

How It's Made: Emulsion (Organ) Tanned Leathers

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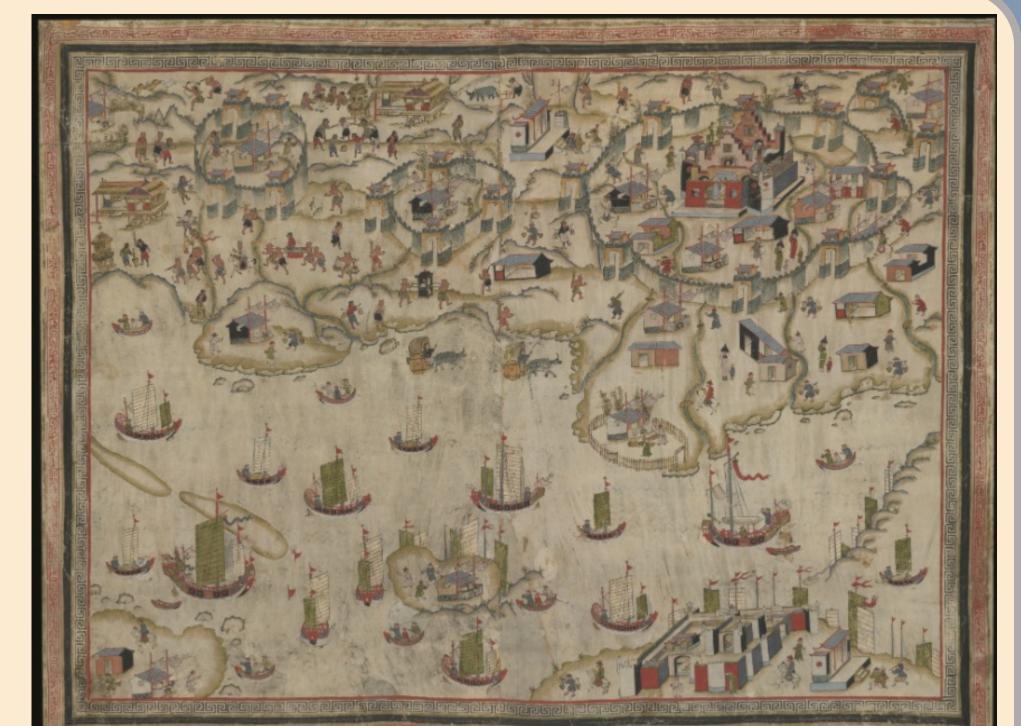
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INTRODUCTION

Significance and Background

- Leather tanning strengthens the collagen protein matrix of skin, creating a durable and malleable material
- Emulsion tanning is a traditional method using natural emulsifying agents (oils/lipids), generally from the organs (brain or liver) of skinned animals, to preserve skin
- Emulsion tanning has been used by cultures globally because of its efficiency
- This technique has not been industrialized because it is labor intensive



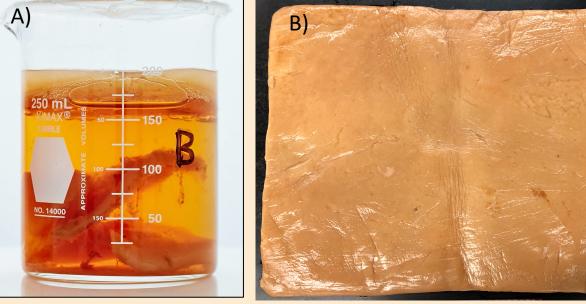
TRADITIONAL BRAIN TANNING vs. BIO-LEATHER

What is Our Bio-leather Made Of?

- Microbial Cellulose (MC) is comprised of numerous interconnected metabolic pools involving substrates and enzymes that are produced by bacteria. Like the SCOBY which produces kombucha!
- Versatile in application ranging from biomedical implants to fashion textiles.

Modern Day Organ Tanning

1) Smear brain paste onto MC 2) Massage to absorb for 15 minutes 3) Let sit for 2 hours the rinse off 4) Air dry



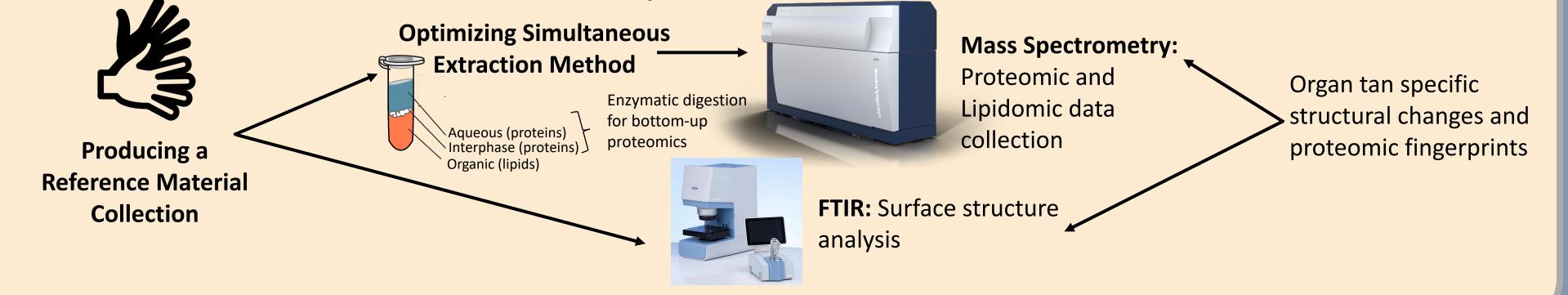
MC in culture) Sheet of MC after two weeks of growth period

The Object

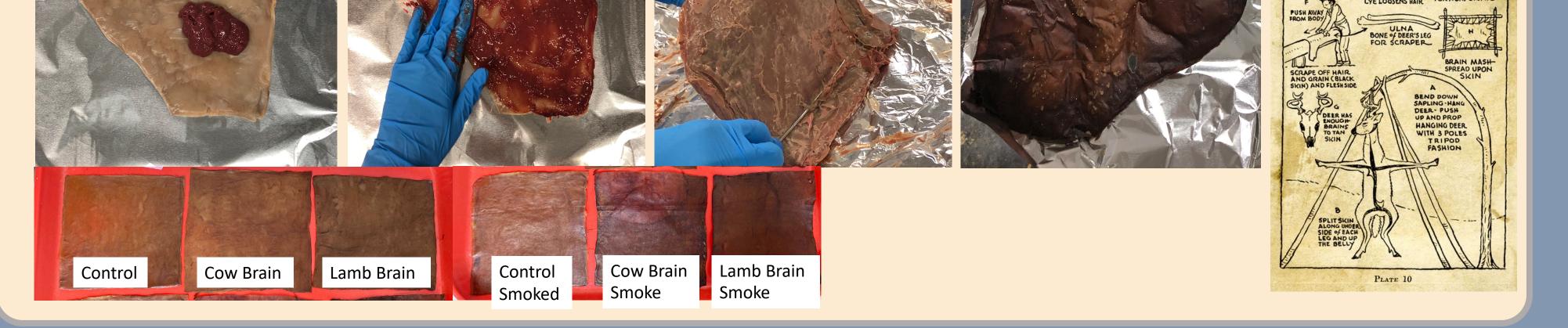
• The pictorial map entitled Forts Zeelandia and Provinitia and the City of Tainan (Met Accession 09.3) depicts the Taiwanese town of Tainan painted on leather, a technique that may have been imported from Europe by the Dutch.

Study Objective and Hypothesis

- There is currently no scientific method to identify emulsion tanned leathers. Determining the effects of tanning agents on animal skin at the molecular level is critical to understand how structural changes induced by the tanning process change the chemical and mechanical properties, and preservation of the leather product.
- We are optimizing liquid-liquid extraction methods to allow for analysis of the proteins and lipids for the leather substrate and the tanning emulsion all from a single sample.
- We have developed a set of reference materials using bio-leather, which acts as a collagen-free reference material, and allows us to study modern emulsion tanned materials.



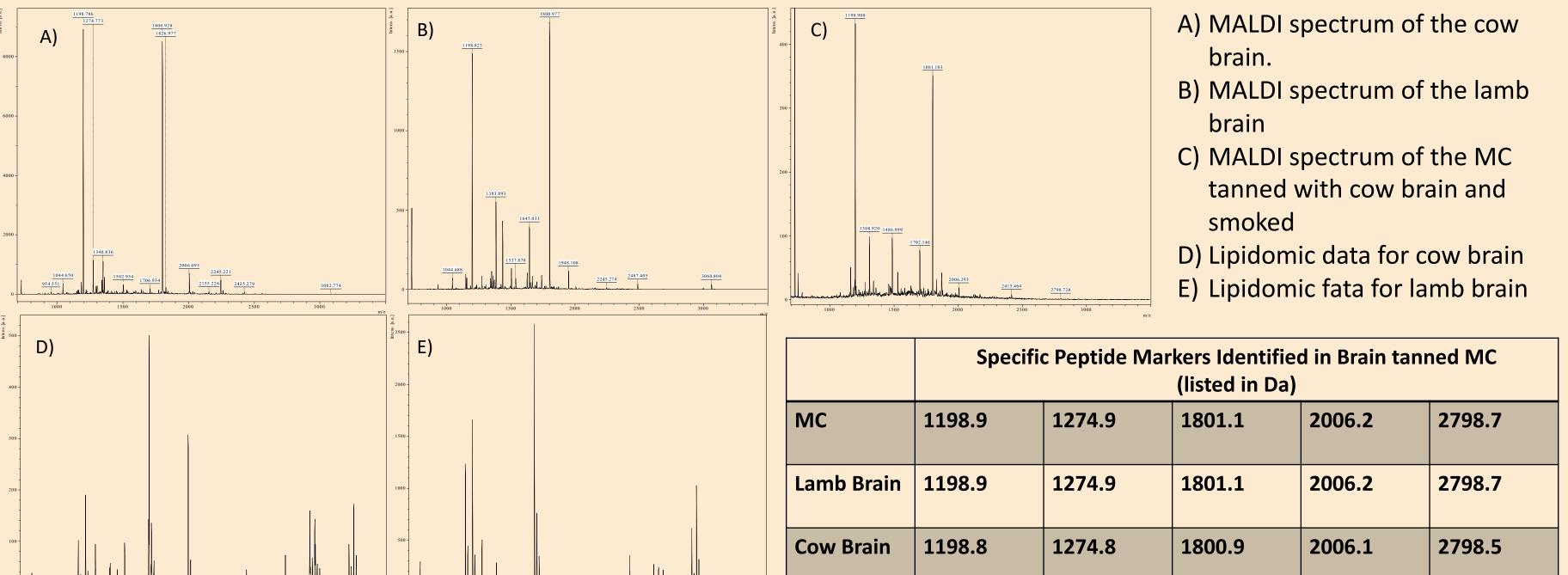
ANALYZING THE LEATHER MALDI of the Leather



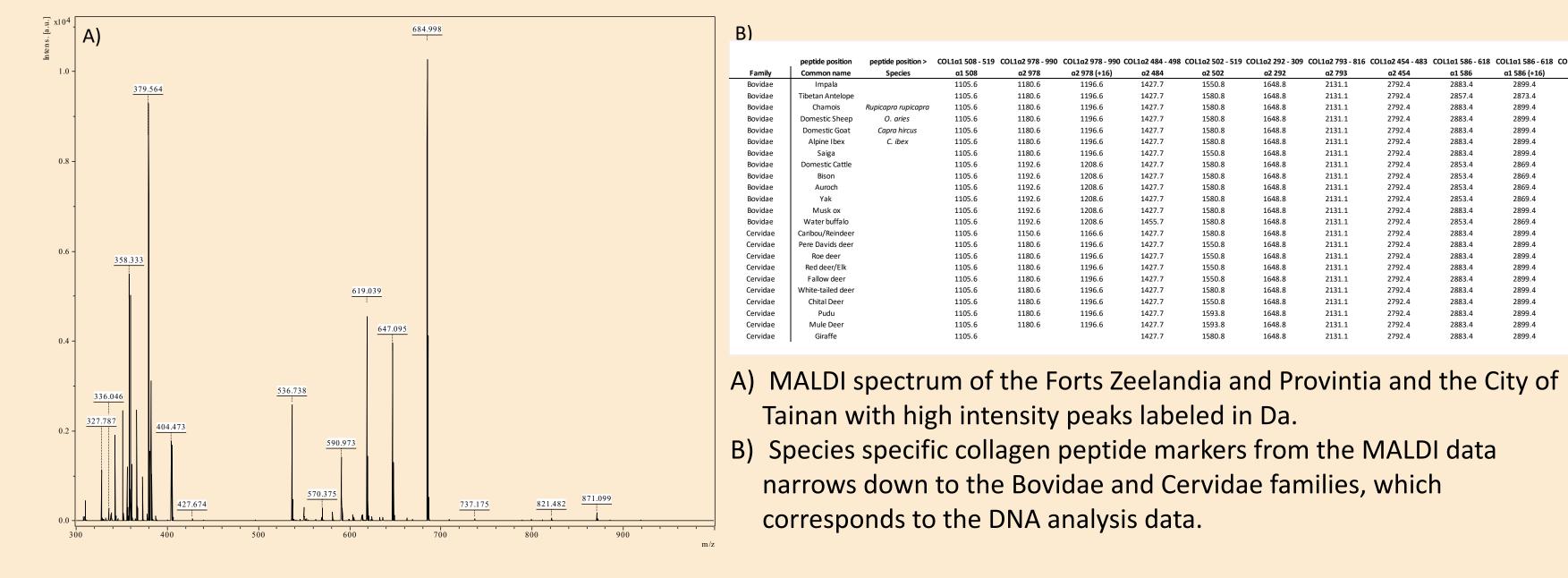
ANALYZING THE BRAIN TANNING EMULSION

MALDI of the Tanning Emulsion

- MC is a great negative control: it eliminates the dominating signal of skin collagen from the data.
- Five peptides of interest were identified that are not seen in any of the brain-tanned leather samples because the MALDI spectra were dominated by skin collagen
- Liver proteomics and lipidomic data are still being analyzed in order to further eliminate peptides that are not specific to brain tanning but may represent more general organ tanning methods
- These markers will be confirmed by LC-MS/MS to build a peptide mass fingerprinting database for brain tanning. Methods to improve the extraction of these peptides over skin collagen will then be developed to study leather samples.



- Using proteomics, a specific technique commonly referred to as ZooMS, collagen peptides fingerprints are identified by matrix assisted laser desorption/ionization (MALDI) mass spectrometry to determine the animal origin of the leather.
- The Method: Samples are extracted using the simultaneous lipid/protein extraction protocol, then the protein fraction undergoes a standard proteomics trypsin digestion prior to analysis by MALDI-MS.
- **Expected Output:** species or taxonomic family specific collagen peptide mass fingerprints. Comparing to known protein sequences and peptide markers to identify the leather's animal origin.



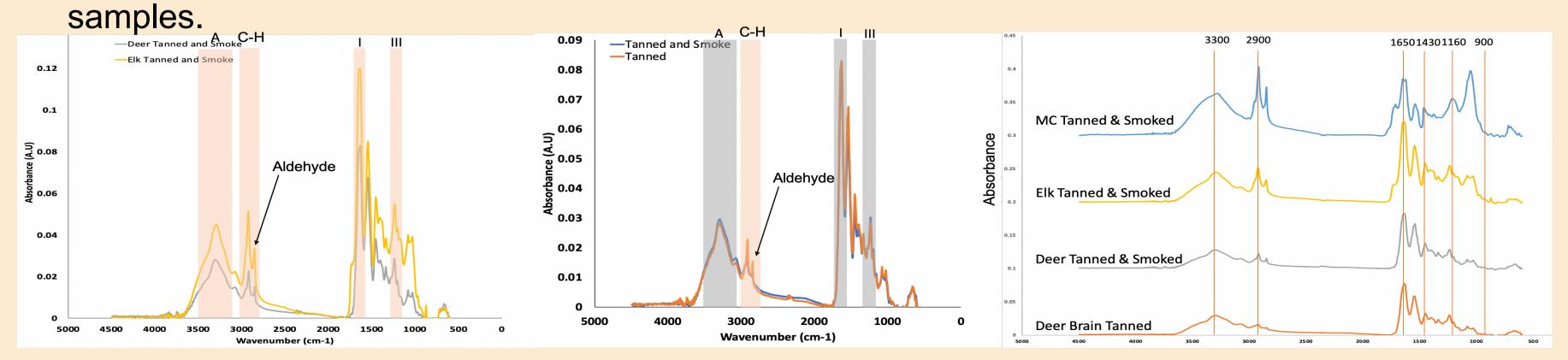
Conclusion

MALDI has narrowed the leather source taxonomy down between the *Bovidae* (cow) and *Cervidae* (deer) families for the wall hanging. This corresponds with the DNA analysis which also narrowed the leather origin to the Artiodactyla order (even-toed hooved mammals), which includes both of these families. To

FTIR (Fourier Transform Infrared Spectroscopy)

Difference in signal intensity were observed across species

• Moving forward, ATR-FTIR imaging of cross sections will allow for characterization of change in chemistry, chemical distribution, and stratigraphy of the brain tanned bio-leathers and leathers. The aldehyde emitted by the smoking process gave rise to an increase in aldehyde signal in the smoked



Conclusion

have more specific results, further extractions protocols will be optimized, and LC-MS/MS experiments

will be undertaken, which can provide species specific results.

REFERENCES Elliss et al., 1999; Ramana et al., 2000; Ishihara et al., 2002. Environ et al., 1984.



- In addition to gathering a collection of brain tanned leathers, we have produced a reference collection of cow brain tanned, cow brain tanned smoked, lamb brain tanned, and lamb brain tanned smoked MC samples. Liver tanned samples have also been prepared.
- Peptide markers will be identified by LC-MS/MS and confirm the species of origin of the leather samples.
- The same series of tanned references have been made with liver to study the use of other organs for

tanning leather. Liver, spinal cord, bone marrow have all been mentioned as emulsion tanning agents

in historic records