Burial-Induced Deterioration in Leather – A FTIR-ATR, DSC, TG/DTG, MHT and SEM study

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Abstract

In this study we used an analytical approach based on complementary techniques that targets all structural levels of collagen to investigate the effects of burial tests on vegetable-tanned leather. For the first time, a group of deterioration markers specific to molecular, fibrillar and fibrous structure of collagen in leather was associated with the anaerobic condition of buried leather. Gelatin was identified both through the FTIR-ATR spectral markers (molecular alteration of collagenous triple helix structure) and SEM imaging (alteration of fibrils and fibres morphology). Loosening of collagen-tannin interaction and de-tanning were detected by the application of second derivative spectral analysis. Collagen denaturing profile was evidenced by DSC analysis and documented by SEM observations at high magnification, while TG/DTG analysis gave information on the leather thermo-oxidative deterioration. Our results confirmed that the shrinkage temperature measured by MHT method could be misleading. However, this method proved its usefulness in assessing the structural inhomogeneity of collagen in buried leather and identifying gelatin. The complementary FTIR-ATR, DSC, TG/DTG, MHT and SEM analytical protocol proved its high effectiveness and could thus be extended to the challenging study of archaeological leather.

1. Introduction

Materials, whether natural or created by humans, sooner or later undergoes alterations as a consequence of the interaction between their structure and environment. Leather can be considered as the first biomaterial ever produced. It was one of the major materials in the past, and its use continues into the present. Its wide usability is the reason why leather artefacts are frequently found during archaeological excavations. The varied properties of leather that provide such a wide use are due to the intrinsic chemical, morphological and physical properties of collagen, hence to the species from which it originates, and to the method with which hide was processed. The vital importance of collagen as a scaffold for animals demands a manifold of essential characteristics. These characteristics, including thermal stability, mechanical strength, and the ability to engage in specific interactions with other biomolecules lies on its hierarchical structure based on the collagen fibril as an elementary building block. Collagen fibril is a networked, nanoscale rope in which cross-links are both within and between microfibers. At the lowest level, there are the collagen molecules, which are triplehelical structures, and at the higher level there are fibers and tissues. Through tanning (namely collagen binding with tanning agents), thermal stability and mechanical strength of hides are enhanced allowing leather to well survive even under extreme condition such as arid waterless area, waterlogged area, permafrost, peat bogs etc. Vegetable tannage with organic materials obtained from different parts of plants including woods, barks, fruits, fruit pods, seeds and leaves left to macerate in water was mostly used up to 19th century. Although vegetable-tanned leather is a highly durable material, its integrity is influenced by external factors which may alter its physical, chemical and mechanical properties. Yet, physical and chemical properties, but also the aesthetic appeal of leather, gradually deteriorate as a result of interaction with its environment until the loss of the function of the object or even until its total loss. Deterioration may occur at any level

of collagen structure from where, due to its hierarchy, damage is distributed to the other structural levels [1, 2]. In the case of archaeological leather the kinetics of deterioration may also be influenced by the sudden change of the environmental conditions through exposure to the atmosphere. The archaeological leather damage condition should therefore be evaluated with utmost urgency to define the optimal conservation conditions and prioritize possible conservation interventions. It should be mentioned that the few studies on archaeological leather reported so far are dealing with the origin of neolithic leather by microstructural, chemical and isotopic evidence [3], identification of archaeological leather by mass spectrometry (ZooMS) [4], evaluation of archaeological leather through amino acid composition and thermal analysis [5], red stain degradation [6] or linseed oil emulsion used in dressing archaeological leather [7]. However, multi-technical approaches have been already used to characterize historical leathers which are very heterogeneous mixtures of tanned (leather-like), un-tanned (parchment-like), gelatinised collagen, amorphous collagen, tannins and deterioration compounds [2, 8–12]. Multi-analytical approaches have also been used in the case of other archaeological materials such as ceramics [13–15]. To facilitate such an analytical approach for the study of archaeological leather remains, burial tests were performed on vegetable tanned leather. Non-destructive and micro-destructive techniques such as Fourier transform infrared spectroscopy in attenuated total reflection mode (FTIR-ATR), thermal microscopy (MHT method), differential scanning calorimetry (DSC), thermogravimetry (TG/DTG) and scanning electron microscopy (SEM) were employed to provide the best possible insight into the deterioration pattern with special focus on collagen-tannin matrix de-tanning and collagen gelatinisation. Such approach has the advantage of combining surface and bulk analyses while targeting the different structural levels of collagen, from molecules to fibrils and fibres.

FTIR-ATR is a surface non-invasive and non-destructive technique widely used to characterise the molecular changes in the collagen secondary structure, while providing the identification of tannins and other organic and inorganic compounds used in the fabrication process of collagen-based materials or formed during deterioration [12, 16–22].

Thermogravimetry (TG/DTG), differential scanning calorimetry (DSC) and micro hot table (MHT) methods have been also employed to assess the thermo-oxidative, and hydrothermal stability, and deterioration of collagen-based materials [10–11, 23–31]. Both TG/DTG and DSC analyses are micro-destructive (only a few milligrams samples are required) but fundamental to determine the long-term stability of leather and validate the results provided by the MHT method. It is worth mentioning that MHT method is widely used by conservators due to the fact that only a few microfibrils are required to determine the shrinkage temperature in an inexpensive, easy to do and, apparently, easy to interpret way [1–2, 16, 32–33].

Scanning electron microscopy (SEM) is a non-destructive high-resolution technique used for imaging a wide range of materials including the collagenous materials. Depending on the magnification, the surface microstructure of leather, i.e. morphology of the fibre network, fibres and microfibrils, can be observed. Micrographs can thus reveal relevant morphology alterations at micro- and mesoscopic levels as a result

of either natural or induced deterioration [16, 23, 34–35]. SEM proved its usefulness in detecting the microbial attack, too [36].

2. Materials And Methods

2.1. Materials

Four newly manufactured vegetable tanned leathers purchased from the Gara TZL PLUS s.r.o., Otrokovice, Czech Republic were used to perform the burial tests. Variation of animal species (bovine and sheep) and age (calf and cattle) as well as tannin type (hydrolysable and condensed) were considered (Table 1). Leather samples were buried to a depth of 50 cm, at 454 m altitude, in the western part of Czech Republic, a region characterised by the mild central-European climate [37]. The average annual temperature is (6.6–8.0) °C and the average rainfall per year is (550–650) mm [38]. The soil has a loamy texture and neutral pH around 6.5. The samples were dug up and analysed after one- and two-year burial periods. Only the cattle sample was kept buried for a four-year period. Before performing the analytical measurements, all leather samples were washed with deionised water and dried at room temperature ($21 \pm 1^\circ\text{C}$, $45 \pm 5\%$ RH) under light pressure to avoid wrinkling.

2.2. Methods

2.2.1. Fourier Transform Infrared Spectroscopy in Attenuated Total Reflection mode (FTIR-ATR)

FTIR-ATR measurements were carried out using an ALPHA FTIR (Bruker Optics, Germany) equipped with a Platinum ATR module. The penetration depth, depending on the wavelength, the refractive indices of ATR crystal typically amount to a few microns (ca. 0.5–3 μm). Spectra were recorded by co-adding of 64 scans in the range from 4000 to 400 cm^{-1} with a spectral resolution of 4 cm^{-1} . Six different replicates of each sample were measured and averaged before data preprocessing. Opus 7.0 software (Bruker Optics, Germany) was used for the acquisition and elaboration of the spectra, while their graphic presentation was done with Origin 7.5 software [39–41].

2.2.2. Differential Scanning Calorimetry (DSC)

DSC measurements were performed with a DSC 204 F1 Phoenix instrument (Netzsch, Germany) in nitrogen flow ($20\text{ mL}\cdot\text{min}^{-1}$), in the temperature range (25–280) °C at a scanning rate of 10 K/min, in open aluminium crucibles [27–28]. Three measurements were performed using fresh subsamples of about 5 mg for each leathers sample.

2.2.3. Thermogravimetry / Differential Thermogravimetry (TG/DTG)

TG/DTG curves were obtained using a STA 449 C Jupiter instrument (Netzsch, Germany). Leather samples of about 5 mg were heated in open aluminium pans in the temperature range (25–500) °C in

nitrogen flow ($100 \text{ mL} \cdot \text{min}^{-1}$), at a scanning rate of 10 K/min . The rate v_{310} ($\text{d}\% \Delta m / \text{dt}$) of the first process of thermo-oxidation was calculated at 310°C from the first derivative of TG curve (DTG curve) [27–28].

2.2.4. Micro Hot Table (MHT)

MHT measurements were carried out using a homemade MHT equipment consisting of a micro-heating plate (Caloris Group, Romania) controlled by a temperature processor and a stereomicroscope (Leica S4E) assisted by a homemade software F.L.T.K. 1.1.X. for data collection. Samples composed of a few fibres from corium (flesh) side of parchment were wetted with demineralised water and kept for 10 min on a microscope slide with concavity. After fibres separation, samples were covered with a slide, placed on the heating table and heated at $2 \text{ C} \cdot \text{min}^{-1}$. Shrinkage activity was observed at 40X magnification. At least two measurements were made for each sample [1–2, 16, 32–33].

2.2.5. Scanning Electron Microscopy (SEM)

Examination of the leather surface morphology before and after soil burial tests was performed using a Tescan MIRA3 (Brno, CZ) scanning electron microscope. Samples mounted on carbon stubs and covered by a 20 nm AuPd (80/20) layer were observed at different locations at increasing magnification (from 500X to 50000X). Only the grain surface of leather samples was AuPd covered. SEM observation was performed at 15 keV accelerating voltage and 15 mm working distance.

2.2.6. pH measurement

Surface pH measurements were carried out according to the ISO 10390 standard [42] using a surface electrode WTW SenTix Sur and a pHmeter Mettler Toledo SevenCompact S220. Each measurement spot ($1 \times 1 \text{ cm}^2$) was damped with a droplet of water using a pipette. After 3 min extraction, the electrode was placed on the spot surface and the pH of the water film was measured. The pH value was obtained as the average between 3 measurements.

3. Results And Discussion

3.1. Effects of leather burial on collagen shrinkage activity by MHT method

The MHT method provides the shrinkage activity of collagen fibres which is described by a sequence of temperature intervals: no activity – A1 – B1 – C – B2 – A2 – complete shrinkage [32]. In the first two intervals, A1 and B1, shrinkage discretely occurs in individual fibres, but shows a more intense activity (i.e. higher amount of shrinkage per unit of time) in the B1 interval. Most of the fibre mass shrinks in the main interval ΔC called the main shrinkage interval whose starting temperature is considered as the shrinkage temperature, T_s . Generally, the shrinkage in the ΔC is short and intense, and levels off through B2 and A2 intervals. The shrinkage interval is delimited by T_f , the temperature at which the very first

motion is observed, and T_l , the temperature of the very last observed motion. The total shrinkage interval is thus calculated as $\Delta T = T_l - T_f$.

Table 1 List of the buried leather samples, with their symbols, animal origin and burial time

Animal species	Tannin type	Burial time (years)	Symbol
Calf	Hydrolysable	0	CH0
		1	CH1
		2	CH2
Calf	Condensed	0	CC0
		1	CC1
		2	CC2
Sheep	Condensed	0	SC0
		1	SC1
		2	SC2
Cattle	Condensed	0	CaC0
		1	CaC1
		2	CaC2
		4	CaC4

Shrinkage behaviour of new, undamaged vegetable tanned leather was shown to depend on the tannin type, animal species and processing method [8, 11]. As vegetable tanned leather ages and deteriorates its shrinkage activity changes specifically depending on the mechanism of damage. Consequently, the shrinkage temperature has been widely used as a metric for leather damage [2, 8, 27–28, 43]. Some of us have also reported how the macroscopic properties of collagen fibres measured by MHT method (i.e., hydrothermal stability expressed as T_f and T_s , and structural cohesivity and heterogeneity expressed as ΔC and ΔT , respectively) correlates with natural or artificially induced ageing pattern [2, 8–9, 16, 44].

For the investigated vegetable tanned leathers, the variation of shrinkage parameters with burial times is reported in Fig. 1. The most important changes we observed were the following:

1. T_s increased for all leather samples, regardless of the type of tannin (hydrolysable or condensed) and the duration of treatment, except for CaC4 sample which no longer shows activity in the main shrinkage interval.
2. T_f decreased for all samples, but a dramatic decrease occurred for the CH leather tanned with hydrolysable tannin.

3. The shrinkage interval ΔC generally increased except for the CaC4 sample which no longer shows activity in the main shrinkage interval.
4. The total shrinkage interval ΔT generally increased as a result of the increase of both (A1 + B1) and (A2 + B2) intervals. This increase was observed for SC2 sample although it no longer shows activity in the (A2 + B2) interval.

We can say therefore state that burial experiments led to an increase in the degree of inhomogeneity. For the CH leather samples, the high inhomogeneity is due to a partial surface gelatinisation evidenced by the T_f sharp decrease below 30°C. On the other side, the CH samples show an apparent thermal stabilisation which could be explained by a mechanical strengthening (cementing) of collagen fibres after the prolonged contact with soil minerals. In the case of CC leather samples, the increase of structural inhomogeneity is equally due to the increase in the number of collagen fibres showing discrete shrinkage of individual fibres in the (A1 + B1) and (A2 + B2) intervals. CaC1 and CaC2 leather samples behaves similarly to CC1 and CC2, respectively. SC1 leather sample behaves similarly to CC1, while the SC2's behaviour is much different in that the (A2 + B2) interval fully disappear and (A1 + B1) interval is greatly diminished. These differences can be attributed to a different burial test resistance over time of the sheep leather compared to calf and cattle leather.

Interestingly, the more coherent collagen fibres (namely those showing shrinkage in the main shrinkage interval ΔC) showed a slight increase of their thermal stability (indicated by the T_s increase) for all leather samples (except for CaC4), while the length of ΔC interval increased. This is in contrast to what was reported so far: Larsen et al. [43] observed that ΔC interval decreased as leather deterioration increased and Carsote and Badea [2] found shorter ΔC intervals in historical leathers compared to new ones and, in some cases, the absence of ΔC interval. A dual behaviour was observed for vegetable tanned leather exposed to gamma irradiation: low-dose irradiation caused an increase of ΔC length, whereas high-dose irradiation led to a progressive shortening of ΔC interval [44]. The authors explained it by distinct deterioration patterns, i.e., the formation of molecular cross-links at low-dose irradiation while the peptide bonds cleavage was promoted by high-dose irradiation. It can be assumed that the mechanical strengthening (cementing) of collagen fibres after the prolonged contact with soil minerals may have similar effects and hence it explains the concomitant increase of T_s value and ΔC interval.

3.2. Effects of leather burial on collagen thermal stability by DSC

The DSC curves for the new leather samples measured in open crucibles and gas flow (Fig. 2) displayed a broad endotherm ranging from room temperature to about 120°C associated with the loss of moisture, followed by two small endotherms in the temperature intervals (135–150) °C and (220–240) °C, respectively (Table 2). The first small peak was related to the thermal denaturation of collagen-tannin matrix, whereas the second peak at $T > 220^\circ\text{C}$ was ascribed to the thermal denaturation of the more stable crystalline collagen embedded in the amorphous matrix [26, 41]. These curves are typical for collagenous materials and the two small endotherms are in good agreement with the literature data [26,

45–47]. Budrugaec et al. [28] however, assigned these two endotherms to thermal denaturation of the crystalline collagen embedded in the amorphous matrix. Considering our previous results on parchment thermal behaviour corroborated by the literature data, we are of the opinion that the endotherm at $T < 220^{\circ}\text{C}$ is related to the denaturation of collagen-tannin matrix while the endotherm at $T > 220^{\circ}\text{C}$ is related to the denaturation of crystalline collagen. It is known that crystalline collagen fraction has a much lower degree of hydration being hence much more thermostable.

Table 2

Animal species	Tannin type	Burial time (years)	Symbol
Calf	Hydrolysable	0	CH0
		1	CH1
		2	CH2
Calf	Condensed	0	CC0
		1	CC1
		2	CC2
Sheep	Condensed	0	SC0
		1	SC1
		2	SC2
Cattle	Condensed	0	CaC0
		1	CaC1
		2	CaC2
		4	CaC4
DSC and TG/DTG parameters of leather samples before and after the soil burial tests: denaturation temperature of the collagen matrix (T_{d1}), denaturation temperature of the crystalline fraction (T_{d2}) and the rate ($V_{310}/\%min^{-1}$) of the first process of thermal oxidation.			

The DSC curves for the 1-year buried samples no longer had either of the two small peaks. Such a behaviour was previously observed for parchments exposed to the combined action of polluting gases (50 ppm NO_x + SO₂) and dry heat (100°C) [26]. This indicates that the burial tests we performed had a strong denaturing effect on collagen, which is somewhat contradictory to the MHT measurements results. We may therefore infer that the increased thermal stability observed by MHT method is due to soil mineral penetration into leather structure having a fibre cementing effect.

3.3. Effects of leather burial on collagen susceptibility to thermo-oxidative deterioration by TG/DTG

It was reported that the non-isothermal deterioration of leather occurs through three processes accompanied by mass loss: (i) water loss at $T < 150^{\circ}\text{C}$, (ii) pyrolytic thermo-oxidation and (iii) thermal decomposition of the material [27–28]. These processes are evidenced in the DTG curves by the peaks I, II and III (Fig. 3). The rate of the thermo-oxidation process corresponds to the maximum of the thermo-oxidation peak, namely the peak II (Fig. 3). For various collagen-based materials it was shown that the rate $d\%Dm/dt$ of the pyrolytic thermo-oxidation process calculated at 310°C well correlates with the collagen matrix cross-linking degree. Accordingly, Budrugaec et al. [28] used this parameter as an indicator of the damage level in historical collagen-based materials relating the high rates of the thermo-oxidation process to not-damaged/well conserved leather, and the lower rates to various damage levels. In fact, the lower the rate the higher the deterioration degree. The rates $d\%Dm/dt$ of the pyrolytic thermo-oxidation process for the investigated leather samples reported in Table 2 show a progressive decrease of the oxidation rate as burial time increases. This thermo-oxidation susceptibility increase clearly indicates an increase of the damage level with burial time.

3.4 Effects of leather burial on molecular alteration of collagen-tannin matrix by FTIR-ATR

The following aspects were considered when studying the FTIR-ATR spectra of the investigated samples: the effect of burial tests on the behaviour of the complex spectral bands resulted by the overlapping of collagen and vegetable tannins bands, and identification of added during manufacturing or formed during burial [12, 17, 21].

In Fig. 4, the FTIR-ATR spectra of the buried leather samples (CaC1, CaC2 and CaC4) are illustrated and compared with those of newly manufactured leather (CaC0). For all leather samples, the main infrared absorption bands of collagen were clearly detected: (i) the amide A (A_A) at $\sim 3300\text{ cm}^{-1}$ related to stretching vibrations of the amide N-H bonds; (ii) the amide B (A_B) at $\sim 3080\text{ cm}^{-1}$ attributed to the Fermi resonance overtone of the amide II vibration; (iii) the amide I (A_I) at $\sim 1633\text{ cm}^{-1}$ arising mainly from the C = O stretching vibration; (iv) the amide II (A_{II}) at $\sim 1538\text{ cm}^{-1}$ attributed to NH in plane bend and CN stretching vibration and (v) amide III (A_{III}) at $\sim 1235\text{ cm}^{-1}$ corresponding to the NH bending, CN stretching vibration and small contributions from both CO in plane bending and CC stretching vibration [48–49].

There are FTIR-ATR bands specific of all vegetable tannins (hydrolysable and condensed) detectable at: 1606 cm^{-1} ($\nu_{C=C}$; aromatic ring), 1507 cm^{-1} ($\nu_{C=C}$; skeletal ring), 1445 cm^{-1} ($\nu_{C=C}$; aromatic ring), 1198 cm^{-1} (ν_{C-OH} ; aromatic) and 1030 cm^{-1} (β - CH deformation). The hydrolysable tannins are specifically identified through typical bands occurring at 1721 cm^{-1} ($\nu_{C=O}$; phenolic esters lactones or phenolic esters), 1322 cm^{-1} (ν_{C-O} ; lactones), 1080 cm^{-1} (ν^s_{C-O-C} ; aryl phenolic ester), 872 cm^{-1} (γ_{OH} and γ_{C-H} ; tetrasubstituted aromatic) and 758 cm^{-1} (ν^s skeletal - sugar ring, breathing vibration). On the other hand, condensable tannins also present typical bands at 1282 cm^{-1} (ν_{C-O} ; pyran ring), 1159 cm^{-1} (ν^s_{C-O-C} ; cyclic ether), 1110 cm^{-1} (ν^{as}_{C-O-C} ; cyclic ether), 976 cm^{-1} and 842 cm^{-1} (γ_{C-H} ; tetrasubstituted aromatic) [18–19]. Due to the

tannin presence, the spectra of leather are characterized by bands overlapping in the amide I, amide II, and amide III regions making it rather difficult to specifically attribute the spectral variation as a result of deterioration.

For example, in Fig. 4 is observed a reduction of the band intensity in the amide I and amide II region suggesting some changes in the structure of collagen–tannin matrix. However, for a more accurate assessment of the alterations that occurred in the secondary structure of collagen, the second derivative of FTIR spectra was calculated in the spectral range of amide I and amide II (Fig. 5). The second derivative of the FTIR-ATR spectra of new leathers were first characterized and we found the followings (Fig. 5):

1. two components of amide I centered near 1660 cm^{-1} and $1622\text{--}1628\text{ cm}^{-1}$, respectively;
2. two components of amide II centered near 1550 cm^{-1} and $1502\text{--}1515\text{ cm}^{-1}$, respectively;
3. one tannin band at $1602\text{--}1605\text{ cm}^{-1}$, visible as a shoulder of the second component of amide I;
4. a tannin band that fully overlaps the second component of the amide II.

As a result of the burial tests, both amide I components began to decreased after the first year for CC, SC and CaC leathers. In the same time, the tannin band at $1602\text{--}1605\text{ cm}^{-1}$ decreased, too, suggesting a loosening of the collagen – tannin interactions. This destabilization led to de-tanning after the second year of the burial tests as indicated by the net separation of the amide I and tannin bands. The loosening and then breaking of collagen-tannin interactions during burial is also supported by the changes in the amide II region characterized by the shift of the component at 1550 cm^{-1} towards lower wavenumbers ($1546\text{--}1538\text{ cm}^{-1}$), related to α -helix structure conversion to disordered structures [50], and progressive depletion of the complex band observed at $1502\text{--}1515\text{ cm}^{-1}$. Interestingly, for CH leather samples, the intensity of the 1660 cm^{-1} component decreased while the intensity of the 1628 cm^{-1} component increased. This behaviour may be explained by the unfolding of the native left-handed triple α helix into a random coil structure, resulting in the formation of gelatine. It is thus confirmed the gelatinisation of CH leather evidenced by the MHT method. A similar spectral behaviour was previously explained by Stani et al. [51] through the formation of larger number of water-mediated hydrogen bonds in denatured collagen. They assigned the component near 1630 cm^{-1} mainly to the carbonyl stretching of hydroxyproline and of the others amino acids involved in water mediated hydrogen bonds in the native state [52]. Recently, some of us reported an number of these carbonyl moieties in denatured α -chains for collagen (in parchment) exposed to dry-heat ageing, causing the component at 1632 cm^{-1} to increase in intensity [40].

The analysis of FTIR-ATR spectra after 1-year burial indicate the bands of fatliquoring oils/ fats at ~ 2920 and 2850 cm^{-1} ($\nu^{\text{as}}_{\text{CH}_2}$ and $\nu^{\text{s}}_{\text{CH}_2}$) and at ~ 1735 ($\nu^{\text{as}}_{\text{C=O}}$), while the soil aluminosilicates were identified by their specific bands at $\sim 1030\text{ cm}^{-1}$ ($\nu_{\text{Si-O}}$), 525 ($\delta_{\text{Al-O-Si}}$) and 465 cm^{-1} ($\delta_{\text{Si-O-Si}}$) [53] (Fig. 4). Calcium carbonate was identified by its main absorption bands at 1405 cm^{-1} ($\nu^{\text{as}}_{\text{CO}_3}$) and 874 cm^{-1} (δ_{CO_3})

(Fig. 4). Its presence could be attributed to the leaching of the mineral components driven either by soil humidity or microbial attack [36].

3.5 Effects of burial tests on leather surface morphology by SEM

Collagen structural damage in the fibrillar structure and network documented by SEM imaging on all the buried samples confirmed the above illustrated findings. The newly manufactured leather showed ordered fibrillar networking with the fibrils grouped in bundles (Fig. 6a) and their typical periodic structure clearly visible at higher magnification (Fig. 7a). The buried samples displayed discontinuities and loosening of the fibre matrix cohesion, progressive shrinkage and extended melted/gelatinised areas (Fig. 6b and 6c). The loss of fibrillar structure and massive presence of amorphous and gelatinised structures are already evident at higher magnification after 1-year burial period (Fig. 7b). Bozec and Odlyha [54] related the loss of the repeated periodicity of collagen fibril ultrastructure to collagen entirely structurally denatured. Collagen structural denaturation is in accordance with the DSC results presented above.

In addition, a microbial attack with the leaching of mineral component on the leather surface (most probably calcium carbonate, as indicated by FTIR-ATR results) was observed (Fig. 8). The chains of bacterial spores and filaments resemble the typical structure of some *Actinomyces* [36], aerobic spore forming gram-positive bacteria, which are the most abundant organism that form thread-like filaments in the soil and play a major rôle in the cycling of organic matter [55].

3.6 pH variation as a result of the burial tests

The pH value of the new vegetable tanned leathers was in the range 3.5–4.6, depending on the tannin type and manufacturing process. The pH values of the buried leather increased to 6.5–7.3, which is actually the pH value of the soil. In fact, the soil acts as a buffer, so the pH value of the archaeological leather does not depend on the type of damage, but on the type of soil in which it was buried.

Conclusions

This work demonstrates how the combination of sensitive and chemical specific techniques can be used to study the progress of deterioration in complex materials such as vegetable tanned leather. For the first time, burial tests were applied on newly manufactured vegetable tanned leather and clear evidences have been obtained to help understanding the denaturation pattern of collagen in archaeological leather.

The second derivative of the FTIR-ATR leather spectra allowed us to differentiate between collagen and tannin spectral components and identify the spectral markers useful for detecting the loosening of the collagen-tannin interaction and their breakdown (de-tanning). Furthermore, we were able to discriminate between collagen and gelatine on the surface of the studied samples. The identification of calcium carbonate on the buried leather surface was related to the leaching of the mineral components driven by the microbial attack documented by SEM imaging.

DSC analyses have indicated a strong denaturing effect on collagen induced by the burial tests which was further confirmed by the loss of the repeated periodicity of collagen fibril ultrastructure by SEM. In addition, TG/DTG analyses demonstrated the increase of leather susceptibility to thermo-oxidative deterioration as a result of burial tests.

MHT method was suitable to evaluate the variation of the inhomogeneity degree of collagen in leather as well as to detect the gelatinisation phenomenon. It is however not recommended to use the shrinkage temperature as a metric of deterioration due to the misleading effect of fibre cementing through soil mineral penetration into leather structure.

SEM images provided useful information concerning the loosening of the fibre matrix cohesion, progressive shrinkage and formation of melted/gelatinised areas. At higher magnification, the loss of the repeated periodicity of collagen fibril ultrastructure was detected as well as the presence of bacterial spores and filaments, and calcium carbonate crystals.

These findings improve our understanding of the burial-induced deterioration in vegetable tanned leather and could be of great assistance in assessing archaeological leather. Noteworthy that the analytical protocol based on MHT, DSC, FTIR-ATR and SEM demonstrated high potential as a routine approach for a rapid qualitative characterization of the collagen matrix structure and stability from molecular to macroscopic level.

Abbreviations

T	Temperature
TG	Thermal gravimetry
DTG	Differential Thermal Gravimetry
DSC	Differential Scanning calorimetry
MHT	Micro Hot Table
FTIR	Fourier transform infrared spectroscopy
ATR	Attenuated Total Reflection
SEM	Scanning Electron Microscopy

Declarations

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Contributions

GV: Methodology, FTIR-ATR Investigation, MHT Investigation, SEM Investigation, TG/DTG Investigation, DSC investigation, Validation, Writing – original draft. **RS:** Methodology, Supervision. **CC:** Data analysis, Validation, Writing – review & editing. **EB:** Data analysis, Validation, Supervision, Writing – review & editing. All authors read and approved the final manuscript.

Competing interests

Not applicable

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References

1. Badea E, Poulsen Sommer DV, Muhlen Axelsson K, Larsen R, Kurysheva A, Miu L, Della Gatta G. Damage ranking of historic parchments: from microscopic studies of fibre structure to collagen denaturation assessment by micro DSC. *e-Preservation Science*. 2012;9:97–109.
2. Carsote C, Badea E. Micro differential scanning calorimetry and Micro Hot Table method for quantifying deterioration of historical leather. *Herit Sci*. 2019;7(48):1-13. doi: 10.1186/s404 94- 019-0292- 8.
3. Spangenberg JE, Ferrer M, Tschudin P, Volken M, Hafner A. Microstructural, chemical and isotopic evidence for the origin of late neolithic leather recovered from an ice field in the Swiss Alps. *J Archaeol Sci*. 2010;37(8):1851-65. <https://doi.org/10.1016/j.jas.2010.02.003>.
4. Ebsen JA, Haase K, Larsen R, Poulsen Sommer DV, Ørsted Brandt L. 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. <https://doi.org/10.1016/j.culher.2019.04.008>.
5. Plavan V, Miu L, Gavriluk N. Evaluation of the Amino Acid Composition, Structure and Properties of Archaeological Leather. *Procedia Chem*. 2013;8:279-

83. <https://doi.org/10.1016/j.proche.2013.03.034>.
6. Koochakzaei A, Achachluei MM. Red stains on archaeological leather: Degradation characteristics of a shoe from the 11th-13th centuries (Seljuk period, Iran). *J Am Ins Conserv*. 2015;54(1):45-56. [10.1179/1945233014Y.0000000033](https://doi.org/10.1179/1945233014Y.0000000033).
7. Hassan RRA. A preliminary study on using linseed oil emulsion in dressing archaeological leather. *J Cult Herit*. 2016;21:786–95.
8. Badea E, Şendrea C, Carsote C, Adams A, Blümich B, Iovu H. Unilateral NMR and thermal microscopy studies of vegetable tanned leather exposed to dehydrothermal treatment and light irradiation. *Microchem J*. 2016;129:158–65. <https://doi.org/10.1016/j.microc.2016.06.013>
9. Carsote C, Badea E, Miu L, Della Gatta G. Study of the effect of tannins and animal species on the thermal stability of vegetable leather by differential scanning calorimetry. *J Therm Anal Calorim*. 2016;124:1255–66. <https://doi.org/10.1007/s10973-016-5344-7>.
10. Sebestyén Z, Czégény Z, Badea E, Carsote C, Şendrea C, Barta-Rajnai E, János B, Miu L, Jakab E. Thermal characterization of new, artificially aged and historical leather and parchment. *J Anal Appl Pyrol*. 2015;115:419-27. doi: [10.1016/j.jaap.2015.08.022](https://doi.org/10.1016/j.jaap.2015.08.022).
11. Sebestyén Z, Jakab E, Badea E, Barta-Rajnai E, Şendrea C, Czégény Z. Thermal degradation study of vegetable tannins and vegetable tanned leathers. *J Anal Appl Pyrol*. 2019;138:178–87. <https://doi.org/10.1016/j.jaap.2018.12.022>.
12. Proietti N, Di Tullio V, Carsote C, Badea E. ¹³C solid-state NMR complemented by ATR-FTIR and micro-DSC to study modern collagen-based material and historical leather. *Magn Reson Chem*. 2020;58:840–59. <https://doi.org/10.1002/mrc.5024>.
13. Canevali C, Gentile P, Orlandi M, Modugno F, Lucejko JJ, Colombini MP, Brambilla L, Goidanich S, Riedo C, Chiantore O, Baraldi P, Baraldi C, Gamberini MC. A multi-analytical approach for the characterization of powders from the Pompeii archaeological site. *Anal Bioanal Chem*. 2011;401:1801–14. doi [10.1007/s00216-011-5216-8](https://doi.org/10.1007/s00216-011-5216-8).
14. Raja Annamalai G, Ravisankar R, Rajalakshmi A, Chandrasekaran A, Rajan K. Spectroscopic characterization of recently excavated archaeological potsherds from Tamilnadu, India with multi-analytical approach. *Spectrochim Acta A*. 2014;133:112-8. <https://doi.org/10.1016/j.saa.2014.04.188>.
15. Ribechini E, Colombini MP, Giachi G, Modugno F, Pallecchi P. A multi-analytical approach for the characterization of commodities in a ceramic jar from Antinoe (Egypt). *Archaeometry*. 2009;51(3):480–94. doi: [10.1111/j.1475-4754.2008.00406](https://doi.org/10.1111/j.1475-4754.2008.00406).
16. Badea E, Carsote C, Hadimbu E, Sendrea C, Lupas MC. The effect of halloysite nanotubes dispersions on vegetable-tanned leather thermal stability. *Herit Sci*. 2019;7:68. <https://doi.org/10.1186/s40494-019-0310-x>.
17. Carsote C, Badea E, Caniola I, Paunescu S, Lupas MC, Sendrea C, Miu L. The Homiliary of Varlaam: Scientific investigation of the leather bookbinding. *Rev Chim*. 2020;71(3):51-8, <https://doi.org/10.37358/RC.20.3.7973>.

18. Falcão L, Araújo MEM. Tannins characterization in historic leathers by complementary analytical techniques ATR-FTIR, UV-Vis and chemical tests. *J Cult Herit* 2013;14:499–508.
19. Falcão L, Araújo MEM. Vegetable tannins used in the manufacture of historic leathers. *Molecules*. 2018;23:8–10. <https://doi.org/10.3390/molecules23051081>.
20. Sendrea C, Carsote C, Badea E, Adams A, Niculescu M, Iovu H. . Non-invasive characterisation of collagen-based materials by NMR-MOUSE and ATR-FTIR. *UPB. Sci Bull Ser Chem*. 2016;78:27–38.
21. 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;3:7150–57.
22. Vyskočilová G, Ebersbach M, Kopecská R, Prokeš L, Příhoda J. Model study of the leather degradation by oxidation and hydrolysis. *Herit Sci*. 2019;7:1-13. <https://doi.org/10.1186/s40494-019-0269-7>.
23. Badea E, Miu L, Budrugeac P, Giurginca M, Mašić A, Badea N, Della Gatta G. Study of deterioration of historical parchments by various thermal analysis techniques, complemented by SEM, FTIR, UV-VIS-NIR and unilateral NMR investigations. *J Therm Anal Calor*. 2008;91:17-27.
24. Badea E, Della Gatta G, Budrugeac P. Characterisation and evaluation of the environmental impact on historical parchment by DSC, *J Therm Anal Calorim*. 2011;104: 495–506.
25. Badea E, Della Gatta G, Usacheva T. Effects of temperature and humidity on fibrillar collagen within parchment: A micro differential scanning calorimetry (micro DSC) study. *Polym Degrad Stabil*. 2012;97:246-53.
26. Budrugeac P, Badea E, Della Gatta G, Miu L, Comanescu A. A DSC study of deterioration caused by environmental chemical pollutants to parchment, a collagen-based material. *Thermochim Acta*. 2010;500:51-62.
27. Budrugeac P, Cucos A, Miu L. The use of thermal analysis methods for authentication and conservation state determination of historical and/or cultural objects manufactured from leather. *J Therm Anal Calorim*. 2011;104:439–50.
28. Budrugeac P, Carsote C, Miu L. *Application of thermal analysis methods for damage assessment of leather in an old military coat belonging to the History Museum of Braşov-Romania*. *J Therm Anal Calorim*. 2017;127(1):765-72. doi: [10.1007/s10973-016-5343-8](https://doi.org/10.1007/s10973-016-5343-8).
29. Chahine C. *Changes in hydrothermal stability of leather and parchment with deterioration: a DSC study*. *Thermochim Acta*. 2000;365:101-10.
30. Della Gatta G, Badea E, Ceccarelli R, Usacheva T, Mašić A, Coluccia S. Assessment of damage in old parchments by DSC and SEM. *J Therm Anal Calorim*. 2005;82:637-49.
31. Popescu C, Budrugeac P, Miu L, Wortmann FJ, Demco DE, Baias M. Assessment of collagen-based materials which are supports of cultural and historical objects. *Polym Degrad Stabil*. 2008;93(5):976-82.
32. Larsen R, Vest M, Nielsen K. *Determination of hydrothermal stability (shrinkage temperature) of historical leathers by Micro Hot Table technique*. *J Soc Leather Technol Chem*. 1993;77:151–6.

33. Larsen R. *Experiments and observations in the study of environmental impact on historical vegetable tanned leathers*. *Thermochim Acta*. 2000;365:85–99.
34. Bicchieri M, Biocca P, Colaizzi P, Pinzari F. Microscopic observations of paper and parchment: the archaeology of small objects. *Herit Sci*. 2019;7(47):1-12. <https://doi.org/10.1186/s40494-019-0291-9>.
35. Vadrucchi M, Cicero C, Parisse P, Casalis L, De Bellis G. Surface evaluation of the effect of X-rays irradiation on parchment artefacts through AFM and SEM. *App Surf Sci*. 2020; 513:145881. <https://doi.org/10.1016/j.apsusc.2020.145881>.
36. Piñar G, Sterflinger K, Pinzari F. Unmasking the measles-like parchment discoloration: molecular and microanalytical approach. *Environ Microbiol*. 2015;17(2):427–43. doi:10.1111/1462-2920.12471
37. Geoportal. *The Czech National Geoportal*. 2019 <https://geoportal.gov.cz/web/guest/map?permalink=3ce4d8ab3c6bc1ee95a090c4f411668f>
38. CHMI. *Czech Hydrometeorological Institute*. 2020 <http://portal.chmi.cz/historicka-data/pocasi/uzemni-srazky?l=en>.
39. Cappa F, Paganoni I, Carsote C, Badea E, Schreiner M. Studies on the effects of mixed light-thermal ageing on parchment by vibrational spectroscopy and micro hot table method. *Herit Sci*. 2020;8(15):1-12. doi: 10.1186/s40494-020-0353-z.
40. Cappa F, Paganoni I, Carsote C, Schreiner M, Badea E. Studies on the effect of dry-heat ageing on parchment deterioration by vibrational spectroscopy and micro hot table method. *Polym Degrad Stab*. 2020;182(109375):1–11. <https://doi.org/10.1016/j.polymdegradstab.2020.109375>
41. Carșote C, Budrugaec P, Decheva R, Haralampiev NS, Miu L, Badea E. Characterization of a byzantine manuscript by infrared spectroscopy and thermal analysis. *Rev Roum Chim*. 2014;59:429–36.
42. ISO, Č., *ČSN ISO 10390: Soil quality - Determination of pH*. 2011, Český normalizační institut. p. 12.
43. Larsen R, Vest M, Nielsen K. Determination of hydrothermal stability. In: Larsen R, Vest M, Kejser UB, editors. STEP Leather Project. Evaluation of the correlation between natural and artificial ageing of vegetable tanned leather and determination of parameters for standardization of an artificial ageing method. Bjarnholt Repro, Copenhagen; 1994. p. 151–64.
44. Sendrea C, Carsote C, Radu M, Badea E, Miu L. The effect of gamma irradiation on shrinkage activity of collagen in vegetable tanned leather. *Rev Chim*. 2017;68:1535–38. <https://doi.org/10.37358/rc.17.7.5711>.
45. Badea E, Usacheva T, Della Gatta G. The use of differential scanning calorimetry to characterise collagen deterioration in parchment. *Russian Chemistry Journal*. 2015;59(1):28-41.
46. [46] Miles CA, Ghelashvili M. Polymer-in-a-Box Mechanism for the Thermal Stabilization of Collagen Molecules in Fibers. *Biophys J*. 1999;78(6):3243-52.
47. Samouillan V, Delaunay F, Dandurand J, Merbahi N, Gardou JP, Yousfi M, Gandaglia A, Spina M, Lacabanne CJ. The Use of Thermal Techniques for the Characterization and Selection of Natural Biomaterials. *Funct Biomater*. 2011;2:230-48, doi: 10.3390/jfb2030230.

48. Barth A. Infrared spectroscopy of proteins. *Biochim Biophys Acta – Bioenerg.* 2007;1767:1073–101. <https://doi.org/10.1016/j.bbabbio.2007.06.004>.
49. Glassford SE, Byrne B, Kazarian SG. Recent applications of ATR-FTIR spectroscopy and imaging to proteins. *Biochim Biophys Acta.* 2013;1834:2849–58.
50. Rabotyagova OS, Cebe P, Kaplan DL. Collagen structural hierarchy and susceptibility to degradation by ultraviolet radiation. *Mater Sci Eng C.* 2008;28:1420-29. <https://doi.org/10.1016/j.msec.2008.03.012>
51. Stani C, Vaccari L, Mitri E, Birarda G. FTIR investigation of the secondary structure of type I collagen: new insight into the amide III band, *Spectrochim Acta A Mol Biomol Spectrosc.* 2020;229:118006.
52. De Campos Vidal B, Mello MLS. Collagen type I amide I band infrared spectroscopy. *Micron.* 2011;42:283–9. doi: 10.1016/j.micron.2010.09.010.
53. Spence A, Kelleher BP. FT-IR spectroscopic analysis of kaolinite–microbial interactions. *Vib Spectrosc.* 2012;61:151– 5.
54. Bozec L, Odlyha M. Thermal denaturation studies of collagen by microthermal analysis and atomic force microscopy. *Biophys J.* 2011;101:228-36.
55. Bhatti AA, Haq S, Bhat RA. Actinomycetes benefaction role in soil and plant health. *Microb Pathog.* 2017;111:458-67. <http://dx.doi.org/10.1016/j.micpath.2017.09.036>

Figures

Figure 1

Stacked column charts displaying the shrinking intervals for collagen fibres for the investigated leathers before and after the soil burial tests. The main shrinkage temperatures (T_f , T_s and T_l) and intervals (ΔC and ΔT) are indicated.

Figure 2

DSC curves obtained in open crucibles and nitrogen flow for leather samples before and after the 1-year soil burial: (a) CH; (b) CC; (c) SC and (d) CaC. The dehydration peak, denaturation peak of the collagen-tannin matrix (showing a maximum at Td1), and denaturation peak of the crystalline fraction (showing a maximum at Td2) are indicated.

Figure 3

TG and DTG curves showing the typical decomposition pattern for a newly manufactured vegetable-tanned leather characterised by three successive exothermic processes: water loss (peak I), pyrolytic thermal oxidation (peak II) and thermal decomposition (peak III).

Figure 4

FTIR-ATR spectra for CaC, before and after the soil burial tests. The main absorption bands of collagen (AA, AB, AI, AII and AIII), as well as those corresponding to calcium carbonate (1405 and 874 cm⁻¹), fatliquoring oils/fats (2920, 2850 and 1735 cm⁻¹) and aluminosilicates (1030, 525 and 465 cm⁻¹) are highlighted.

Figure 5

The second derivative FTIR-ATR spectra in the 1700 – 1450 cm^{-1} region for (a) CH; (b) CC; (c) SC and (d) CaC, before and after soil burial tests.

Figure 6

SEM images showing (a) the ordered fibrillar networking with the fibriles grouped in bundles for CaC0; (b) and (c) loss of the fibre matrix cohesion, progressive shrinkage and melted/ gelatinised areas for CaC1 and CaC2. Magnification 5000X.

Figure 7

SEM images showing (a) the typical periodic structure of the collagen fibril for CaC0 and (b) complete loss of the surface fibrillar structure for CaC1. Magnification 50000X.

Figure 8

SEM images of CC1 (a) at 5000X magnification and (b) at 15000X magnification showing the presence of CaCO_3 crystals (1), bacterial spores (2) and filaments (3) resembling the typical structure of Actinomycetes.