

RESEARCH

Open Access



Comparison of various test methods to quantify the deterioration degree of archaeological leather

Shuli Yao^{1,2}, Yirui Lin¹, Chao Lei², Ya-nan Wang^{1,2}, Yue Yu^{1,2} and Yunhang Zeng^{1,2*}

Abstract

Quantifying the degree of deterioration is crucial for developing prevention strategies for archaeological leather. In this work, the morphology, chemical compositions, and physical properties of artificially aged leather samples were analyzed using a series of test methods to determine non-destructive or micro-destructive technologies for quantifying the degree of deterioration in archaeological leather. Results show that the hydroxyproline (Hyp) content of leather decreases with the increase of deterioration degree because deterioration leads to the gradual destruction of the collagen fiber network of leather. Moreover, the Hyp content of leather can be micro-destructively determined by high-performance liquid chromatography with fluorescence detection. Therefore, the determination of Hyp content is recommended to quantify the degree of deterioration in archaeological leather.

Keywords Archaeological leather, Artificially aged leather, Deterioration, Quantification

Introduction

Animal skin/hide, an important collagen-based biomass resource, has been used for making clothes to protect against the cold since the early stages of human civilization [1]. Increasing leather products had been produced to meet the needs of daily life and military with the rapid development of technology and science in ancient time [2]. Archaeological leather products such as clothes, footwear, belts, and armor have shown great research importance as they reflect the cultural characteristics and social customs of their time [3, 4]. Archaeological leather products are influenced by their surroundings, and they may experience stiffness, discoloration, and fractures [5]. The quantification of deterioration degree plays an important

role in guiding the formulation of prevention strategies for archaeological leather products.

Non-destructive and micro-destructive testing technologies are essential for quantifying the deterioration degree of archaeological leather because of the preciousness of archaeological artifacts. Spectroscopies are widely used to analyze the structure of archaeological artifacts because they rarely cause damage to the testing samples. For example, attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) can be used to observe changes at the molecular level of the archaeological leather in a non-destructive way [6]. Halldorsdottir et al. [7] investigated the changes in functional groups related to collagen, tannins, and lipids in archaeological leather and buried vegetable-tanned leather by using ATR-FTIR. Their results showed a rapid decrease in lipid peaks and detanning of leather that was buried in water-saturated soil. Koochakzai et al. [8] identified the type of tannin in leather from Iran by using FTIR and principal component analysis. Microscopies are often used to observe the morphology of leather with little or no damage to the leather samples. Ebsena et al. [9] used a

*Correspondence:

Yunhang Zeng
zengyunhang@scu.edu.cn

¹ College of Biomass Science and Engineering, Sichuan University, Chengdu 610065, China

² Ministry of Education, Key Laboratory of Leather Chemistry and Engineering (Sichuan University), Chengdu 610065, China

stereo microscope to observe archaeological leather and identified their species based on the grain patterns. Scanning electron microscopy (SEM) can be used to observe the fiber network, fibers, and microfibrils of leather and obtain changes in the micromorphology of leather during deterioration. By using SEM, Vadrucchi et al. [10] observed that the collagen fibers of irradiated leather showed fraying and fragmentation, and some fibers lost their ordered fibrillary structure. Vyskočilová et al. [11] also used SEM to observe buried leather and found the discontinuities and loosening of the collagen structure of buried leather, with large areas of amorphous and gelatinized structures appearing on the collagen after 1 year of burial. Differential scanning calorimetry (DSC) can quantitatively analyze the thermal behavior of leather to distinguish old and new leathers, and only a small amount of leather sample is needed for this analysis [12–14]. Chahine et al. [15] reported that the thermal denaturation temperature (T_d) of the artificially aged leather and historical leather measured by DSC were lower compared with a new leather.

Other analyses of physical and chemical properties have also been used to study the change in archaeological leather. For example, Abdel-Maksoud et al. [16] found that leather bookbinding from the nineteenth century had a low pH and shrinkage temperature caused by airborne sulfur dioxide pollution. Vyskočilová et al. [11] found that leather had a pH value that gradually approached the soil environment and low mechanical strength. Leather color also changed when buried underground for a long time. Moreover, many standards are used to evaluate the properties of leather, such as the determination standards for tensile strength (ISO 3376), softness (ISO 17235), matter soluble in dichloromethane (ISO 4048), volatile matter (ISO 4684), and pH (ISO 4045). In addition, hydroxyproline (Hyp) content [17, 18] and porosity [19] are usually used to evaluate the quality of leather. However, whether these determination standards or methods can obtain accurate analysis results of archaeological leather still needs to be verified under non-destructive or micro-destructive conditions. Most previous studies detected archaeological leather using some of these analytical technologies. However, a systematic comparison is lacking among various analytical methods for leather. Therefore, a comprehensive understanding of various analytical methods has a substantial implication for selecting suitable technologies to quantify the degree of deterioration of archaeological leather.

This paper aims to systematically compare different analytical methods for leather, including stereo microscopy, SEM, and micro-computed tomography (Micro-CT) for observing the morphology of leather; component analyses for determining moisture, Hyp, fat, and metal

elements of leather; pH analysis; DSC for determining the thermal property of leather; and analyses for measuring the tensile strength, softness, and color of leather. In brief, artificially aged leather samples with different degrees of deterioration were prepared and measured by using the above-mentioned methods. Then, the damage to the leather sample caused by these analytical methods was evaluated based on the weight of the sample required for testing, and these methods were classified into non-destructive, micro-destructive (sample weight < 5 mg), and destructive (sample weight > 5 mg) methods. The precision of these analytical methods was evaluated according to the relative standard deviation (RSD) of the analytical results. Finally, the non-destructive or micro-destructive methods that are suitable for quantifying the deterioration degree of archaeological leather were identified, and a deterioration assessment was proposed based on the recommended methods and resulting data.

Materials and methods

Materials

Sheep leather was produced in accordance with the procedures shown in Table S1 for the preparation of artificially aged leather samples. Compost (detailed information is shown in Section S2 of the supporting information) was purchased from Chengdu Huahong Biotechnology Co., Ltd., Sichuan, China. Hyp was of analytical grade and purchased from Shanghai Macklin Biochemical Co., Ltd. (China). *p*-Dimethylaminobenzaldehyde (DMAB), hydrogen peroxide (30%), nitric acid (68%), hydrochloric acid (36%), and dichloromethane were of analytical grade and purchased from Chengdu Kelong Chemical Reagent Co., Ltd. (China). 7-Chloro-4-nitrobenzol-2-oxa-1,3-diazole (NBD-Cl) was of analytical grade and purchased from Titan Scientific Co., Ltd. (China). Other chemicals and reagents were of analytical or chromatographic grade.

Preparation of artificially aged leather samples

The deterioration rate of leather is closely related to hide species and tanning methods, but the changes in different types of leather caused by deterioration are reflected in the main components of leather, such as collagen, fats, and moisture, as well as in the collagen fiber network. This study focuses on investigating non-destructive and micro-destructive methods for analyzing the deterioration of leather, rather than examining the trends of change in specific types of aged leather. Hence, only one type of leather was selected in our study to investigate the deterioration of leather. In addition, Fe-tanned leather degrades more rapidly during deterioration [20], and the change in Fe tanning agent can be determined more easily compared with vegetable-tanned leather. Therefore,

Fe-tanned sheep leather was used in this study. Sheep leather was buried in compost with a depth of 4 cm, and the compost was six times the weight of the sheep leather. Composting was performed in a thermostatic cabinet (ST 5 CS Smart, Pol-Eko, Poland) at 58 °C for 7 days to obtain artificially aged leather samples (Fig. 1). The main components and properties of the compost are listed in Tables S2 and S3. Given the microorganisms in the compost, artificially aged leather samples with a high degree of deterioration can be obtained in a short period of time. Macroscopic changes of leather were continuously observed for 7 days. The un-aged leather sample numbered 1 and artificially aged leather samples with five degrees of deterioration numbered 2–6 were collected (Fig. 2). The deterioration degrees of the six samples increased from sample no. 1 to sample no. 6.

Morphological analyses of leather samples

The morphology of artificially aged leather samples was observed using a stereo microscope (M205C, Leica, Germany) and SEM (Nova Nano SEM450, FEI, USA). Furthermore, the leather samples were scanned using a Micro-CT scanner (Viva CT 80, Scano Medical, Switzerland) to obtain images of their internal structure. The three-dimensional structure of the sample with a voxel size of 6.0 μm was reconstructed using the Scanco μCT Evaluation Program (V6.6), and the porosity of the sample was calculated using Formula (1).

$$\text{Porosity}(\%) = \frac{TV - SMV}{TV} \times 100 \tag{1}$$

where *TV* represents the total volume of the sample (mm³), and *SMV* denotes the solid matter volume of the sample (mm³).

The collagen fiber networks of the samples were analyzed using Masson’s trichrome staining [21]. The detailed procedures are shown in Section S3 of the supporting information.

Components analyses of leather samples

Moisture content

The moisture contents of the artificially aged leather samples were determined through oven drying (Fig. 3a) in accordance with ISO 4684 [22], Karl Fischer titration equipped with a Karl Fischer moisture tester (S-300, Heye, China; Fig. 3b), and the capacitance method equipped with a skin moisture tester (CM 825, Courage and Khazaka, Germany) (Fig. 3c) [23]. The details of the experiments are listed in Section S4 of the supporting information. For the capacitance method, the test probes of the skin moisture tester were kept in close contact with the leather surface, and they contained tiny electrodes that can form a capacitor when they closely contact with the leather surface (Fig. 3c). An electric field was formed between the metal electrodes, with one metal electrode accumulating a negative charge and the other accumulating a positive charge. The stray electric field can penetrate the leather surface. Considering that

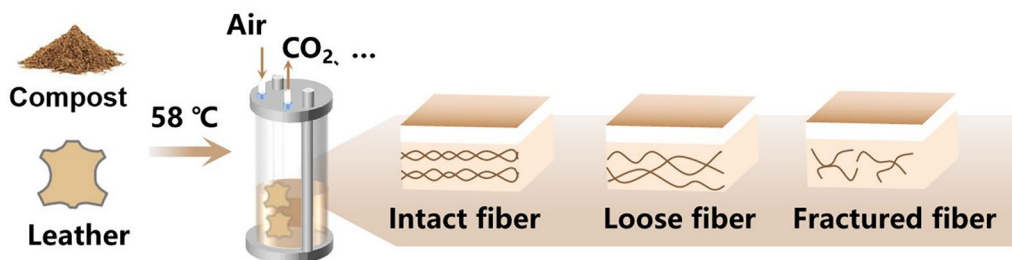


Fig. 1 Schematic of the preparation of artificially aged leather samples

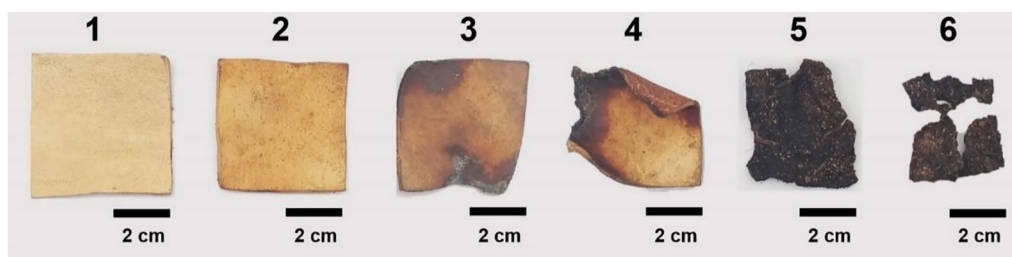


Fig. 2 Artificially aged leather samples with different degrees of deterioration

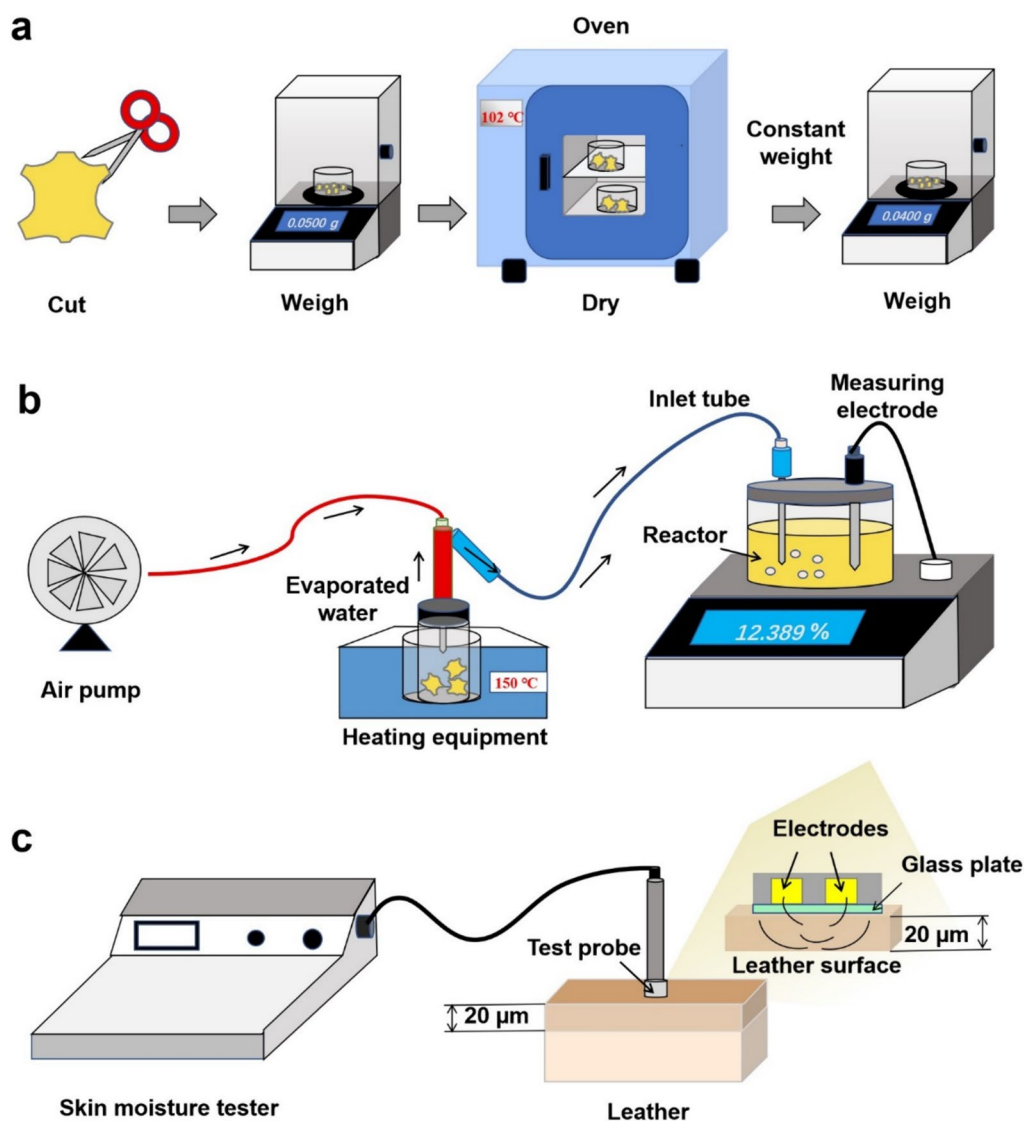


Fig. 3 Schematic of the measuring moisture contents of samples: **a** oven drying, **b** Karl Fischer titration, and **c** capacitance method

the permittivity of water in leather is higher than that of fat, protein, and other substances, the moisture content of the leather sample can be obtained by measuring its capacitance. The tester detected and recorded the capacitance of the leather to evaluate the moisture content of the leather sample.

Hyp content

The artificially aged leather samples were sampled and hydrolyzed with 6 mol/L hydrochloric acid at 110 °C for 10 h. The hydrolysate was used to determine the Hyp content of the leather sample using an ultraviolet–visible (UV–Vis) spectrometry based on the reaction of oxidized Hyp with DMAB [17] and high-performance liquid

chromatography with fluorescence detection (HPLC–FLD) [18]. HPLC–FLD used NBD–Cl as a fluorescent reagent to react with Hyp. The details of the experiments are listed in Section S5 of the supporting information, and the chromatograms of Hyp are shown in Fig. S4.

Fat content

The fat content of the samples was determined using Soxhlet extraction with dichloromethane as the solvent in accordance with ISO 4048.

Other components

The artificially aged leathers were sampled and dried to a constant weight at 102 °C. Then, the dried sample

(50 mg) was digested with 5 mL of nitric acid and 1 mL of hydrogen peroxide in a microwave digestion instrument (Multiwave PRO, Anton Paar, Austria). The Fe concentration of the digestion solution was measured by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 2100DV, PerkinElmer, USA). The Fe content of the leather sample was calculated using Formula (2):

$$\text{Fe content (mg/g)} = (c \times V) / w \quad (2)$$

where c is the Fe concentration of the digestion solution (mg/L), V is the volume of the digestion solution (L), and w is the dry weight of the leather sample (g) [24].

The element content of the samples was analyzed by using an energy-dispersive X-ray spectrometer (EDX; X-MaxN, Oxford, UK).

The total carbon (TC) and total nitrogen (TN) contents of the leather samples were analyzed using a Dumas nitrogen analyzer (Primacs100, Skalar, Netherlands) with helium as the carrier gas and oxygen as combustion gas at a heating temperature of 1000 °C [25]. Details of this method are described in Section S6 of the supporting information.

Analysis of the chemical structure of leather samples

The chemical structures of the samples were measured using ATR-FTIR (Nicolet iS50, Thermo Fisher, USA) with a diamond crystal in the wavenumber range of 2000–800 cm^{-1} with 64 scans and a resolution of 4 cm^{-1} . Baseline correction of the spectra was performed using EZ OMNIC software, version 7.2a. The curve-fitting analyses of the overlapping peaks in the wavenumber range of 1715–1485 cm^{-1} , along with graphical presentation, were conducted using Origin 8.0 software.

Analysis of pH of leather samples

The pH of the leather samples was analyzed in accordance with ISO 4045, with a minor modification. The leather sample (50 mg) was mixed with 1 mL of ultrapure water and incubated in a shaking water bath (ZWY-2102C, ZhiCheng, China) at 25 °C for 6 h. The pH of the extraction solution was determined at 25 °C using a precise pH meter (FE28-Standard, Mettler-Toledo, Switzerland) equipped with a micro-electrode.

Analyses of the thermal property of leather samples

The thermal stability of the artificially aged leather samples was analyzed using DSC (204-F1, Netzsch, Germany) at a heating rate of 10 °C/min from 20 °C to 200 °C under N_2 atmosphere [26].

Analyses of the mechanical and sensory properties of leather samples

Tensile strength and percentage elongation

The tensile strength and percentage elongation caused by a specified load (10 N/mm^2) of the samples were measured in accordance with ISO 3376.

Softness

The softness of the samples was measured using a leather softness tester in accordance with ISO 17235 (GT-303, Gotech, China).

Compressibility

Compressibility is a sensory performance of leather. The thickness of the leather sample was measured using a digital thickness gauge (GT-313-A1, Gotech, China) and recorded as the initial thickness. A load of 0.013 N/mm^2 was added to the leather sample every 10 s until the load reached 0.065 N/mm^2 , and the thicknesses of the leather sample corresponding to different loads were determined. The thickness change rates of the leather sample under different loads were calculated using Formula (3) to evaluate the compressibility.

$$\text{Thickness change rate (\%)} = (T_0 - T_i) / T_0 \times 100 \quad (3)$$

where T_0 is the initial thickness of the leather sample, and T_i is the thickness of the leather sample under a certain load [27].

Color parameters

The color parameters (L , a , and b) of the samples were measured using a colorimeter (CR-13, Konica Minolta, Japan). The color difference (ΔE) was calculated using Formula (4).

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

where L is the brightness, which describes the color in the range from black to white, a is the red/green value, and b is the yellow/blue value [28]. For comparison, ΔL , Δa , and Δb were calculated using the parameters shown in sample no. 1.

Results and discussion

Effect of deterioration on the morphology of leather

The morphology of the un-aged leather sample (no. 1) and the five artificially aged leather samples (nos. 2–6) were observed using a stereoscopic microscope. As shown in Fig. 4a and b, the leather surface becomes fuzzy; the color of the leather gradually changes to black, which may be due to the oxidation of Fe tanning

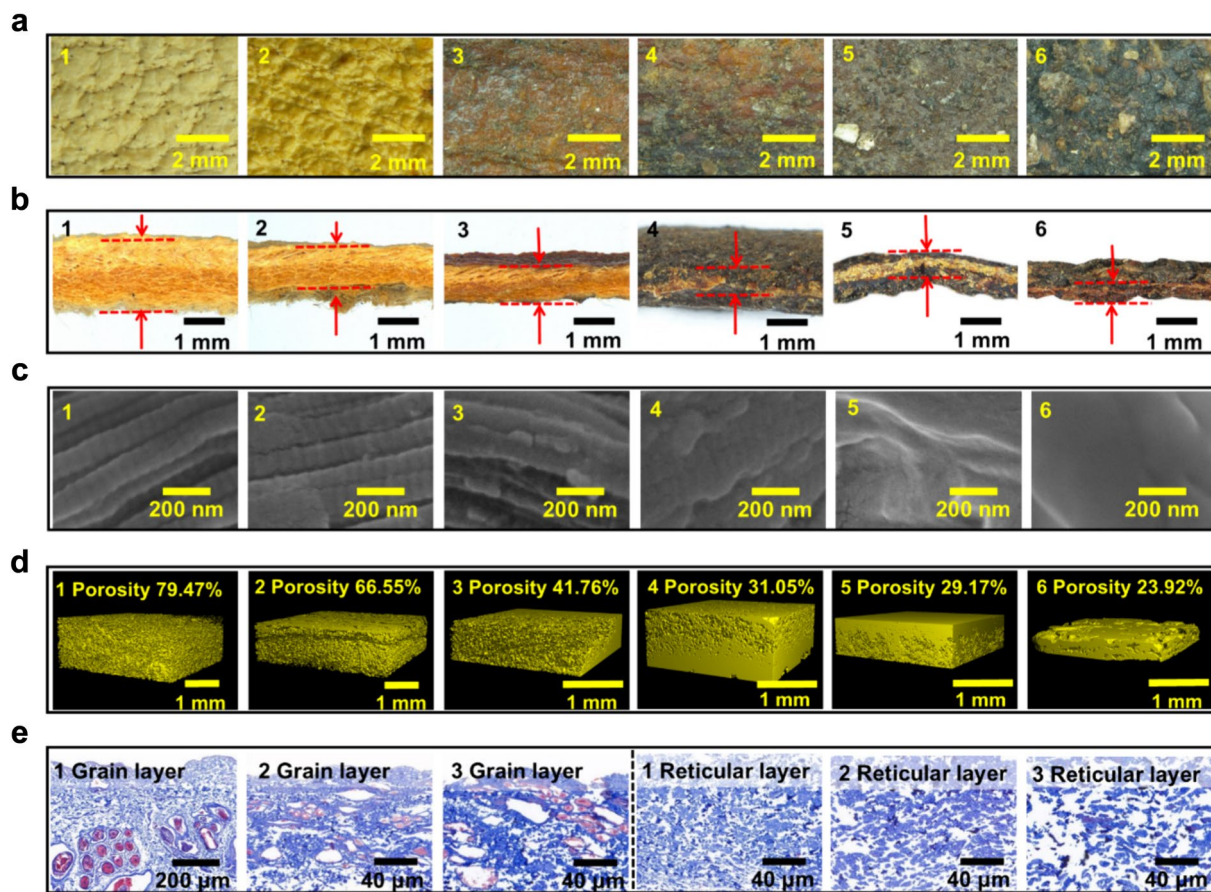


Fig. 4 a–b Stereo micrographs of the grain surfaces **a** and cross-sections **b** of samples; **c** SEM images of the cross-sections of samples; **d** Micro-CT images and porosity of samples; **e** optical microscopic images of the cross-sections of samples after Masson's trichrome staining

agent and contamination of compost, and the thickness decreases with the increase of leather deterioration. The SEM images shown in Fig. 4c indicate that the collagen fiber of sample no. 1 was complete and clear, where a distinct “D-period” can be observed (Collagen molecules are arranged head-to-tail within microfibrils, with a 67 nm gap, in a quarter-stagger arrangement [29]. This arrangement results in the formation of a typical cross-striation structure on the surface of the microfibrils, and the repetition period of this structure is D-period.). The damage degree of collagen fiber bundles increased with the increase of the deterioration degree of leather. The absence of the “D-period” in sample no. 4, as shown in Figs. 4c and S2, indicated that the fibril structures were damaged, and this damage becomes more severe in samples no. 5 and no. 6. Deterioration leads to the gradual destruction of the structure of collagen fibers in leather, which is consistent with the results of previous studies [26, 30].

The micro-CT images of the leather samples, along with their porosity calculated using the μ CT Evaluation

Program, are shown in Fig. 4d. Sample no. 1 has a porous structure with a porosity of 79.47%, indicating its high dispersion of collagen fibers. The porosity of the samples decreases, and the porous structure gradually disappears from sample no. 1 to sample no. 6. Sample no. 6 exhibits a block-like stacking structure with the lowest porosity. This result may be attributed to the adhesion among collagen fibers and the collapse of fiber network.

Samples nos. 1–3 were further observed using Masson's trichrome staining (Figs. 4e and S3), whereas samples nos. 4–6 were difficult to slice and stain because of their severe deterioration. After Masson's trichrome staining, the collagen fibers are blue; the cytoplasm and muscle are red, and the nucleus is black. Collagen fibers change from being coherent and dense to being defective and sparse, indicating a gradual destruction of the fiber network from sample no. 1 to sample no. 3. In addition, the grain surface of sample no. 3 showed serious damage, with parts of fat glands, sweat glands, and hair follicles (red substances) in the grain layer disappearing.

In summary, the artificially aged leather samples are gradually destroyed from the outward to the inward layers. The damage caused by deterioration initially occurs on collagen fiber bundles and then affects collagen fibers and fibrils at the microscopic level, causing the collagen to lose its characteristic structure. The changes in the morphology and porosity of leather become evident with aging. Therefore, the morphology and porosity of leather can be used to evaluate the deterioration degree.

Effect of deterioration on the components of leather

Moisture content

Water contributes to the maintenance of the natural state of the collagen fiber network [31]. The moisture content of the whole leather sample was first determined using oven drying and Karl Fischer titration. The data measured by the two methods are similar, and they decrease with the increase of deterioration degree (Fig. 5a). This trend may be due to the damaged collagen fiber network caused by deterioration, which weakens the connections of water molecules to leather. Therefore, finding a non-destructive method for testing the moisture content of leather is crucial.

A skin moisture tester is a convenient and non-destructive tool for determining the moisture content of the surface of human skin. Leather is made from animal skin/hide and has a dermal structure. The distribution of water in the leather is uniform across the papillary and reticular layers. Here, the moisture content of the artificially aged leather samples was measured using a skin moisture tester, which characterizes the moisture content based on the capacitance measurement of the sample. Resulting data exhibit a downward trend from sample no. 1 to sample no. 6 (Fig. 5b). The relationship between the moisture content of the whole leather and the leather surface was investigated by linear regression analysis (X-coordinate, the moisture content of the leather surface measured

by the skin moisture tester [unit: AU]; Y-coordinate, the moisture contents of whole leather measured by oven drying and Karl Fischer titration [unit: %]). Notably, the unit of the result tested by the skin moisture tester is different from the unit of the results tested by the oven drying method and Karl Fischer method. However, the correlation coefficient (R^2) values are greater than 0.95 (Fig. 5c), indicating good linear regression fitting. The results indicate that the skin moisture tester can be used for non-destructive testing of the moisture content of leather.

Hyp content

Hyp is the characteristic amino acid of the collagen, and it can be used to estimate collagen content. The UV-Vis spectrometry is a common method for determining Hyp content, which has a high level of accuracy but requires a large amount of sample, usually more than 50 mg [32]. HPLC-FLD has high sensitivity, and it only requires a small amount of sample, typically just a few milligrams [33]. Comparing Fig. 6a and b, although HPLC-FLD has a higher error than UV-Vis spectrometry, the results obtained by using HPLC-FLD are close to those obtained using UV-Vis spectrometry. The results indicate that HPLC-FLD can be used for micro-destructive analysis of the Hyp content of samples. The Hyp contents of the artificially aged samples determined by the UV-Vis spectrometry (Fig. 6a) and HPLC-FLD (Fig. 6b) decreased gradually with the increase of deterioration degree. This result may be due to the microbial degradation of collagen fibers during composting. As ideal nutrient sources, collagen, fat, and other organic substances in leather are likely decomposed by bacteria under suitable temperature and humidity [34].

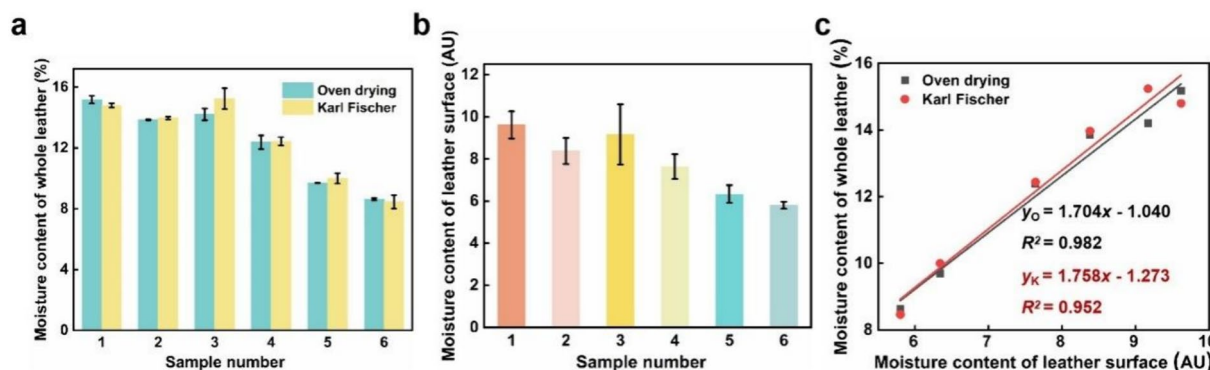


Fig. 5 a Moisture content of the whole leather determined using oven drying and Karl Fischer titration; b moisture contents of leather surface determined using a skin moisture tester; c relationship between moisture content of the whole leather and leather surface

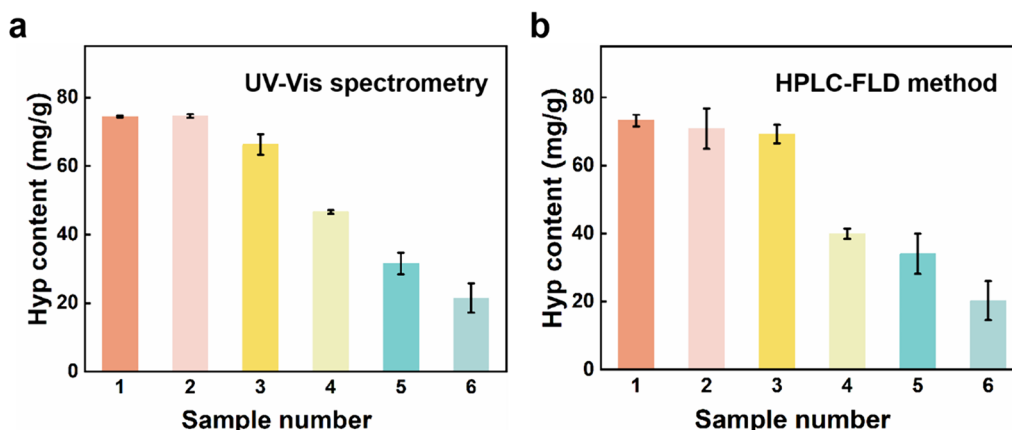


Fig. 6 Hyp contents of samples determined using the UV-Vis spectrometry (a) and HPLC-FLD (b)

Fat content and other components

The fat content of samples nos. 1–4 was tested through Soxhlet extraction, whereas that of samples nos. 5 and 6 was not tested because of their high levels of fine impurities from the compost. In addition, the fat contents of samples nos. 1–4 decreased with the

increase of deterioration degree (Fig. 7a). This trend may be due to the fats in leather being decomposed by microorganisms.

Changes in the contents of Fe and other elements in the samples were analyzed by using ICP-OES, Dumas combustion method, and EDX to investigate

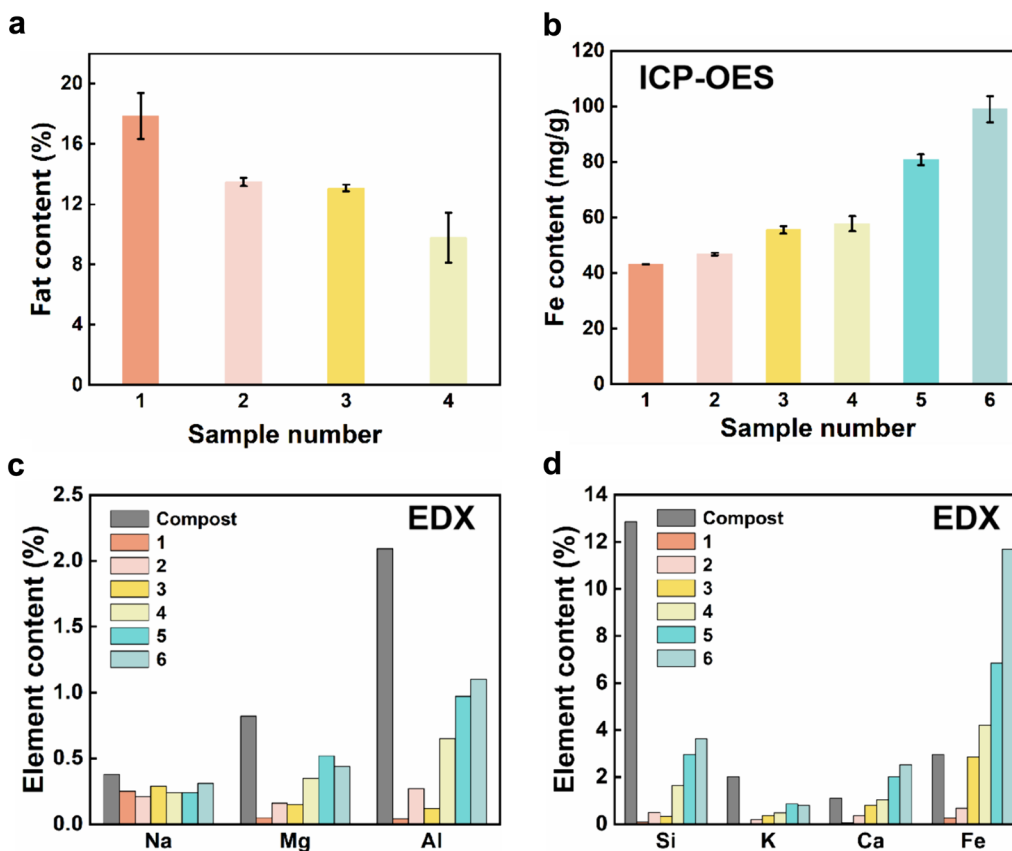


Fig. 7 Fat contents (a), Fe contents (b), and element contents (c–d) of samples

Table 1 TC and TN contents of compost and leather samples

Content (%)		Compost	Leather samples					
			1	2	3	4	5	6
TC	Mean	14.85	42.87	42.11	42.11	40.10	35.26	28.62
	Standard Error	1.85	0.29	0.01	0.46	0.31	0.67	1.92
	RSD ^a	12.43	0.67	0.01	1.09	0.76	1.89	6.69
TN	Mean	0.63	13.47	13.39	13.37	12.61	9.89	4.99
	Standard Error	0.08	0.08	0.04	0.09	0.25	0.21	0.25
	RSD ^a	12.39	0.58	0.30	0.67	1.97	2.09	4.98

^a Relative standard deviation of the test results

the influence of deterioration on the tanning agent and elemental composition of the leather. As shown in Tables 1 and S4 and Fig. 7b–d, the content of C and N decreased; the content of Fe increased, and the content of Si and Ca increased with the increase of sample deterioration. Some metal elements, such as Na, Mg, Al, and K, were also detected in low concentrations (almost less than 1%). The results indicated the decomposition of collagen and other organic materials in the leather, which led to the enrichment of Fe. It is worth noting that the Fe content of sample no. 1, determined by ICP-OES, is approximately 4% (Fig. 7b), while the content detected by EDX is less than 1% (Fig. 7d). Obviously, ICP-OES provides more accurate results than EDX, with the Fe content of sample no. 1 being approximately 4%. We speculate that the low Fe content detected by EDX may be due to the limitations of the technique. EDX is considered a semi-quantitative test. It can only detect the distribution and content of elements on the surface and is not accurate enough for elements present in low concentrations. In addition,

the adhesion of compost materials (vermiculite and perlite) to the leather increased the contents of Si and other metal elements.

Effect of deterioration on the chemical structure of leather

The artificially aged samples were analyzed by ATR-FTIR to investigate the changes in collagen peptide bonds. The absorption peaks of samples 1–3 are similar (Fig. 8a), with an amide A band (3310–3280 cm^{-1} , ν N–H), amide I band (1640–1620 cm^{-1} , ν C=O), amide II band (1550–1540 cm^{-1} , 40% ν C–N and 60% δ N–H), and amide III band (1240–1230 cm^{-1} , 30% ν C–N, 30% δ N–H, 20% ν C–C, and 10% ν C–H) [35, 36]. However, the absorption peaks of the amide I and amide II bands of samples 4–6 overlap, and the intensity of the amide III band is too low to be distinguished. For further comparison of amide I and amide II bands, curve-fitting analyses of the overlapping peaks (1715–1485 cm^{-1}) was conducted, and the fitting results are shown in Fig. 8b. The wavenumbers of the characteristic peaks in ATR-FTIR spectra and Amide I/Amide

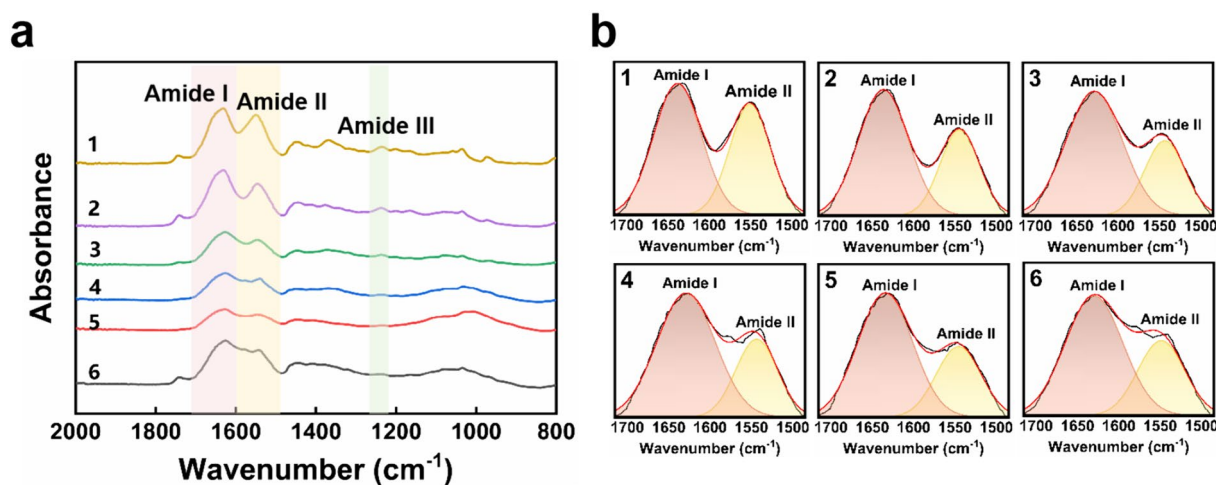


Fig. 8 ATR-FTIR spectra (a), and curve-fitting analyses of amide I and amide II in the ATR-FTIR spectra (b) of samples

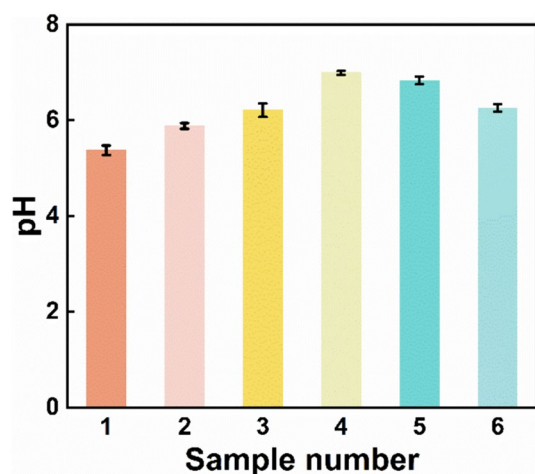
Table 2 Characteristic peaks in ATR-FTIR spectra and Amide I/Amide II value

Sample	Peak wavenumber (cm ⁻¹)		Amide I/ Amide II
	Amide I	Amide II	
1	1638	1550	1.19
2	1637	1544	1.47
3	1629	1543	1.66
4	1629	1544	1.59
5	1634	1545	1.76
6	1630	1549	1.60

II values are shown in Table 2. The amide I/amide II values of the five artificially aged leather samples (nos. 2–6) were higher than that of the un-aged leather sample (no. 1), and those of samples nos. 3–6 reached over 1.5. This change suggests the hydrolysis of the peptide chains of collagen during deterioration [36]; thus, the original regular triple helix structure of collagen gradually transforms into a disordered random coil structure [37, 38]. This phenomenon also explains the disappearance of the D-period observed by SEM in severely deteriorated leather (Fig. 4c).

Effect of deterioration on the pH of leather

The pH value of aged leather is affected by the pH of the surroundings and the degradation level of leather. As shown in Fig. 9, the pH of the un-aged leather sample (no. 1) was 5.37; the pH of the five artificially aged leather samples initially increased (nos. 2–4) and then decreased (nos. 5–6). The pH of sample no. 3 was 6.99, which was close to the pH of the compost (7.24). These results indicate that the pH value of leather was mainly

**Fig. 9** pH values of leather samples

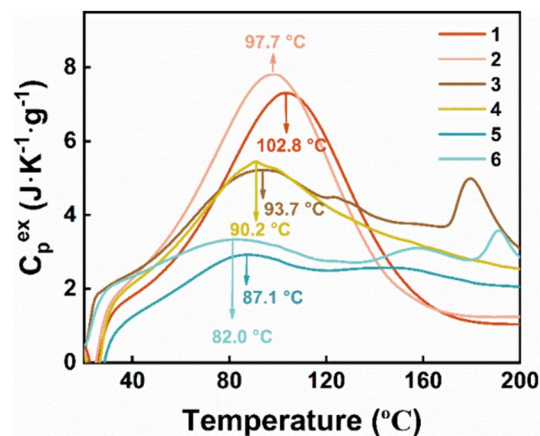
influenced by the compost at the initial stage of aging. In the later stage of aging, the pH of leather gradually dropped because of the increased deterioration of the leather and the hydrolysis of many peptide bonds into carboxyl groups.

Effect of deterioration on thermal stability

Damage to the fiber structure and the degradation or loss of leather components can affect the thermal stability of leather. In this section, the effect of deterioration on leather was investigated by measuring the thermal property. For the six samples, the DSC curves show peaks at 82 °C–102.8 °C (Fig. 10), which indicate the collagen denaturation peak [29, 39]. The T_d values of the samples decreased with the increase of leather deterioration. This trend might be due to the structure that allows the leather to maintain high stability being destroyed during aging, causing the leather to denature at a low temperature during the DSC testing.

Effect of deterioration on mechanical and sensory properties

The tensile strength of samples 1–3 decreased, and the percentage elongation increased with the increase of deterioration (Fig. 11a). The softness (Fig. 11b) and compressibility (Fig. 11c) of samples 1–3 exhibited a decreasing trend. Samples 4–6 were too fragile to determine these properties. These results were related to the hydrolysis of collagen fibers and the loss of hide substances such as proteins, fats, and water. As shown in Fig. 11d, the absolute ΔL and ΔE values of the samples increased gradually with the increase of leather deterioration. This result indicated that the brightness of the samples reduced, and their color difference increased as the leather aged. The leather was tanned with iron sulfate

**Fig. 10** DSC curves of leather samples

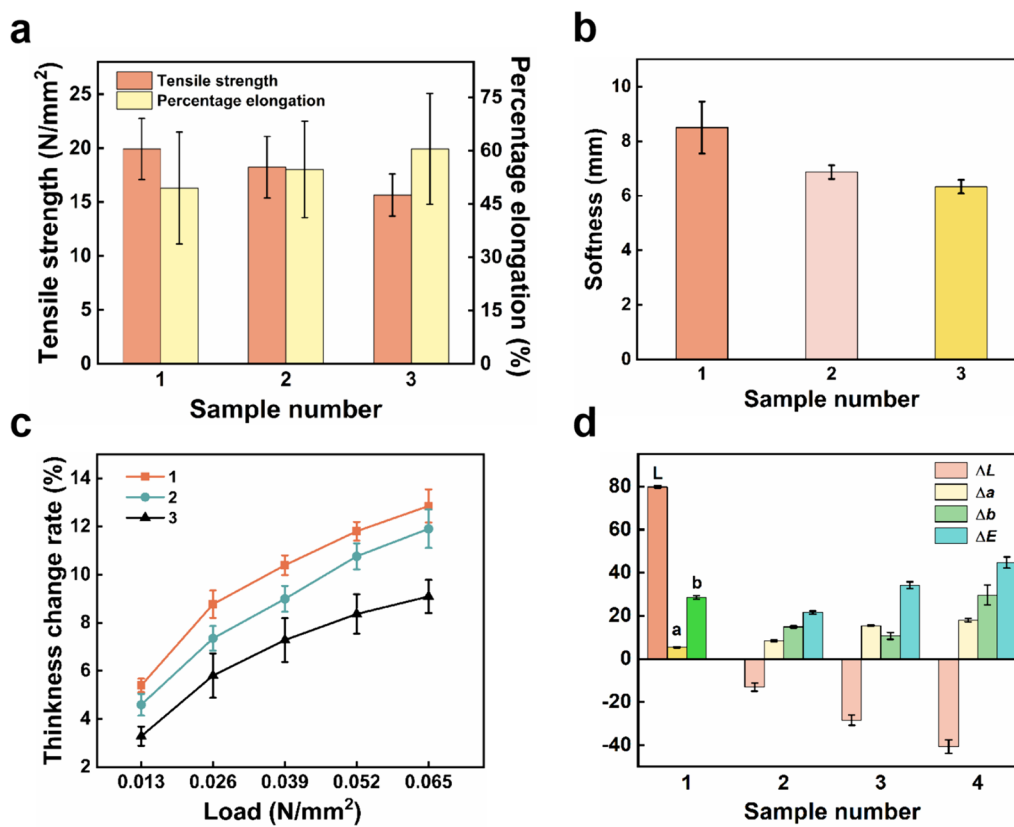


Fig. 11 Tensile strength, percentage elongation (a), softness (b), thickness change rates (c), and color parameters (d) of samples

and was not dyed. Thus, the colors of the samples were mainly from the Fe tanning agent. The changes in color may be attributed to the oxidation of the Fe tanning agent in the leather and some dark-colored and Fe-rich compost adhering to the surface of the leather.

Comparison of analytical methods and quantification of deterioration degree

Damage to the leather caused by the test methods, the average RSD of the test results (calculated using the data in Table S5), and the factors affecting the test results are shown in Table 3. The testing methods or instruments that are non-destructive include a stereo microscope, Micro-CT, a skin moisture tester, ATR-FTIR, a leather softness tester, compressibility test, and a colorimeter. These methods or instruments can be recommended for the analysis of archaeological leather. Micro-destructive (sample weight ≤ 5 mg) methods or instruments include SEM, Hyp detection by HPLC-FLD, EDX, and DSC. These methods or instruments can obtain important information about the sample with minor damage, and they are recommended for the analysis of archaeological leather.

Notably, most of the results obtained by these testing methods may not be suitable to quantify the deterioration degree of leather. For example, the softness, compressibility, and color of different types of leather are different, and the initial values of these indicators cannot be obtained. The microscopic morphology, element content, moisture content, and thermal properties are easily influenced by leather-making processes or burial environments; therefore, determining the deterioration degree of leather based on changes in these indicators is difficult. However, these indicators can be used as auxiliary indicators to verify the effect of deterioration on the properties of archaeological leather.

Hyp is a characteristic amino acid of collagen, and its proportion in collagen is basically constant. The Hyp content of Fe-tanned leather is hardly affected by the leather-making process, and it decreases because of the damage caused by leather deterioration. This result indicates a negative correlation between the Hyp content of leather and its degree of deterioration. Therefore, Hyp content analysis using HPLC-FLD can also be used to quantify the deterioration degree of leather.

Masson's trichrome staining for microscopic morphology, Soxhlet extraction for fat content, ICP-OES analysis,

Table 3 Comparison of damage degree and precision of testing methods

No	Parameter	Testing method or instrument	Damage to leather	RSD ^b (%)	Factors affecting the test result
1	Macroscopic morphology	Stereo microscope	Non-destructive	–	Leather-making process; Surroundings
2	Microscopic morphology	SEM	Micro-destructive	–	–
3	Microscopic morphology	Micro-CT	Non-destructive	–	–
4	Microscopic morphology	Masson's trichrome staining	Destructive	–	–
5	Moisture content	Oven drying	40 mg ^a ; Destructive	1.00	Surroundings
6	Moisture content	Karl Fischer titration	40 mg ^a ; Destructive	1.88	Surroundings
7	Moisture content	Skin moisture tester	Non-destructive	4.18	Surroundings
8	Hyp content	UV-Vis spectrometry	10 mg ^a ; Destructive	6.07	–
9	Hyp content	HPLC-FLD method	1 mg ^a ; Micro-destructive	10.70	–
10	Fat content	Soxhlet extraction method	100 mg ^a ; Destructive	7.30	Leather-making process; Hide/skin type
11	Fe content	ICP-OES	50 mg ^a ; Destructive	2.57	Leather-making process; Surroundings
12	Element content	EDX	Micro-destructive	–	Leather-making process; Surroundings
13	TC and TN contents	Dumas Combustion method	50 mg ^a ; Destructive	2.74	Leather-making process
14	Chemical structure	ATR-FTIR	Non-destructive	–	–
15	pH	pH meter	50 mg ^a ; Destructive	1.36	Leather-making process; Surroundings
16	Thermal stability	DSC	5 mg ^a ; Micro-destructive	–	Leather-making process
17	Tensile strength	Tensile testing machine	Destructive	14.05	Leather-making process; Leather type
18	Percentage elongation	Tensile testing machine	Destructive	27.42	Leather-making process; Leather type
19	Softness	Leather softness tester	Non-destructive	7.00	Leather-making process; Leather type
20	Compressibility	Desktop thickness gauge	Non-destructive	7.79	Leather-making process; Leather type
21	Color change	Colorimeter	Non-destructive	5.82	Leather-making process; Surroundings

^a Weight of leather samples required for testing

^b Average relative standard deviation of the test results

Dumas combustion for TC and TN contents, pH determination, and a tensile strength test require a large dosage of samples to obtain accurate results. Therefore, these methods are not available for the analysis of archaeological leather. However, these methods can provide useful information about the composition and structure of artificially aged leather, thereby providing comprehensive understanding of the deterioration mechanism of archaeological leather.


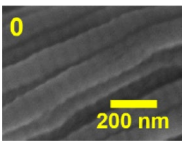

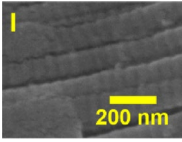

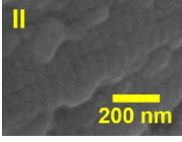

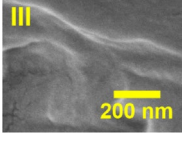
In summary, the Hyp content and microscopic morphology of the leather can be detected using micro-destructive methods, and the results can be used synergistically to provide a comprehensive and accurate assessment on the deterioration degree of the leather. Hence, the artificially aged leather samples were classified into four deterioration degrees based on the above-mentioned results (Table 4). Deterioration degree of 0 refers to no deterioration, and the deterioration degrees increase from I to III, which is positively related to the loss of Hyp and the change in the morphology of collagen

fibers. Notably, the deterioration grading parameters were proposed in accordance with the test results of Fe-tanned leather. Although the conclusions were supported by the artificially aged leather samples, the archaeological leather sample was not assessed. Therefore, future work should include follow-up studies designed to evaluate whether the recommended methods are applicable to archaeological leathers tanned with various tanning agents.

Conclusions

Artificially aged leather samples with different deterioration degrees were rapidly prepared through composting at a high temperature of 58 °C. The test results of various methods showed that the collagen fiber network was gradually destroyed. The contents of moisture, Hyp, and fat decreased, and the Fe content increased with the increase of leather deterioration. In addition, the thermal stability, tensile strength, softness, and compressibility of leather decreased, and the color gradually changed to

Table 4 Deterioration grading of artificially aged leather samples

Degree of deterioration	Digital photo	Description	Hyp content (mg/g)	SEM image
0		Non deterioration	≥ 70	
I		Slight deterioration	60–70	
II		Moderate deterioration	40–60	
III		Severe deterioration	≤ 40	

black. The Hyp content determined by HPLC-FLD can be considered to quantify the deterioration degree of archaeological leather because of its micro-destructive treatment of leather samples.

Abbreviations

Hyp	Hydroxyproline
ATR-FTIR	Attenuated total reflection-Fourier transform infrared spectroscopy
SEM	Scanning electron microscope
DSC	Differential scanning calorimetry
T_d	Denaturation temperature
Micro-CT	Micro-computed tomography
DMAB	P-Dimethylaminobenzaldehyde
UV-Vis	Ultraviolet-visible
NBD-Cl	7-Chloro-4-nitrobenzol-2-oxa-1,3-diazole
HPLC-FLD	High-performance liquid chromatography with fluorescence detection
ICP-OES	Inductively coupled plasma optical emission spectrometer
EDX	Energy-dispersive X-ray energy spectrometer
TC	Total carbon
TN	Total nitrogen
RSD	Relative standard deviation

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40494-024-01435-7>.

Additional file 1

Acknowledgements

We thank Dr. Jinwei Zhang for technical assistance in the leather-making process. We thank Dr. Xiu He and Jie Tang for technical assistance in the testing work.

Author contributions

Shuli Yao: Methodology, Investigation, Visualization, Formal analysis, Writing—original draft. Yirui Lin: Visualization, Formal analysis, Validation. Chao Lei: Visualization, Writing—review & editing. Ya-nan Wang: Methodology, Formal analysis. Yue Yu: Methodology, Formal analysis. Yunhang Zeng: Conceptualization, Methodology, Supervision, Writing—review & editing.

Funding

This work was supported by National Key R&D Program (2022YFF0904100).

Availability of data and materials

The data presented in this study are available from the corresponding author upon request. No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 9 April 2024 Accepted: 26 August 2024

Published online: 04 September 2024

References

- Doyon L, Faure T, Sanz M, Daura J, Cassard L, d'Errico F. A 39,600-year-old leather punch board from Canyars, Gavà, Spain. *Sci Adv*. 2023;9(15):0834.
- Clarkson LA. The organization of the English leather industry in the late 16th-century and 17th-century. *Econ Hist Rev*. 1960;13(2):245–56.
- Wertmann P, Xu DL, Elkina I, Vogel R, Yibulayinmu M, Tarasov PE, et al. No borders for innovations: a ca. 2700-year-old assyrian-style leather scale armour in Northwest China. *Quat Int*. 2022;623:110–26.
- Carsote C, Şendrea C, Micu MC, Adams A, Badea E. Micro-DSC, FTIR-ATR and NMR MOUSE study of the dose-dependent effects of gamma irradiation on vegetable-tanned leather: the influence of leather thermal stability. *Radiat Phys Chem*. 2021;189:109712.
- Elnaggar A, Leona M, Nevin A, Heywood A. The characterization of vegetable tannins and colouring agents in ancient Egyptian leather from the collection of the Metropolitan Museum of Art. *Archaeometry*. 2017;59(1):133–47.
- Vichi A, Eliazyan G, Kazarian SG. Study of the degradation and conservation of historical leather book covers with macro attenuated total reflection-fourier transform infrared spectroscopic imaging. *ACS Omega*. 2018;3(7):7150–7.
- Halldorsdottir HH, Williams R, Greene EM, Taylor G. Rapid deterioration in buried leather: archaeological implications. *RSC Adv*. 2024;14(6):3762–70.
- Koochakzaei A, Sabaghian M. Tannin characterization and sourcing in historical leathers through FTIR spectroscopy and PCA analysis. *Collagen Leather*. 2023;5(1):21.
- Ebsen JA, Haase K, Larsen R, Sommer DVP, Brandt LO. Identifying archaeological leather—discussing the potential of grain pattern analysis and zooarchaeology by mass spectrometry (ZooMS) through a case study involving medieval shoe parts from Denmark. *J Cult Herit*. 2019;39:21–31.
- Vadrucci M, Bellis GD, Mazzuca C, Mercuri F, Borgognoni F, Schifano E, et al. Effects of the ionizing radiation disinfection treatment on historical leather. *Front Mater*. 2020;7:21.
- Vyskočilová G, Kopecká R, Pavliňák D, Laichmanová M, Sedláček I, Orlita A, et al. The influence of soil environment on the degradation of archaeological leather. *Archaeometry*. 2021;64(2):483–99.
- Budrugaec P, Miu L, Popescu C, Wortmann FJ. Identification of collagen-based materials that are supports of cultural and historical objects. *J Therm Anal Calorim*. 2004;77:975–85.
- Budrugaec P, Miu L. The suitability of DSC method for damage assessment and certification of historical leathers and parchments. *J Cult Herit*. 2008;9:146–53.
- Popescu C, Budrugaec P, Wortmann FJ, Miu L, Demco DE, Baias M. Assessment of collagen-based materials which are supports of cultural and historical objects. *Polym Degrad Stabil*. 2008;93:976–82.
- Chahine C. Changes in hydrothermal stability of leather and parchment with deterioration: a DSC study. *Thermochim Acta*. 2000;365:101–10.
- Abdel-Maksoud G. Analytical techniques used for the evaluation of a 19th century quranic manuscript conditions. *Measurement*. 2011;44(9):1606–17.
- Reddy GK, Enwemeka CS. A simplified method for the analysis of hydroxyproline in biological tissues. *Clin Biochem*. 1996;29(3):225–9.
- Narayanan P, Sethurajan S, Vedhanayagam M, Sreeram KJ. A rapid quantification of hydroxyproline in leather using high-performance liquid chromatograph—fluorescence detection (HPLC-FLD) method. *J Am Leather Chem Assoc*. 2023;118(5):183–92.
- Yi YD, Zhang Y, Mansel B, Wang YN, Prabakar S, Shi B. Effect of dialdehyde carboxymethyl cellulose cross-linking on the porous structure of the collagen matrix. *Biol Macromol*. 2022;23(4):1723–32.
- Covington AD. *Tanning chemistry: the science of leather*. Cambridge: The Royal Society of Chemistry; 2009. p. 263–76.
- Goldner J. A modification of the masson trichrome technique for routine laboratory purposes. *Am J Pathol*. 1938;14(2):237–43.
- Soyer S, Gürlek G, Kilic E. Valorization of leather industry waste in polyurethane composites for reduced flammability. *J Mater Cycles Waste Manage*. 2023;25(1):314–23.
- Barel AO, Clarys P. In vitro calibration of the capacitance method (Corneometer CM 825) and conductance method (Skicon-200) for the evaluation of the hydration state of the skin. *Skin Res Technol*. 1997;3(2):107–13.
- Mekuria DM, Kassegne AB, Asfaw SL. Assessing pollution profiles along Little Akaki River receiving municipal and industrial wastewaters, Central Ethiopia: implications for environmental and public health safety. *Heliyon*. 2021;7:e07526.
- Etheridge RD, Pesti GM, Foster EH. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. *Anim Feed Sci Technol*. 1998;73(1–2):21–8.
- Vyskočilová G, Carsote C, Ševčík R, Badea E. Burial-induced deterioration in leather: a FTIR-ATR, DSC, TG/DTG MHT and SEM study. *Herit Sci*. 2022;10:7.
- Lei C, Lin YR, Zeng YH, Wang YN, Yuan Y, Shi B. A cleaner delimiting technology with glycine for ammonia-nitrogen reduction in leather manufacture. *J Clean Prod*. 2020;245:118900.
- Yang TQ, Zeng YH, Sun QY, Chao L, Shi B. Effect of pickling materials on leather quality from a hide surface charge perspective. *J Am Leather Chem Assoc*. 2022;117(7):279–87.
- Badea E, Gatta DG, Budrugaec P. Characterisation and evaluation of the environmental impact on historical parchments by differential scanning calorimetry. *J Therm Anal Calorim*. 2011;104(2):495–506.
- Hu YD, Liu J, Han GH, Li XM, Zhang ZH, Zheng XJ, et al. Artificial deterioration of vegetable-tanned leather under synergistic effect of temperature and humidity. *J Cult Herit*. 2022;53:118–26.
- Mogilner IG, Ruderman G, Grigera JR. Collagen stability, hydration and native state. *J Mol Graph*. 2002;21(3):209–13.
- Kolar K. Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat-products - NMKL collaborative study. *J Assoc Off Anal Chem*. 1990;73(1):54–7.
- Kakinuma M, Watanabe Y, Hori Y, Oh-i T, Tsuboi R. Quantification of hydroxyproline in small amounts of skin tissue using isocratic high performance liquid chromatography with NBD-F as fluorogenic reagent. *J Chromatogr B*. 2005;824(1–2):161–5.
- Zhang MR, Hu YD, Liu J, Pei Y, Tang KY, Lei Y. Biodeterioration of collagen-based cultural relics: a review. *Fungal Biol Rev*. 2022;39:46–59.
- Boyatzis SC, Velivasaki G, Malea E. A study of the deterioration of aged parchment marked with laboratory iron gall inks using FTIR-ATR spectroscopy and micro hot table. *Herit Sci*. 2016;4:13.
- Vyskočilová G, Ebersbach M, Kopecká R, Prokeš L, Příhoda J. Model study of the leather degradation by oxidation and hydrolysis. *Herit Sci*. 2019;7:26.
- Rabotyagova OS, Cebe P, Kaplan DL. Collagen structural hierarchy and susceptibility to degradation by ultraviolet radiation. *Mater Sci Eng C-Mater Biol Appl*. 2008;28(8):1420–9.
- Friess W, Lee G. Basic thermoanalytical studies of insoluble collagen matrices. *Biomaterials*. 1996;17(23):2289–94.
- Onem E, Yorgancioglu A, Karavana HA, Yilmaz O. Comparison of different tanning agents on the stabilization of collagen via differential scanning calorimetry. *J Therm Anal Calorim*. 2017;129(1):615–22.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.