Institution: University of York
Unit of Assessment: 17, Geography, Environmental Studies and Archaeology
Title of case study: ZooMS: archaeozoology, food security, traceability and authentication

1. Summary of the impact

Demand for cheap meat has increased the potential for fraudulent food labeling, which exploded in public debate in 2009 and 2013. Zooarchaeology by Mass Spectrometry (ZooMS) is a technology originally developed to identify bone fragments from archaeological sites by determining the sequence of the bone protein, collagen. By applying this research to the food industry we have provided evidence of fraud. In 2009 ZooMS identified pig and cow gelatin being pumped into chicken meat to increase weight. Action taken by the food producers when confronted with our research respected the beliefs of up to 3.8million[a] people in the UK who choose to avoid pig and cow products.

2. Underpinning research (indicative maximum 500 words)

ZooMS is a procedure developed at York by Professor Matthew Collins (Archaeology), in collaboration with Professor Jane Thomas-Oates (Chemistry), to identify the animal origin of worked bone and bone fragments from archaeological sites[1]. Bone is composed of two major constituents, mineral (apatite) and protein (collagen). Most ancient proteins decay, by cross-linking to other molecules (humification) and by cleavage (hydrolysis) into random fragments. These twin processes make analysis of ancient proteins a challenging research area.

We have been researching the application of protein mass spectrometric methods to analyse proteins in archaeological bones[2]. The highly conserved main collagen chain is a robust triple helix which (we believe) is stabilised by radial compression - the so-called Link-Lock hypothesis[3]. Upon heating or treatment with strong acid or alkali, the chains unravel to form gelatin. The structural features that make collagen so stable were, for a long time, considered to make collagen useless as a species indicator. Our research illustrated that the 'conserved' repetitive collagen sequence has sufficient variation to fingerprint not only ancient bone but other collagen based tissues - a method we term ZooMS (illustrated in Figure 1 below). The method has been reported widely (*e.g. Chemistry World,* Nov 2010, 44; *Science*, 2010, **330**, 28-29), and a large database of collagen amino acid sequences has been assembled.

Our development of robust protein mass spectrometric methods has many potential archaeological applications. It also offers great potential in other areas. This case study reports the detection of food fraud in gelatin-based plumping agents used to rehydrate chicken meat; gelatin is produced from collagen in bone on boiling The ZooMS technique is superior to DNA to test the animal origin of this type of highly processed material as DNA can be detected in trace amounts, whilst ZooMS tests the product (the protein itself). In highly processed materials the original DNA is destroyed, but minute quantities of fresh tissue (e.g. blood) from another animal can be used to mask the original source. Alongside ZooMS, our research used

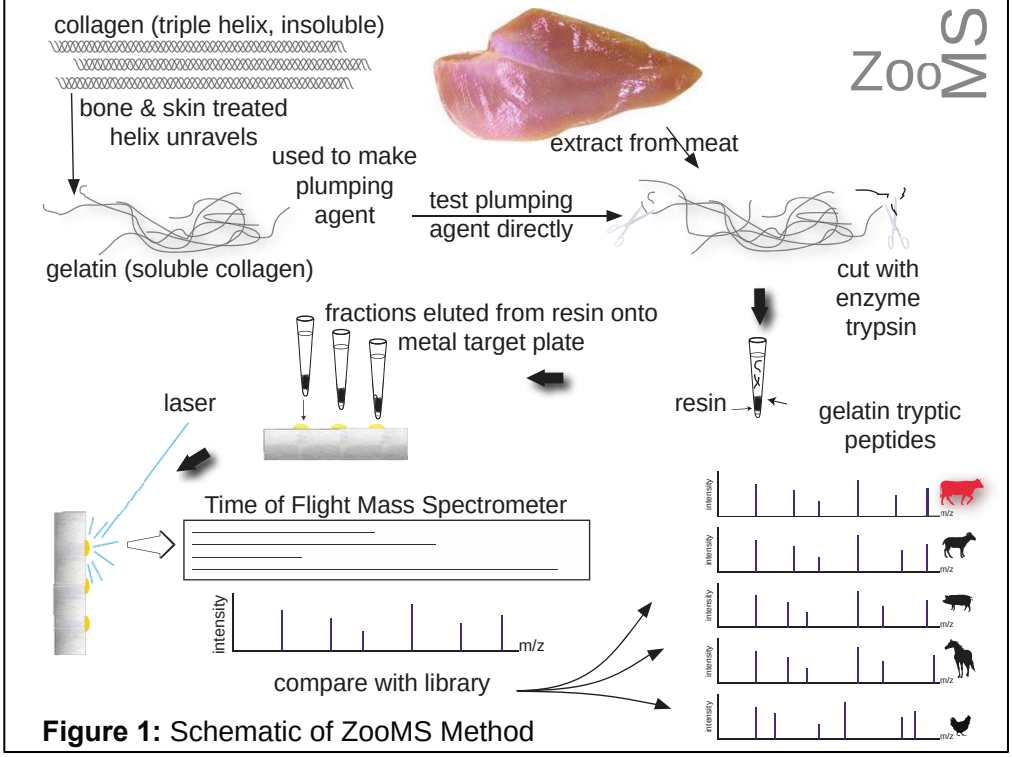
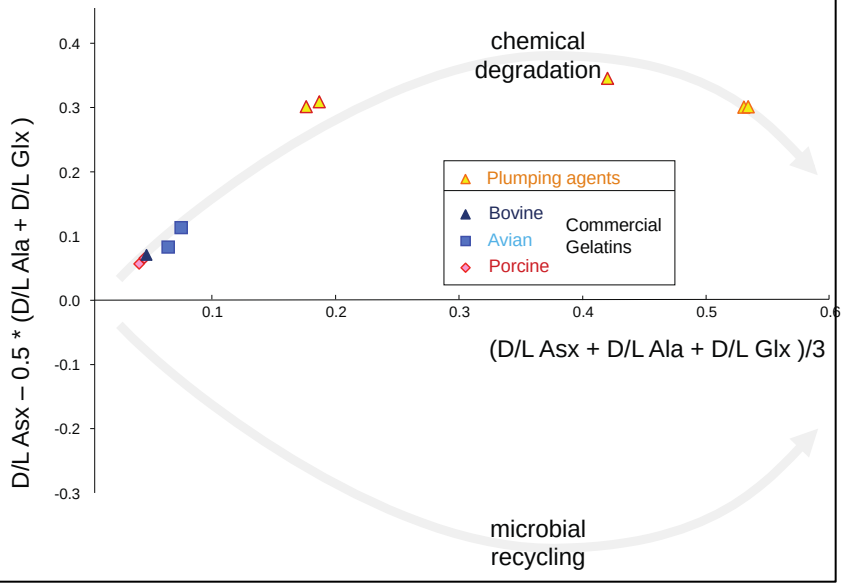


Figure 1: Schematic of ZooMS Method

racemization analysis (developed by Collins[5], York is home to the NERC recognised NEAAR facility) to measure the extent of processing in plumping agents and commercial gelatins. Analysis of the plumping agent revealed that they were more aggressively treated than the gelatins (Fig. 2), to a point well beyond the survival of DNA.

Figure 2. Plumping agents are much more highly processed than normal gelatins. The more highly altered the protein is, the further towards the right of the graph the values will lie. The plot discriminates microbial recycling from physicochemical processing; see [5] for further details of plot. DNA was recovered in plumping agents *more* easily than from the less processed gelatin, which suggests that the DNA in the plumping agents was not from the original protein source. (Grundy HH, Collins MJ. *et al., (submitted)* A method for the determination of the species of origin of gelatin in foods and pharmaceutical products.)



Key BioArCh Researchers and Positions Held

Prof. Matthew Collins, Reader then Professor, Founder and head of BioArCh; Dr Mike Buckley, October 2005–May 2010. PhD student then NERC PDRA; Dr Enrico Cappellini, PDRA October 2005-8; Dr Hannah Koon, October 2003–June 2010, PhD student, then Wellcome Trust Fellow then AHRC PDRA.

3. References to the research (indicative maximum of six references)

1. Buckley, M., Collins, M., Thomas-Oates, J., & Wilson, J.C. (2009). Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid communications in mass spectrometry*, **23**(23), 3843-3854. **47 citations** – DOI: 10.1002/rcm.4316 (Available in REF2, Chemistry).
2. Cappellini, E., Jensen, L.J., Szklarczyk, D., Ginolhac, A., da Fonseca, R.A.R., Stafford, T.W., Holen, S.R., Collins, M.J., Orlando, L., Willerslev, E., Gilbert, M.T.P., Olsen, J.V., 2012. Proteomic analysis of a pleistocene mammoth femur reveals more than one hundred ancient bone proteins. *J. Proteome Res.* **11**, 917–926. doi: 10.1021/pr200721u International media coverage. **11 citations**
   "*The study...unleashes the field of palaeoproteomics by identifying prehistoric protein sequences that could be used to help identify species, evolutionary relationships and even, perhaps, ancient diseases*" *Nature* doi:10.1038/nature.2011.9601
3. Covington, A.D., Song, L., Suparno, O., Collins, M.J., & Koon, H.E.C., (2008). Link-Lock: the mechanism of stabilising collagen by chemical reactions. *J. Soc. Leather Technol. Chem,* 92, 1-7. **10 citations.** Available upon request
   "[The link-lock hypothesis] *has made it possible to take quantum steps forward in developments in tanning technology*" (*Tanning Chemistry: The Science of Leather*. p. 464).
4. Reece, P., Chassaigne, H., Collins, M., Buckley, M., 2012. Proteomic analysis of meat and bone meal and animal feed, in: Jorgensen J.S., B.V. (Ed.), *Detection, Identification and Quantification of Processed Animal Proteins in Feedingstuffs*. Presses Universitaires de Namur, Namur, pp. 113–124. Available on request.
5. Willerslev, E., Cappellini, E., Boomsma, W., et al., Collins, M.J., 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* **317**, 111–114. **157 citations** doi: 10.1126/science.1141758

Citation data: SCOPUS unless journal not indexed, then Google Scholar 17.10.2013

Funding

Decoding domesticate DNA in archaeological bone and manuscripts (EC ERC-2011-AdG 295729-CodeX 1/04/12 - 31/03/17, £390,000) Collins Co-I

Fish farming tracked from fragmentary remains using a universal bone barcode (AHRC Speculative Research Award, AH/G011281/1) 05/09 – 04/10, Collins, Thomas-Oates, £137,000

A new method for detecting the animal origin of collagen, (NERC NE/G000204/1) Collins, Thomas-Oates, 20/10/08 - 19/01/10 £129,093,

Paleoproteomics: a revolution in ancient biomolecular studies? (NERC NE/C511148/1) project grant to Collins, Thomas-Oates, Genever, 2006-2008. £192,726

Optimisation of bone reactivity for use in the remediation of toxic metals (NERC, GR9/01656, CONNECT B) Valsami-Jones, Wess, Collins, 1/04/2004 - 31/03/2007, £129,000.

4. Details of the impact (indicative maximum 750 words)

The new research has had impacts in the detection of food contaminants. As ready meals containing meat have become more popular (now estimated at 5% of the UK food budget), the potential for fraudulent food labelling has increased. The potential for mislabelling came to the fore in 2009 and again in 2013. The Food and Environment Research Agency (Fera), part of DEFRA, guides the science evidence base in the food and environment sectors to ensure that UK policy-makers are well informed, undertaking both surveillance and research and development activities. Its services underpin regulatory frameworks as well as supporting religious and cultural factors such as food provenance (e.g. halal or kosher).

Fera was member of the European SAfe FEED-Processed Animal Proteins (SAFEED-PAP), alongside our NERC CONNECT B partner, Stephen Woodgate, then of Prosper De Mulder Ltd (PDM) and Technical Director of the European Fat Processing and Renderers Association. Our findings that collagen was thermally stable and that animal origin could be identified by a peptide fingerprint were reported by Woodgate to the SAFEED-PAP team and Collins was invited to become an advisor to the project. Working with Fera scientists we demonstrated that ZooMS was able to discriminate (thermally treated) meat and bone meal particles. Dr Vincent Baeten, coordinator of SAFEED-PAP and Head of the Food and Feed Unit for the EU Reference Laboratory, applauded *"…the innovative analytical solution that the Department has proposed for detection of animal bone, using protein mass-spectrometry...[and] the effort made...to adapt this method coming from archaeology to feed safety…"*[b].

Collins was subsequently invited by Fera to apply the technique within a Food Standards Agency (FSA) investigation into the suspected use of pig and cow-derived gelatin (so-called plumping agents) used to re-hydrate air-freighted chicken meat. ZooMS confirmed that the FSA's suspicions were correct.

The results of the investigation were broadcast on 14 July 2009 to an estimated audience of 5 million (*What's really in our food*?)[c]. Dr Shuja Shafi of the Muslim Council of Britain stated that Muslims would be extremely annoyed and extremely distressed to learn that chicken sold as halal contains protein from prohibited species and they would be extremely angry if this turned out to be a deliberate deception as Muslims rely heavily on accurate food labelling (minutes 53 -55 of programme[c]). The discovery of the fraud was widely publicised in the UK press and reported in the Annual Report of the Chief Scientist of the FSA[d]. Secretary general of the Hindu Forum of Britain, Bharti Tailor, was quoted on 04/06/2009 as saying "*Eating beef is expressly forbidden because cows are considered to be sacred as they are a representation of the bounty of the gods, even unknowingly. The fact that the protein powders injected into chickens served in restaurants and cafes contain even traces of beef or pork is horrific*"[c].

As a direct consequence of the investigation, food industry suppliers in Spain, Germany and the Netherlands were inspected and the largest supplier of poultry to the Asian market in the UK (Euro Foods Group) changed their practices. An announcement on their website (consulted July 2009) stated that in order to '*eradicate any future question marks and/or confusion over non-chicken*

protein detection levels in our product range, Euro Foods Group has decided to switch to a vegetable derived protein instead, a move which was completed by 29th June 2009', (see also minutes 51-52 of programme in reference 2). Due to the sensitive nature of the issue we do not know the full impact of our work.

We signed a license agreement with Fera in 2011 giving them commercial access to our collagen databases for use in the future forensic work for a fee of 5% of income generation. In a DEFRA-funded study (£91k contract, 01/03/11 to 31/03/12), they validated the technique and found it to be superior to commercially-available immunochemical and DNA based assays (Grundy *et al.* submitted).

The impact of this research extends internationally through the Fera contract, which included an inter-laboratory trial, during which the analytical method was successfully transferred to alternative food enforcement laboratories in Europe and North America (including the US FDA). York provided training in the methodology, species-specific marker peptide sequences and data assessment from these trials, our choice of mammoth gelatin as the positive control (CBC News: Jan 03, 2011), was in recognition of the archaeological origins of the method. A further DEFRA-funded project (FA0126) is transferring the method to other food matrices for Fera customers to screen foods destined for the Halal markets, products such as gelatin capsules (for therapeutic/supplement formulations) aimed at vegetarian customers and in the wake of the 2013 controversy, horse. This research therefore has impact on public and commercial services[e] as well as public debate.

Writing about the impact of the team's relationship with FERA, Adrian Charlton, Head of Chemical and Biochemical Profiling at FERA, wrote: "…*FERA's close collaboration with Professors Thomas-Oates and Collins has led to a number of technologies that we routinely exploit to deliver ongoing project work and to underpin project proposals, papers and publicity material. In particular, we have undertaken a number of studies to determine peptide sequences that can be used for the species origin determination in collaboration with you and your colleagues. These projects have led to Fera offering an* ***international service*** *for the species identification of gelatin and Meat and Bone Meal in food and feed, respectively…*".

5. Sources to corroborate the impact (indicative maximum of 10 references)

a. 263,000 Jews, 817,000 Hindus, 2.7M Muslims, 2011 Census (England and Wales)
b. Head of the Food and Feed Unit, EU Reference Laboratory
c. BBC programme http://www.bbc.co.uk/programmes/b00lrjk4 audience figures from http://www.tomheap.com/?cat=4 - broadcasts

   4 June 2009, *The Independent*, 'Chicken injected with beef waste sold in UK' http://www.independent.co.uk/life-style/food-and-drink/news/chicken-injected-with-beef-waste-sold-in-uk-1696407.html

   5 June 2009 *The Mail* 'Chicken secretly injected with beef and pork products served in UK restaurants' http://www.dailymail.co.uk/news/article-1190796/Chicken-secretly-injected-beef-pork-products-sold-UK-restaurants.html

   17 Sept 2009 *The Sun*, 'Chicken fill it' http://www.thesun.co.uk/sol/homepage/news/ 2464679/Chicken-fill-it.html
d. Annual Report of the Chief scientist of the Food Standards Agency 2009/10. p. 66 http://www.food.gov.uk/multimedia/pdfs/csr0910.pdf. Also FSA website 16 Sept 2009 '*New study highlights undeclared ingredients in chicken products*'
e. Head of Chemical and Biochemical Profiling at FERA