Human blood and bird egg proteins identified in red paint covering a 1,000-year-old gold mask from Peru

Elisabete Pires[1], Luciana da Costa Carvalho[2], Izumi Shimada[3] and James McCullagh[1*]*

[1]Mass Spectrometry Research Facility, Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK

[2]School of Archaeology, University of Oxford, Oxford OX1 3TG, UK

[3]Department of Anthropology, Southern Illinois University, Carbondale IL 62901-6899, USA

ABSTRACT

We analyzed a red paint sample from the surface of a gold mask excavated from a Middle Sicán elite tomb in Peru. The mask covered the face of the principal male and dates from ca. 1,000AD, a period when large quantities of painted precious metal objects were produced. The paint's inorganic pigment was identified more than 30 years ago as cinnabar (a mercury and sulfur scarlet-red to brown-red mineral) but the identity of the effective organic binder remained a mystery. Fourier Transform Infrared (FTIR) analysis of the sample indicated a proteinaceous composition and no lipids were recovered from a N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatized extract of the sample analyzed by gas chromatography-mass spectrometry (GC-MS).

Proteomics analysis nanoLC-MS/MS identified unique peptides belonging to human blood and bird egg proteins in the sample suggesting it may have been involved in ritualistic practices. Cinnabar-based paints were used in the context of social elites and ritually important items. The presence of human blood would support previous ideas that cinnabar paint could represent 'life force' intended to support 'rebirth'. As the red paint sample came from the first scientifically excavated Sicán mask, the results suggest a method to authenticate similar un-provenanced masks now in private and museum collections. Proteomics dataset identifier: https://doi.org/10.5287/bodleian:1ajYbBgQP

KEYWORDS

mass spectrometry, bottom-up proteomic analysis, archaeological proteomics, Fourier-transform infrared, Sicán mask, cinnabar paint, Middle Sicán

INTRODUCTION

The Sicán was a prominent pre-Hispanic culture that existed during the 9th-14th centuries in the Lambayeque region in the northern coast of modern Peru. The Middle Sicán Period (ca. 900-1,100AD) is characterized by the large-scale production of gold alloy objects. The sample submitted for analysis was of a red paint finish on a gold mask found at the bottom of a deep shaft tomb known as East Tomb at the north base of the monumental Huaca Loro temple mound within the Pomác Forest National Historical Sanctuary (ca. 800 km from Lima). Excavated in 1991-1992

by the Sicán Archaeological Project, the site represents the first scientifically excavated Middle Sicán elite tomb.[1] For decades prior to this work numerous similar Sicán elite tombs were looted.[2]

The central personage of the tomb was a 40-50-year-old man, his tightly flexed skeleton entirely painted red and intentionally placed upside down at the centre of the 3 x 3m burial chamber. Nearby were two young women (their skeletons arranged in parturition and midwifing poses) and two crouched child-skeletons placed at a higher level. Around 1.2 tons of diverse grave goods accompanied the bodies, approximately a third of which were metal artefacts including some 100 personal ornaments made of a 14-18 karat gold-silver-copper alloy[3] (equivalent to modern gold jewelry). Amongst these objects was a crumpled red-painted gold mask covering the face of the man, whose skull was found intentionally detached from his body and placed right side up (**Figure 1A**).

Figure 1. The golden Sicán mask as found **[A]** and during reshaping (location of the sample marked by the arrow) **[B]** (Photographs courtesy of Izumi Shimada)

Shortly after excavation, archaeologists and conservators started to wonder how a thick (ca.1-2 mm) paint layer remained largely attached to the polished metal surface of the mask despite being buried 1,000 years 12 m underground. During the mask's reshaping (**Figure 1B**) some loose paint fragments were collected for characterization. The elemental composition of the paint sample was

determined to contain mercury and sulfur using X-ray fluorescence (portable). Cinnabar was subsequently identified by X-ray diffraction (unpublished results) but the identity of the binding material in the red paint that had been so effective, remained a mystery. Having been given a small sample of the red paint we decided to analyze it using a multi-technique approach comprising FTIR, lipid profiling and proteomics.

METHODS

The cinnabar control sample was supplied by the British Museum, UK.

All reagents were analytical or chromatography grade and purchased from Sigma-Aldrich or Fisher (UK Ltd). Deionized water was obtained from a Milli Q reverse osmosis system (typically 18.2Ω resistivity and <4ppb carbon).

Fourier-Transform Infrared

KBr pellets of the cinnabar control and the red paint sample were analyzed in transmittance mode using an Excalibur Series Varian UMA600. Measurements were taken between the 4000-400cm^{-1} range, with 64 scans for each measurement which included background subtraction. Data was processed using Digilab Resolutions Pro 4.0 software and figures created with Spectragryph v.1.2.12. Band assignments for the red paint spectra were made according to published FTIR reference tables.[4,5]

Sample preparation for GC-MS analysis

Around 4mg of the red paint sample was extracted with 2:1 chloroform/methanol under sonication and blown down under nitrogen (repeated 3 times with the supernatant always being blown down into the same vial). 50µL of hexane was added to the vial alongside 5µL of 3.51 ×

10^{-4} M tridecanoic acid solution in hexane (internal standard) added before derivatization with BSTFA at 70ºC for 30 minutes.

GC-MS Analysis

The derivatized extract was transferred to a vial containing a restrictor and 10µL of 1.44 x 10-4 M hexadecane solution in hexane (instrument standard) and injected in an Agilent 7820A gas chromatograph system fitted with an Agilent HP-5MS column (30m length × 0.25mm i.d. × 0.25m film thickness, 5%-phenyl-methylpolysiloxane stationary phase) coupled with an Agilent 5975 quadrupole mass spectrometer, operating in electron ionization mode (70eV) and the scan range was m/z 50–650 amu. The inlet temperature was set at 280°C, flow rate 1.2 mL/min, transfer line temperature 280°C. Helium was used as the carrier gas. The temperature program for the GC oven started with 2 minutes at 50°C, followed by a ramp from 50°C to 300°C at 10°C/min and finishing by 10 minutes isothermally held at 300°C. Injections were made by an Agilent 7693A autosampler and sample injection volume was 1µL in splitless mode.

Sample preparation for protein analysis

Around 2mg of sample was partially dissolved in 2:1 v/v chloroform/methanol under sonication. After centrifugation, the supernatant was transferred to a clean tube with ice-cold acetone added prior to overnight incubation at -20ºC. After centrifugation, the supernatant was removed, and the remaining sample extracted with RIPA Buffer prepared in the laboratory. The protein extract was digested using LysC and trypsin protease mixture followed by desalting and sample concentration using C18 ZipTip.

Protein Analysis

The resulting tryptic digest was re-suspended in 40µL milli-Q water with 2% acetonitrile and 0.1% formic acid. 2uL was analyzed by nanoLC-MS/MS using a Waters NanoAcquity-UPLC system interfaced with a Thermo LTQ Velos Orbitrap Elite mass spectrometer possessing an EASY-Spray ion source. Initial peptide trapping on a packed guard column (75µm i.d. x 20 mm, Acclaim Pep-map100 C18, 3µm, 120 Å) took place using solvent A (0.1% Formic Acid in water) at a pressure of 140 bar. Peptides were separated on an EASY-spray Acclaim Pep-Map® analytical column (75µm i.d. x 15mm, RSLC C18, 3µm, 100Å) using a 120 minutes linear gradient ranging from 3 to 97% of solvent B (0.1% formic acid in acetonitrile). The flow was set to 300nL/min and the column was heated to 40°C. The nanoESI source was operated at 1600 V needle voltage and the ion transfer tube temperature set to 275°C. The separated peptides were ionized via electrospray directly into the mass spectrometer operating in a data-dependent acquisition mode using a CID-based method. Full scan MS spectra (scan range 350-1500m/z, resolution 120000, AGC target 1e6, maximum injection time 250ms) and subsequent CID MS/MS spectra (AGC target 5e4, maximum injection time 100ms) of 10 most intense peaks were acquired in the Ion Trap. CID fragmentation was performed at 35% of normalized collision energy and the signal intensity threshold was kept at 500 counts. The CID method used performs beam-type CID fragmentation of the peptides. Brand new guard and separation columns were used to avoid contamination from previous samples analyzed in the laboratory. Bovine Serum Albumin standard was also digested and analyzed in parallel using the same conditions.

Data Analysis

Data analysis was performed using PEAKS 8.5v (Bioinformatics Solutions Inc., Waterloo, ON, Canada). Raw MS data was searched against the general Uniprot database and identifications confirmed with Uniprot taxonomy databases with LysCTryp selected as the protease. Carbamidomethylation (Cysteine) was set as a fixed modification, Oxidation (Methionine), Deamination (Asparagine, Gluta-mine) and +12Da on N-terminal proline were set as variable modifications. Precursor mass tolerance was set as 15ppm and fragment mass tolerances for CID was set to 0.8Da.

RESULTS AND DISCUSSION

Characterization by FTIR indicated the presence of proteins

Some of the absorbance bands in the cinnabar spectrum can be seen reflected in the red paint sample spectrum (overlaid in **Figure 2**).

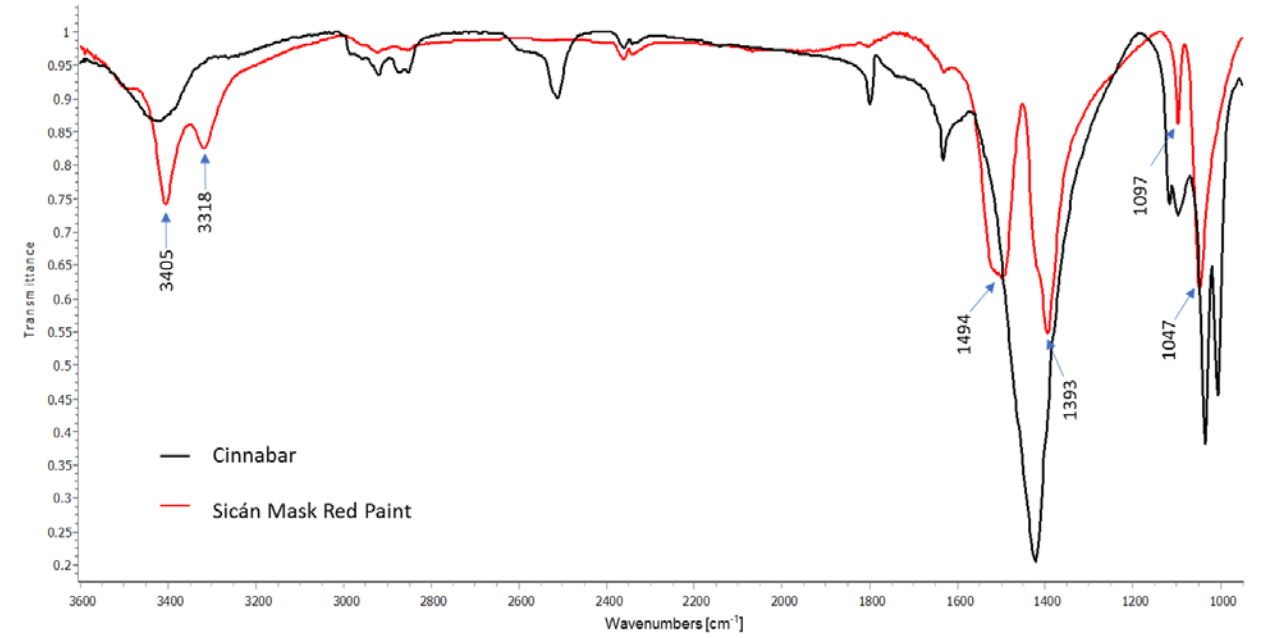


Figure 2. FTIR spectra for cinnabar (control) and Sican Mask Red Paint between 3600-950 cm^{-1} (baseline corrected)

Diagnostic bands[4,5] in the red paint sample are at 3,405 and 3,318cm^{-1} (N-H stretching bands of primary and secondary amines), a strong wide peak at 1494cm^{-1} (C=C stretching bands of aromatic ring system), a strong peak at 1393cm^{-1} (C-H aliphatic deformations), and a medium peak at 1097cm^{-1} (C-O-C stretching vibration from aromatic, olefinic or aliphatic ethers). A strong band at 1047 cm^{-1} (C=S stretching from thioamides and thioureas). The absence of strong bands at 2,900-2,880cm^{-1} indicates that waxes, resins or oils are either absent[6] or present at quantities below the detection limit of the technique. GC-MS analysis of the BSTFA derivatized extract of the red paint sample did not yield any compounds other than the internal standards.

Human blood and bird egg proteins identified in red paint from the Sicán Mask

A selection of the proteins identified in the sample are listed in **Table 1**. Complementary information about the highest scoring peptides of the selected human blood and bird egg proteins of the Sicán Mask Red Paint sample identified with Uniprot database searches are included in the Supporting Information (S1-7).

Table 1. Selected human blood and bird egg proteins identified in the Sicán Mask Red Paint sample using proteomics. Further analytical information about for each peptide (S1-7) can be found in Supplementary Information.

Protein name	Uniprot Accession Code* taxa	-10lgP**	Coverage (%)	#Peptides***	Highest scoring peptides [peptide score (-10lgP**)]	AScore**** (-10lgP)
Serum albumin	P02768 (S1) *Homo sapiens*	124.5	15	8(8)	YIC(+57.02)ENQDSISSK [65.2]	C3c1000.0
Immunoglobin G	S6B2B0 (S2) *Homo sapiens*	48.5	5	1(1)	AGVETTTPSK [54.5]	
Immunoglobin Kappa constant	P01834 (S3) *Homo sapiens*	92.3	36	2(2)	TVAAPSVFIFPPSDEQLK [71.9]	
Ovoalbumin (fragment)	P01013 (S4) *Gallus gallus*	19.6	5	1(1)	ERIEKTIN(+.98)FEK [19.6]	N8d1000.0
Ovomucoid	A0A663E3H3 (S5) *Aquila chrysaetos*	63.7	14	8 (8)	C(+57.02)RLERAQA [28.9]	C1c1000.0
Ovotransferrin	A0A087VGQ5 (S6) *Balearica regulorm gibbericeps*	84.2	37	21 (10)	ITWNNLQ(+.98)GK [24.7]	Q7d20.5
Vitellogenin-2	P02845 (S7) *Gallus gallus*	163.30	60	149 (149)	S(+43.01)PYEDIQAK [45.7]	S1c145.3

Note: d = Deamidation NQ(+0.98), c = Carbamidomethylation C (+57.02), c = Carbamylation any amino acid at N-terminal (+43.01)

* Each code denotes a different biological source. (S1-7) refers to spectral data included in the Supporting Information Figures S1-7.

**The -10lgP score indicates the statistical significance of the protein identification.

***The peptides with a -10lgP > 19 are listed in the Supporting Information Table S1

**** AScore is the Localization score assigned to modifications on the peptide. It is the -10 log of a p-value. In this case, the p-value is the probability that the modification occurs at the reported position compared to other possible positions. Scores are listed in the format amino acid, peptide position, modification, Ascore. (for example, a -10lgP of 20 is equal to a p-value of 0.01).

Searching against the *Uniprot All proteins* database returned 56 protein hits, mostly representing human keratin, skin and saliva-associated proteins (the results of this search are included in the Supporting Information Table S2). It cannot be ruled out that keratin, skin and potentially saliva contamination may have occurred during sample handling and processing and these proteins were discounted from further analysis. Indeed, other archaeological samples processed in the laboratory also contained similar keratin profiles to the red paint sample where the intensity of the keratin peptides was approximately two-fold higher than peptides from the remaining proteins. One of these samples also contained saliva proteins. It is therefore possible that saliva proteins may also be present as a result of contamination of the sample during excavation and/or conservation of the mask.

Interestingly amongst the remaining proteins six are found in human blood, including Serum Albumin which had a protein identification -10lgP score >100 with 8 identified peptides.

To investigate the protein associations further, filtered data searches were conducted using the *Uniprot Blood* and *Uniprot Egg* databases. Out of the top 10 proteins with the highest coverage identified with *Uniprot Blood* nine were assigned to *pan troglodytes* (chimpanzee) initially. As chimpanzees are not found in Peru, and given their genetic similarities to humans,[7,8] another search was conducted against the *Uniprot Human Blood* database.

The *Uniprot Human Blood* database search returned twenty protein assignments ranging from 12-82% peptide coverage. Three human blood proteins (with multiple peptide coverage ranging from 5-36%) were matched to the peptide sequences from the red paint digest and are listed in **Table 1**. Serum albumin, the most abundant protein in mammals, was identified with one of the higher protein identification scores after keratin proteins which had a score > 200. Immunoglobulin

G is a type of human anti-body representing approximately 75% of serum antibodies in humans.[9] It is the most common type of antibody found in blood circulation and was identified in this search with peptide AGVETTTPSK. Immunoglobulin kappa constant, a region of immunoglobulin light chains, was identified by two peptides including TVAAPSVFIFPPSDEQLK representing 36% coverage. The highest scored protein was cytokeratin-1, found in epithelial cells. As this protein belongs to the keratin family, its source was assumed to be contamination.

The search using *Uniprot Egg* returned twenty-one proteins, all from bird species. The most intense protein matches to peptide sequences measured in the red paint digest are listed in Table 1 and are Ovalbumin, Ovotransferrin, Vitellogenin-2 and Ovomucoid. Ovalbumin, despite being the major egg-white protein, was only identified as a protein fragment as only one peptide contributed to its identification. Whilst small sample size, low protein extraction yields, digestion are some of the factors that could have contributed to this result, the most important contributing factor is degradation over time. A study of egg-white proteins demonstrated that ovalbumin (and clusterin) undergo degradation during 40 days of egg storage. It is probable that the unprotected ovoalbumin in the egg-white used to make the red paint has markedly decreased due to processing in the past and degradation over the last 1,000 years. Ovotransferrin is the second most intense protein in egg white.[10] Ovomucoid is also one of the major egg white proteins – one of its identification (Uniprot Accession Code A0A663E3H3) contains the peptide sequence C(+57.02)RLERAQA identified with a Carbamidomethylation on cysteine. Vitellogenin is the major yolk precursor protein found in the liver of oviparous vertebrates and detected in egg whites,[11] was also identified in the sample. To test the validity of these identifications a further search was performed against *Uniprot EggWhite* database. Sixteen proteins were identified, all assigned to g*allus gallus* (chicken). Although evidence for pre-Columbian chicken has been recovered from archaeological sites in

Chile,[12] the consensus is still that chicken was brough to the Americas by European colonizers[13] making this source of the egg proteins in the red paint untenable. A close indigenous Andean avian domesticate to chicken that was raised and eaten by ancient Sicáns is the Muscovy duck. Although a protein for Anas platyrhynchos *platyrhynchos* (mallard duck) was initially identified in the red paint using *Uniprot Egg* it is impossible at this stage to ascertain the species of bird whose egg was used in the red paint as the proteomes of chicken and duck eggs[14] are practically indistinguishable from one another and given the highly degraded state of the archaeological proteins.

It is worth pointing out that the identified proteins described in **Table 1** were only found in the Sicán sample and not identified in the BSA standard processed and analyzed in parallel; we were not able to find any evidence that the presence of the blood or egg proteins was likely to have resulted from the excavation, sample handling or preparation of sample in the laboratory.

Cystatin A was another protein with high protein identification score and coverage >70%. As this protein is mainly present in epidermis tissue[15] we cannot rule out the possibility of its source being skin contamination.

The significance of human blood identified in the cinnabar paint sample: past and present

Other reddish paints from Sicán metal objects were found to be a mixture of iron oxides and *prosopis pallida* (locally abundant and known as *algarrobo*; carob tree) resin and another substance that appear to have been made from fish bones.[16] The cinnabar paint was clearly restricted to elite use, while ochre paint was available to non-elites. The identification of proteins associated with human blood in the cinnabar paint sample lends weight to a hypothesis that application of this red paint on the entire deceased skeleton symbolized intensely red, oxygenated

blood, "life force" essential for the desired effect to take place. The unique inverted placement of the skeleton next to the two young adult women in parturition and midwifing poses suggests that the desired effect was the rebirth of the deceased leader.[17] From an archaeological perspective, the use of human blood in the paint would not be surprising. A recent osteological study of human sacrificial victims from Sicán indicated that many were cut particularly on the neck and in a systematic manner to maximize bleeding. [18]

Practically all Middle Sicán artefacts in museums and private collections were looted from archaeological sites in modern times. Once within illicit trade networks these objects are commonly subject to various undocumented interventions to enhance their aesthetic qualities. Sicán gold objects often had their paint stripped off and pigments, feathers and bangles removed as well as ancient tool marks smoothed over due to careless cleaning.[19] Some masks were flattened to facilitate display. Consequently, attempts to understand the cultural significance of these artefacts are significantly hampered.

An artifact with a well-documented archaeological context, carefully excavated and with a detailed conservation history is rare and provides unique opportunities. Although it was not possible to analyze other artefacts or the surrounding sediments as negative controls, and therefore there remains the possibility that the paint sample was contaminated from other materials in the tomb, the identification of evidence for the presence of human blood from the cinnabar paint sample has the potential to enable the authentication of unprovenanced masks that still contain remains of red paint; it also opens up the possibility of using forensic techniques where no traces of red paint are visible.[20]

CONCLUSION

In this study we analyzed a red paint sample from a 1,000-year-old Sicán gold mask excavated from a tomb Peru, sing FTIR, lipid analysis and proteomics by mass spectrometry. Initial characterization by FTIR indicated the sample was dominated by proteins and did not appear to contain lipids. Lipids were not detected using a standard lipid analysis method[21] corroborating the FTIR results. Proteomic analysis identified multiple peptides associated with human blood and bird egg proteins. Given this evidence, it is unclear why no-lipids were identified given their prevalence in both human blood and avian eggs[22]. A number of factors that may have contributed to the absence of lipids from our results include degradation during burial, limited sample size and potential interactions between the lipid molecules and the cinnabar, as in the case between lipids within porous ceramics, where higher recovery yields have been obtained from 200mg of sample and extraction with acidified methanol.[23]

Although the number of archaeological investigations using proteomics is increasing,[24] its adoption in the field of organic residue analysis[25,26] is limited by the high cost of analysis and the absence of sampling protocols specifically designed for archaeological samples and the ability to implement them in the field. The proteomics data presented here were obtained using standard methods designed for analysis of cell extracts. The results are highly intriguing, and suggest that the paint covering the Sicán gold mask included human blood and avian egg in its making, however, given the lack of additional samples from the tomb itself or sediments it was not possible to rule out whether these also contained such proteins. Often, archaeological materials are highly precious and what is available for analysis are relatively few samples passed thought a number of stakeholders, and these limitations highlight the importance of using multiple samples to make

identifications as secure as possible and to include positive and negative control samples from excavations and archaeological sites where possible. Nevertheless, the specificity and selectivity of the proteomics workflow and its results provides a powerful analytical capability for archaeological science applications with the potential to both complement and support archaeological theories as well as we provide (sometimes surprising) new information about the way people lived their lives in the past.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Figure S1. Spectral Information: Serum albumin (Homo Sapiens) - YIC(+57.02)ENQDSISSK

Figure S2. Spectral Information: Immunoglobin G (Homo Sapiens) - AGVETTTPSK

Figure S3. Spectral Information: Immunoglobin Kappa constant (Homo Sapiens) - TVAAPSVFIFPPSDEQLK

Figure S4. Spectral Information: Ovoalbumin fragment (Gallus gallus) - ERIEKTIN(+.98)FEK

Figure S5. Spectral Information: Ovomucoid (Aquila chrysaetos) - C(+57.02)RLERAQA

Figure S6. Spectral Information: Ovotransferrin (Balearica regulorm gibbericeps) - ITWNNLQ(+.98)GK

Figure S7. Spectral Information: Vitellogenin-2 (Gallus gallus) - S(+43.01)PYEDIQAK

Table S1 – Peptides with -10lgP value > 19 used for protein matches listed in Table 1

Table S2 – Results of the search with *Uniprot All Proteins* database

AUTHOR INFORMATION

Corresponding Author

*lucy@petros.co.uk

*james.mccullagh@chem.ox.ac.uk

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Dr John Merkel from the Institute of Archaeology, University College London, UK, the conservator who initially worked on the mask, for providing the red paint sample and for the invaluable insights during data analysis. We also thank the British Museum, UK for providing the cinnabar control sample.

ABBREVIATIONS

BSTFA, N,O-Bis(trimethylsilyl)trifluoroacetamide; GC-MS, gas chromatography coupled with mass spectrometry; FTIR, Fourier-Transform infrared; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LDF, linear discriminative function score;

REFERENCES

(1) Shimada, I.; Merkel, J. F. A Sicán tomb in Peru. *Minerva*. **1993**, 4 (1), 18-25.

(2) Carcedo, P.; Shimada, I. Behind the Golden Mask: Sicán Gold Artifacts from Batán Grande, Peru. In *Art of Pre-Columbian Gold: Jan Mitchell Collection*. Julie Jones (ed.). **1985**, 60 -75. London: Weidenfeld & Nicolson.

(3) Shimada, I.; Gordus, A.; Griffin, J-A. Technology, Iconography, and Significance of Metals: A Multi-Dimensional Analysis of Middle Sicán Objects. In Pre-Columbian Gold: Technology, Iconography, and Style. Colin McEwan (ed). **2000**, 28-61. London: The British Museum Press.

(4) Cross, A. D.; Jones, R. A. *An Introduction to Practical Infrared Spectroscopy*. 3rd Ed. **1969**. London: Butterworth & Co.

(5) Socrates, G. *Infrared and Raman Characteristic Group Frequencies – Tables and Charts*. 3rd Ed. **2001**. Chichester: Wiley.

(6) Derrick, M. R.; Stulik, D.; Landry, J. M. *Infrared Spectroscopy in Conservation Science – Scientific Tools for Conservation*. **1999**. Los Angeles: The Getty Conservation Institute

(7) Cagan, A.; Theunert, C.; Laayouni, H.; Santpere, G.; Pybus, M.; Casals, F.; Prüfer, K.; Navarro, A.; Marques - Bonet, T.; Bertranpetit, J.; Andrés, A. M. Natural Selection in the Great Apes. *Mol. Biol. Evol*. **2016**, 33 (12), 3268-3283

(8) Dumas, G.; Malesys, S.; Bourgeron, T. Systematic detection of brain protein-coding genes under positive selection during primate evolution and their roles in cognition. *Genome Res.* **2021**, 31 (3), 484-496

(9) Adkins, Joshua N ; Varnum, Susan M ; Auberry, Kenneth J ; Moore, Ronald J ; Angell, Nicolas H ; Smith, Richard D ; Springer, David L ; Pounds, Joel G. Toward a Human Blood Serum Proteome. *Mol. Cell. Proteom.* **2002**, 1 (12), 947-955

(10) Mann, K. The chicken egg white proteome. *Proteomics*. 2007, 7(19), 3558-3568

(11) Cavallero, G. J.; Landoni, M.; Couto, A. S. In depth N-glycoproteomics shows glyco-features of chicken egg white. *Food Biosci.* **2020**, 35, 100590

(12) Storey, A. A.; Ramírez, J. M.; Quiroz, D.; Burley, D. V.; Addison, D. J.; Walter, R.; Anderson, A. J.; Hunt, T. L.; Athens, J. S.; Huynen, L.; Matisoo-Smith, E. A. Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. *PNAS*. **2007**, 104 (25), 10335

(13) Storey, A. A.; Daniel Quiroz, N. B.; Matisoo-Smith, E. Polynesian Chickens in the New World: A Detailed Application of a Commensal Approach. *Archaeol. Ocean*. **2013**, 48 (2), 101-19

(14) Zhang, F.; Yin, Z-T.; Zhang, J-F.; Zhu, F.; Hincke, M.; Yang, N.; Hou, Z-C. Integrating transcriptome, proteome and QTL data to discover functionally important genes for duck eggshell and albumen formation. *Genomics*. **2020**, 112 (5), 3687-3695.

(15) Shamsi, A. ; Bano, B. Journey of cystatins from being mere thiol protease inhibitors to at heart of many pathological conditions. *Int. J. Biol. Macromol.* **2017**, 102, 674-693

(16) Shimada, I. *Cultura Sicán: Dios, Riqueza y Poder en la Costa Norte del Peru*. **1995**. Lima: Edu-Banco Continental.

(17) Shimada, I.; Shinoda, K.; Farnum, J.; Corruccini, R.; Watanabe, H. An Integrated Analysis of Pre-Hispanic Mortuary Practices: A Middle Sicán Case Study. *Curr. Anthropol.* **2004**, 45 (3), 369-402.

(18) Klaus, H. and Shimada, I. Bodies and Blood: Middle Sicán Human Sacrifice in the Lambayeque Valley Complex (A.D. 900-1100). In *Ritual Violence in the Ancient Andes: Reconstructing Sacrifice on the North Coast of Peru*. Haagen D. Klaus and J. Marla Toyne (eds). **2016**, 120-149. Austin: University of Texas Press.

(19) Shimada, I. and Griffin, J. A.; Precious Metal Objects of the Middle Sicán. *Sci. Am*. **2005**, 15 (1), 80-89.

(20) Merkel, J. F. *perscomm* **2021**

(21) Evershed, R. P; Heron, C.; Goad, L. J. Analysis of organic residues of archaeological origin by high-temperature gas chromatography and gas chromatography-mass spectrometry. *Analyst.* 1990. 115 (10), 1339-1342

(22) Belitz, H.-D.; Grosch, W.; Schieberle, P. Eggs. In *Food Chemistry*. **2009**. 546-616. Berlin: Springer

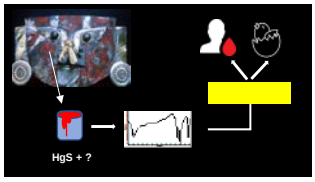
(23) Correa-ascencio, M. and Evershed, R. P. High throughput screening of organic residues in archaeological potsherds using direct acidified methanol extraction. *Analytical Methods*. **2014**, 6 (5), 1330-1340

(24) Hendy, J. Ancient protein analysis in archaeology. *Sci. Adv*. **2021**, 7, 1-11

(25) Greco, E.; El-Aguizy, O.; Ali, M. F.; Foti, S.; Cunsolo, V.; Saletti, R.; Ciliberto, E. Proteomic Analyses on an Ancient Egyptian Cheese and Biomolecular Evidence of Brucellosis. *Anal. Chem*. **2018**, 90, 9673-9676

(26) Hendy, J.; Colonese, A. C.; Franz, I.; Fernandes, R.; Fischer, R.; Orton, D.; Lucquin, A.; Spindler, L.; Anvari, J.; Stroud, E.; Biehl, P. F.; Speller, C.; Boivin, N.; Mackier, M.; Jersie-Christensen, R. R.; Olsen, J. V.; Collins, M. J.; Craig, O. E.; Rosenstock, E. Ancient proteins from ceramic vessels at Çatalhöyük West reveal the hidden cuisine of early farmers. *Nat. Commun*. **2018**, 9 (1), 4064

"For TOC Only"


HgS + ?