid test. Photo: Ina Halvorsen.

ATR-FTIR

The interpretation of FTIR spectra is based on the reference data provided by Falcão and Araújo (2012) in their study on analytical methods for the characterisation of vegetable tannins in modern and historic leathers. See previous Table 3 for which chemical bonds the different bands are assigned to. The results from the ATR-FTIR analysis are presented in Table 5.

Specimens EIBB, HisA, HisB and HisD had to be re-extracted and re-run because of weak signals. These results are therefore not from sample #1 of the leather specimens, but from sample #2 – except specimen HisA, where sample #5 was used because it was the sample with the least amount of dye visibly present. This sample was therefore preferred, as the presence of dye might have given inconclusive results. The other leather specimens are analysed from sample #1 of each specimen.

The bands for general tannins are identified in nearly every specimen, except specimen HisD, where

the bands at 1518-1507 and 1211-1196 are absent. Specimen ConA and ConB both show the presence of the bands characteristic for condensed tannins, although the band at 1288-1282 is absent in ConA. They both also show the presence of a band at 1731-1704, which is attributed to hydrolysable tannins, but the presence of bands for condensed tannins most likely indicate that the leather contains condensed tannins. See Figure 8 for the spectra of sample ConA

GalA shows a complete absence of condensed tannins, and every band for hydrolysable tannins and gallotannins are present, which indicates that this leather most likely contains gallotannins – see Figure 9. GalB only displays one gallotannin band at 763; however, the bands for hydrolysable tannins are present, which indicates that the leather contains hydrolysable tannins, most likely also gallotannins. Specimen EIIA displays both bands for hydrolysable tannins, although the band at 1325 appears as a shoulder. This specimen also displays all bands for gallotannins, the band 1093 also appearing as a shoulder. No bands for condensed tannins are detected, which indicates that this leather most likely is tanned with hydrolysable tannins, although the spectrum indicates that it was tanned with gallotannins – see Figure 10. Specimen EIBB displays both bands for hydrolysable tannins, the band at 1325 as a shoulder. No bands for either condensed tannins or gallotannins are detected, which suggests that the leather contains ellagitannins, even though the presence of ellagitannins only can be confirmed by the absence of gallotannins and condensed tannins (Falcão and Araújo 2012).

Specimen HisA displays every band for both hydrolysable tannins and gallotannins and no bands for condensed tannins, which means that this leather might contain gallotannins – see Figure 11. Specimen HisB displays weak bands in the entire spectrum, which might indicate that very small amounts of vegetable tannins are present in the leather. The four bands at 1609, 1514, 1200 and 1042 indicating general tannins are all apparent as shoulders on the side of more prominent peaks, one of them being a band at 1112 from what appears to be a condensed tannin. Weak bands also appearing as shoulders at 872 and 763 might indicate the presence of gallotannins; however, the results from the ATR-FTIR analysis must be considered inconclusive because of the weak bands. Specimen HisC displays three bands at 1286, 1166 and 979, all which indicate the presence

Table 5: Registered bands from ATR-FTIR analysis. Numbers in paranthesis indicates that the registered band appeared as a shoulder on a more prominent peak.

	<i>MARKERS cm⁻¹</i>	ConA #1	ConB #1	GalA #1	GalB #1	ElIA #1	ElIB #2	HisA #5	HisB #2	HisC #1	HisD #2
Tannins	1615-1606	1615	1613	1612	1610	1608	1611	1608	609)	1626	1613
		1508	1508	1516	1510	(1514)	1511	1513	(1514)	1536	-
	1518-1507	1453	1453	1448	1449	1447	1448	1446	1452	1439	1452
	1452-1446	1198	1203	1202	1204	1181	(1206)	1198	(1200)	(1198)	-
		1031	1038	1034	1037	1047	1040	1030	(1042)	1040	1042
	1211-1196										
Condensed	1043-1030										
	1288-1282	-	1286	-	-	-	-	-	-	1286	(1285)
		1159	1160	-	-	-	-	-	-	1166	-
	1162-1155	1115	1113	-	-	-	-	-	1112	-	1115
	1116-1110	976	976	-	-	-	-	-	-	979	-
	976	843	843	-	-	-	-	-	-	-	-
Hydrolysable	844-842										
	1731-1704	1708	1707	1709	1715	1728	1729	1704	1711	(1707)	1711
		-	-	1322	1325	(1325)	(1325)	1318	-	-	-
Gallotannins	1325-1317										
	1088-1082	-	-	1087	-	(1093)	-	1088	-	1079	-
		-	-	872	-	860	-	870	(872)	-	-
	872-870	-	-	759	763	756	-	760	(763)	-	-
	763-758										

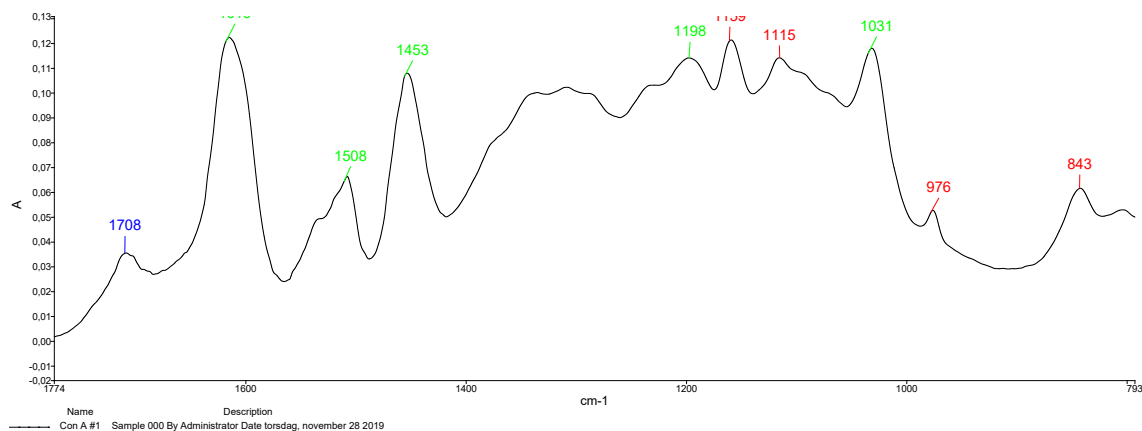


Figure 8: ATR-FTIR spectra of sample ConA. Blue peaks are indicators for hydrolysable tannins, green peaks indicate general tannins, red peaks indicate condensed tannins.

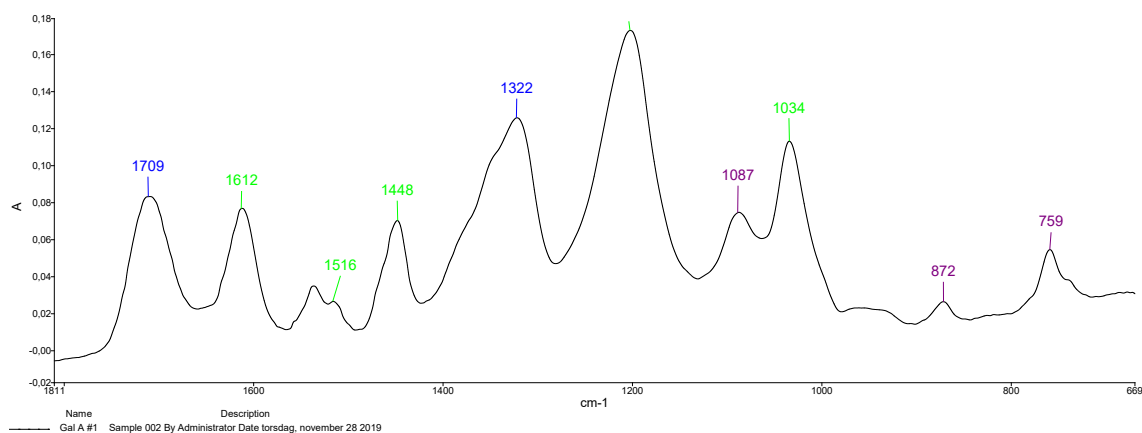


Figure 9: Spectra of sample GalA. Blue peaks indicate hydrolysable tannins, green peaks general tannins and purple peaks gallotannins.

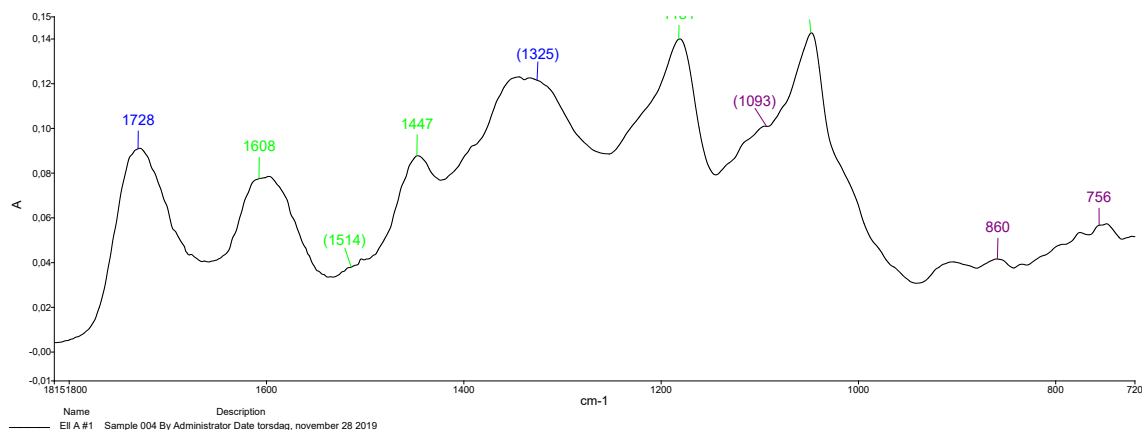


Figure 10: Spectra of sample EIIA. Blue peaks indicate hydrolysable tannins, green peaks general tannins and purple peaks gallotannins.

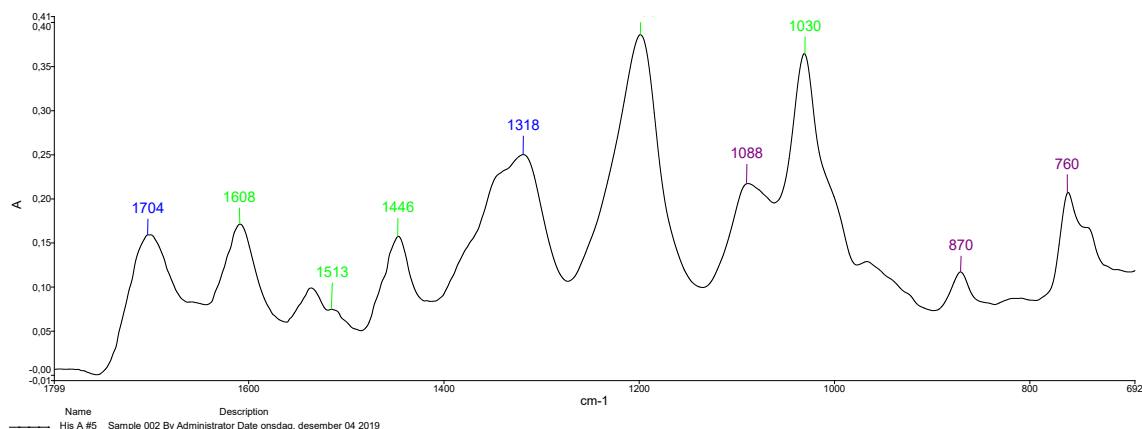


Figure 11: Spectra of sample HisA. Blue peaks indicate hydrolysable tannins, green peaks general tannins and purple peaks gallotannins.

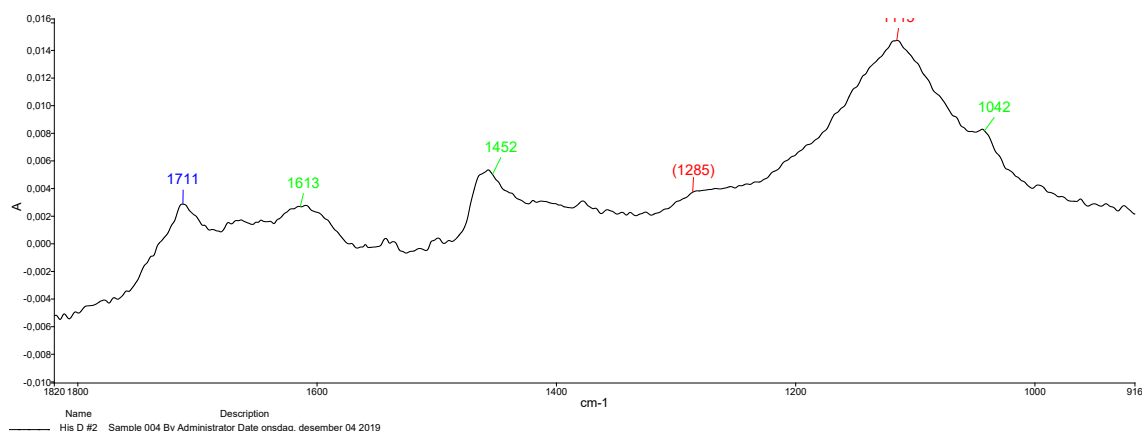


Figure 12: Spectra of sample HisD. Blue peaks indicate hydrolysable tannins, green peaks general tannins and red peaks condensed tannins.

of condensed tannins. The specimen also shows bands at 1707 and 1079 for hydrolysable tannins and gallotannins respectively, although 1707 appears as a shoulder. The bands in HisD are in general weak and only the bands at 1613, 1452 and 1042 can be contributed to the general tannins. A peak at 1115 and a shoulder at 1285 might indicate the presence of condensed tannins, although a peak at 1711 might indicate hydrolysable tannins – see Figure 12. The results from this leather must be viewed in light of its unique history, which will be discussed later.

Discussion

Specimen HisB yielded inconclusive results in every test method and ATR-FTIR, most likely because of the leather being either too degraded or tanned with

another tannage. This specimen will not be discussed further. The results from the nitrous acid test were inconclusive. Discussion of the nitrous acid test will therefore be limited to the overall practicability of the test.

Test methods

Ferric test for vegetable tannins

As presented in the results, specimens HisA, HisC and HisD indicated being tanned with vegetable tannins. This test will only determine whether the leather was tanned with vegetable tannins or not, and may therefore only be useful in cases where there is uncertainty as to the general tanning method of the leather. However, it has been pointed out that the

presence of other phenolic compounds in the leather also might yield a positive result, for example in the case of organic dyes (Poulsen 2002; Falcão and Araújo 2011). Leathers that may be tanned with minerals and dyed with organic dyes, might therefore yield a positive result for vegetable tannins, although the ferric test only tests for phenolics present in the leather. Regardless, in this case it is likely that the results from the ferric tests are reliable, as the ATR-FTIR results also indicates vegetable tannins of specimens HisA, HisC and HisD.

Vanillin test for condensed tannins

As presented in the results, specimens ConA and ConB were the only specimens that yielded positive results with the vanillin test, supporting the information from the suppliers, indicating leather specimens tanned with condensed tannins. This was also supported by the ATR-FTIR analysis. However, Falcão and Araújo (2011) state that the vanillin test is unreliable for the detection of condensed tannins for four main reasons: 1) the test may react with widely distributed flavanols such as catechin and epicatechin, which may result in a higher colour yield than that of condensed tannins. 2) The test is very sensitive to the presence of water that may quench colour yield. 3) Some proanthocyanidins, like quebracho, do not produce an intense colour with this reagent. 4) It has been reported that vanillin reacts reluctantly with aged leathers, making it challenging to identify the presence of condensed tannins. The last reason may have proved to be true in this case, where the HisA, HisC and HisD specimens yielded positive results in the acid butanol test for condensed tannins, and HisC and HisD indicated the presence of condensed tannins by ATR-FTIR. Consequently, these specimens should in theory have yielded positive results with the vanillin test. However, it is a test that is cheap and easy to conduct, and might be useful on modern leathers which have not been tanned with quebracho, and where catechin and epicatechin are known to be absent.

Rhodanine test for gallotannins/gallic acid

The results confirmed tannage by gallotannins for specimens GalA and GalB, and indicated the presence of gallotannins and gallic acid in specimens EIIA, EIIB and HisA. The colour formed during the procedure proved difficult to see in deteriorated and dyed leather. Additionally, free gallic acid is a common deterioration product from both gallotannins, ellagitannins and

condensed tannins, so the positive characterisation of gallotannins can only be determined after comparing the results with the results from other tests (Poulsen 2002; Florian 2006; Falcão and Araújo 2011).

This means that although EIIA and EIIB indicated the presence of gallic acid, it might be due to the deterioration of ellagitannins and does not necessarily mean that they are tanned with gallotannins. However, a faint red colour formation was also seen in the gallotannin-samples of specimen EIIA and EIIB, and the presence of gallotannins cannot be disregarded - especially considering the ATR-FTIR results from specimen EIIA, showing peaks indicating the presence of gallotannins. The results from HisA, indicating a red colour formation for both gallotannins and gallic acid in reaction to rhodanine reagent, is supported by the results from the ATR-FTIR which clearly shows peaks indicating gallotannins.

Acid butanol test for condensed tannins

All the historical specimens tested positive for condensed tannins, as did ConA and ConB, as expected. However, the red colour developed might vary significantly, depending on which anthocyanidin formed (Falcão and Araújo 2011). It was also clear that the colour formation varied considerably through the different samples of each specimen, such that the colour formation ranged from soft pink to burgundy. This was an interesting observation, as this might indicate that even though a standard procedure was followed, there was an unknown factor contributing to the colour formation. This unknown factor could be the amount of tannins present in each sampling area of the specimen, or the amount of deterioration taken place in each area.

This test proves more useful in the testing of historical leathers than the vanillin test, as the vanillin test did not provide positive results in any of the historic specimens. The presence of condensed tannins in HisC by ATR-FTIR, confirms the results from the acid butanol test. However, the positive result in the acid butanol test in HisA is difficult to explain. The positive reaction to gallotannins in both the rhodanine test and ATR-FTIR should indicate the leather being tanned with gallotannins. The acid butanol test might therefore have rendered ambiguous results in specimen HisA. Regarding the practicability of the test, it has to be mentioned that this method is tedious and time consuming, which might discourage the average conservator from using it.

Nitrous acid test for ellagitannins/ellagic acid

This test has proved to give unsuccessful results with historic leathers in the literature (Falcão and Araújo 2012). Although the test gives the possibility of directly assessing the ellagitannin content in leather, something that has not previously been possible, it is unclear how this knowledge can be of use in leather conservation.

When it comes to the practicability of the test, it is worth including that this method uses harmful and unpleasant chemicals. Pyridine is highly flammable and requires precautionary measures against static discharge, like wearing antistatic protective clothing. It's fumes causes irritation to the eyes and may be harmful if inhaled, which requires the use of respiratory protection. The vapour has a distinct smell of rotten fish, in addition to having a flash point of 17°C. For this reason, the bottle of pyridine is delivered with a SureSeal® cap, which requires the use of a syringe with needle for outtake, something that complicates use, as the bottle will have to be turned upside down every time when used. Sodium nitrite is toxic and needs to be disposed of correctly, as it is harmful to the environment.

It became clear during the procedure and afterwards, that this test is not under any circumstances worth undertaking. The experiment was tedious and unpleasant due to the smell and harmfulness of pyridine, in addition to yielding an exothermic reaction in combination with HCl, which caused heat and vapour to develop in the test vials. The procedure should have been aborted immediately after it became clear that the results were inconclusive – or better yet, not have been undertaken at all.

ATR-FTIR

As stated in the results, the spectra from ConA, ConB, GalA and EllB appear to match the information from the suppliers regarding the tannage of the specimens. Both ConA and ConB also show the presence of a band at 1731-1704, which is attributed to hydrolysable tannins. This may be due to the use of oak bark – a tanning source known to contain complex tannins, in which a catechin unit is bound glycosidically to a gallotannin or ellagitannin unit (Khanbabaee and van Ree 2001: 643). The absence of two gallotannin bands in specimen GalB might be explained by information from the supplier: the specimen was tanned with a mixture of tanning sources: synthetic tan, sumac

and tara. This could imply only a minor presence of gallotannins. Based on the spectrum for EllA and the results from the chemical tests, this specimen appears to be tanned with gallotannins, and not ellagitannins as stated by the supplier. The spectrum of specimen HisA indicated the presence of gallotannins. This does not correspond with the results from the acid butanol test; however, it does correspond with the results from the rhodanine test, indicating gallotannins and gallic acid, and it can therefore be stated with a fair amount of certainty that this specimen is tanned with gallotannins. Regarding specimen HisC, the acid butanol test indicated the presence of condensed tannins, but the vanillin test did not. The rhodanine test was negative, but the ATR-FTIR indicated both hydrolysable and condensed tannins. This might indicate that the specimen has been tanned with complex tannins, as may be the case when oak bark is used as a tanning agent (Khanbabaee and van Ree 2001: 643; Falcão and Araújo 2018). Specimen HisD has a unique history, which might explain the weak results in the ATR-FTIR analysis. The leather stems from the Metta Catharina shipwreck, which sank in Plymouth Sound in 1786, and from which leather finds were retrieved at the end of the 20th century. Upon retrieval, samples of the leather were sent to the British Leather Manufacturer's Research Association and identified as Russia leather from reindeer – a Russian tanning method with the use of willow bark, which is later curried with birch oil (Skelton 2010: 239). This leather has therefore been waterlogged and the tannins might have leached out of the leather, making tannin characterisation difficult – a reoccurring issue with marine archaeological leather (Bickley 1991). Both willow and birch contain condensed tannins – as seen in Table 1 – so the analysis of specimen HisD should therefore in theory be positive for condensed tannins. The acid butanol test rendered positive results for condensed tannins, which might confirm this.

Although time consuming, this method requires only minimal preparation. It does require access to an ATR-FTIR spectrometer and some knowledge of how to use the software, but with the help of the reference data provided in Falcão and Araújo (2012), interpretation of the spectra was straightforward. However, the use of this method for tannin characterisation of deteriorated leathers may be challenging because of spectral bands resembling peptide bands formed during the deterioration of collagen, which overlap with the specific tannin bands (Falcão and Araújo 2012). This might be the cause of the ambiguous

results in specimens HisB and HisD. The extraction of fibres prior to the analysis may also extract fats used in lubrication, which in turn may interfere with the results (Falcão and Araújo 2012). Last, but not least: the relatively large sample size of 10 mg fibre is hard to justify and conflicts with ethical requirements to minimise invasive and destructive sampling. All the above-mentioned limitations have, since these experiments were undertaken, proven solvable. ATR-FTIR analysis have been undertaken directly on archaeological and historical leather fibres, without the need for solvent extraction on beforehand, making it possible to undertake the analysis using a much smaller sample size (Warming et. al. 2020). The problem with overlapping bands in deteriorated leathers can be solved by calculation of the second derivative of the spectra scan data, making tannin characterisation possible in even highly degraded leather (Odlyha et. al. 2009; Baldassarre et. al. 2015; Vichi et. al. 2018; Warming et. al. 2020). This could potentially have solved the issues with the ambiguous results in sample HisB and HisD.

The use of ATR-FTIR on vegetable tanned leathers is therefore a reliable and valuable method which renders important information about the tanning chemistry of the leather, and the method may also be used to obtain information about the structural changes in the collagen molecule during deterioration. The analysis is easily conducted with the use of available reference data, although it is worth mentioning that the heterogenous nature of historical leathers may yield ambiguous results.

Conclusion

By characterising the tannins present in historic leather objects, valuable information can be drawn, which can be useful in the conservation of leather objects tanned with vegetable tannins. This can be done by performing different kinds of chemical tests and analysis. Five chemical test methods and ATR-FTIR were compared, with varying results. When testing modern and moderately deteriorated leather, the test methods and ATR-FTIR proved reliable. However, when badly deteriorated leathers were tested, the results proved to be inconclusive.

New research has established a method for the use of ATR-FTIR on highly degraded leathers, making ATR-FTIR analysis even more relevant in the conservation of vegetable-tanned leathers.

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Competing interests

The authors declare that they have no competing interests.