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EXPERIMENTAL OBSERVATIONS ON PROCESSING LEATHER, SKIN, AND PARCHMENT FOR RADIOCARBON DATING

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ABSTRACT. Skin-based samples (leather, skin, and parchment) in archaeological, historic and museum settings are among the most challenging materials to radiocarbon (^{14}C) date in terms of removing exogenous carbon sources—comparable to bone collagen in many respects but with much less empirical study to guide pretreatment approaches. In the case of leather, the ^{14}C content of materials used in manufacturing the leather can vary greatly. The presence of leather manufacturing chemicals before pretreatment and their absence afterward is difficult to demonstrate, and the accuracy of dates depends upon isolating the original animal proteins and removing exogenous carbon. Parchments differ in production technique from leather but include similar unknowns. It is not clear that lessons learned in the treatment of one are always salient for treating the other. We measured the ^{14}C content of variously pretreated leather, parchment, skin samples, and extracts, producing apparent ages that varied by hundreds or occasionally thousands of years depending upon sample pretreatment. Fourier Transform Infrared Spectroscopy (FTIR) and C:N ratios provided insight into the chemical composition of carbon reservoirs contributing to age differences. The results of these analyses demonstrated that XAD column chromatography resulted in the most accurate ^{14}C dates for leather and samples of unknown tannage, and FTIR allowed for the detection of contamination that might have otherwise been overlooked.

KEYWORDS: AMS dating, FTIR, hide, leather, pretreatment.

INTRODUCTION

Animal skins have long been chemically treated by humans to maintain pliability, increase water resistance, and retard decay. Many human cultures had some way of treating animal skin for a variety of uses including making clothing, armor, horse tack, rope, tents, and tools (Kamper 2020; Kite and Thomson 2005). Because these skin-based technologies were so vital to human societies around the world and throughout time, it is imperative to be able to accurately radiocarbon (^{14}C) date such materials.

Most existing studies on ^{14}C dating skin-based technologies focus on parchment. Prior studies show that an initial solvent wash is necessary (Rasmussen et al. 2001, 2009; Donahue et al. 2002) and further treatment—either gelatinization or dilute Acid Base Acid (ABA) pretreatment—is generally recommended (Donahue et al. 2002; Brock 2013). Recent work has focused on minimizing the size of parchment samples needed to obtain an accurate ^{14}C date (Kasso et al. 2021) and using Fourier-transform infrared spectroscopy (FTIR) to identify remaining contaminants when samples pretreated with solvent washes and dilute ABA still result in ^{14}C ages that are not consistent with paleographic estimates (Kasso et al. 2023).

Leather and skin-based samples where production techniques are unknown have been studied less. One existing study compared the ^{14}C dates measured on demineralized, gelatinized, and acid insoluble remnants of skin-based samples from the Americas (Tate et al. 2020), but studies tend to focus on ^{14}C dating parchment rather than leather or samples with unknown production techniques. This is partially because leather is rare in the archaeological record,

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even in places such as the Desert West of North America where plant fiber-based technologies are relatively common in dry rockshelters (Connolly et al. 2016; Leach 2018). As a result, there are few studies evaluating the difficulties of ^{14}C dating skin-based mediums.

In this paper, we compare two pretreatment approaches for ^{14}C dating skin-based samples: ABA, in which successive acid, base and acid washes are used to remove contaminating carbonates and base soluble (e.g., humic) material, and XAD column chromatography purification, in which collagen and contaminants are hydrolyzed in concentrated acid to individual amino acids and other molecules, and passed through a column that retains polar compounds to isolate the eluted collagen amino acid. The former method is typically used for wood or charcoal samples. The latter is a technique developed to purify heavily contaminated bone gelatin (Stafford et al. 1988). In theory, XAD should be an appropriate pretreatment for ^{14}C dating leather because XAD was developed to purify amino acids from bone proteins, primarily type I collagen. Type I collagen is the most abundant type of collagen. Its densely packed triple helixes provide structure for skin and bone tissues. Leather can be viewed as an analog for heavily contaminated bone collagen because the process of turning skin into leather involves complexing the collagen with tanning agents and coating the collagen fibers in lipids to ensure pliability (Groenman-van Waateringe et al. 1999; Kite and Thomson 2005; Covington 2009). Further, because skin is much more porous than bone and the collagen molecule is unprotected by a crystalline inorganic matrix, it can also exchange organic molecules with its environment more rapidly than bone and these contaminants can more easily bond with the collagen molecule. Thus, we tested XAD column chromatography purification rather than ultrafiltration which retains contaminants larger than the nominal molecular weight cutoff (MWCO) of the filter e.g., 30kDa. We analyzed C:N ratio measurements and used Fourier Transform Infrared Spectroscopy (FTIR) to detect exogenous components and explain differences in F^{14}C values for different pretreatments of the same leather samples (DeNiro 1985; DeNiro and Weiner 1988; van Klinken 1999).

BACKGROUND

Manufacturing Skin-Based Materials

Across space and time, human cultures have subjected animal skins to a variety of chemical and physical treatments to create durable materials for specific functions. When attempting to ^{14}C date skin-based samples, any information known about the processing method should be considered, as different chemicals with potentially varying ^{14}C ages will be introduced into the skin, and the chemical structure of the skin collagen will change depending on the manufacturing method. A few widely used and relevant manufacturing processes are summarized below. This is not an exhaustive list of skin manufacturing techniques, and the described techniques may vary widely. Skin manufacturing methods are discussed in greater detail in specialist sources (Covington 2009; Kite and Thomson 2005).

Brain and Smoke Tanning

Brain and smoke tanning, while separate techniques, can be used in concert and have been used to produce leather worldwide since at least the late Pleistocene (Gilligan 2010; Ruth 2013; d'Errico et al. 2018). Leather produced in the Americas is often tanned with brains and/or smoke (Riggs 2017; Hayden 2022). First, the skin is defleshed to remove any adhering fat, muscle, or connective tissue. The skin may also be dehaired, during which time the epidermis of the skin is also removed. The skin may be soaked in water at this point. An animal brain paste is then worked into the skin and the skin is manually worked to soften it. Brain tanning is a type

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of oil tanning, where phospholipids from the brain lubricate the skin, but the exact chemical change that occurs in the collagen is not understood (Covington 2005); some research has suggested that oils added to skin do not create chemical bonds to the collagen (Covington 2005; Rasmussen 2009). Because brain tissue cannot be easily stored for long periods of time without rotting, the ^{14}C age of the brain used should be contemporary with the skin and this tanning process should have no effect on the ^{14}C date. Other types of oil tanning could affect the apparent ^{14}C age if the oil used has a different ^{14}C age than the skin. After softening, the skin is stretched and allowed to dry. Sometimes the skin will be scraped again to remove more hair or thin it to increase flexibility. Smoke tanning is usually performed after brain tanning. The skin is stretched over a smoking fire and aldehydes and phenols from the smoke covalently bond with amine groups in the collagen (Covington 2005). Smoke tanning waterproofs the leather, adds color, and preserves the skin against microbial degradation (Doyal and Kite 2005). This step could change the apparent ^{14}C age of the skin, as carbon from the aldehydes and phenols in the wood smoke could derive from wood older than the skin, i.e., scavenged relict wood (Schiffer 1987), or inner wood (Dee and Bronk Ramsey 2014).

Vegetable Tanning

Vegetable tanning has been used worldwide for centuries. Vegetable and chrome tanned leathers are created by complexing the collagen molecules with either vegetable tannins or metal ions to delay microbial degradation and preserve pliability (Groenman-van Waateringe et al. 1999; Thomson 2005). Skins are defleshed mechanically, then limed. Liming a skin involves immersing it in an alkaline solution (usually calcium hydroxide ($Ca(OH)_2$), sodium sulfide (NaS_2) or sodium hydroxide (NaOH)) which helps to dehair the skin by breaking disulfide bonds in keratin and the epidermis, makes the skin whiter and more absorbent by removing ~50% of the subcutaneous lipids through saponification of the triglyceride esters, and deamidates some aspartic and glutamic acid amino acids of the collagen which opens up the collagen structure so other solutions (such as tannins and fatliquors) can more easily penetrate the collagen triple-helix (Menderes et al. 1999; Doherty et al. 2021). Liming can introduce contamination into the skin through a reaction with atmospheric CO_2, which is incorporated into the skin as $CaCO_3$, $NaCO_3$, $NaHCO_3$, though the added carbon should be contemporary with the liming process. Liming also makes the skin more susceptible to future gelatinization and hydrolysis of the amino acid backbones of collagen's triple helix (Menderes et al. 1999). After liming, the skin is defleshed again mechanically and the epidermis and hair is completely removed. Thick skins (such as cow) may be split into two different layers, then the skin is delimed via a water wash (decalcination and deliming agents such as acid may be added), then hides are immersed in solution of water and vegetable tannins. Before the invention of rotating drums in the nineteenth century, hides were soaked in vats over a period of weeks or months, with the hides being moved gradually into vats with higher concentrations of tannins (Thomson 2005). After the nineteenth century, a rotating drum was used to decrease the amount of time necessary for the skins to absorb tannins to days. Tannins replace some water molecules from the skin, forming hydrogen bonds with collagen and stabilizing the triple helix structure of the collagen (Covington 2009). Tannins, often derived from plant bark, introduce exogenous carbon into the skin, but the ^{14}C age of the tannins should be contemporary with the tanning process (albeit skewed as an averaged ^{14}C content of the last several years of inbuilt age). After tanning, hides are then pressed to remove excess water, a second tanning with a different tannin could be carried out, the leather could be dyed, and finally the leather undergoes fatliquoring (adding oil to make leather soft and smooth) and is pressed and dried again. The final step, fatliquoring, is a source of potential contamination. Oils used for fatliquoring may be

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contemporary with the leather if they are plants or animal in origin, but petroleum-based oils can introduce carbon of a vastly different ^{14}C age and oiling may be an ongoing practice of leather maintenance for the life of the object.

Parchment Production

Skin processed to form a writing surface is known as parchment. First, the skin is defleshed, then it is limed. Liming for parchment is similar to the liming processing for vegetable tanned leather and can introduce the same contamination (Doherty et al. 2021). After liming, the skin is defleshed again to remove the epidermis and adipose tissue, then soaked in water (sometimes with added acid) to delime the skin. Further manual processing: stretching, scraping, pouncing (abrading with pumice stone to produce a uniform surface) is carried out as needed to turn the skin into an even-surfaced and thin material ideal for writing.

Reuse and Conservation

Of equal concern to the accuracy of the ^{14}C date are contaminants introduced in re-use and or conservation of skin-based materials. Because processing skin is labor intensive, the resulting products are often reused. Parchments become palimpsests, leather is oiled to restore pliability, and all types of skin-based material can be cut into smaller pieces and reused. These processes can introduce ^{14}C contamination of a younger (or older) age into a skin, as when a parchment is written on with much younger ink, or a saddle is oiled with the ^{14}C dead petrochemical neatsfoot oil. Repairs made to a book with younger parchment or scraps of older leather used to patch or sew a hide can create a minefield of ^{14}C treachery that confounds attempts to date the creation of a book, saddle, or hide.

SAMPLE DESCRIPTION

In the spring of 2022, Penn State Radiocarbon lab was asked to ^{14}C date several skin-based samples (see Table 1). Archaeologists from the University of Oregon and the University of Nevada, Reno were working to ^{14}C date four skin-based samples from museum collections excavated from Cougar Mountain Cave, Oregon, and a bookseller asked the Penn State Radiocarbon Lab to ^{14}C date parchment from a late medieval text. To test pretreatment protocols ahead of processing these unknowns, we acquired two contemporary vegetable tanned leather samples from Pergamena (a manufacturer of traditional vegetable tanned leathers) and an untanned rabbit skin.

Sample Description and Context

Contemporary Vegetable Tanned Leather

We obtained two contemporary leather samples from Pergamena. These leather samples are undyed vegetable tanned crusts from a goat and cow. The exact year when these animals were slaughtered is unknown, but it was within the past twenty years. The goat was tanned with mimosa bark and the cow with chestnut bark. We also obtained samples of these two tannins from Pergamena.

Contemporary Untanned Skin

We also obtained a rabbit skin from central California dating between 1985 and 1990 CE. The skin was part of a handmade craft object and attached to a wooden shaft with tacks (no obvious adhesives). It shows no sign of microbial degradation when examined under a stereoscope. This

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Table 1 Sample list.

Sample	Material	Age	Source	Provenance
Goat leather	Vegetable-tanned leather (mimosa)	AD 2005 to present	Pergamena	Unknown
Cow leather	Vegetable-tanned leather (chestnut)	AD 2005 to present	Pergamena	Unknown
Rabbit skin	Untreated skin	AD 1985–1990	Rabbit skin collected in the late 1980s by Culleton	Central California
CMC21-1	Pelt	Sagebrush cord (RR28) in hide dated: 10,315 ± 35 BP	Favell Museum	Cougar Mountain Cave, Oregon
CMC21-3	Pelt	Elk hair (RR30) on hide dated: 1040 ± 15 BP	Favell Museum	Cougar Mountain Cave, Oregon
CMC21-4	Pelt	Unknown	Favell Museum	Cougar Mountain Cave, Oregon
CMC21-5	Pelt	Fiber cord (RR32) in leather dated: 8085 ± 30 BP	Favell Museum	Cougar Mountain Cave, Oregon
IM2	Parchment	Late Medieval	Private collection	Unknown

rabbit skin was included in the study to understand how an untanned, undegraded skin would respond to various pretreatments.

Archaeological Samples

The four archaeological samples (CMC21-1, CMC21-3, CMC21-4, and CMC21-5) are from Cougar Mountain Cave (35LK55), located in the Fort Rock Basin of central Oregon. It was excavated by an amateur in the 1950s (Cowles 1960) and until recently the materials he recovered remained unstudied first in a private collection and then in museum display cases. The relative dryness of the cave meant that an astonishing array of organic materials spanning the late Pleistocene and throughout the Holocene survived—including seed piles, hide strings, fur, feathers, wooden tools, and many fiber-based textiles. After Cowles' death, most of the materials were transferred to the Favell Museum in Klamath Falls, Oregon. Recently, a team from the University of Oregon Museum of Natural and Cultural History, University Nevada, Reno, and the Penn State Radiocarbon lab began a collections-based project of Cougar Mountain Cave from which the materials in this paper are derived (Rosencrance et al. 2019).

We describe the Cougar Mountain Cave artifacts in this study as skin-based samples of unknown tanning technique because the animals' species has not yet been determined and whether these skin-based samples underwent any tanning procedures (or what kind) is unknown. These skin-based samples are very well preserved; some are still pliable with identifiable hair follicles (see supplemental Figure 1 for picture and stereoscope images of these and other samples). All samples had more than 200mg of material which could be used for this analysis. We selected three of the four samples (CMC21-1,3 and 5) because they had organic material, either cord or hair, incorporated as stitching (cord) or still present on one surface (hair) which could be ^{14}C dated for comparison. CMC21-4 has some surface hairs, and we thought these might provide a similar comparison. However, the hairs did not survive pretreatment, and could not be ^{14}C dated. RR28 is a 2-ply s-twist fiber cord made of sagebrush (*Artemisia* spp.) bark sewn into CMC21-1. RR32 is a 2-ply s-twist fiber cord made of dogbane (*Apocynum*) sewn into CMC21-5. RR30, the hair on the dorsal side of CMC21-3, compares most favorably with elk (*Cervus canadensis*).

Parchment Sample

The final sample was a piece of parchment from a medieval manuscript sent to the Penn State Radiocarbon Lab by a private individual. No ink or conservants were visible on the sample surface, but it was still possible that this manuscript might have been treated with a conservant.

METHODS AND CHEMICAL CHARACTERIZATION

Sample Pretreatment Prior to ^{14}C Analysis

Sample pretreatment and AMS ^{14}C measurements were performed at the Penn State Radiocarbon Lab, except for the three organic samples paired with the archaeological hides. These paired organic samples were mechanically and chemically cleaned and combusted under vacuum to yield CO_2 in the University of Nevada, Reno Human Paleoecology and Archaeometry lab, and then graphitized and measured at Penn State.

All sample vessels were baked before use, and ASTM Type I water (18.2MOhms/cm resistivity and Total Organic Content: 1–5 ppb) was used for water washes and to make reagents for sample pretreatment. At each processing step, subsamples were retained for analysis (see Figure 1). Around 1 gram of the contemporary veg-tanned leathers were initially processed and

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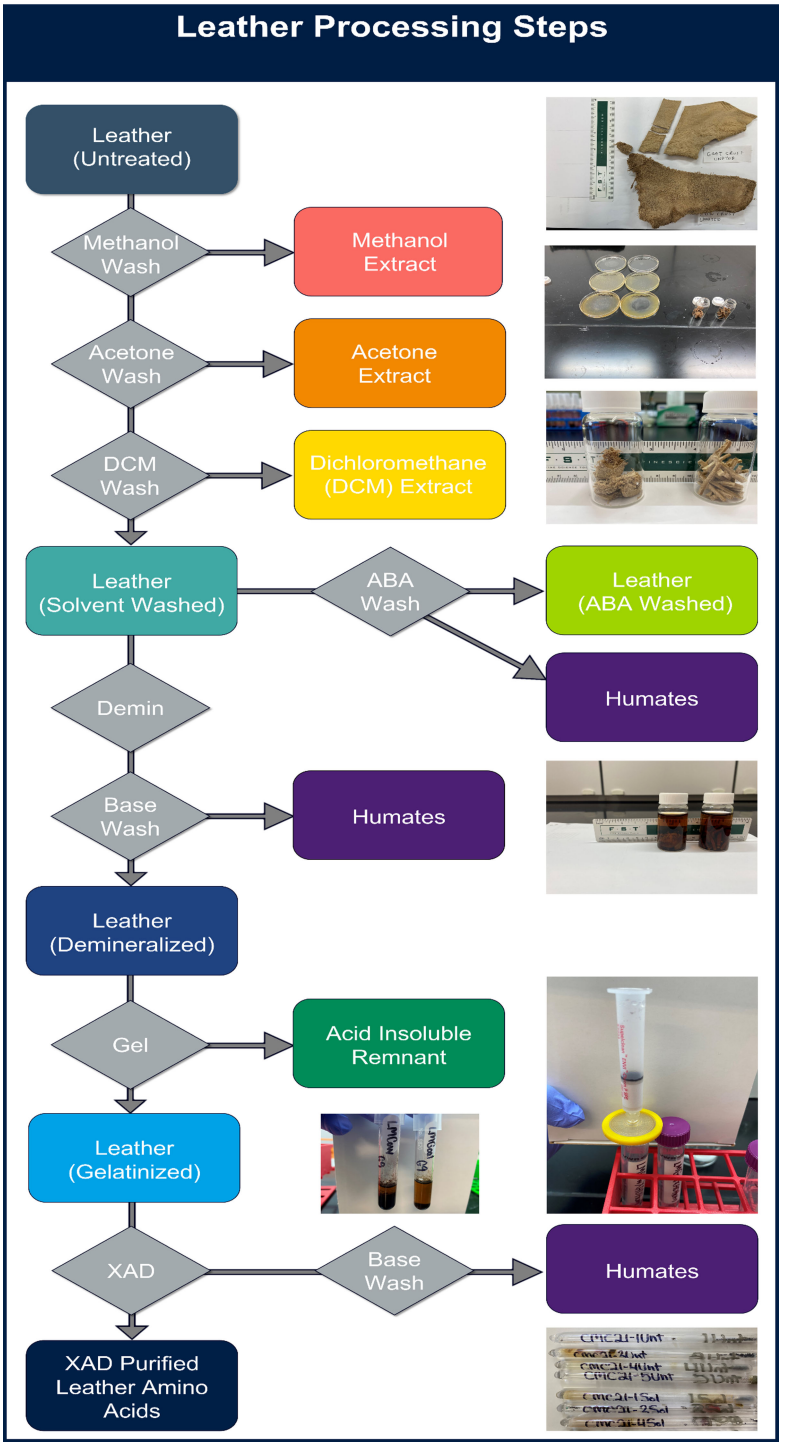

Figure 1 Flowchart of sample processing, with diamonds indicating chemical pretreatment steps, and rectangles indicating subsamples or extracts whose radiocarbon ratio, FTIR spectrum, stable isotopes, and C:N ratio were measured when sample mass permitted.

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around 100 mg of the untanned rabbit skin and archaeological samples were processed. The large sample size was intended to provide enough material for subsampling at each step and analysis of extracts. Samples were manually surface cleaned and sonicated in water, solvent washed through sonication in successive 20-min washes of methanol, acetone, and dichloromethane at room temperature (except for the parchment sample, which was sonicated in each solvent for an hour). Solvents were retained and evaporated to collect extracted material. An organics-style dilute ABA was performed on roughly 40-mg subsamples of the two contemporary leather samples and RR30 (elk hair), with 0.1N hydrochloric acid (HCl) wash at 70°C for 20 min, repeated 0.1N sodium hydroxide (NaOH) washes at 20°C for 20 min each, a second 0.1N hydrochloric acid (HCl) wash at 70°C for 20 min, and two water washes at 70°C for 20 min each. Base washes were retained, and any humates precipitated with acid for additional analyses. This organics style ABA pretreatment was not carried out on any other skin-based samples due to poor yields of the contemporary leather samples. The fiber cord samples (RR28 and RR32) were subjected to a standard organics style ABA, with 1N NaOH at 70°C for 20 min and 1N HCL at 70°C for 20 min.

All other skin-based samples continued to be pretreated following a bone pretreatment protocol. Samples were demineralized in 0.5N HCl at 4ºC for 16 hr, water washed to neutrality, washed in 0.1N NaOH for 10 min at 20°C at least once, washed in 0.1N HCl for 10 min at 20°C, then water washed again to neutrality. The base wash was again retained and any humates precipitated with acid. Samples were then gelatinized, with 0.01N HCl at 60°C for 10 hr (Longin 1971). Gelatinization was repeated until either enough collagen gel had been collected, or no collagen was present in previous gel. Sample gels were frozen with liquid nitrogen and lyophilized so the gels could be weighed and the quality of gelatin assessed. All samples yielded enough gelatin to proceed to XAD purification.

Gelatin was hydrolyzed at 100°C with 6N HCl for 24 hr. The amino acid hydrolysate was then purified on an equilibrated SigmaAldrich ENVI-Chrom styrene divinylbenzene column, using 6N HCl as the mobile phase (adapted from Stafford et al. 1988). Purified material was concentrated with a LabConco CentriVap and freeze dried. The columns were then flushed with 1N NaOH, and compounds flushed from the column with the base were precipitated with acid and concentrated for analysis.

All subsamples and extracts were packed for ^{14}C dating in baked quartz tubes with 60mg of CuO wire and Ag wire to trap sulfur and chlorine compounds. Friable or viscous samples were loaded into the quartz tubes using baked Ag capsules. Sample tubes were sealed under vacuum and combusted at 900°C for 3 hr except for XAD processed samples, which were combusted at 800°C. (NB: XAD samples are combusted at a lower temperature to reduce the likelihood of CuO and Ag wire/capsules melting as a eutectic alloy [e.g., Cu28%/Ag72% = 777°C] influenced by the presence of chlorides from the XAD process (pers. comm. J. Southon 2007). Resulting CO_2 was cryogenically purified and graphitized through the Bosch reaction on baked iron powder catalyst at 550°C for 3 hr (Vogel et al. 1984) with an excess of hydrogen gas and $Mg(ClO_4)_2$ desiccant to draw off reaction water (Santos et al. 2004).

Sample graphite was pressed into aluminum targets for AMS measurement along with graphite of 6 OXII primary standards, and corresponding process backgrounds and secondaries. Process backgrounds were intended to replicate the pretreatment conditions each unknown or extract was subject to, and to reflect the net removal and introduction of exogenous carbon to the sample inclusive of each processing step and any background introduced by the glassware

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or reagents (e.g., Ag wire, Ag capsules, and CuO wire during combustion; H_2 gas and Fe powder during graphitization). Machine backgrounds on the AMS, determined by measuring unprocessed Alfa Aesar graphite (Lot# W24B025) averaged $F^{14}C = 0.0011$; combustion and graphitization backgrounds on APCS coal (POC#3) averaged $F^{14}C = 0.0020$; ABA and solvent wash backgrounds on Pleistocene Prophet River, BC, wood averaged $F^{14}C = 0.0026$ (these were also applied to the solvent extracts themselves); and Latton Mammoth bone used for XAD hydrolyzates had a background of $F^{14}C = 0.0063$. All background $F^{14}C$ values are assigned a 30% error to account for variations from run to run; in practice per run counting errors are on the scale of 3–8%. In addition, IAEA-C5 was processed as ABA and solvent wash checks (n=3; measured $F^{14}C = .2308 \pm 0.0010$; consensus value 0.2305 ± 0.0002).

AMS ^{14}C measurements were made on a modified NEC 500kV 1.5SDH-1 compact AMS at the Penn State AMS ^{14}C laboratory. $F^{14}C$ and conventional ^{14}C ages were corrected for fractionation occurring during graphitization and measurement with $\delta^{13}C$ values measured on the AMS following the conventions of Stuiver and Polach (1977) and Reimer et al. (2004).

Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectra were measured on all extracts and leather sample fractions of the two contemporary leather samples and on the untreated, gelatinized, acid insoluble, and XAD pretreated fractions of the archaeological hide and rabbit skin, and sample fractions of the parchment, where sample mass permitted. Attenuated total-reflectance (ATR) FTIR spectra were measured with a Bruker 70 Vertex Spectrometer equipped with a Diamax DGTS detector with a zinc selenium coated diamond crystal. Spectra were recorded from 4000–500 cm^{-1} with a scan speed of 10Hz for 100 scans at a resolution of 6 cm^{-1}.

C:N Ratios

C:N ratios were calculated from measured abundances of carbon and nitrogen (DeNiro 1985; DeNiro and Weiner 1988; van Klinken 1999) on all extracts and leather sample fractions of the two contemporary leather samples, on the untreated, gelatinized, acid insoluble, and XAD pretreated fractions of the archaeological hide and rabbit skin, and on sample fractions of the parchment, where sample size permitted. These measurements were made at YASIC (Yale Analytical and Stable Isotope Center) on a Costech ECS 4010 Elemental Analyzer. Measurements are reported as atomic C:N ratios.

RESULTS

ABA Yields

Vegetable tanned samples pretreated with organics style dilute ABA (0.1N HCl at 70°C for 20 min; repeated 0.1N NaOH washes at 20°C for 20 min until base was clear; 0.1N HCl at 70°C for 20 min; dH_2O at 70°C for 20 min twice) had very low yields. For the cow leather, the ABA yield was 3.9% and for the goat leather the ABA yield was 5.3% (Supplemental Table 1). Usually, the base washes are repeated until the last base wash remains clear and colorless, indicating that no more material is being extracted from the sample by the base. For the contemporary vegetable tanned leather samples, this change never occurred. Repeated base washes continued to be opaque dark brown until they were stopped because of sample degradation. Due to the extremely poor yields, this process was not used on any other samples.

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An ABA step was also performed in the bone style processing. Demineralization and base wash are effectively an ABA pretreatment: 0.5N HCl at 4°C overnight; 0.1N NaOH at 20°C for 10 min; 0.1N HCl at 20°C for 10 min; two dH_2O washes at 20°C for 10 min. Yields for this processing were higher at 62% for the vegetable-tanned goat leather, 49% for the vegetable-tanned cow leather, and 72% for the medieval parchment IM01 (Supplemental Table 1). The parchment yield from the bone style ABA is consistent with previous yields of ABA processed parchment, 69.3–96.7% observed by Brock (2013).

Gelatin Yields

Skin-based samples should have high gelatin yields because most of the sample weight is composed of collagen. While bone gelatin yields below 2–4% indicate that the bone gelatin is degraded (DeNiro and Weiner 1988; van Klinken 1999), previous tests of gelatinizing historical parchment show gelatin yields by mass ranging from 46.7–71.3% (Brock 2013) and 32.4–98.3% (Doherty et al. 2023). Experimental results have also shown that contemporary unprocessed skin samples have average gelatin yields of 70%, slightly higher than the average 63% yield of parchment (Doherty et al. 2021). For skin-based samples of unknown tanning technique, gelatin yields ranged from 8–71% (Tate et al. 2020).

We calculated gelatin yields (Table 2) by comparing dry mass of the initial samples with the dry mass of extracted gelatin. We lyophilized samples to ensure they were dry. The gelatin yields for the contemporary vegetable tanned samples are low: 20% gelatin yield for the goat leather and 16% for the cow leather. These low yields are largely due to incomplete gelatinization. Because the initial sample size was large, time constraints meant that gelatinization was not taken to completion. However, the samples also lost 13% (goat leather) and 39% (cow leather) of their mass in the solvent washes (Supplemental Table 1), which decreased the gelatin yield. The untreated rabbit skin had a gelatin yield of 69%, CMC21-1 had a gelatin yield of 45%, CMC21-3 had a gelatin yield of 49%, CMC21-4 had a gelatin yield of 61%, and CMC21-5 had a gelatin yield of 38%. The parchment sample (IM01B) had a gelatin yield of 36%. None of these samples had gelatin yields that were low enough to raise concerns about the quality of the gelatin for ^{14}C dating.

We did not calculate the yields of XAD purified amino acids because the hydrolysis of the gelatin introduces water molecules into the sample.

C:N Ratios

C:N ratios were calculated from the measured abundances of carbon and nitrogen (see Table 2 and Supplemental Table 1). Our measurements are compared to the "acceptable" bone collagen value of 2.9–3.6 (as established by DeNiro 1985; DeNiro and Weiner 1988; van Klinken 1999), and a theoretical expected value of 3.243 (Schwarcz and Nahal 2021). In general, a higher C:N ratio is due to contamination with carbon-rich material like humic acids from soil (Schwarcz and Nahal 2021), or in the case of leather, chemicals used in tanning or later conservation of samples.

The C:N ratios of several of the untreated samples are within the acceptable C:N ratio: the untreated rabbit skin; CMC21-4; CMC21-5; and the parchment sample. For all other samples, the C:N ratio of the untreated sample was too high. Processing steps generally decreased the C:N ratios of the samples, and material removed from the samples by processing was, with a few exceptions, too carbon rich to be pure collagen, indicating that the processing steps did remove

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Table 2 Sample yields, radiocarbon content, and C:N ratio for untreated, gel, and XAD processing steps.

PSU AMS#	Sample ID	Description	% Yield	$F^{14}C$	±	^{14}C age (BP)	±	C:N Ratio	%C	%N
10757	GoatUnt	Surface cleaned goat leather	990 mg	0.9164	0.0015	700	15	5.44	44.1	9.5
10888	GoatGel	Gelatin from goat leather	20%	1.0008	0.0016	0	15	3.25	42.5	15.2
10960	GoatXAD	XAD purified amino acids		1.0071	0.0017	–50	15	3.07	26.0	9.9
10758	CowUnt	Surface cleaned cow leather	1120 mg	0.8981	0.0015	865	15	7.27	54.2	8.7
10887	CowGel	Gelatin from goat leather	16%	1.0201	0.0015	–155	15	3.79	40.5	12.4
10959	CowXAD	XAD purified amino acids		1.0148	0.0018	–115	15	3.10	28.8	10.9
10831	RabUnt	Surface cleaned rabbit skin	100 mg	1.126	0.0018	–950	15	3.33	40.0	14.0
10892	RabGel	Gelatin from rabbit skin	69%	1.2022	0.0017	–1475	15	3.12	46.0	17.2
10961	RabXAD	XAD purified amino acids		1.2025	0.0017	–1475	15	2.99	27.1	10.6
10691	RR28	Fiber cord in CMC21-1		0.2768	0.001	10315	35	NM	NM	NM
10893	CMC21-1Unt	Surface cleaned sewn hide	98 mg	0.3103	0.0019	9400	50	4.04	26.5	7.7
10905	CMC21-1Gel	Gelatin from sewn hide	45%	0.2752	0.0009	10365	30	3.23	34.3	12.4
10970	CMC21-1XAD	XAD purified amino acids		0.2733	0.0016	10420	50	3.20	27.3	10.0
10693	RR30	Elk hair from CMC21-3		0.8786	0.0014	1040	15	NM	NM	NM
10894	CMC21-3Unt	Surface cleaned hide	92 mg	0.8834	0.0013	995	15	4.32	51.1	13.8
10906	CMC21-3Gel	Gelatin from hide	49%	0.8794	0.0012	1035	15	3.26	40.5	14.5
10971	CMC21-3XAD	XAD purified amino acids		0.8875	0.0015	960	15	3.16	27.7	10.2
10895	CMC21-4Unt	Surface cleaned hide string	128 mg	0.282	0.0009	10170	30	3.27	34.7	12.4
10907	CMC21-4Gel	Gelatin from hide string	61%	0.275	0.0009	10370	30	3.29	42.9	15.2
10972	CMC21-4XAD	XAD purified amino acids		0.2727	0.0017	10440	50	3.06	27.0	10.3
10694	RR32	Fiber cord in CMC21-5		0.3655	0.0012	8085	30	NM	NM	NM
10896	CMC21-5Unt	Surface cleaned sewn hide	125 mg	0.3773	0.0009	7830	20	3.40	27.9	9.6
10908	CMC21-5Gel	Gelatin from sewn hide	38%	0.3709	0.0011	7970	25	3.29	33.1	11.7
10973	CMC21-5XAD	XAD purified amino acids		0.369	0.0016	8010	35	3.15	22.9	8.5
11206	IM02	Surface cleaned parchment		0.9600	0.0017	330	15	3.31	39.4	13.9
11205	IM01	ABA'd parchment	72%	0.9512	0.0015	400	15	NM	NM	NM
11368	IM01B	Gelatin from macerated parchment sample	36%	0.9580	0.0015	345	15	3.20	41.3	15.1

non-collagen material from the samples. Material we extracted from the contemporary leather with solvents had very high C:N ratios, ranging from 43.67 for the GoatMethExt to 138.19 for the CowMethExt. Base washes have previously been shown to improve the C:N ratios of historical parchment (Brock 2013), and that result is corroborated here. Not only did base washes lower the C:N ratios of samples, but C:N ratios of the material extracted by the base washes after demineralizing the archaeological samples range from 4.78–6.41. All the samples had acceptable C:N ratios after gelatinization, except for the contemporary cow leather, which at 3.79, was anomolously higher than expected. The C:N ratios of the acid insoluble material that remained after gelatinization ranged from 3.83–6.84 and were too high to be pure collagen, except for the acid insoluble material from the goat leather. This is probably due to incomplete gelatinization of the goat leather, leaving behind a large amount of gelatin in the acid insoluble fraction. The XAD purified amino acids from all the skin-based samples had C:N ratios that were consistent with collagen, and the material rinsed from the XAD column with base had C:N ratios that were too high. The relatively lower C:N ratios of the XAD purified material is partially due to selective retention of certain amino acids (Doherty et al. 2021; Schwarcz and Nahal 2021), on the XAD column but is primarily due to retention of nonpolar and carbon rich contamination (Stafford et al. 1988).

Radiocarbon Measurements

Fraction Modern ($F^{14}C$) was measured on subsamples of each processing step (Table 2 and Supplemental Table 1). We chose to report and discuss $F^{14}C$ (i.e., the normalized and $\delta^{13}C$-corrected ^{14}C concentration of the sample with respect to the modern standard; Reimer et al. 2004) because it is more meaningful than ^{14}C age for many of these samples. Because the ^{14}C in these samples comes from a mixture of carbon from many differently aged reservoirs—skin collagen and other proteins, soil humates, potentially $CaCO_3$ from liming, skin lipids, tanning lipids, tannins, and many other tanning chemicals—we want to think in terms of the ^{14}C concentration of the observed mixture rather than its apparent age, which we assume *a priori* to be incorrect.

Contemporary Skin-Based Samples $F^{14}C$

The untreated contemporary samples: veg-tanned leather from a cow (Figure 2) and goat (Supplemental Figure 2), and untanned rabbit skin (Supplemental Figure 3) had a lower $F^{14}C$ than expected, and as we pretreated the samples the $F^{14}C$ increased. This change reflects the pretreatment steps' removal of tanning chemicals with a lower $F^{14}C$ from the leather, resulting in more carbon endogenous to the animal skin being measured and a higher $F^{14}C$.

The degree of change in $F^{14}C$ is large: hundreds of years in apparent ^{14}C age. The untreated goat leather (PSUAMS-10757: $F^{14}C = 0.9164 \pm 0.0015$) had an apparent ^{14}C age of 700 ± 15 BP while the XAD purified amino acids from this leather (PSUAMS-10960: $F^{14}C = 1.0071 \pm 0.0017$) were apparently –50 ± 15 BP. A similar change ($F^{14}C = 0.8981 \pm 0.0015$ to 1.0148 ± 0.0018 or 865 ± 15 BP to –115 ± 15 BP) occurs in ^{14}C measurements of the untreated cow leather (PSUAMS-10758) to the XAD purified amino acids from the cow leather (PSUAMS-10959). The degree of variation in the contemporary vegetable tanned leather is caused by ^{14}C dead petrochemicals ($F^{14}C=0$) used in the tanning process. Pergamena's vegetable tanned leathers are manufactured using fatliquors (which help the leather remain soft and pliable) with a range of petrochemical content from 2–70% depending on the specific product, and a surfactant composed entirely of petrochemicals (pers. comm., J Meyer, Oct. 2022). The 865 ± 15 BP ^{14}C date on untreated contemporary cow leather is a measurement of the mixture of dead carbon from the tanning chemicals, additional contemporary

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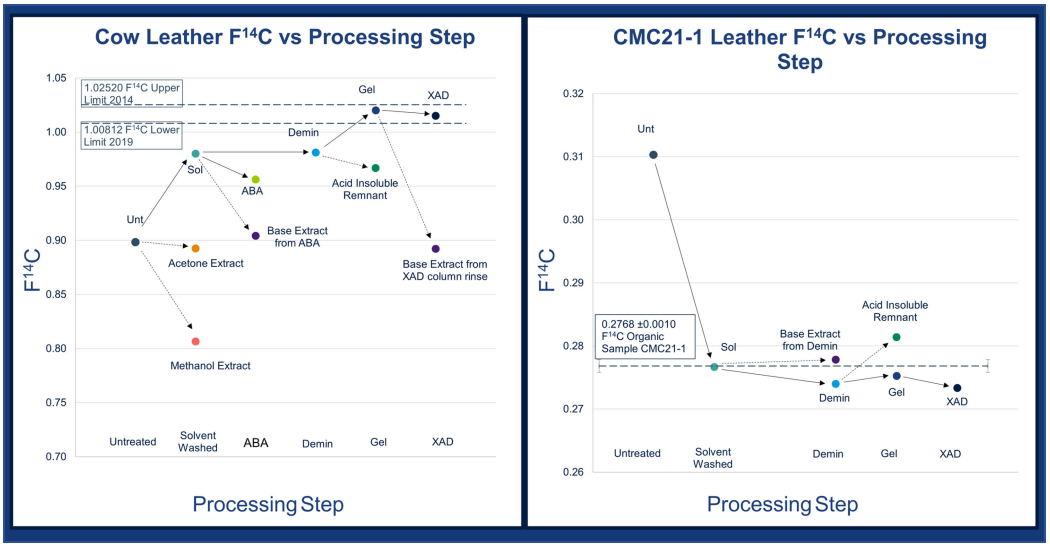

Figure 2 $F^{14}C$ at each processing step for contemporary vegetable-tanned cow leather (left) and archaeological hide sample CMC21-3 (right). Changes in $F^{14}C$ with each processing step are shown with arrows, and any known $F^{14}C$ sample values for the samples are plotted.

carbon like tannins used to manufacture leather, and the carbon from the cow skin. The ^{14}C measurements of material extracted from the contemporary leather in methanol washes, acetone washes, base washes, gelatinization, and XAD purification similarly show that ^{14}C depleted material is being removed from the leather by these processing steps.

Archaeological Samples $F^{14}C$

In contrast to the contemporary leather, the untreated archaeological samples had higher $F^{14}C$ values than $F^{14}C$ values of the paired organic samples, and the $F^{14}C$ of the hide samples generally decreased with each processing step (Figure 2 and Supplemental Figures 4–6). The contamination removed from the samples by these processing steps is likely younger (more ^{14}C enriched) carbon that sank down into lower layers of the cave sediments where the samples were deposited (Rosencrance et al. 2019). The change in the $F^{14}C$ value that occurs between the untreated and XAD purified amino acids is generally much smaller than the change observed in the contemporary leather samples. The archaeological sample with the largest change in value is CMC21-1. The $F^{14}C$ for the untreated sample (PSUAMS-10893 $F^{14}C= 0.3103 \pm 0.0019$) was greater than XAD purified amino acids from the sample (PSUAMS-10970 $F^{14}C=0.2733 \pm 0.0016$). For three of the four archaeological samples, the solvent washing step produced the largest change in ^{14}C content, reinforcing how important solvent washing is for the pretreatment of skin-based samples. Contrary to the general trend of decreased $F^{14}C$ value with each additional processing step, the gelatin was slightly more ^{14}C enriched than the demineralized samples. However, only CMC21-5's $F^{14}C$ value varied significantly between demineralized and gelatinized samples: CMC21-1Demin (PSUAMS-10901) vs. CMC21-1Gel (PSUAMS-10905) ($T=3.8414$; $X^2=0.2967$; $df=1$); CMC21-3Demin (PSUAMS-10902) vs. CMC21-3Gel (PSUAMS-10906) ($T=3.8414$; $X^2=0.7188$; $df=1$); CMC21-4Demin (PSUAMS-10903) vs. CMC21-4Gel (PSUAMS-10907) ($T=3.8414$; $X^2=2.722$; $df=1$); CMC21-5Demin (PSUAMS-10904) vs. CMC21-5Gel (PSUAMS-10908) ($T=3.8414$; $X^2=4.5098$; $df=1$) (Ward and Wilson 1978).

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XAD purifying the amino acids of the samples decreased the $F^{14}C$ value for all the archaeological samples except CMC21-3. Two of the samples, CMC21-1XAD and CMC21-5XAD, had $F^{14}C$ values that were not statistically different from the $F^{14}C$ values of their paired organic samples RR28 and RR32: CMC21-1XAD (PSUAMS-10970) vs. RR28 (PSUAMS-10691) (T=3.8414; X^2=3.4410; df=1); CMC21-5XAD (PSUAMS-10973) vs. RR32 (PSUAMS-10694) (T=3.8414; X^2=3.0625; df=1). CMC21-4XAD did not have a paired organic sample for comparison, and CMC21-3XAD was statistically different from its paired organic sample: CMC21-3XAD (PSUAMS-10972) vs. RR30 (PSUAMS-10693) (T=3.8414; X^2=18.8147; df=1) (Ward and Wilson 1978).

Parchment $F^{14}C$

The $F^{14}C$ values of this sample fluctuated during processing. Initially, this sample (IM01; PSUAMS-11205 $F^{14}C$= 0.9512 ± 0.0015) was solvent washed and demineralized and had a lower $F^{14}C$ value than the untreated parchment sample (IM02; PSUAMS-11206 $F^{14}C$= 0.9600 ± 0.0017). FTIR testing, described below, revealed the IM01 sample was contaminated, and redoing the solvent washes, demineralization, and then continuing to gelatinization resulted in sample IM01B's $F^{14}C$ value of 0.9580 ± 0.0015 (PSUAMS-11368). This $F^{14}C$ value was slightly lower than the untreated parchment (IM02), but the $F^{14}C$ values of 0.9600 ±0.0017 (IM02; PSUAMS-11206) and 0.9580 ± 0.0015 (IM01B; PSUAMS-11368) were not statistically different (T=3.8414; X^2=0.7782; df=1) (Ward and Wilson 1978).

Fourier-Transform Infrared Spectroscopy Results

In our analysis of the FTIR spectra, we first established what the FTIR spectra of processed collagen should be based on the spectra of bone samples and the untanned rabbit skin. Because skin and bone gelatin both contain type I collagen, we expected that a skin or leather spectrum should resemble the gelatin spectrum from bone: with a broad OH peak around 3300 cm^{-1}, methyl type peaks around 2950 cm^{-1}, three strong protein peaks with a characteristic "stepping-down" appearance at 1650–1660 cm^{-1} (Amide I), 1540 cm^{-1} (Amide II), and 1455 cm^{-1} (Proline), and a final amide peak at 1250 cm^{-1} (Weiner et al. 2010). We compared the FTIR spectra from subsamples of the leather and skin to spectra we measured on bone gelatin from a contemporary caribou (*Rangifer tarandus*) (Supplemental Figure 9) and XAD purified amino acids from a sample of the Beaufort Whale bone used as a process background at PSUAMS (*Eschrictius robustus*; SR-5156; cf. Stafford et al. 1987:32) to identify which peaks were characteristic of type I collagen after various processing steps and which peaks were anomalous and might be due to contamination.

FTIR Spectra of Rabbit Skin

FTIR spectra measured on the contemporary rabbit skin confirmed skin collagen has a similar spectrum (Supplemental Figure 7) to bone collagen. Processing the rabbit skin removed two strong methyl peaks (2917 cm^{-1} and 2854 cm^{-1}) which could reflect lipids being removed. Additionally, the peak at 1031 cm^{-1}, which was as intense as the peak at 1074 cm^{-1} in the untanned rabbit skin, became relatively less intense in both the solvent washed rabbit skin and gelatin spectra. Once the expected spectra of the type I collagen present in skin had been established, we observed four general changes in the spectra of the skin-based samples after processing (Figure 3).

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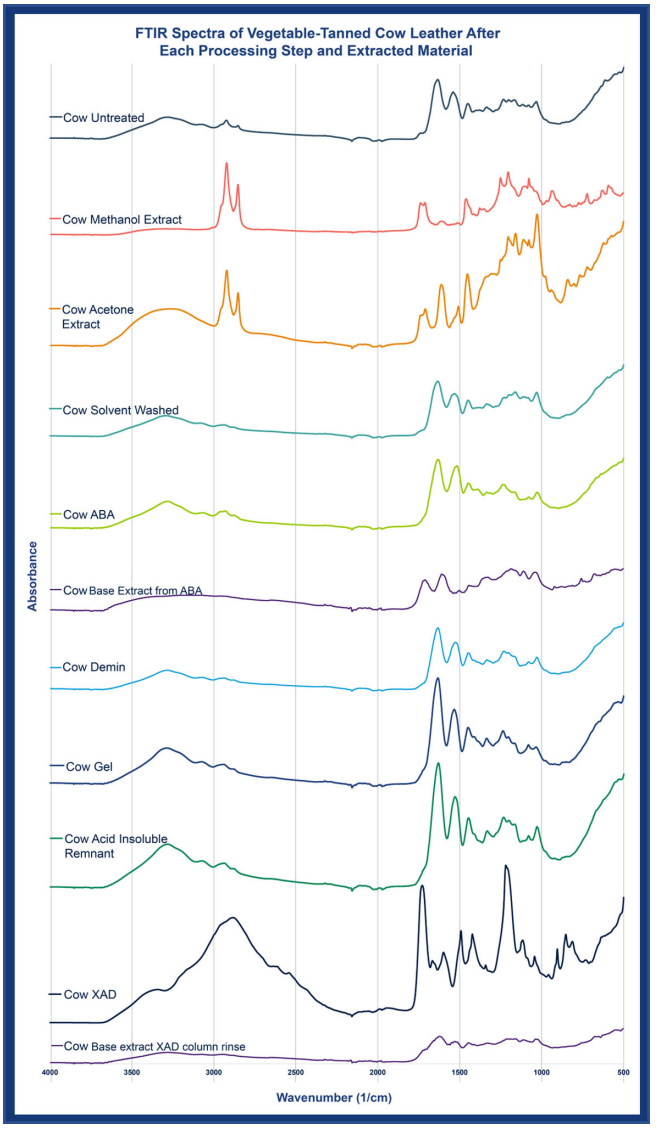

Figure 3 FTIR spectra of subsamples and extracts from each step of processing contemporary cow leather from untreated leather (top) to XAD purified amino acids and the material flushed from the XAD column (bottom).

FTIR Observed Changes during Sample Processing

First, FTIR spectra confirmed that lipids were removed from the skin-based samples by the solvent washes (Figure 4). The spectrum of the material extracted with methanol has an OH or NH peak around 3280 cm^{-1}, two strong hydrocarbon peaks at 2958 cm^{-1} and 2922 cm^{-1}, and a double peak at 1741 cm^{-1} and 1712 cm^{-1}. These peaks are consistent with lipids and match the position of peaks which were present in the untreated leather spectra but had disappeared in the spectra of the solvent washed leather. The FTIR spectra of the acetone-extracted material indicates that lipids and tannins were extracted by the acetone solvent wash. The same lipid

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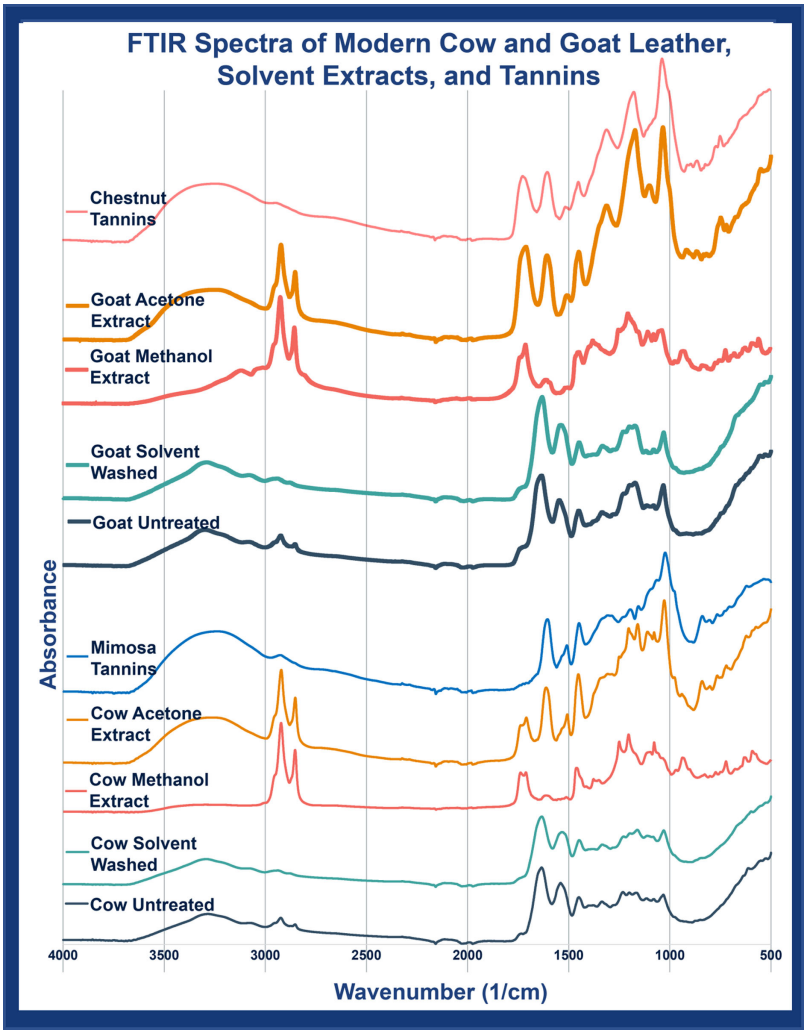

Figure 4 FTIR spectra of chestnut and mimosa tannins, acetone extracts, and methanol extracts compared to the spectra of solvent washed and untreated subsamples of goat and cow modern leather (top to bottom).

peaks previously listed for the methanol-extracted material are present in the acetone-extracted material. We compared the spectra of chestnut and mimosa tannins to the acetone-extracted spectrum of the goat and cow leather, respectively, and saw similar peaks, especially in the fingerprint region. FTIR of acetone extracts from leather can be used to identify unknown leather tannins (Falcão and Araújo 2014).

Observations on the FTIR spectra of the parchment sample (Supplemental Figure 8) confirm the importance of complete solvent washes. The FTIR spectra of the parchment samples shows several anomalous peaks in the untreated parchment sample (IM02) remaining in the parchment sample that underwent a solvent wash and bone demineralization style ABA washes (IM02). These peaks include the remnant of a methyl peak around 2900 cm^{-1}, a strong peak in

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the fingerprint region around 1030 cm^{-1}, and a carbonate peak at 875 cm^{-1}. These peaks show that non-protein material was not completely removed by the pretreatment. In response to this, the remainder of the parchment surface was cleaned, many small holes were punched through the sample, and it was left to macerate in water for several days until the parchment was much more pliable. Solvent washes, demineralization and gelatinization were then repeated. FTIR spectra of these subsamples showed that the anomalous peaks were removed by the additional solvent washes and demineralization.

Second, FTIR spectra of ABA (either organic style or bone style) pretreated samples shows that ABA pretreatment can degrade the collagen and retain contamination. FTIR spectra of the ABA processed contemporary vegetable tanned leather shows a decrease in the height of the Amine I peak relative to the height of Amine II peak indicating collagen degradation due to alkaline hydrolysis (Vyskocilova et al. 2019). FTIR spectra of the humates extracted from the base washes of the cow leather (Figure 3) show that degraded gelatin was also being extracted by the base wash. There is clearly an Amine I and an Amine II peak in that spectrum. Because the bond strength between collagen and contaminating humates and the bond strength of the collagen is similar, base washes can degrade the collagen while leaving a measurable amount of humic material behind (van Klinken and Hedges 1995). While the contemporary leather is not contaminated with humates, it does contain tanning material that is complexed with collagen. Several peaks are present at 1210 cm^{-1}, 1160 cm^{-1}, and 1030 cm^{-1} in the FTIR spectra of the ABA processed and demineralized vegetable tanned leather samples that are not consistent with collagen.

Third, gelatinizing the skin-based samples removes the peaks between 1300–900 cm^{-1} that were present in ABA'd and demineralized samples (Figure 3 and Supplemental Figure 9). Peaks in this region are present in FTIR spectra of many old parchments (Carşote et al. 2014; Kasso et al. 2023) and in contaminated bone samples (D'Elia et al. 2007). For the contemporary vegetable tanned leather samples, the spectra of the demineralized cow and goat leather had more absorption in the 1300 cm^{-1} to 1000 cm^{-1} range of the FTIR spectra than seen in the gelatin spectra, and peaks around 1210 cm^{-1}, 1160 cm^{-1} and 1030 cm^{-1}, which were removed or significantly decreased in intensity in the spectra of gelatin but were present in the spectra of the acid insoluble remnant. Similarly, the untreated archaeological hide samples have significant IR absorption between 1300 cm^{-1} and 1000 cm^{-1}, and CMC21-1 and CMC21-5 have strong peaks around 1030 cm^{-1} (Supplemental Figure 9). This peak seemed to be largely removed and IR absorption in the 1300 cm^{-1}-1000 cm^{-1} range reduced by gelatinization. Only one gelatin spectra had an anomalous peak: CMC21-5 retained a stronger peak at 1030 cm^{-1} than is typical for gelatin. This peak (1030 cm^{-1}) is intense in the spectra of the Acid Insoluble Residue of CMC21-1 and CMC21-5 and is present in the other two acid insoluble archaeological hide spectra. These changes in the spectra of skin-based samples show there is a significant amount of non-collagen material retained in the acid-insoluble residue, thus gelatinized skin-based samples contain less contaminating material than samples that have only undergone demineralization.

Fourth, FTIR spectra of gelatin and XAD purified amino acids are very consistent across multiple types of skin-based samples. FTIR shows no evidence of contamination remaining in the XAD purified amino acids when the FTIR spectra of the skin-based samples is compared to the FTIR spectrum we measured on XAD purified amino acids from the Beaufort whale (cf. Stafford et al. 1987; Figure 5).

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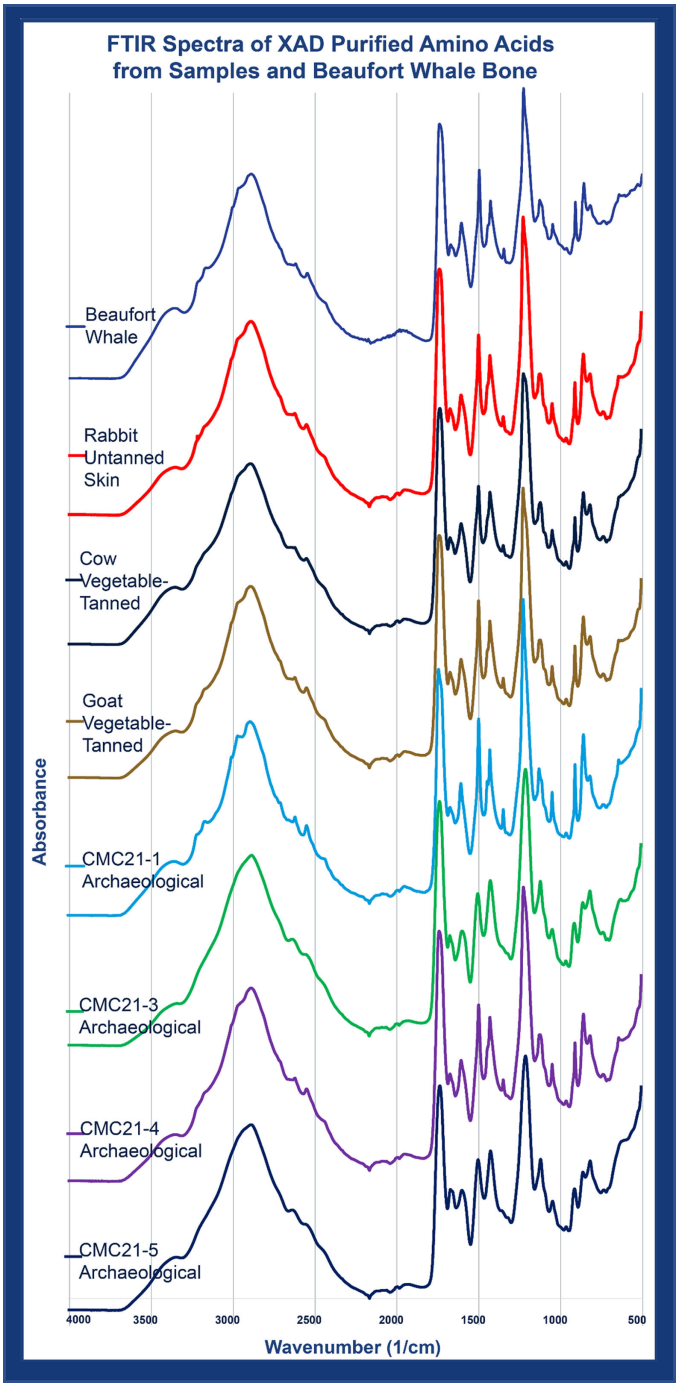

Figure 5 FTIR spectra of XAD purified amino acids from the Beaufort Whale, rabbit skin, modern leathers, and archaeological hide (top to bottom).

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DISCUSSION

Skin-based samples can absorb contamination that dramatically changes their apparent ^{14}C age. The untreated goat and cow leather had apparent ^{14}C ages of 700 ± 15 BP and 865 ± 15 BP, respectively, ages which reflected the mixture of ^{14}C dead petrochemicals used in tanning these samples and the contemporary skin collagen. For the contemporary skin-based samples, processing steps removed mostly older contaminating carbon, increasing the $F^{14}C$ after most processing steps. For the archaeological samples, processing steps removed mostly younger contaminating carbon, (probably from humates filtering down from younger cave sediments) and generally decreased the $F^{14}C$. With a few interesting exceptions, as we completed more processing steps, the $F^{14}C$ value of the sample moved closer to the samples' theoretical known $F^{14}C$ value, or the $F^{14}C$ value of a paired organic sample. This change in ^{14}C age was accompanied by decreasing C:N ratios into the 2.9–3.6 range expected for collagen, and disappearance of contaminant peaks in the FTIR spectra as we completed more processing steps.

Our experiments confirmed that solvent washes are necessary (Brock 2013; Donahue et al. 2002; Rasmussen 2009), but not sufficient, to remove contamination from skin-based samples. The disappearance of contaminant peaks in the FTIR spectra, improved C:N ratios, and changes in the $F^{14}C$ values of the solvent washed samples showed that solvent washing removed exogenous lipids from vegetable-tanning. The vegetable tanned samples had absorbed ^{14}C-dead petrochemicals during the tanning process, changing the apparent ^{14}C age of these samples by hundreds of years, and solvent washing removed a large component of this contamination. However, solvent washing samples alone did not consistently yield accurate ^{14}C dates, acceptable C:N ratios, or clean FTIR spectra. Solvent washes can also remove endogenous skin lipids, as can be seen in the loss of methyl peaks in the FTIR of the untanned rabbit skin, but we do not have a method to distinguish between endogenous and exogenous lipids when trying to remove contaminating carbon from the sample. We also note that none of the skin-based samples in this experiment had conservants applied, side-stepping potential ^{14}C contamination of all kinds and ages. See Larsen (1996) for an exhaustive—if certainly not comprehensive—list of concoctions that have been applied to leather. Further study is needed to test how and if conservants can be removed from skin-based samples, and the first step of this further study is determining which solvents should be used for targeted removal of conservants.

We observed that base washes do improve C:N ratios (with respect to expected C:N ratios of collagen) and accuracy of ^{14}C ages by extracting non-collagenous material from samples as previously noted by Brock (2013). However, base washes degrade collagen as they are removing contamination (e.g., humic acids, humates, tannins) that is bonded to the collagen molecule (Menderes et al. 1999; van Klinken and Hedges 1995). Our ABA processing of leather samples resulted in very low sample yields, unacceptable C:N ratios, FTIR spectra showing retained contamination despite alkaline hydrolysis of the collagen (Vyskocilova et al. 2019), and $F^{14}C$ values that were less accurate than the previous step of solvent washed leather. Demineralizing the samples with a weak and brief base wash was more successful as it improved the accuracy of the $F^{14}C$ value in comparison to the $F^{14}C$ value of the solvent washed samples and had a higher yield. However, the base washes could not be continued until all contamination was removed, and FTIR spectra still showed contamination was present after the base wash. Additionally, C:N ratios of the demineralized samples were not consistently within the 2.9–3.6 range considered acceptable, and the $F^{14}C$ values of the vegetable tanned leather samples differed from the known $F^{14}C$ value of those samples.

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Gelatinization has not been routinely used for skin-based samples, perhaps because its mass yield is typically lower than ABA, and researchers are often trying to minimize sampling of historic parchments. The gelatin yields of our skin-samples were less than the yields of our demineralized samples or previous results for ABA pretreatment of parchment (Brock 2013) but this was partly because gelatinization separated collagen from non-collagenous material. The FTIR spectra of our demineralized samples had contamination peaks present between 1300 cm^{-1} and 900 cm^{-1}, which were undetectable or decreased in intensity in the FTIR spectra of the gelatinized samples. C:N ratios of the gelatinized samples were generally improved over demineralized samples, with the anomolous exception of the vegetable-tanned cow leather. The gelatin from the archaeological hide samples was more ^{14}C enriched than the demineralized samples, but as discussed in the Results section, this difference was not statistically significant. Further, analysis of the acid-insoluble remnant showed that the C:N ratios and FTIR spectra were inconsistent with collagen. The $F^{14}C$ value of the acid insoluble fraction of vegetable tanned leathers were much lower than the gelatin $F^{14}C$ values, and the archaeological skin-based samples showed the reverse pattern—higher $F^{14}C$ value of the acid insoluble remnant relative to gelatin. For both the contemporary vegetable-tanned leather and the archaeological leather, contaminating carbon was concentrated in the acid insoluble remnant.

Finally, all the samples that we purified with XAD had acceptable C:N ratios and FTIR spectra consistent with the XAD processed spectrum of the Beaufort whale bone background. Only one sample, CMC21-3XAD (PSUAMS-10971), had a $F^{14}C$ value which had a statistically significant difference from the $F^{14}C$ value of the paired hair sample. We suspect that the paired hair sample (RR30; PSUAMS-10693) still retained contamination. A dilute ABA (0.1N HCl and 0.1N NaOH at room temperature) was used to purify that sample rather than the standard ABA (1N HCl and 1N NaOH at 70ºC) because of the friable nature of the hair sample. Contamination might still be present in this hair sample which could explain this age difference, and RR30 will be redated. The material extracted from the skin-based samples by XAD was also non-collagenous. If gelatinization was sufficient to remove contamination from skin-based samples, then material retained on the XAD column for the vegetable-tanned leather samples should not have had much lower $F^{14}C$ values and C:N ratios that were too high. These results suggest that vegetable-tanned leather, or any skin-based samples where contamination with conservants or humic acids is suspected, should be purified with XAD.

Additionally, we found FTIR spectroscopy indispensable in evaluating whether contamination was present in skin-based samples. The utility of FTIR in selecting parchment samples and checking for contamination has previously been described (Kasso et al. 2023). The FTIR spectrum of the solvent-washed and demineralized parchment sample (IM02) showed the sample was still contaminated with lipids (methyl peaks around 2900 cm^{-1}), an unknown material (peak around 1030 cm^{-1}) and carbonates (875 cm^{-1}). Despite the contamination evident from the FTIR spectrum, IM02's yield and C:N ratio were acceptable, and the ^{14}C age was older than the pretreated sample. The parchment sample was reprocessed and FTIR spectra could no longer detect this contamination in IM01B. FTIR spectra also revealed contamination in the demineralized samples which was removed by gelatinization and confirmed that contamination was not detectable in the XAD samples. Because skin-based samples can more readily absorb contamination than bone, we suggest that two FTIR spectra—of untreated samples and processed samples—be published along with the C:N ratios and yields. These spectra should show that any contamination detectable in the untreated sample is no longer detectable in the processed sample.

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CONCLUSION

Careful surface cleaning, solvent washing, and demineralization of skin-based samples removed an amount of contaminating carbon sufficient to alter ^{14}C measurements. ABA processing of the vegetable tanned leather degraded the skin proteins, resulting in a low sample yield. Even contemporary leather can be easily degraded by too many base washes applied at too high temperatures. However, all the samples survived a bone style demineralization, which is the same series of chemical steps as ABA, applied below room temperature, with a very limited number of base washes. The $F^{14}C$ values of the material precipitated from those base washes are also very different that the $F^{14}C$ values of their respective samples. Base washes do remove contamination from leather and using low temperature base washes can help prevent degradation of skin proteins.

Additional contamination could still be detected in the FTIR spectra and C:N ratios of the solvent washed and demineralized fractions, so we used gelatinization and XAD purification of hydrolyzed amino acids to remove exogenous carbon to produce more accurate ^{14}C dates. XAD pretreatment of the leather samples produced accurate ^{14}C dates (except for CMC21-3) with FTIR spectra consistent with hydrolyzed amino acids from bone and C:N ratios consistent with collagen. Only CMC21-3XAD (PSUAMS-10971) had a $F^{14}C$ value that was statistically different than the $F^{14}C$ value of a paired hair sample (RR30; PSUAMS-10693) (Ward and Wilson 1978). Additionally, we observed that non-collagenous material was visibly retained on the XAD column for the vegetable tanned contemporary samples. XAD may not be necessary for all skin-based samples, but more research is needed to verify which type of leather or skin samples do not need XAD to remove contaminants interlinking with the collagen protein chain. Collagen is a complicated sample matrix that is capable of absorbing organic materials with varying ^{14}C contents and more research is needed to verify if XAD, checking C:N ratios, and analyzing FTIR spectra is sufficient pretreatment for heavily contaminated skin-based samples.

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LAND ACKNOWLEDGMENT

This research was conducted in State College, Pennsylvania, which is part of the unceded land of the Susquehannock. We would like to acknowledge the Susquehannock and pay our respects to their past, present, and future elders. Cougar Mountain Cave is located on the ancestral

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homelands of the Klamath, Modoc, and Northern Paiute peoples who are today primarily members of The Klamath Tribes, Burns Paiute Tribe, Confederated Tribes of Warm Springs, and the Fort Bidwell Indian Community. We are very grateful for the opportunity to conduct this research on objects that belonged to their ancestors.

COMPETING INTEREST DECLARATION

The authors declare no competing interests.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/RDC.2023.88

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