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Analytical Characterization of the Leather Binding of a Precious 17th-Century Chaghatay Medical Manuscript from Xinjiang, China

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Abstract

Zhìliáo Zhǐnán (Treatment Guide) is a 17th-century Chaghatay medical manuscript included in China's National Catalogue of Precious Ancient Books. Its leather binding is severely deteriorated, with extensive wear, blurred decorative patterns, fire damage, deformation, and multiple tears. In this study, colorimetry, near-infrared (NIR) spectroscopy, X-ray fluorescence (XRF) spectroscopy, extended depth-of-field microscopy, scanning electron microscopy (SEM), and proteomics were combined to characterize the cover, spine, back cover, and endpapers. Microscopic examination showed that the grain-surface pore pattern and cross-sectional fibrillar architecture were morphologically similar to modern goat leather; however, aging and surface loss prevent definitive species identification from morphology alone. SEM revealed pronounced collagen gelatinization in the cover. Proteomic analysis detected abundant type I collagen and identified casein-family peptides in the spine, supporting the use of a casein-based adhesive. NIR and XRF further distinguished leather from cellulose-based components and revealed marked spatial differences in elemental composition across the binding. Together, these results provide material evidence for the construction and deterioration of a 17th-century Chaghatay manuscript binding and establish an analytical basis for its conservation and restoration.

Keywords: Leather heritage; Material identification; Bookbinding technology; Collagen degradation; Proteomics

Introduction

Zhìliáo Zhǐnán was compiled in the 17th century by Arif Khojani (A.D. 1556–1662) in the Chaghatay script and was included in the second batch of China's National Catalogue of Precious Ancient Books in 2009. The manuscript comprises three major parts addressing basic medical theory, health care and diagnosis; understanding and treatment of organ diseases; and recognition and treatment of systemic diseases. It therefore has substantial value for research on the history of medicine. Beyond its textual importance, the manuscript is also a composite organic heritage object whose binding preserves evidence of historical materials and craftsmanship. Its advanced age and imperfect past storage conditions, however, have resulted in severe deterioration of the leather cover.

Scientific investigation of leather cultural heritage generally addresses two closely related issues: manufacturing technology and deterioration. Optical and electron microscopy can document pore morphology, fiber organization, cracking, shrinkage, and other structural changes. Spectroscopic and chromatographic methods, including FTIR, nuclear magnetic resonance (NMR), and high-performance liquid chromatography (HPLC), have been used to investigate tanning-related materials, whereas differential scanning calorimetry (DSC), thermogravimetry (TG), and coupled techniques such as TG-FTIR-MS and GC-MS provide information on thermal behavior and degradation products[1]. Species identification can additionally be approached through biomolecular methods. DNA analysis targets mitochondrial DNA (mtDNA) by PCR, while proteomic methods use peptide mass fingerprints or tandem mass spectrometry and can require less sample preparation and lower analytical cost than conventional DNA-based approaches[2-4].

Historical bookbinding leather has been examined using increasingly integrated analytical approaches. Vichi et al. characterized leather covers from 16th–19th century manuscripts in the Matenadaran Institute of Ancient Manuscripts (Yerevan, Armenia) by ATR-FTIR imaging and mapped collagen deterioration, particularly gelatinization, while also reporting calcium stearate[5]. Hassan et al. combined pH measurement, SEM, XRD, and FTIR to study the leather cover and paper leaves of a 10th-century Ottoman Qur'an manuscript; the authors reported a leather pH of 4.5, goat-like pore morphology, surface cracking, corrosion, insect damage, and changes associated with disruption of peptide and hydrogen bonding in collagen[6]. Studies of 19th-century historical leather bindings from the National Archives of Cairo have also shown that degradation alters the abundance of polar amino acids, including arginine, lysine, glutamic acid, and aspartic acid[7].

By comparison, systematic material studies of ancient books associated with ethnic-minority cultures in western China remain limited. Research on Chaghatay manuscripts from Xinjiang has concentrated primarily on textual philology and interpretation of medical content, whereas the material composition, binding technology, and deterioration of surviving leather covers have rarely been investigated scientifically. This gap limits reconstruction of regional bookbinding practices and also reduces the

evidence available for selecting conservation treatments for related manuscript collections.

Accordingly, the historically transmitted leather binding of *Zhìliáo Zhǐnán* was examined using a combination of non-invasive and micro-destructive analytical techniques. The study aimed to distinguish the principal binding materials, evaluate the spatial pattern and severity of deterioration, and assess evidence related to leather processing and adhesive use. By integrating morphological, spectroscopic, elemental, and proteomic information, the work seeks to expand the material record of book production in the Chaghatay cultural sphere and to provide a scientific basis for conservation of this manuscript.

1 Materials and Methods

1.1 Materials

Samples were obtained from the *Zhìliáo Zhǐnán* manuscript held by the Xinjiang Uygur Autonomous Region Academy of Pharmaceutical Sciences, China. The manuscript dates to the 17th century and has binding dimensions of 32 cm × 22 cm × 8 cm. All micro-samples were collected with authorization from the cultural relics conservation authority. To minimize intervention, sampling was restricted to non-display edge areas of the leather cover and followed ethical principles for micro-destructive analysis of cultural heritage. Because the manuscript is a museum-held historically transmitted object rather than an archaeological find, no stratigraphic context is available; its dating is based on combined codicological, textual, and binding-style evidence.

1.2 Methods

1.2.1 Colorimetry

Colour measurements were performed on the front and back covers using a Spectra Magic NX spectrophotocolorimeter (CM-700d1, Konica Minolta, Japan) in the CIELAB colour space. L denotes lightness, a denotes red-green chromaticity and b denotes yellow-blue chromaticity. The colour difference ΔE was calculated against a standard white reference, according to the formula:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

1.2.2 Extended depth-of-field microscopy

A HIROX RH-2000 digital three-dimensional microscopy system with a low-magnification lens (MXB-2016Z) and a high-magnification lens (MXB-2500REZ) was

used to observe and analyse the surface morphology of the front and back covers. Observations included degradation types, leather grain surface morphology and cross-sectional structure. Only cross-sections were prepared via micro-destructive sampling, taken from the fore-edge of the front cover; all other observations were performed in situ. Microscopic identification was performed in accordance with the national standard GB/T 38408-2019 *Leather—Identification—Method of microscopy*[8].

1.2.3 Scanning electron microscopy

Micro-morphological observations were performed on three micro-samples: the bottom-left fold of the front cover (F1), the top of the spine (F2) and the fore-edge fold of the back cover (F3), using a Thermo Scientific Apreo 2C field-emission scanning electron microscope at accelerating voltages of 5.00 kV and 10.00 kV, at magnifications of 2000×, 5000× and 2000×. Prior to observation, samples were sputter-coated with gold to improve electrical conductivity and imaging quality. Sample preparation was straightforward: samples were cut to dimensions less than 1 cm × 1 cm × 0.5 cm and mounted on sample stubs.

1.2.4 Near-infrared spectroscopy

Near-infrared spectra were acquired at five points on the cover (Table 1) using a MicroPHAZIR Rx handheld NIR spectrometer. The scanning range was 1600–2400 nm with a pixel sampling interval of 8 nm.

Table 1 Positions of NIR measurement points

Number	Position	Images
A1	Top-right corner of front cover	
A2	Upper part of spine	
A3	Damaged area on lower spine	
A4	Lower middle of back cover (with endpaper)	 
A5	Lower right of back cover (with endpaper)	

1.2.5 X-ray fluorescence spectroscopy

Elemental composition analysis was performed using a Niton XL3t handheld X-ray fluorescence spectrometer in TestAll-Geo mining mode, with an acquisition time of 30 s and a confidence level of 2σ. Results are reported in ppm and converted to mass fraction (wt%), with a conversion factor of 1 wt% = 10 000 ppm.

1.2.6 Proteomic analysis

(1) Sample preparation

Three micro-samples (F1, F2, F3) from the cover and spine were analysed.

- **Protein extraction**: Each sample was ground in liquid nitrogen and mixed with SDT lysis buffer. Samples were incubated in boiling water for 3 min, followed by ultrasonic disruption for 2 min. After centrifugation at 16 000 g for 20 min at 4 °C, the supernatant was collected and protein concentration was determined using the BCA assay. Protein samples were mixed with 5× loading buffer at a volume ratio of 5:1, incubated in boiling water for 5 min, and separated by 8–16% SDS-PAGE with Coomassie brilliant blue staining.
- **Protein digestion**: Filter-aided sample preparation (FASP) was applied for digestion. DTT was added to each sample to a final concentration of 100 mM, followed by incubation in boiling water for 5 min and cooling to room temperature. 200 µL of UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.0) was added and mixed, then transferred to a 10 kD ultrafiltration tube and centrifuged at 1200 g for 15 min. The washing step with UA buffer was repeated once and the filtrate was discarded. 100 µL of 50 mM IAA in UA buffer was added, followed by shaking at 600 r/min for 1 min and incubation in the dark at room temperature for 30 min, then centrifugation at 1200 g for 10 min. Two additional washes with UA buffer were performed. 100 µL of NH_4HCO_3 buffer was added, followed by centrifugation at 14 000 g for 10 min, repeated twice. 40 µL of trypsin buffer (6 µg trypsin in 40 µL NH_4HCO_3 buffer) was added, followed by shaking at 600 r/min for 1 min and digestion at 37 °C for 16–18 h. After digestion, peptides were collected by centrifugation at 1200 g for 10 min, desalted using a C18 cartridge and lyophilised. Dried peptides were reconstituted in 0.1% formic acid for LC-MS analysis.

(2) LC-MS data acquisition

Peptide separation was performed on a Vanquish Neo UHPLC system, and mass spectrometric analysis was performed on an Orbitrap Astral Zoom mass spectrometer (Thermo Scientific) in data-independent acquisition (DIA) mode. The analysis duration was 8 min, with an electrospray voltage of 2.2 kV in positive ion mode. The precursor scan range was 380–980 m/z, with a first-stage MS resolution of 240 000, AGC target of 500% and maximum injection time of 3 ms. The MS/MS resolution was 80 000, AGC target of 500%, maximum injection time of 3 ms, RF lens of 40%, HCD activation, isolation window of 2 Th, normalised collision energy of 25% and cycle time of 0.6 s.

(3) Database searching

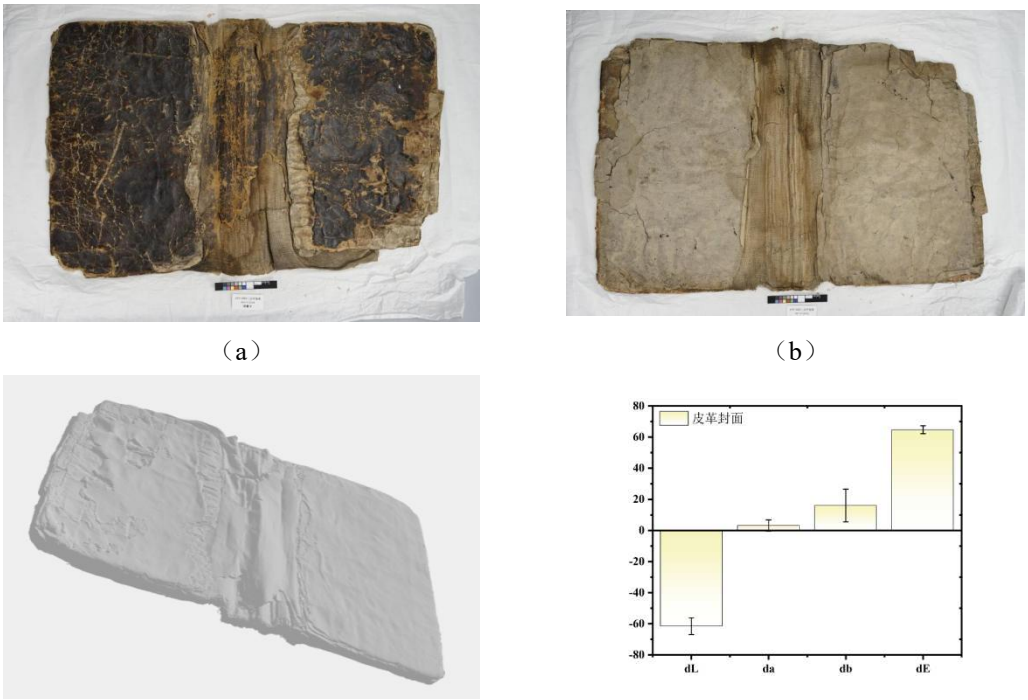
DIA-NN software was used to process the raw spectra and perform database searching and label-free quantification. Database searches were performed against the UniProt

Bos taurus (bovine) and *Ovis aries* (ovine) reference databases[9], with a false discovery rate threshold of $Q \leq 0.01$. Due to sampling constraints, this study focused only on the identification of collagen and exogenous adhesive proteins.

2 Results

2.1 Morphology and color

Macroscopic examination and 3D scanning of the binding (Figure 1a–c) documented extensive deterioration. Fire damage is visible on the front cover, spine, and back cover, and the leather surface shows pronounced undulation, cracking, peeling, and local deformation. At the spine, loss of covering material exposes the lining fabric, while partial tearing reveals the layered arrangement of leather, linen lining, and paper. The 3D scan records the spatial distribution of warping, wrinkling, and tears and provides a morphological reference for subsequent microscopic observation and selection of analytical points. Colorimetric results are presented in Figure 1d. Relative to the standard white reference, the cover exhibits negative ΔL together with positive Δa and Δb values, corresponding to reduced lightness and an overall reddish-yellow color shift.



（a） （b）

（c） （d）

Figure 1. Cover morphology and colorimetry of the *Zhìliáo Zhǐnán* manuscript. (a) Front cover (left: cover, middle: spine, right: back cover); (b) back cover (left: cover, middle: spine, right: back cover); (c) three-dimensional scanning image; (d) color-difference values of the front cover.

2.2 Extended depth-of-field microscopy observations

Extended depth-of-field microscopy identified six principal forms of surface deterioration: wear, soiling, stiffening, tearing, deformation, and lining fracture (Figure 2). Wear occurs widely across the cover, whereas tearing is concentrated at the spine, fore-edge, and corners. The exposed lining and cardboard at the spine carry abundant yellowish-brown deposits that may include residual adhesive together with accumulated environmental particulates. The lower portion of the back cover is strongly stiffened, warped, and wrinkled and also shows localized burn-like deterioration. Across the binding, damage is greatest at the spine, less severe on the front cover, and least pronounced on the endpapers.

Grain-surface and cross-sectional observations of the historical leather (Figure 3) show round to oval pores arranged predominantly in groups of 3~5 along linear or curved rows. Fine pore detail is poorly preserved on the heavily worn back-cover sample. In cross-section, collagen fiber bundles form a layered interwoven network, with a denser upper region and a looser lower region; this stratification is less clearly defined in the spine sample. Modern goat leather and schematic pore patterns of common bookbinding leathers were examined as morphological references (Figure 4).

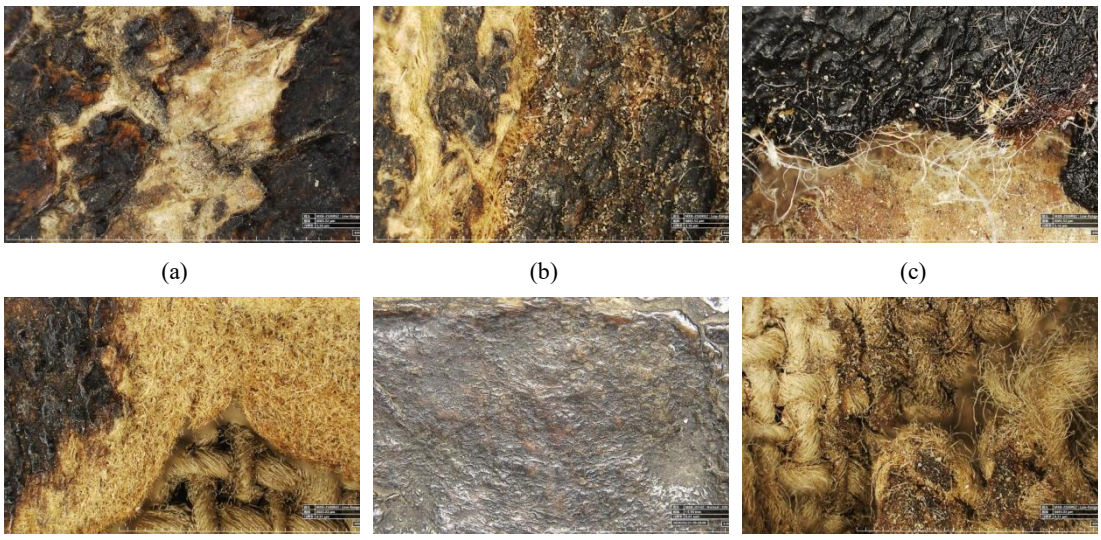
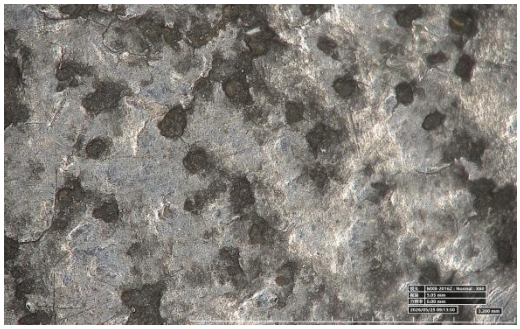


Figure 2. Principal types of surface deterioration on the cover. (a) Wear; (b) soiling; (c) stiffening; (d) tearing; (e) deformation; (f) lining fracture.



(a) Grain surface of front cover (60×)



(b) Cross-sectional morphology of front cover (100×)



(c) Grain surface of back cover (20×)



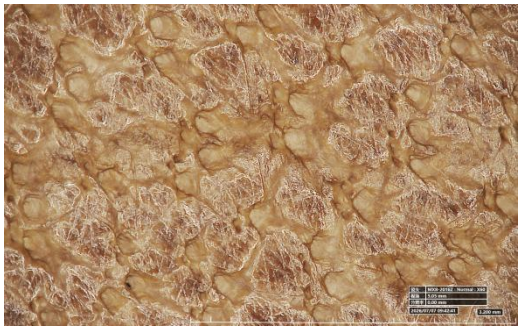
(d) Cross-sectional morphology of back cover (100×)



(e) Grain surface of spine (50×)



(f) Cross-sectional morphology of spine (100×)



(g) Grain surface of modern goat leather reference (60×)



(h) Cross-sectional morphology of modern goat leather reference (100×)

Figure 3. Grain-surface and cross-sectional morphology of historical and reference leather. (a–f) Front cover, back cover, and spine; (g–h) modern goat leather reference.

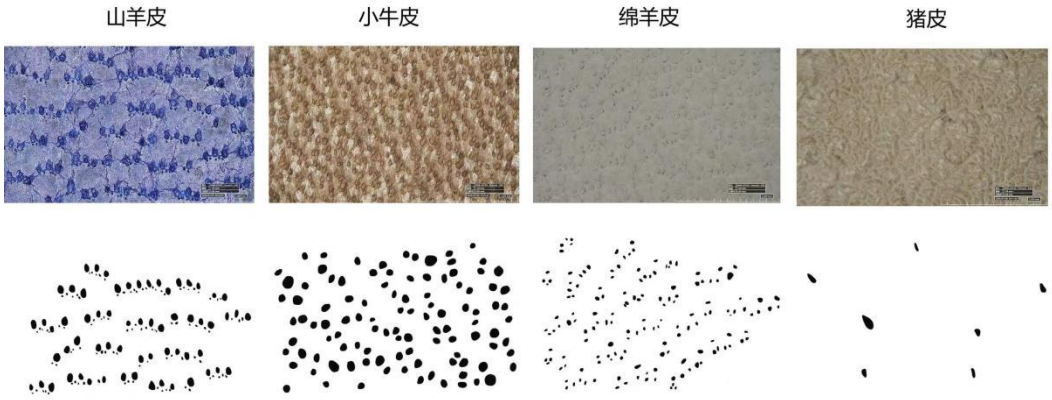
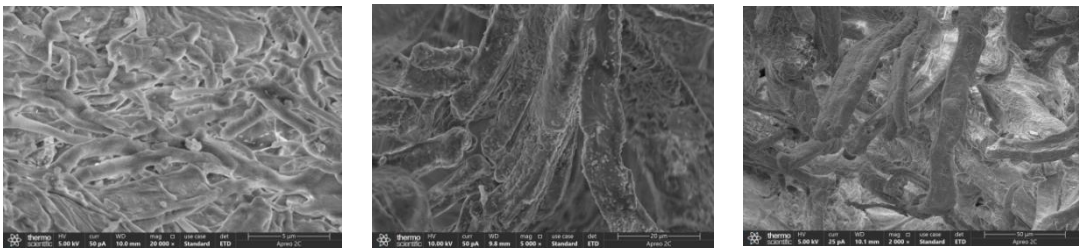


Figure 4. Schematic comparison of pore patterns in common bookbinding leathers.

2.3 SEM observations

SEM revealed markedly different preservation states among micro-samples F1, F2, and F3 (Figure 5). In F1, collagen fibrils are agglutinated and locally fused, and their boundaries are poorly defined, features consistent with gelatinization. F2 is dominated by irregular granular and agglomerated surface deposits, and an intact fibrillar network is no longer clearly visible. By contrast, F3 retains a recognizable three-dimensional reticulate arrangement of interwoven collagen fibrils, indicating comparatively better preservation of the leather microstructure.



(a) (b) (c)

Figure 5. SEM micrographs of leather from the cover and spine. (a) F1: bottom-left fold of front cover, 2000×; (b) F2: top of spine, 5000×; (c) F3: fore-edge fold of back cover, 2000×.

2.4 NIR spectroscopy results

Near-infrared spectra collected at the five measurement points are shown in Figure 6. Points A1 and A2 display a broad split doublet at 2050–2200 nm, assigned to combination bands of protein amide groups[10], together with a shoulder at 1700–1760 nm corresponding to the first overtone of C–H stretching in lipid-related components[11]. In contrast, A3, A4, and A5 show a strong narrow absorption near 2100 nm and a secondary band around 2270 nm, attributed to combined O–H and C–H vibrations of cellulose macromolecules[12]; no distinct lipid band is evident in the 1700–

1800 nm interval. All five locations show strong absorption near 1930 nm, consistent with the O–H stretching/bending combination band of water[13].

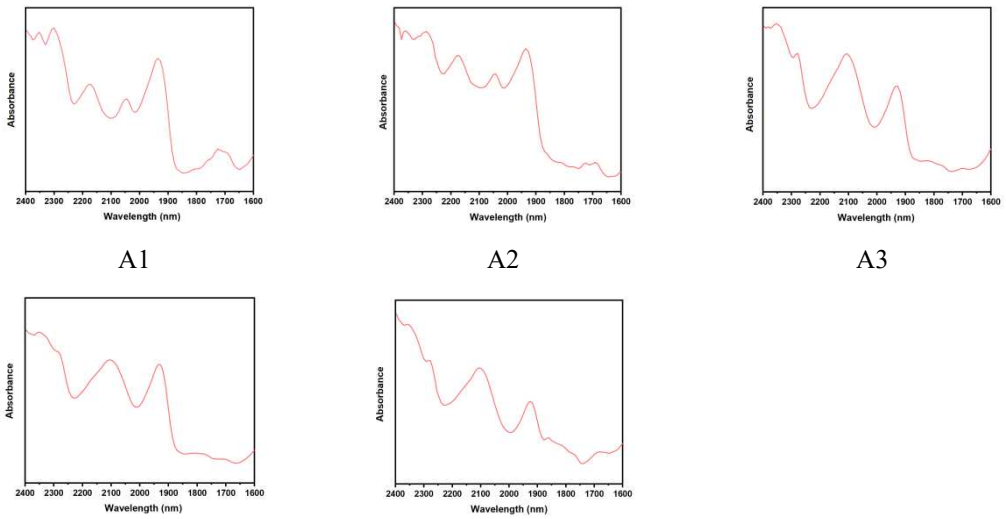


Figure 6. Near-infrared spectra obtained from different positions on the manuscript binding.

2.5 XRF results

Elemental mass fractions measured at the five points are summarized in Table 2. The balance fraction (BAL), representing the predominantly organic matrix, exceeds 94 wt% at every location. A1 contains relatively high Ca and K, no detectable S, and 0.40 wt% Fe. A2 has comparable K and Fe but higher Ca and contains 3.08 wt% S. At A3, Ca, K, and S are below detection, whereas Fe reaches 1.01 wt%. The endpaper points A4 and A5 contain Ca and K, no detectable S, and relatively low Fe; compared with A4, A5 contains higher Ca and Fe. The marked positional variation indicates that the different binding components carry distinct elemental signatures.

Table 2. Elemental composition of the book cover (wt%)

Sample	Mass fraction (wt%)						
	Al	S	K	Ca	Ti	Fe	Bal
A1	-	-	1.27	2.24	0.16	0.40	96.07
A2	-	3.08	1.20	3.73	-	0.53	94.20
A3	-	-	-	-	-	1.01	98.96

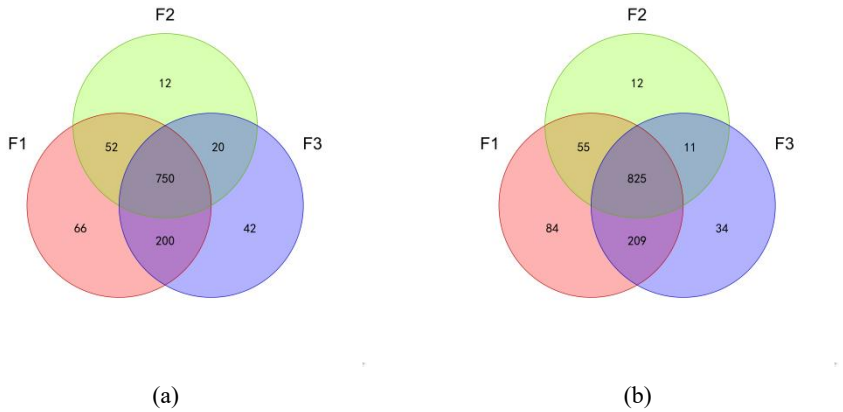
A4	-	-	1.39	2.48	-	0.25	96.28
A5	-	-	0.66	3.30	-	0.53	95.37

2.6 Proteomic results

Proteomic analysis of F1, F2, and F3 was performed by LC-MS/MS in DIA mode. Table 3 summarizes the numbers of identified proteins and unique peptides, and Figure 7 shows shared and unique proteins obtained from bovine and ovine database searches. Type I collagen was abundant in all three samples, together with keratin proteins. F2, collected from the spine, yielded fewer proteins and unique peptides than F1 and F3. Peptides from the complete casein family (α-S1, α-S2, β, κ-casein) were identified in both bovine and ovine database searches[14], and the ovine search additionally returned sheep milk-derived casein peptides.

Table 3 Protein and peptide counts from proteomic analysis

Sample	Sampling position	Number of proteins	Number of unique peptides
F1	Bottom-left fold of front cover	1068	2438
F2	Top of spine	834	1713
F3	Fore-edge fold of back cover	1012	2313



(a) (b)

Figure 7. Shared and unique proteins identified in database searches. (a) Bovine database; (b) ovine database.

3 Discussion

Macroscopic morphology and colorimetry provide the first level of evidence for assessing the manuscript's conservation state. Extensive fire damage, surface deformation, cracking, peeling, tearing, and exposure of the spine indicate severe cumulative deterioration of the binding. These changes probably reflect the combined effects of long-term use, environmental fluctuations, storage history, and localized thermal damage rather than a single aging process. Three-dimensional surface documentation complements this assessment by recording deformation and helping guide subsequent microscopic examination and targeted sampling.

The darkened reddish-yellow appearance recorded by colorimetry agrees with visual observation, but color alone cannot identify a historical tanning or dyeing process. Leather color can be influenced by original tanning and coloring materials as well as by oxidation, soiling, thermal alteration, and previous interventions. Historical tanning systems can produce characteristic color tendencies, including white tones for alum tanning, yellowish-brown hues for iron-related processing, light brown for vegetable tanning, and pale products from some sulfate-based treatments[15]. The reddish-yellow color of this cover may therefore provide contextual evidence, but technological interpretation requires confirmation from microscopy and chemical analysis.

The distribution of deterioration observed by extended depth-of-field microscopy follows the approximate order spine > front cover > endpapers. The concentration of damage at the spine is mechanically plausible because this region repeatedly experiences flexing during opening and closing and also contains adhesive layers that can age, shrink, and accumulate contaminants[7]. The observed pattern therefore reflects both the structural function of the spine and its more complex material composition.

Micromorphology provides useful but non-definitive evidence for leather-species assessment. Pore size, follicle arrangement, and cross-sectional fiber organization are established comparative criteria[8]. Pig leather generally shows large, open pores arranged in triangular groups; cow leather has smaller and denser pores and a cross-sectional gradient from compact to looser fibers[16]; sheep leather is characterized by fine pores and clustered secondary follicles[8, 17]; and goat leather commonly shows oval follicle openings arranged in groups of 3–5 in linear or curved rows, with alternating coarse and fine pores[8, 17]. The historical samples most closely resemble the goat-leather reference in these features. Nevertheless, extensive wear has removed part of the diagnostic grain pattern, and individual biological variation can further complicate visual classification[4]. Therefore, morphological comparison can only be used as a reference for identifying leather type and cannot provide definitive identification.

SEM observations further reveal the nanoscale degradation characteristics of collagen fibrils. The adhesion and fusion of fibrils observed in sample F1 are typical features of gelatinization degradation caused by fluctuations in temperature and humidity and prolonged exposure to acidic environments[18]. This finding is consistent with the degradation pattern commonly reported for historical leather from arid and semi-arid regions, where collagen gelatinisation is a major form of deterioration and repeated

temperature–humidity cycling is considered an important driving factor[5, 7]. The multi-origin granular deposits observed on the spine sample are likely composed of accumulated environmental dust, exuded lipids, and residual adhesive, which is consistent with the structural and mechanical characteristics of the spine region.

NIR spectral characteristics enable broad classification of the constituent materials. Points A1 and A2 exhibit both protein amide and lipid absorption features, consistent with animal leather. In contrast, points A3, A4, and A5 show characteristic cellulose absorption bands without clear protein or lipid signals, supporting their identification as plant-fiber materials such as linen and paper. The water absorption band detected at all positions is considered a normal feature of porous organic materials preserved for long periods under museum conditions.

Based on this material classification, XRF elemental data provide additional information regarding historical processing, post-depositional contamination, and possible restoration interventions. The high-Ca, high-K, and sulfur-free pattern at point A1 is consistent with residual products derived from traditional liming and ash-softening processes before tanning, while the presence of Fe may indicate the use of iron mordants during dyeing[15, 19]. At point A2, the substantially elevated S content, which greatly exceeds natural levels in organic materials, may be associated with sulfate-based tanning, sulfide pre-treatment, or ammonium sulfate deliming[19, 20]. This suggests technological variation across the cover or a later sulfur-containing treatment applied to the spine.

The high level of free Fe at point A3, together with the absence of elements related to tanning or papermaking additives, is most likely associated with iron gall ink staining and supports identification of this area as exposed paper substrate. The Ca and K detected in the endpapers at A4 and A5 may mainly originate from ash cooking, liming, and calcium carbonate fillers used in traditional papermaking[20-22]. The relatively high Ca and Fe contents at A5 may result from ion migration and environmental dust accumulation caused by prolonged contact with the leather cover. Overall, the elemental differences indicate that the book cover is a composite object produced from multiple materials and technologies and that its individual regions experienced different processing, aging, and intervention histories.

It should be noted that the investigated object is a historically transmitted museum collection without archaeological stratigraphy, and its dating is based only on codicological, textual, and binding-style evidence. Furthermore, XRF provides only surface elemental information and cannot distinguish clearly among original production residues, post-depositional enrichment, and later restoration materials. Therefore, all technological interpretations based on XRF should be regarded as hypotheses rather than definitive conclusions.

Proteomic analysis further supports the interpretation of tanning and binding characteristics at the molecular level. The abundant detection of type I collagen and

keratin indicates that the leather retains considerable follicular residues from the dermis and suggests that liming and unhairing were incomplete, which is consistent with traditional hand-crafted leather production. Lower protein and peptide yields at the spine are also consistent with the thick adhesive coating and severe frictional wear observed microscopically. Importantly, casein-family peptides matching both bovine and ovine databases were identified. Because casein is not endogenous to leather, its detection provides direct molecular evidence for the use of a casein-based protein adhesive in the spine binding and offers valuable molecular evidence for 17th-century Chaghatay bookbinding technology.

Sampling limitations restricted the present study to micro-samples collected from the cover edges. The DIA non-targeted acquisition mode provides broad screening capability for unknown proteins and enabled detection of the exogenous casein adhesive. However, its sensitivity and coverage for low-abundance, species-specific peptides derived from the leather itself were insufficient for definitive species identification. In future studies, if further sampling is permitted, ZooMS could be applied. This method was specifically developed for species identification of archaeological and heritage leather based on species-specific peptides from type I collagen and requires only milligram-scale samples, making it particularly suitable for museum objects subject to strict ethical sampling constraints[17].

Taken together, the morphological, spectroscopic, elemental, and molecular evidence indicates that the leather of this Chaghatay manuscript underwent incomplete unhairing, was assembled using a characteristic casein-based adhesive, and experienced different tanning and repair treatments in different regions, suggesting distinct features of regional craft practice.

From the perspective of material culture, this leather binding represents rare physical evidence of 17th-century book-production technology within the Chaghatay cultural sphere of Xinjiang. Previous research on Chaghatay manuscripts has focused mainly on philology and medical content, whereas systematic material investigation of their leather bindings remains limited. As a result, current understanding of regional bookbinding practices has depended largely on textual records and has lacked direct physical and molecular evidence.

Cross-regional comparison with international studies reveals both shared degradation patterns and possible regional differences. Leather manuscript covers from the Matenadaran collection in Armenia also exhibit collagen gelatinization and lipid accumulation, demonstrating the widespread influence of arid and semi-arid storage environments on leather deterioration[5]. However, degradation products such as calcium stearate reported in those manuscripts were not detected in the present study, suggesting possible differences in tanning procedures or adhesive formulations between regions. Similarly, 19th-century Islamic manuscript leather from Cairo shows more severe deterioration at the spine than on the cover, closely corresponding to the “spine most degraded” pattern identified here. This similarity reflects a general

mechanical characteristic of historical bookbindings: repeated opening stress and adhesive accumulation make the spine one of the most vulnerable regions of the binding structure[6, 7].

Animal-skin glue has been widely reported as a binding adhesive in Central Asian and Ottoman manuscripts. In contrast, a casein-based milk-protein adhesive was clearly detected in the present study, suggesting a potentially notable regional technological feature. Although casein adhesives were used in various ancient Eurasian crafts, direct physical and molecular evidence from 17th-century manuscripts from Central Asia and Xinjiang remains scarce. This raises an important question regarding technological transmission: whether this adhesive practice developed independently among local Chaghatay craftspeople or was introduced through cultural exchange with Persia or other Central Asian regions. Because comparable material studies of Chaghatay manuscripts remain limited, this issue cannot yet be resolved. The present dataset may therefore serve as a baseline for future regional comparative studies.

Furthermore, the manuscript should not be regarded as a homogeneous leather object but as a composite binding system consisting of leather, linen, paper, and protein adhesive. This composite structure itself increases degradation risk because the constituent materials differ in their hygrothermal expansion behavior. Aging and shrinkage of the protein adhesive may also generate additional tensile stress on the leather, thereby accelerating cracking and deformation. These interactions provide an internal explanation for the concentration of deterioration at the spine and lining interfaces.

These findings have practical implications for the conservation of similar historical books belonging to ethnic minority collections in Xinjiang. First, because collagen gelatinization is strongly affected by environmental fluctuations, strict control of temperature and relative humidity is required to avoid alternating high-temperature and high-humidity conditions and to slow collagen hydrolysis and fibril adhesion. Second, because the spine represents the weakest part of the binding system, appropriate spine supports should be considered during storage and handling to reduce tensile damage caused by repeated opening and closing. Third, high humidity and contact with acidic pollutants should be minimized to reduce metal-ion migration, fiber hydrolysis, and acid-induced deterioration. Conservation treatment should therefore consider the entire binding structure rather than focusing only on the leather, and isolated reinforcement of the leather without assessment of the linen, endpapers, and adhesive should be avoided.

This study is based on a single historically transmitted manuscript. For such collections, the possibility of historical replacement of the cover or local repair cannot be completely excluded. In addition, because of the limited sample size, the results cannot be considered representative of all 17th-century Chaghatay manuscripts and should instead be interpreted as a case study. Examination of additional comparable objects will be required to define regional bookbinding practices more reliably.

Overall, a systematic multi-analytical characterization of the leather binding of a 17th-century Chaghatay medical manuscript was conducted. The leather cover and cellulose-based endpapers were identified, molecular evidence for a casein adhesive at the spine was obtained, collagen gelatinization was confirmed, and the coupled degradation behavior of the composite binding structure was revealed. These findings provide a systematic scientific basis for the conservation of this manuscript and related heritage objects.

The current dataset provides useful evidence regarding possible pre-tanning treatment and mordant dyeing but remains insufficient for definitive species identification or direct confirmation of historical tanning agents. The study also highlights an inherent methodological challenge in the analysis of historically transmitted organic heritage: original production signals must be distinguished from post-depositional enrichment and later restoration interventions. Comparison with international studies suggests both universal degradation patterns under arid conditions and possible regional differences in adhesive formulation. Because few comparable material studies of Chaghatay manuscripts are currently available, the historical origin of the identified casein adhesive technology remains unresolved. In future work, subject to ethical sampling permission, expanded micro-sampling combined with targeted mass spectrometry could be used to further determine leather species and clarify historical tanning practices, thereby providing stronger evidence for research on Chaghatay craftsmanship and for the conservation of related manuscript collections.

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