Polymer Degradation and Stability 230 (2024) 111076

Contents lists available at ScienceDirect

Polymer Degradation and Stability

journal homepage: www.journals.elsevier.com/polymer-degradation-and-stability

Assessing the effect of minimally invasive lipid extraction on parchment integrity by artificial ageing and integrated analytical techniques

Marc Vermeulen [a,*], Samuel P. Johns [b], Gwen dePolo [c], Pedro Maximo Rocha [a], Matthew J. Collins [d,e], Lora Angelova [a], Mélanie Roffet-Salque [b,*]

[a] Collection Care Department, The National Archives, Richmond, TW9 4DU, UK
[b] Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK
[c] Department of Materials Science and Engineering, Northwestern University, Evanston, IL, 60208, USA
[d] Section for Evolutionary Genomics, The GLOBE Institute, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen N, Denmark
[e] McDonald Institute for Archaeological Research, University of Cambridge, Cambridge CB2 3ER, UK

ARTICLE INFO

Keywords:
Parchment
Lipid extraction
SEM
FTIR
Mechanical properties
Pure shear fracture

ABSTRACT

To assess the short and long-term effect of a newly developed minimally invasive lipid extraction method on parchment, sacrificial pieces of parchments were subjected to artificial ageing and investigated using various analytical methods. Lipids were extracted using our novel vacuum-aided extraction method and characterised by high-temperature gas chromatography (HTGC-FID). Lipids were identified as arising from degraded animal fats. The physical, molecular, and mechanical properties of the parchment samples before/after lipid extraction, and before/after ageing were assessed using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and pure shear single notch fracture testing. SEM imaging allowed for an assessment of potential structural changes of the collagen fibres while FTIR was used to investigate the possible molecular changes indicated by changes in amide I and II bands. Mechanical tests were used to record the changes in brittleness and stiffness occurring in the materials through lipid extraction and ageing. The multimodal investigation did not highlight measurable changes in the structural, molecular, and mechanical properties of the lipid-extracted parchment, thus indicating the suitability for the minimally invasive lipid extraction method to be applied to historical parchments.

1. Introduction

Parchment is an animal membrane that has been used as a writing material for millennia in the form of books, scrolls and folded sheets. It is made from untanned animal skin, usually from sheep, calves, and goats, that has been scraped and dried under tension to obtain a thin and flat surface, suitable for writing [1–4]. It was prized for its durability and capacity to preserve written text over time. As animal skin, parchment is mainly composed of the fibrous triple helical protein collagen [1,5–8], which has been the subject of many studies [5,9–13]. However, parchment is a complex material that also contains fats in the form of lipids, and mineral salts, among other substances [14] that may play a role in parchment degradation [15,16]. More complexity arises from its natural heterogeneity and is further exacerbated by natural degradation [17,18].

The stable isotope composition of lipids extracted from parchments holds the prospect of providing insights into past environments and historical climate events [19]. There are millions of well-dated parchment documents held in archives in Britain and elsewhere, and the potential of lipids as a high-resolution environmental signal has not yet been fully appreciated. To be analysed isotopically by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) [20], lipids must be extracted from parchment. The analysis of lipids from parchment has relied on solvent extraction of parchment fragments [15,18,21,22]. Such an invasive approach is unrealistic for most documents in which fragments cannot be removed or soaked in organic solvents for extended periods of time. To increase the utility of lipid analysis, a minimally invasive lipid extraction procedure was developed in order to study archival parchment documents from The National Archives [19]. However, the effects of the extraction procedure on the integrity of the parchment needed to be assessed before any sampling on historical parchments could be realised.

* Corresponding authors.
E-mail addresses: marc.vermeulen@nationalarchives.gov.uk (M. Vermeulen), Melanie.Salque@bristol.ac.uk (M. Roffet-Salque).

https://doi.org/10.1016/j.polymdegradstab.2024.111076
Received 14 November 2023; Received in revised form 28 October 2024; Accepted 4 November 2024
Available online 5 November 2024

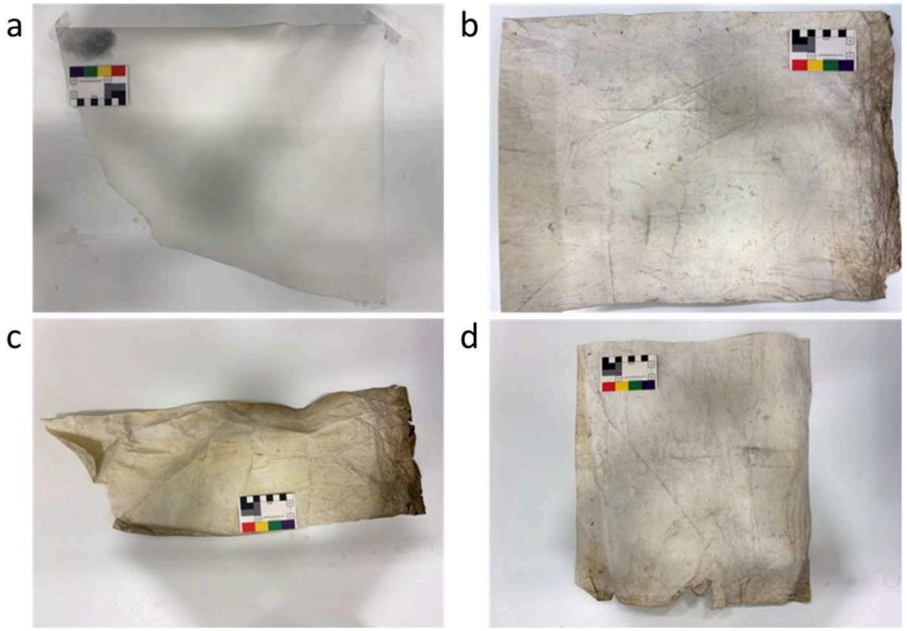

Fig. 1. Photographs of the sacrificial parchments used for the study. (a) P1, modern 21st century clean parchment with artificial soiling (top left); (b) P2; (c) P3; and (d) P4, all older naturally soiled membranes with various amounts of dust deposition.

Table 1
Description of the lipid extraction experimentation performed on each of the parchments (P1-P4). Where: F to H: Flesh to Hair; H to F: Hair to Flesh.

Experiments No.	Analyses	Volume of solvent / mL	Direction of Extraction	Clean/ Soiled*
V1	Full	3	F to H	Clean
V2	Full	3	H to F	Clean
V3	Only lipid	20	F to H	Clean
V4	Only lipid	3	F to H	Soiled
V5	Only lipid	3	H to F	Soiled

* Natural soiling P2-P4; P1 artificially soiled.

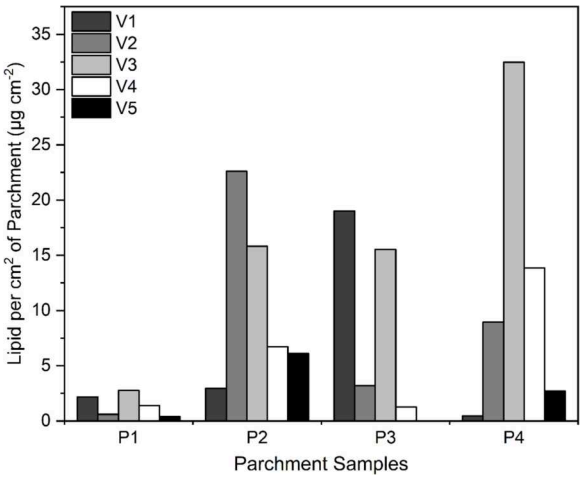

Fig. 2. Mass of lipid per cm^2 (area density, µg cm^{-2}) recovered using the vacuum-aided extraction method for each of the four sacrificial parchments (P1-P4) and extraction experiments (V1-V5).

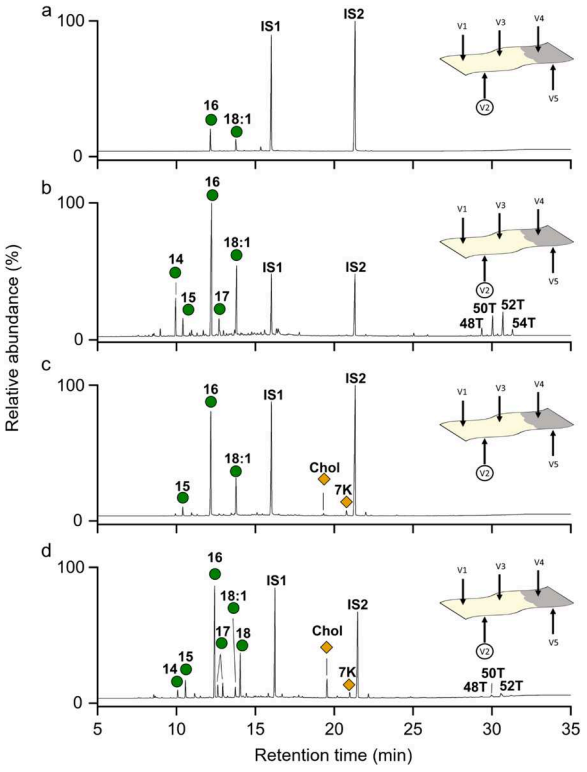

Fig. 3. Partial gas chromatogram of trimethylsilylated (TMS) total lipid extracts obtained from vacuum extraction experiment 2 (V2) for each of the four sacrificial parchments (a) P1, (b) P2, (c) P3 and (d) P4. Green circles indicate fatty acids; orange diamonds, sterols; IS1/2 internal standard (heneicosanoic acid and *n*-tetratriacontane, respectively). Number *n* and *n:i*, acyl carbon number with zero or *i* degrees of unsaturations. *n*T is indicative of triacylglycerols (TAGs) containing *n* number of carbon atoms.

This work primarily utilises Fourier Transformed Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) due to their widespread availability, ease of use and combination of molecular and structural information. However, the structure of collagen within parchment and the understanding of its composition, ageing and

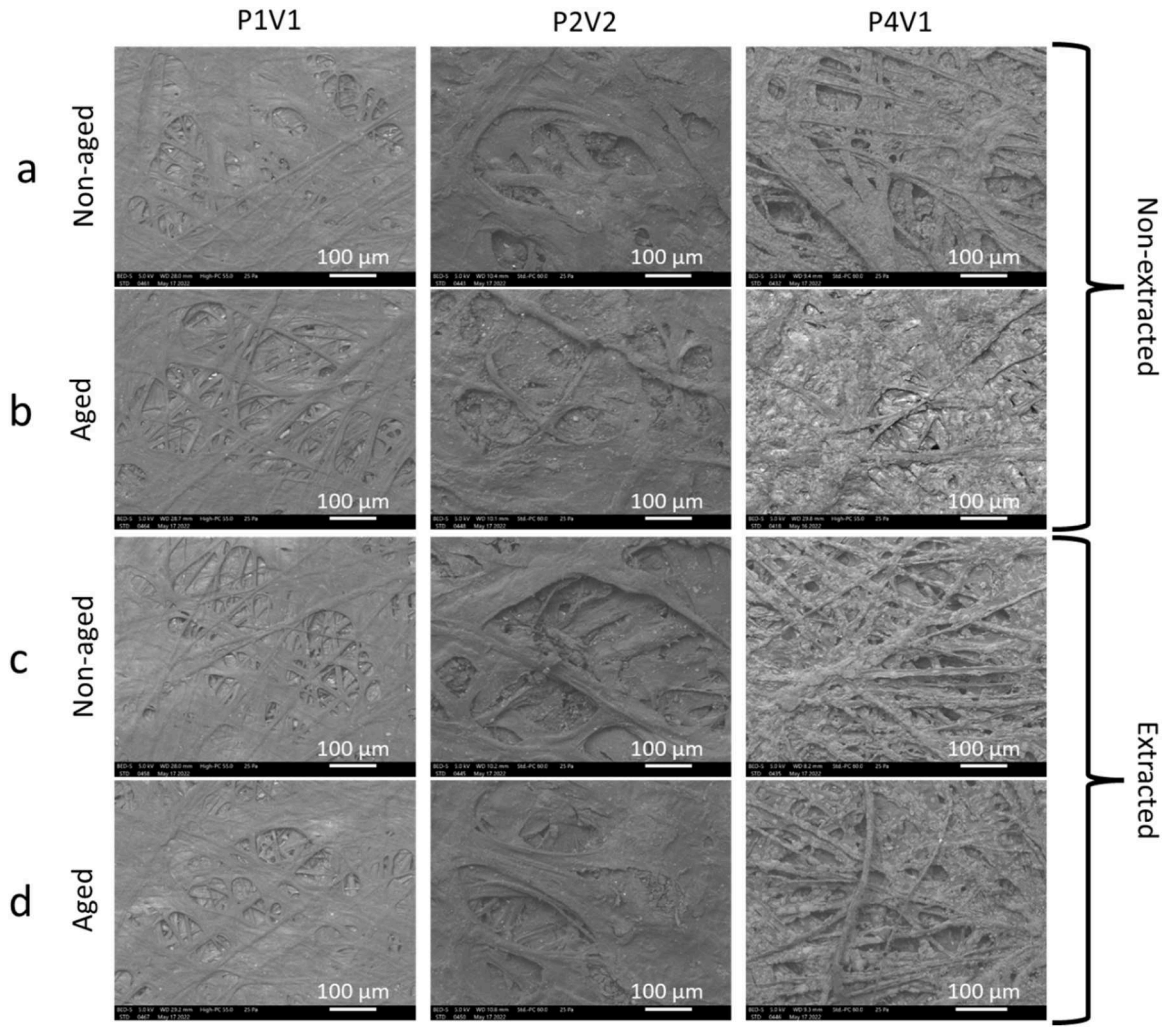

Fig. 4. Representative SEM images obtained on (a) non-extracted and non-aged, (b) non-extracted and aged, (c) extracted, non-aged, and (d) extracted aged parchment samples for parchment P1 (P1V1, flesh-to-hair extraction direction), parchment P2 (P2V2, hair-to-flesh extraction direction) and parchment P4 (P4V1, flesh-to-hair extraction direction).

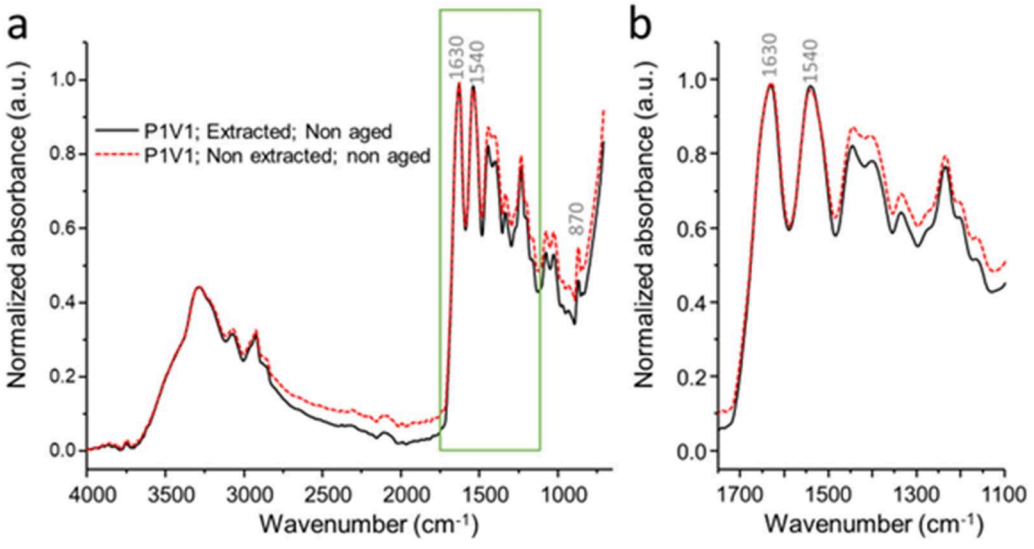

Fig. 5. (a) FTIR spectra representative of all measurements undertaken on the extracted and non-extracted areas of the parchment samples and (b) zoom in of the 1100–1750 cm^{-1} area of interest showing the limited variation observed for the amide I and amide II bands. Spectra were normalised to the amide I band. Dashed red line: non-extracted, non-aged; black line: extracted, non-aged.

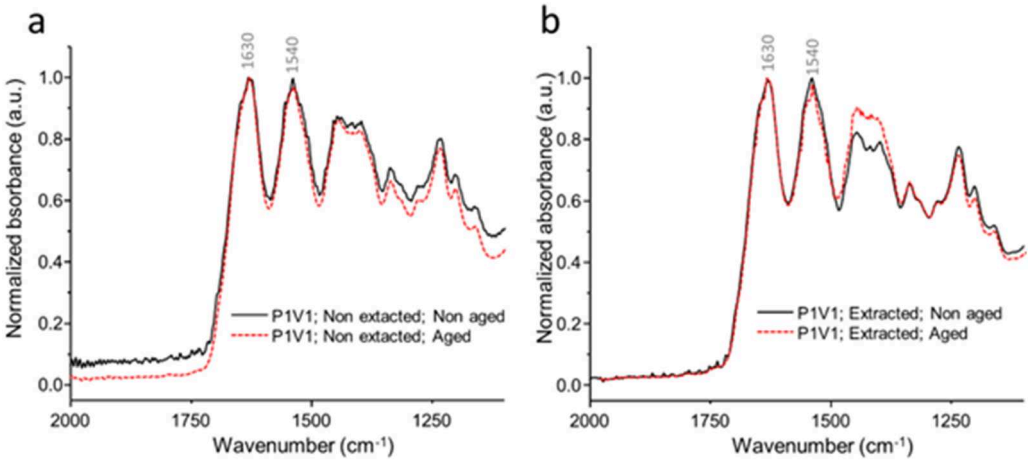

Fig. 6. FTIR spectra representative of all measurements undertaken on the (a) non-extracted areas of the parchment samples before and after artificial ageing and (b) the extracted areas of the parchment samples before and after artificial ageing. Spectra were normalised to the amide I band. Dashed red line: aged; black line: non-aged.

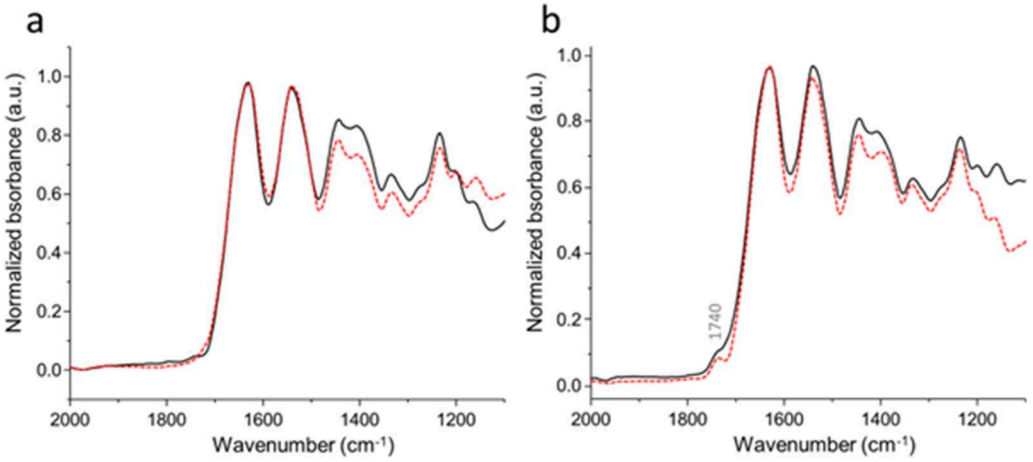

Fig. 7. Selected FTIR spectra for (a) non-aged and (b) aged parchments after lipid extraction: P1V2 (black line) and P4V1 (dashed red line) showing the formation of carbonyl groups at 1740 cm^{-1} during the ageing of the parchment samples. Spectra were normalized to the amide I band.

degradation has been studied by various techniques such as Near Infrared (NIR), NMR (mouse), Small-Angle X-Ray Scattering (SAXS), Differential Scanning Calorimetry (DSC), Micro-Hot Table, Transmission Electron Microscopy (TEM) and mass spectrometry [6,7,11,23–26].

FTIR is a popular spectroscopic method for the study of parchment [8,11,27]. The technique allows for the understanding of the degradation of the collagen by measuring the position and intensity of the absorbance maxima of the amide I and II bands change between 1600–1700 cm^{-1} and 1510–1580 cm^{-1}, respectively [11]. SEM is used here to image potential structural and morphological changes happening during lipid extraction and ageing, natural or artificial, of the parchment. Changes in surface morphology (texture, porosity, etc.) are captured through high-resolution imaging of the fibrillar network of the collagen [23,28,29]. These structures tend to disappear upon degradation of the collagen in parchment, ultimately leading to a flat surface when the collagen in the parchment is considered fully gelatinized making it more vulnerable to changes in humidity and temperature. Damaged parchment is very brittle and prone to fragmenting during handling [23,30]. Consequently, changes in materials can also be addressed by conducting mechanical testing on parchment [31–36]. Tensile fracture testing allows one to gain insights into the parchment's physical properties, structural integrity, and mechanical behaviour by assessing their stiffness and extensibility when subjected to a shearing force [37–40]. The presence of impurities, defects, stress concentrations, and modifications within the material, such as lipid extraction, can also influence the likelihood and nature of shear fracture [40,41]. Tensile fracture tests have been applied in the past to various types of polymeric films [42–44], including natural materials such as cellulose, lignin, keratin, and collagen [45].

In this work, we assess the effect of a novel minimally invasive lipid extraction method [19] on the integrity of the parchment. The investigation seeks to determine immediate and long-term changes to the integrity of parchment by analysing freshly lipid-extracted parchments and artificially aged parchments. This study focused on how the solvent application and removal of the lipid molecular fraction may create a pathway for more rapid embrittlement of the extracted parchment or locally slow down its degradation through the removal of the lipid fraction. The assessment is carried out using a multi-analytical approach making use of FTIR to follow molecular changes, SEM imaging to follow physical changes, and mechanical testing to better understand the potential changes in material brittleness and stiffness. The results of this study help address non-invasive lipid extraction of historical parchment where sampling cannot be carried out, assuring the lipid extraction will not have a long-term impact on the integrity of the parchment sampled.

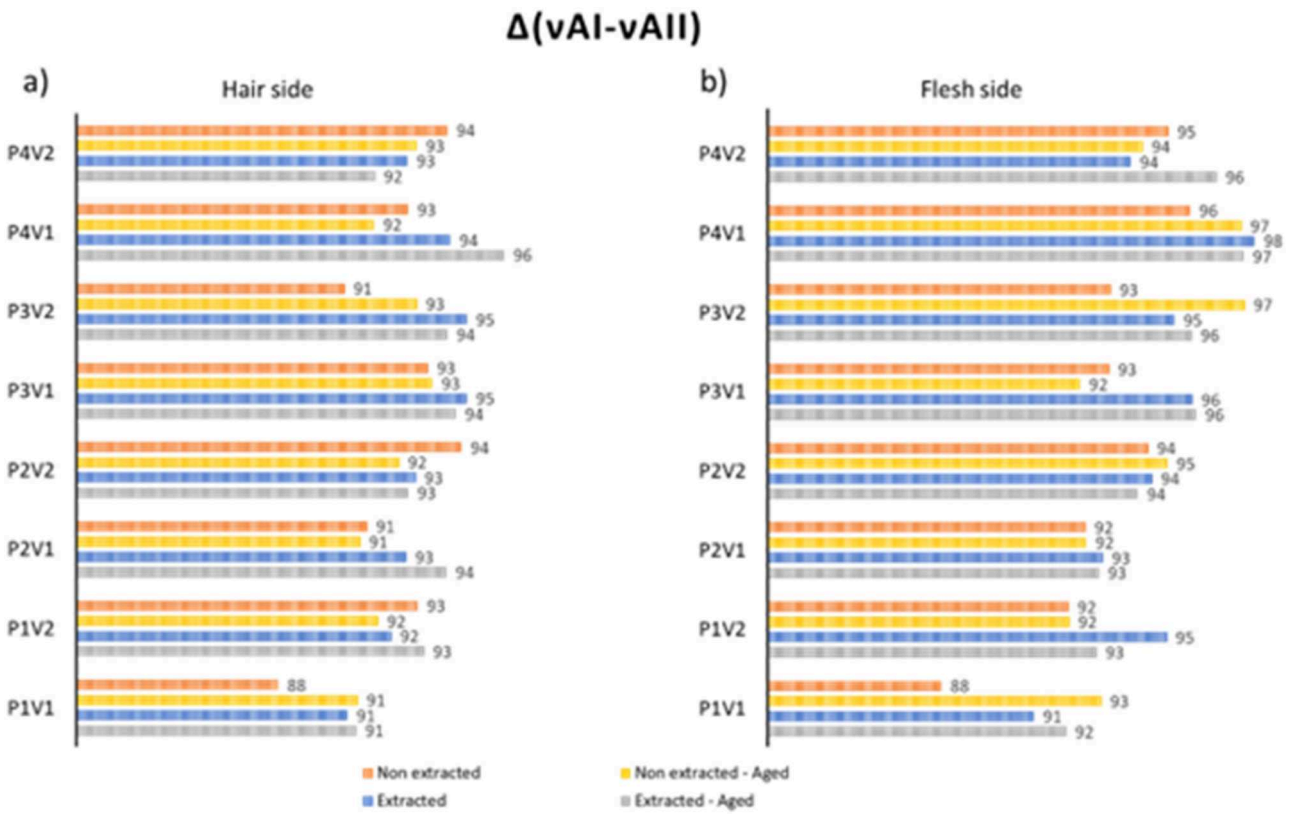

Fig. 8. Differences in the wavenumbers of the amide I and amide II bands (Δ(νAI-νAII)) before and after extraction, and before and after artificial ageing for a) the hair side and b) the flesh side.

2. Materials and methods

1. Glassware, Solvents & Reagents

Reusable glassware was cleaned with Decon 90 (Decon Laboratories) and Mili-Q water then rinsed with acetone before oven drying and furnacing (450 °C; 2 h); disposable glassware was also furnaced (450 °C; 2 h). HPLC grade solvents (≥ 99 % purity; Fisher Scientific/Rathburn) and analytical grade reagents (≥ 98 % purity; Fisher Scientific/Rathburn) were used for all experimentation.

2. Parchment Samples

For this study, four historic non-accessioned parchment documents (P1, P2, P3 and P4) were used (Fig. 1). The parchment selection was representative of the array of parchments that can be found in archival collections, with a clean modern 21st century membrane (P1) that was soiled using an artificial soiling recipe [46] and older naturally soiled membranes with various amount of dust deposition (P2, P3, P4). The samples were sacrificial and not part of the collection, meaning that they were of no documentary or historical value and could be used for destructive analysis for scientific research.

3. Lipid Extraction

Parchment lipids were extracted using a novel vacuum-aided extraction technique described by Johns *et al.* [19] to obtain a total lipid extract (TLE). A total of five extractions were performed on each of the four parchments to initially investigate (a) the direction of sampling, (b) the quantity of solvent used and (c) the effect of soiling upon the lipid recovery and chemical composition. These parameters are outlined in Table 1. A subset of the experiments (V1, V2) were used to investigate the impact of lipid extraction on parchment integrity. The chosen experiments employed 3 mL extraction volumes taken from the flesh to hair side and on clean parchment regions.

4. Lipid Analysis

An aliquot of each total lipid extract (TLE) (P1-P4; V1-V5) was derivatized using 40 μL *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1 % trimethylchlorosilane at 70 °C for 1 h before evaporating excess BSTFA under nitrogen and diluting in hexane. Derivatized TLE samples were analysed using an Agilent Technologies 7890A GC gas chromatograph equipped with a fused silica capillary column (15 m x 0.32 mm) coated with dimethylpolysiloxane stationary phase (DB-1HT; film thickness, 0.1 μm; Agilent Technologies). Samples were injected on-column at an oven temperature of 50 °C (2 min, isothermal hold) before increasing to 350 °C (10 °C min^{-1} ramp; 10 min, isothermal hold). Helium was employed as the carrier gas (flow rate: 4 mL min^{-1}). The temperature of the flame ionising detector (FID) was set to 350 °C. Data acquisition and processing were conducted using Agilent MSD ChemStation software (F.01.01.2317, Agilent Technologies). Extracted lipids were quantified using 10 μg of *n*-tetratriacontane (C_{34}).

5. Artificial Ageing

Following lipid extraction, the parchment samples were cut into two sections using scissors, each containing half of the extracted areas. One-half was kept in the laboratory at ca. 25 °C and 50 % RH while the other half was subjected to mild accelerated ageing.

High temperature and relative humidity levels are often used to create drastic changes in parchments during artificial ageing [47]. In this study, we aimed to accelerate the natural ageing of parchment rather than denature and gelatinise the samples. Furthermore, historical items stored in normal conditions are unlikely to undergo changes reflective of ageing at 80 °C. Consequently, we used mild conditions at

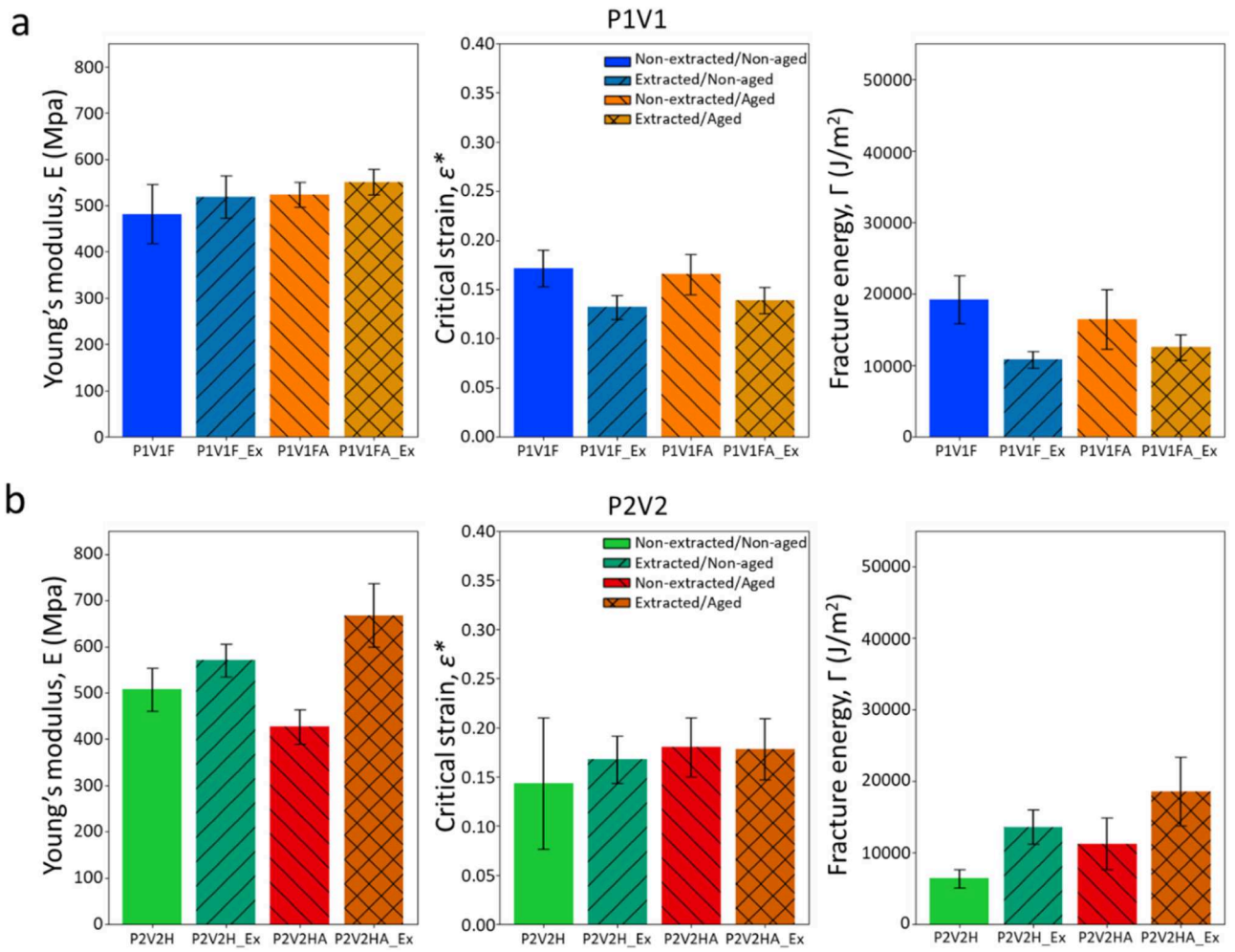

Fig. 9. Fracture data plots (left: Young's modulus; centre: critical strain; and right: fracture energy) obtained for (a) P1V1 and (b) P2V2. F and H respectively refer to the flesh and hair side solvent deposition. Ex refers to samples for which lipids were extracted. A indicates artificial ageing of the samples. The error bars indicate the 95 % confidence interval of the measurements.

60 °C and either 40 or 60 % relative humidity (RH), alternating every 24 h for 32 days as described in [47], with RH cycling known to accentuate the temperature effects [47]. Artificial ageing of the parchment samples was undertaken in a climatic chamber (Binder GmBH, Model KBF 115).

6. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) images were collected with a JSM-IT500 InTouchScope™ Scanning Electron Microscope (JEOL, Tokyo, Japan) equipped with a JED-2300 Analysis Station. The images were acquired at a voltage of 5 kV, in low vacuum (25 Pa) and with a working distance of ca. 25 mm.

7. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was carried out using an Agilent 4300 handheld spectrometer using an Attenuated Total Reflectance (ATR) accessory. A total of 64 scans were averaged in the 650–4000 cm^{-1} range, with 4 cm^{-1} resolution and Happ-Genzel apodization. Data was acquired using Agilent's MicroLab Expert Software and processed using OPUS and Origin Pro 8.

Due to space constraints, five points were measured on non-extracted areas whereas only four were measured on areas where lipid extraction was performed.

8. Mechanical Testing

The pure shear fracture tests were performed using a TA ElectroForce instrument (TA Instruments, New Castle, DE, USA). Samples were cut to be 7–12 mm wide by 20–30 mm long. The width and thickness of each sample was recorded. The gauge length between clamps was set to 4 mm. Samples were loaded into the clamps and pulled at a strain rate of 10 mm min^{-1} to between 0.5–2.5 mm depending on the extensibility of the sample. After the unnotched test, a crack was added to the sample using a pair of scissors. The crack length was recorded, then the sample was loaded into the clamps and pulled again using a strain rate of 10 mm min^{-1} to a maximum value of 0.5–2.5 mm.

3. Results and discussion

1. Lipid Analysis

The mass of lipids recovered per cm^2 of parchment (area density, μg cm^{-2}, Fig. 2) ranged between 1.9 and 11.2 μg cm^{-2} on average for the studied sacrificial parchments, with P1 containing substantially less lipid (0.61 – 2.7 μg cm^{-2}) than P2, P3 and P4 (3.0 – 22.6, 1.3 – 19.0, 0.39 – 32.5 μg cm^{-2}, respectively). The direction of sampling did not appear to influence the lipid recovery with respect to V1 and V2 although in soiled regions, the area density of V4 (flesh to hair) was found to be greater than V5 (hair to flesh).

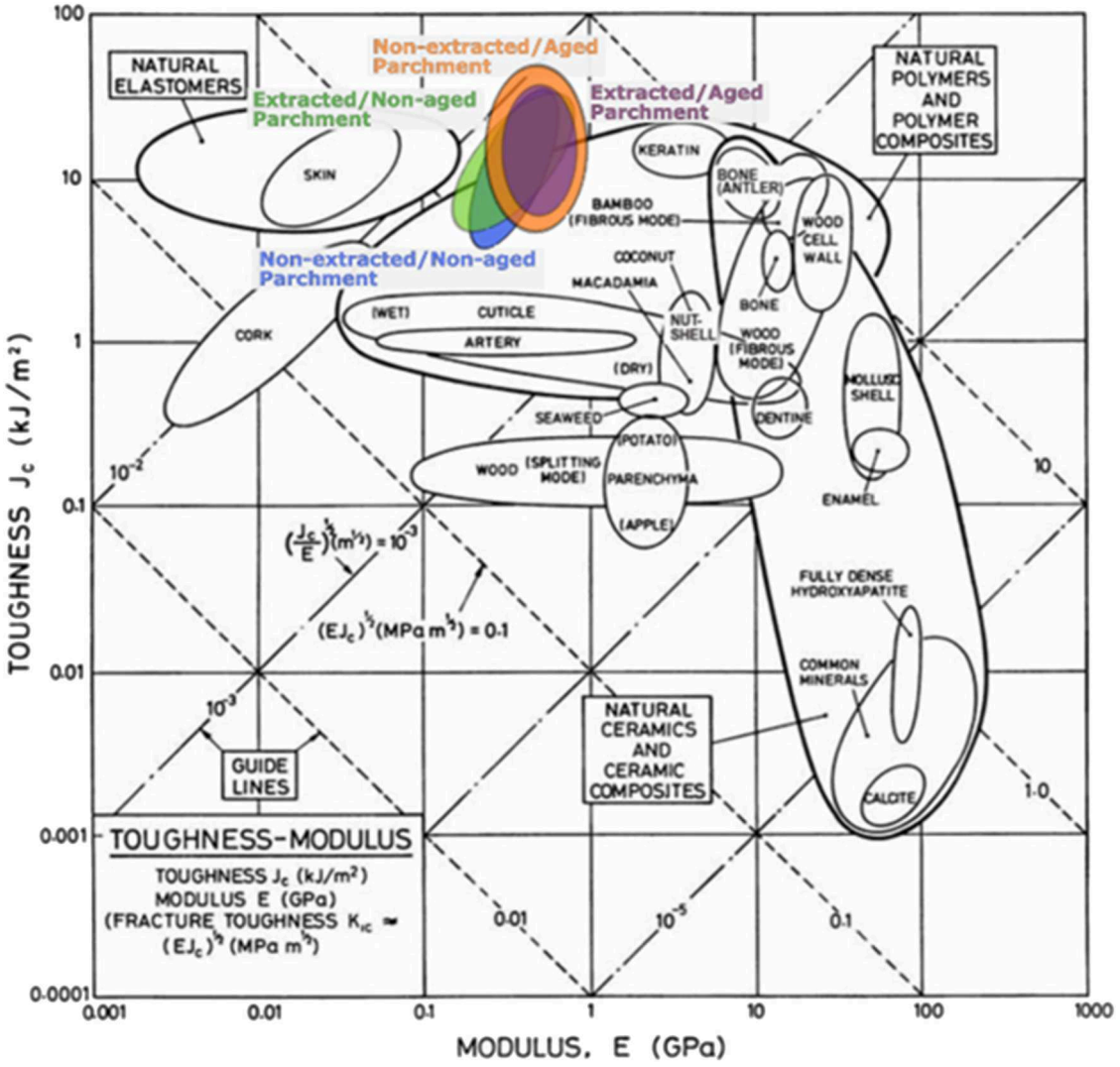

Fig. 10. An Ashby plot which shows the Toughness vs. Modulus of natural materials. The fracture data of the non-extracted and non-aged (blue), non-extracted and aged (orange), extracted and non-aged (green), and extracted and aged (purple) parchment data are summarised using the coloured ellipses on this plot. Adapted with permission from [45].

The varying recoveries of lipids across the parchment samples are likely a product of the heterogeneous nature of parchment and is a commonly reported analytical challenge in non-invasive studies [18]. Studies on parchment folios from a sacrificial collection (Beast2Craft project) [48] using the same vacuum-aided extraction have shown high variation in lipid quantities depending on the region sampled [19]. Previous studies have also highlighted the significant influence of animal species on parchment lipid content [49], with sheepskin being lipid-rich (30–50 % of the dermal dry weight) compared to cattle and goatskin (2–3 % and 3–10 %, respectively) [50]. Species identification via peptide mass fingerprinting (ZooMS) detected peptide markers diagnostic of sheepskin in P4. However, ZooMS analysis could not distinguish sheep from goatskin in P1 and P3 and sheep, calf and goatskin in P2 (S1). The absence of these diagnostic markers likely reflects the protein survival in these parchments which are reduced via hydrolytic, oxidative and biological attack during storage [50–52]. Parchment P1 exhibits a very low amount of lipids. P1 is understood to have been manufactured during the 21st century, it is thus reasonable to hypothesise that this parchment has undergone more sophisticated processing than the older parchments (P2–4), therefore giving rise to greater lipid removal during manufacture [15] and a lower lipid content. Indeed, this aligns with changes in parchment production during mid-19th century whereby skins were regularly bleached and scalded with boiling water to produce whiter parchment with greater capacity to retain ink [15,53].

The lipid composition of total extracts from the same experiment (V2; 3 mL, hair to face) is shown for the four parchments (Fig. 3). The profiles reveal free saturated *n*-alkanoic acids (C_{14}—C_{18}, dominated by $C_{16:0}$ and $C_{18:0}$), consistent with degraded animal fats [52,53] and previous parchment analyses [15,19]. The absence of hydrolysable lipids, such as triacylglycerols (TAGs, T48-T54), highlights lipid degradation from manufacturing, microbial action, and environmental exposure [13, 54]. Cholesterol and its oxidation product 7-ketocholesterol (S2) were identified in P3 and P4 in the absence of handling contaminants such as squalene and vitamin E [55–58]; these are likely of endogenous origin, retained during parchment production and storage [19]. P1′s (Fig. 3a) lipid profile, lacking TAGs and sterols, suggests a more rigorous fat removal process compared to P2–4. Despite differences in composition and lipid recovery, these data demonstrate the extraction technique's effectiveness as witnessed by Johns *et al.* [19]. Given the removal of lipids at the molecular level reflects a permanent change in the structure of parchment, we then investigated the immediate and long-term impact of this extraction technique to validate its use on historically valuable documents. Extractions V1 and V2 were selected over V3–5 for further

investigation since they demonstrated comparable lipid recovery to that of excess solvent volumes (V3, 20 mL). Despite soiled regions (V4-V5) demonstrating comparable lipid recovery and composition in this study, future sampling of historic parchment should prioritise unsoiled areas to minimize the risk of incorporating exogenous contamination into the lipid extract; for this reason, V4 and V5 were excluded.

2. Scanning electron microscopy: surface morphology.

Physical changes in the collagen structure of the parchment were assessed with the use of SEM images. SEM images were captured before and after the lipid extraction process, as well as before and after artificial ageing (Fig. S3-S4). Three examples representative of extraction from flesh-to-hair (P1V1 and P4V1) and hair-to-flesh (P2V2), as well as extractions on clean (P1V1 and P2V2) and soiled (P4V1) parchments, are presented in Fig. 4.

The typical collagen fibrous network characteristic for parchment remains visibly intact on all non-aged samples, regardless of whether they have undergone lipid extraction or not (Fig. 4a and 4c; Fig. S3-S4). Similarly, the fibrillar structure remains visible after artificial ageing (Fig. 4b and 4d). Gelatinization would lead to smooth glass-like material [59] and a loss of the fibrillar structure. This was not observed in any of the samples.

Independently from the extraction process, mineral deposits are seen on the surface of the parchment collagen fibres (particularly visible in P4V1, Fig. 4). Such deposits correspond to the formation of calcium carbonate following the liming of animal skin into a calcium hydroxide solution during parchment-making. The mineral deposits are observed in both non-extracted and extracted areas, further emphasising the minimally invasive nature of the lipid extraction methodology and the limited impact on the parchment structure. The presence of calcium carbonate at the surface of the parchment is also confirmed with FTIR analyses, with the presence of the band at 870 cm^{-1} (Fig. 5a).

3. Fourier transform infrared spectroscopy: molecular information.

Along with identifying the presence of calcium carbonate at the surface of the parchment, FTIR was used to assess the molecular changes in the collagen fibres by monitoring the cumulative displacement of the amide I (AI, 1600–1700 cm^{-1}) and amide II (AII, 1510–1580 cm^{-1}) bands expressed as a change in their separation Δ(νAI–νAII). No significant changes were observed in the position of the amide I and II bands, which remained centred at ca. 1630 and 1540 cm^{-1}, respectively for samples before and after the lipid extraction (Figs. 5 and 6).

A small shoulder centred ca. 1740 cm^{-1} is observed in some aged samples (Fig. 7b). This absorption band can be assigned to carbonyl or carboxyl compounds and is considered a marker of oxidation of the polypeptide chains [11,16,27,32,60,61]. This band is often observed in naturally and artificially aged parchment and can be used to show that artificial ageing has a limited impact on the parchment, independent of whether lipids were extracted.

When looking at the overall parchment set, a slight increase of Δ(νAI-νAII), often in the range of ±4 cm^{-1}, is observed between measurements on lipid-extracted or non-extracted parchments before and after ageing (Fig. 8). This limited ±4 cm^{-1} change in the Δ(νAI-νAII) is observed on both the solvent deposition and solvent collection sides, showing the limited impact of the extended contact between solvent and parchment upon solvent deposition. However, this change cannot unequivocally be associated with the extraction method as this is within the 4 cm^{-1} spectral resolution of the instrument. Furthermore, it is not unusual to see a shift toward lower wavenumber by ca. 20 cm^{-1} for the amide II and a shift toward higher wavenumbers (ca. 15 cm^{-1}) for the amide I upon ageing of parchment, leading to a Δ(νAI-νAII) of up to 35 cm^{-1} [11]. The few changes observed in samples before and after ageing in both non-extracted and extracted areas of the parchments (Fig. 6) demonstrate the limited impact of both the extraction and ageing process on the molecular structure of the parchment.

4. Mechanical testing: physical properties

The short-and long-term influence of the extraction on the parchment was assessed using mechanical testing using pure shear single notch fracture tests, focusing on three types of fracture data: Young's modulus, critical strain and fracture energy [62–64] (Fig. 9). All three capture different aspects of the material properties that govern the response of a parchment sample to applied and environmental stress. Young's modulus, also known as the elastic modulus, describes the stiffness or rigidity of a material and quantifies the material's ability to resist deformation under an applied load. By determining the elastic modulus of parchment, we can evaluate its flexibility, resistance to bending or warping, and potential for dimensional changes over time. The critical strain, also referred to as strain at break, refers to the amount of deformation or elongation a parchment sample undergoes before it fractures. It provides insights into the material's extensibility or ability to deform without breaking. Understanding the strain at break is crucial for assessing the parchment's ability to withstand stress, handling, and environmental conditions. Finally, the fracture energy measures the amount of energy required for a crack to propagate in a parchment sample. Fracture energy provides the most information about the toughness and resistance of the parchment sample to stretching and pulling forces. More simply put, fracture energy can be thought of as the 'tear energy' required for a parchment sample to show a crack. This property can be calculated from a pure shear fracture test by integrating the area under the load vs. displacement curve up to the critical strain.

All fracture data was obtained on extracted and non-extracted parchments, before and after artificial ageing and all four measurements were plotted against each other to visualise the influence of the lipid extraction and artificial ageing of the samples.

No consistent significant changes in the fracture data were observed (Fig. 9, Fig. S5-S8). For P1V1, the Young's modulus evidenced a consistent value of ca. 500 MPa showing limited change in the stiffness of P1 both before and after the extraction and before and after the ageing. In P2V2, a small increase (500 – 600 MPa) in the Young's modulus was seen between the non-extracted and extracted parchment but when compared to the artificially aged parchment, it was found that the extracted parchment evidenced a smaller change (550 – 650 MPa) than the non-extracted parchment (400 – 600 MPa). These data indicate that the ageing has a greater impact on the stiffness of P2 than the lipid extraction. The limited impact of the extraction (and of the artificial ageing) is further ascertained by the limited variations observed for the critical strain and fracture energy, confirming that the material maintains similar levels of extensibility and toughness after lipid extraction and ageing.

The Toughness-Modulus graph, also known as an Ashby plot [45], provides a visual representation of the mechanical properties of natural materials. It presents the trade-off between toughness and modulus (stiffness), allowing engineers and researchers to assess and compare different materials based on their desired mechanical properties for specific applications. When plotting the results of the mechanical testing experiments on non-extracted and extracted parchment before and after artificial ageing within the Toughness-Modulus graphs in (Fig. 10), it becomes clearer that the ageing of the parchment has more impact on the physical properties of the parchment samples than the lipid extraction. As a result of ageing the parchment samples, the modulus values slightly increase while the range of toughness remains similar. The values for the toughness and modulus of the parchment samples also correlate with other reported values for collagen-based materials [65]. However, some variability in the mechanical property data could also be linked to factors such as the anisotropy of the parchment or the fibril alignment which was not accounted for when preparing the samples for the fracture testing. This is beyond the scope of this research and has not been investigated further.

4. Conclusions

Lipids extracted from parchment offer an untapped potential for the reconstruction of past climates and environments. Johns *et* al. devised a novel method for the minimally invasive extraction of lipids from historic parchments [19]. In this study, we focused on understanding the short and long-term impact of this novel minimally invasive methodology for lipid extraction from parchment to facilitate the exploitation of parchment lipids by molecular and isotopic techniques. To do so, lipids were extracted from historical sacrificial parchment samples and the latter analysed before and after extraction as well as before and after artificial ageing. Chromatographic and biomolecular analysis of the four sacrificial parchments evidenced the suitability of the minimally invasive extraction method for the sampling of historic parchment.

The impact of both the extraction and ageing was assessed using a multi-analytical approach, which included Fourier Transform Infrared Spectroscopy (FTIR) to track molecular changes, Scanning Electron Microscopy (SEM) to observe physical changes, and mechanical testing to evaluate changes in the material brittleness and stiffness. The lipid extraction method had no significant short-term or long-term influence on the morphology of the parchment, with the typical collagen fibrous network characteristic of parchment remaining intact in both non-extracted and extracted samples, before and after artificial ageing. The consistent visibility of the fibrillar structure in both extracted and non-extracted parchment samples, before and after artificial ageing, supports the conclusion that the lipid extraction method employed in this study has no significant short-term and long-term influence on the morphology of the parchment.

The presence of mineral deposits, such as calcium carbonate, was also observed on the surface of the parchment, asserting that the extraction process did not affect their presence. FTIR analysis showed no significant changes in the position of the amide I and amide II bands, indicating minimal molecular alterations in the collagen fibres following lipid extraction. Even after artificial ageing, the amide bands remained relatively stable, despite the appearance of carbonyl groups, often associated with natural ageing of parchment. This also tends to show that artificial ageing has a more significant impact on the parchment than the lipid extraction. Finally, the pure shear single notch fracture tests highlighted little variations in material stiffness and brittleness before and after extraction and/or ageing, further supporting the conclusion drawn through SEM and FTIR analyses.

The findings of this study underscore the practicality of employing minimally invasive lipid extraction techniques when faced with historical parchment analysis scenarios where more intrusive methods are unviable. This research significantly contributes to the refinement of minimally invasive procedures for lipid extraction from parchment, offering valuable insights for the scrutiny of historical documents and the exploration of their organic constituents. The non-intrusive nature of this extraction method, with its minimal short- and long-term impact, enables us to access critical information while safeguarding the long-term preservation of historical parchment records. Furthermore, due to the widespread availability of well-dated parchment, the examination of lipids and isotopes in this distinctive material is paving the way for novel opportunities in dietary and climate studies.

CRediT authorship contribution statement

Marc Vermeulen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing, Validation. **Samuel P. Johns:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **Gwen dePolo:** Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing, Funding acquisition. **Pedro Maximo Rocha:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Matthew J. Collins:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. **Lora Angelova:** Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. **Mélanie Roffet-Salque:** Conceptualization, Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Samuel P Johns reports financial support was provided by European Research Council. Matthew J Collins reports financial support was provided by European Research Council. Samuel P Johns reports financial support was provided by Carlsberg Foundation. Matthew J Collins reports financial support was provided by Carlsberg Foundation. Gwen dePolo reports financial support was provided by National Science Foundation. Melanie Roffet-Salque reports financial support was provided by The Royal Society.

Data availability

Data will be made available on request.

Acknowledgements

The National Archives would like to thank Dr Constantina Vlachou-Mogire for granting access to the scanning electron microscope at the Historic Royal Palaces' heritage science facilities. The National Science Foundation is thanked for supporting the work of GdP (OISE-1743748). This study was partially funded by the European Research Council (ERC) Advanced Grant 'Beasts to Craft' (ERC-2020-ADG/787282) and the Carlsberg Foundation Semper Ardens Advance grant 'ArcHives' (No. CF18–1110) to MJC. The Royal Society is thanked for funding a fellowship to MRS (DHF\R1\180064 and RGF\EA\181067).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.polymdegradstab.2024.111076.

Data availability

Data will be made available on request.

References

[1] C.J. Kennedy, T.J. Wess, The structure of collagen within parchment – a review, Restaurator 24 (2) (2003) 61–80.
[2] M.L. Ryder, Parchment—its history, manufacture and composition, J. Soc. Arch. 2 (9) (1964) 391–399.
[3] Gottscher, L., *Ancient Methods of Parchment Making: discussion on Recipes and Experimental Essays*, in *Ancient and Medieval book Materials and Techniques*. Marilena and Paola F. Munafo (eds) 1993;41–56, Studi E Testi.
[4] N.K. Turner, The Materiality of Medieval Parchment: a Response to "The Animal Turn, Revista Hispánica Moderna 71 (2018) 39–67.
[5] L.G. Gonzalez, T.J. Wess, The effects of hydration on the collagen and gelatine phases within parchment artefacts, Heritage Sci. 1 (1) (2013) 14.
[6] Z. Sebestyén, et al., Thermal characterization of new, artificially aged and historical leather and parchment, J. Anal. Appl. Pyrolysis 115 (2015) 419–427.
[7] Bicchieri, M., et al., *Inside the parchment.* 9th International Conference on NDT of Art, 2008.
[8] B. Dolgin, V. Bulatov, I. Schechter, Complex Anal. Method Parchment Characterization 28 (3–4) (2009) 151–307.
[9] D. Fessas, et al., Thermal analysis on parchments I: DSC and TGA combined approach for heat damage assessment, Thermochim. Acta 447 (1) (2006) 30–35.
[10] S.C. Boyatzis, G. Velivasaki, E. Malea, A study of the deterioration of aged parchment marked with laboratory iron gall inks using FTIR-ATR spectroscopy and micro hot table, Heritage Sci. 4 (1) (2016) 13.

[11] F. Cappa, et al., Studies on the effects of mixed light-thermal ageing on parchment by vibrational spectroscopy and micro hot table method, Heritage Sci. 8 (1) (2020) 15.
[12] K.M. Axelsson, et al., Degradation of collagen in parchment under the influence of heat-induced oxidation: preliminary study of changes at macroscopic, microscopic, and molecular levels, Stud. Conserv. 61 (1) (2016) 46–57.
[13] P. Budrugeac, et al., A DSC study of deterioration caused by environmental chemical pollutants to parchment, a collagen-based material, Thermochim. Acta 500 (1) (2010) 51–62.
[14] M. Falkiewicz-Dulík, Leather and Leather Products, in: Falkiewicz-Dulik (Ed.), Handbook of Material Biodegradation, Biodeterioration, and Biostablization, ChemTec Publishing, Toronto, 2015. Editor.
[15] C. Ghioni, et al., Evidence of a distinct lipid fraction in historical parchments: a potential role in degradation? J. Lipid Res. 46 (12) (2005) 2726–2734.
[16] M. Strlič, et al., Autoxidation of lipids in parchment, Polym. Degrad. Stab. 94 (6) (2009) 886–890.
[17] A. Možir, et al., Material properties of historic parchment: a reference collection survey, Stud. Conserv. 59 (3) (2014) 136–149.
[18] A. Možir, et al., A study of degradation of historic parchment using small-angle X-ray scattering, synchrotron-IR and multivariate data analysis, Anal. Bioanal. Chem. 402 (4) (2012) 1559–1566.
[19] S.P. Johns, et al., Minimally invasive vacuum-aided extraction technique for the lipid analysis of historic parchment, Anal. Chem. 96 (34) (2024) 13811–13820.
[20] M. Roffet-Salque, et al., From the inside out: upscaling organic residue analyses of archaeological ceramics, J. Archaeol. Sci.: Rep. 16 (2017) 627–640.
[21] A. Možir, et al., On oxidative degradation of parchment and its non-destructive characterisation and dating, Appl. Phys. A 104 (1) (2011) 211–217.
[22] S. Doherty, et al., Measuring the impact of parchment production on skin collagen stable isotope (δ13C and δ15N) values, STAR: Sci. Technol. Archaeol. Res. 7 (1) (2021) 1–12.
[23] G. Della Gatta, et al., Assessment of damage in old parchments by DSC and SEM, J. Therm. Anal. Calorim. 82 (3) (2005) 637–649.
[24] A. Bartoletti, Nanometrology for damage assessment and preservation of parchment. Division of Biomaterials and Tissue Engineering, UCL, London, 2017.
[25] M. Manfredi, et al., Quantitative multispectral imaging for the detection of parchment ageing caused by light: a comparison with ATR-FTIR, GCMS– and TGA analyses, Int. J. Conserv. Sci. 6 (2016) 3–14.
[26] P. Richardin, et al., Gas chromatography - Mass spectrometry identification of collagen breakdown products in naturally and artificially aged leathers, J. Am. Leather Chem. Assoc. 91 (1996) 2–17.
[27] A. Kamińska, A. Sionkowska, Effect of UV radiation on the infrared spectra of collagen, Polym. Degrad. Stab. 51 (1) (1996) 19–26.
[28] M. Vadrucci, et al., Surface evaluation of the effect of X-rays irradiation on parchment artefacts through AFM and SEM, Appl Surf Sci 513 (2020) 145881.
[29] Y. Maor, P. Shor, Z. Aizenshtat, Parchment browning and the Dead Sea Scrolls – Part I: artificial aging, Polym. Degrad. Stab. 176 (2020) 109109.
[30] E. Badea, et al., 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. Calorim. 91 (1) (2008) 17–27.
[31] N.S. Cohen, M. Odlyha, G.M. Foster, Measurement of shrinkage behaviour in leather and parchment by dynamic mechanical thermal analysis, Thermochim Acta 365 (1) (2000) 111–117.
[32] M. Odlyha, et al., Fourier Transform Infra-Red Spectroscopy (ATR/FTIR) and Scanning Probe Microscopy of Parchment, E-Preservation Science, 2009, p. 6.
[33] E.F. Hansen, S.N. Lee, H. Sobel, The effects of relative humidity on some physical properties of modern vellum: implications for the optimum relative humidity for the display and storage of parchment, J. Am. Inst. Conserv. 31 (3) (1992) 325–342.
[34] A. Facchini, et al., Ancient parchment examination by surface investigation methods, J. Colloid Interface Sci. 231 (2) (2000) 213–220.
[35] M. Odlyha, et al., Dynamic mechanical analysis (DMA), 13c solid state nmr and micro-thermomechanical studies of historical parchment, J. Therm. Anal. Calorim. 71 (3) (2003) 939–951.
[36] M. Odlyha, et al., Damage assessment of parchment at the collagen fibril level using atomic force microscopy and mechanical testing at the macro level, in: ICOM-CC 17th Triennial Conference, 2014. Melbourne.
[37] M.A. Meyers, K.K. Chawla, Fracture: macroscopic Aspects, in: M.A. Meyers, K. K. Chawla (Eds.), Mechanical Behavior of Materials, Cambridge University Press, Cambridge, 2008, pp. 404–444. Editors.
[38] W.W. Gerberich, et al., Scale effects for strength, ductility, and toughness in "brittle" materials, J. Mater. Res. 24 (3) (2009) 898–906.
[39] R.O. Ritchie, Toughening materials: enhancing resistance to fracture, Philos. Trans. R. Soc., A 379 (2203) (2021) 20200437.
[40] G. Li, et al., Fracture toughness of thermoelectric materials, Mater. Sci. Eng.: R: Rep. 144 (2021) 100607.
[41] W. Wan, et al., The size effects of point defect on the mechanical properties of monocrystalline silicon: a molecular dynamics study, Materials (Basel) 14 (2021), https://doi.org/10.3390/ma14113011.
[42] M.d.S.R. Bastos, et al., Physical and mechanical testing of essential oil-embedded cellulose ester films, Polym Test 49 (2016) 156–161.
[43] S. Kahlen, et al., Characterization of physical and chemical aging of polymeric solar materials by mechanical testing, Polym Test 29 (1) (2010) 72–81.
[44] L.A. Felton, P.B. O'Donnell, J.W. McGinity, Mechanical properties of polymeric films prepared from aqueous dispersions, in: L.A. Felton, P.B. O'Donnell, J. W. McGinity (Eds.), Aqueous Polymeric Coatings For Pharmaceutical Dosage Forms, CRC Press, Boca Raton, 2008, pp. 125–148. Editors.
[45] U.G.K. Wegst, M.F. Ashby, The mechanical efficiency of natural materials, Philos. Mag. 84 (21) (2004) 2167–2186.
[46] R. Wolbers, The use of a synthetic soiling mixture as a means for evaluating the efficacy of aqueous cleaning materials on painted surfaces, Conservation-restauration des biens culturels: revue de l'ARAAFU 1992 (4) (1992 Oct) 22–29, n. 4.
[47] E. Badea, G.Della Gatta, T. Usacheva, Effects of temperature and relative humidity on fibrillar collagen in parchment: a micro differential scanning calorimetry (micro DSC) study, Polym. Degrad. Stab. 97 (3) (2012) 346–353.
[48] Collins, M.J. *B2C Parchment Collection*. 2023 [cited 2023; Available from: https://sites.google.com/palaeome.org/ercb2c/get-involved/b2c-parchment-collection.
[49] M. Fourneau, et al., Histological study of sheep skin transformation during the recreation of historical parchment manufacture, Heritage Sci. 8 (1) (2020) 78.
[50] S.P. Doherty, et al., Scratching the surface: the use of sheepskin parchment to deter textual erasure in early modern legal deeds, Heritage Sci. 9 (1) (2021) 29.
[51] E. Badea, et al., Damage ranking in historical parchments: from microscopic study of fibres structure to collagen denaturation assessment by micro DSC, e-Preserv. Sci. 9 (2012) 97–109.
[52] F. Brock, et al., Analysis of bone "collagen" extraction products for radiocarbon dating, Radiocarb. 55 (2) (2013) 445–463.
[53] R. Reed, The Nature and Making of Parchment, Elmete press, Leeds, 1975.
[54] R.P. Evershed, et al., Chemistry of archaeological animal fats, Acc. Chem. Res. 35 (8) (2002) 660–668.
[55] H.R. Mottram, et al., New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animal fats preserved in archaeological pottery, J. Chromatogr. A 833 (2) (1999) 209–221.
[56] F. Pinzari, V. Cialei, G. Piñar, et al., A case study of ancient parchment biodeterioration using variable pressure and high vacuum scanning electron microscopy, in: N. Meeks, et al. (Eds.), Historical Technology, Materials and Conservation: SEM and Microanalysis, Archetype Publications, London, 2012, pp. 92–98. Editors.
[57] M.A. Lyons, A.J. Brown, 7-Ketocholesterol, Int. J. Biochem. Cell Biol. 31 (3) (1999) 369–375.
[58] H.L. Whelton, et al., A call for caution in the analysis of lipids and other small biomolecules from archaeological contexts, J. Archaeol. Sci. 132 (2021) 105397.
[59] A. Mašić, *Applicazione Di Tecniche Innovative Nello Studio Dei Processi Di Degrado Dei Manufatti Di Interesse artistico-culturale*, in *Department of Chemistry*, University of Turin: Italy, 2005.
[60] Á. Csepregi, et al., Possible modifications of parchment during ion beam analysis, Heritage Sci. 10 (1) (2022) 140.
[61] R. Inagi, T. Miyata, Oxidative protein damage with carbohydrates and lipids in uremia: 'carbonyl stress, Blood Purif. 17 (2–3) (1999) 95–98.
[62] R.S. Rivlin, A.G. Thomas, Rupture of rubber. I. Characteristic energy for tearing, J. Polym. Sci. 10 (3) (1953) 291–318.
[63] C. Creton, M. Ciccotti, Fracture and adhesion of soft materials: a review, Rep. Prog. Phys. 79 (4) (2016) 046601.
[64] A.J. Silvaroli, et al., Role of nanoscale heterogeneity in the mechanical performance of hybrid elastomers, Macromolecules 56 (11) (2023) 4075–4086.
[65] A. Khayer Dastjerdi, F. Barthelat, Teleost fish scales amongst the toughest collagenous materials, J. Mech. Behav. Biomed Mater. 52 (2015) 95–107.