Institution: University of York
Unit of Assessment: 8, Chemistry
Title of case study: Food security, traceability and authentication

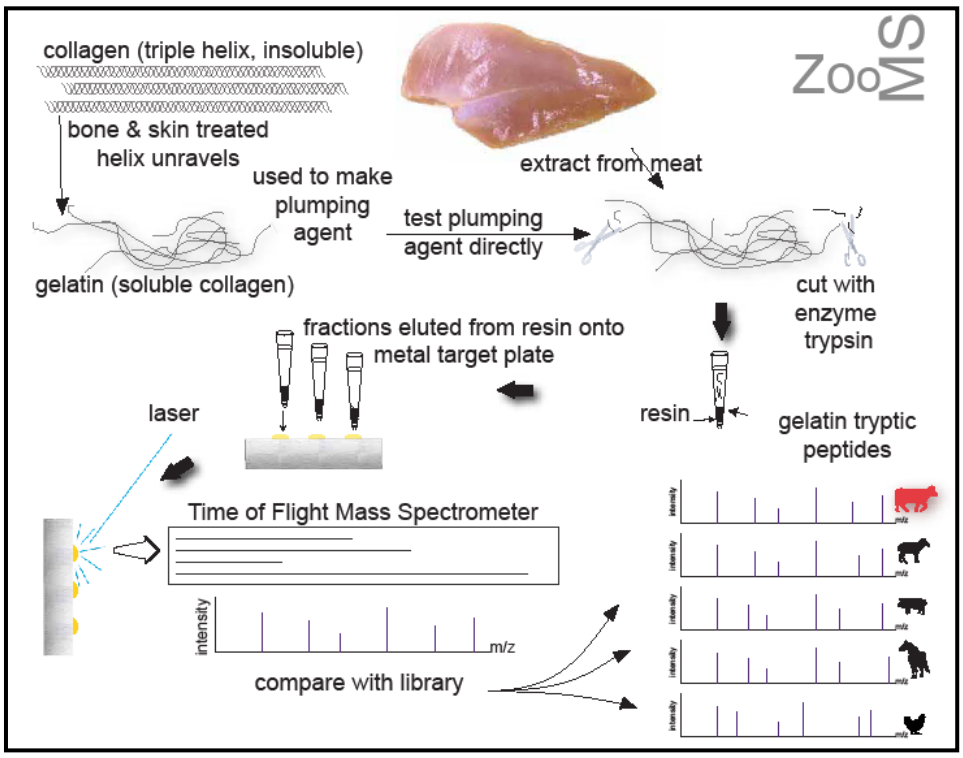
1. Summary of the impact (indicative maximum 100 words)

York's analytical methods have been applied in food authentication, traceability and safety and have been shown to be superior to other methods. Mass spectrometric methods developed in York for the identification of archaeological bone samples rely on analysing surviving fragments of the bone protein, collagen. These techniques also identify collagen fragments present in gelatin-based plumping agents that retain water in meats for human consumption. York's authentication applications disclosed the animal species from which the collagen was derived, and revealed contamination of chicken with pork-derived plumping agents, a significant issue in communities with halal and kosher diets. These results have been disseminated by high-profile media reporting, including a one-hour BBC special, and the press. The Food and Environment Research Agency (FERA, a DEFRA agency) has validated the York analytical method and applied it to processed food and pharmaceutical products. An inter-laboratory trial transferred the method to other food enforcement laboratories across Europe and the USA (including the US FDA). The results were highlighted in the press in 2009 and the debate over food authentication exploded in 2013 highlighting the economic effects of mislabelling. This research therefore has impact on public and commercial services as well as public debate.

2. Underpinning research (indicative maximum 500 words)

Professors Matthew Collins (Archaeology) and Jane Thomas-Oates (Chemistry) have had a joint programme of work since 2003, using mass spectrometry to analyse ancient and other damaged proteins. The protein collagen has extraordinary stability due to its three-dimensional structure, which has led to the idea that collagen can persist for thousands of years essentially intact. However, the structural features that make collagen so stable were for a long time also considered to make collagen useless as a species indicator. The highly conserved main collagen chain is made up largely of a tripeptide repeat (glycine-proline-hydroxyproline), while the chain-terminating 'telopeptides' were believed to be intractable, due to their cross-linking that is crucial for collagen fibril formation. In collaborative work, a postdoctoral fellow and PhD student (both NERC-funded) in the York research groups investigated collagen's preservation and developed approaches to its extraction from fossil bones.

The collaborators have shown that one of the *C*-terminal telopeptides is not involved in cross-linking and has some species-diagnostic value. In addition, and more importantly, the groups have demonstrated that the 'conserved' repetitive collagen backbone polypeptides in fact have a good deal of species-specific amino-acid sequence variation. The work has shown that collagen can be extracted effectively from very small samples of bone and, using the sample



handling strategies developed in this collaboration, employed to identify historical and archaeological bone fragments in the archaeological record. In these studies, as in much archaeological research, a broad network of collaborators is involved, all studying the same assemblages or samples. The role of the York chemists involved mass spectrometry (sample preparation, measurement and data analysis).The NERC-funded PhD studentship was a CASE Award with Prosper de Mulder, a meat and bone meal company, whose interest was in detecting not ancient but heat-treated meat and bone meal, and the ability to identify its animal source. The approach is similarly applicable to the detection and analysis of gelatin, which is produced from the collagen in bone on boiling. One of the outcomes of these projects is a rapid, affordable, medium-throughput method for detecting and determining the species of origin of archaeological bone fragments. The method which York termed Zooarchaeology by Mass Spectrometry or ZooMS, 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 (Figure). Dr Julie Wilson (Chemistry and Mathematics) joined the team more recently, applying data analysis and pattern recognition methods to the raw spectra, allowing species identification where sequence information may not be available. The outcomes of the collaboration also led FERA to invite the York team to join an EU Framework 6 project (SAFEED-PAP, Jan 2007-Dec 2009) to determine the species origin of proteins in animal feed (grant of £143k).

Key researchers

Jane E. Thomas-Oates: Appointed 01/04/2002 as Professor of Analytical Science

Julie C. Wilson: Started 01/10/93 as Research Fellow (RA1A), then RCUK fellow, transfer to Lecturer 01/07/2011 (joint Mathematics and Chemistry appointment).

Matthew Collins, Archaeology, started 01/10/2003, Reader then Chair, head of BioArCh, An interdisciplinary centre between ***Bio***logy, ***Ar***chaeology and ***Ch***emistry

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

This research exceeds the quality threshold as is evident from the journal quality and the number of citations (Scopus 20.9.13).

Grant:

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

Peer-reviewed publications:

M. Buckley, A. Walker, S.Y.W. Ho, Y. Yang, C. Smith, P. Ashton, J. Thomas Oates, E. Cappellini, H. Koon, K. Penkman, B. Elsworth, D. Ashford, C. Solazzo, P. Andrews, J. Strahler, B. Shapiro, P. Ostrom, H. Gandhi, W. Miller, B. Raney, M. I. Zylber, M.T.P. Gilbert, R.V. Prigodich, M. Ryan, K.F. Rijsdijk, A. Janoo, M.J. Collins, Comment on "Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry", *Science*, 2008, **319**, 33. DOI: 10.1126/science.1147046. *19 citations*

M. Buckley, M.J. Collins, J. Thomas-Oates "A method of isolating the collagen (I) α2 chain carboxytelopeptide for species identification in bone fragments", *Anal. Biochem.* 2008*,* **374**, 325-334. DOI: 10.1016/j.ab.2007.12.002. 7 citations

M. Buckley, C. Anderung, K. Penkman, B.J. Raney, A., Götherström, J. Thomas-Oates, M. Collins "Comparing the survival of osteocalcin and mtDNA in archaeological bone from four European sites", *J. Archaeol. Sci.*, 2008, **35**, 1756-1764. DOI: 10.1016/j.jas.2007.11.022. *16 citations*

M. Buckley, M. Collins, J. Thomas-Oates, J.C. Wilson "Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry", *Rapid Commun. Mass Spectrom.* 2009, **23**, 3843-3854. DOI: 10.1002/rcm.4316. *35 citations*

M. Buckley, S. Whitcher Kansa, S. Howard, S. Campbell, J. Thomas-Oates, M. Collins "Distinguishing between archaeological sheep and goat bones using a single collagen peptide", *J. Archaeol. Sci,* 2010, **37**, 13-20. DOI: 10.1016/j.jas.2009.08.020. *28 citations*

K. Korzow Richter, J. Wilson, M. Buckley, N. van Doorn, A. Jones and M. Collins "Fish 'n chips: ZooMS in a 96 well plate format to identify fish bone fragments", *J. Archaeol. Sci.* 2011, **38**, 1502-1510. DOI: 10.1016/j.jas.2011.02.014. *7 citations*

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, an agency of DEFRA close to York) guides the science evidence base in the food and environment sectors to ensure that UK policy makers are well informed, undertaking both surveillance and R&D activities. Its services underpin regulatory frameworks as well as supporting religious and cultural factors such as food provenance (*e.g.* halal or kosher). Five million people in the UK choose to avoid pig and cow products on vegetarian or religious grounds; they include 2.7M Muslims and 817,000 Hindus.[1]

The standard method of identifying the species origin of meat is DNA sequencing, but this is not possible on all samples (*e.g.* gelatin, material that has been heated). Moreover, exogenous DNA can be added fraudulently to mask endogenous signals. The York team of Collins and Thomas-Oates was asked to use ZooMS and other proprietary mass spectrometry approaches for collagen identification, to support a Food Standards Agency investigation into the use of pig- and cow-derived gelatin employed to re-hydrate air-freighted products in foods labelled as containing only chicken (the addition of other animal protein to meat stated to be from a single source is illegal). They were then approached anonymously by an investigative team later revealed to be from the BBC, to test chicken breasts supplied to Asian restaurants. As a result of the BBC TV investigation (broadcast on 14/07/09, entitled 'What's really in our food?'), Euro Foods Group announced on their website (July 2009) 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). Euro Foods (http://www.eurofoods.co.uk) is the largest supplier of poultry to the Asian market in the UK. The discovery of bovine gelatin in chicken was publicised in the UK press (*The Independen*t, *The Daily Mail*, and *The Sun*), and featured in the BBC documentary.[2] The research featured in the Annual Report of the Chief Scientist of the Food Standards Agency 2009/10.[3] 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 in reference 2).

As a consequence of York's work it was evident that a complex process had been developed to dupe DNA testing, enabling low-cost (pork and cattle) gelatin to be sold as 'chicken protein' and used as a plumping agent in chicken breast meat. Two factories (in Germany and Spain) that produced the plumping agents were raided by local inspectors.

FERA has worked with the York team to apply the University of York methods of analysis based on collagen sequencing to their core business of food authentication. Specifically, it has determined the species provenance of gelatin and hydrolysed collagen added to chicken fillets, so-called chicken-plumping agents. In a DEFRA-funded study (£91k contract, 01/03/11 to 31/03/12), it validated the technique against other commercially-available approaches (enzyme-linked immunosorbent assay (ELISA) and PCR) and found it to be superior, with the alternative techniques providing numerous false positive results. This contract involved an inter-laboratory trial of the method, including laboratories across Europe and in the USA, during which the analytical method was transferred successfully to alternative food enforcement laboratories. A further DEFRA-funded project (Defra project code FA0126) is transferring the method to a wide range of processed foods and pharmaceutical products. The method has also been developed and applied to other food matrices for FERA customers to screen foods destined for the halal markets and also for products such as gelatin capsules (for therapeutic/supplement formulations) aimed at vegetarian customers.

Since the outbreak of BSE in 1987, the inclusion of animal proteins has been banned in animal feeds to prevent cannibalism in the food chain. There is scope to relax the extended feed ban, should scientific methods be available to first screen animal feeds and then to provide confirmatory analysis of positive samples. A method was developed by the York team during this partnership to

determine the species origin of bone chips using the collagen method. This method is currently being reported to the EU, and FERA is publicising its availability.

There is now interest from various laboratories in Europe, as well as key government research laboratories to license the method for gelatin identification; the US Food and Drug Administration (FDA) is one of six partners in this development. Training in the methodology has been provided by the York team for FERA and for analytical laboratories from across Europe and North America (including the US FDA). The York team made species-specific marker peptide sequences available to FERA, that are now being used as internal standards in this assay. FERA is also training two Malaysian laboratories in the method.

In 2013, inclusion of horse meat in ready meals affected numerous products in the UK and other EU countries. FERA has demonstrated that, as expected, York's approach is also able to identify horse gelatin. FERA has funding approved to adapt the pork and beef methods for horse gelatin (Defra project code FA0126).

Quotations to support impact:

Collins was co-opted as a consultant to an EU project SAFEED-PAP, 6th EC FP, DG RTD. The archaeological method proved particularly appropriate to the detection of animal bone fragments. Dr Ir Vincent Baeten, Head of the Food and Feed Unit, EU Reference Laboratory said: *"I have to applaud the innovative analytical solution that the Department has proposed for detection of animal bone, using protein mass-spectrometry (ZooMS). I would like also to recognise the effort made by the Department to adapt this method coming from archaeology to feed safety"*.[4]

Dr Adrian Charlton, Head of Chemical and Biochemical Profiling at FERA wrote as follows, corroborating the nature and extent of the collaboration with FERA:

"*FERA's close collaboration with Professors Thomas-Oates and Collins have 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 MBM in food and feed, respectively*."[5]

The research undertaken in York has provided the food standards and enforcement agencies with the ability to determine the animal source(s) of processed foods in which DNA analysis is not possible, by targeting the protein which is the principal component of meat. The York methods have been adopted by the UK agencies and transferred to equivalent bodies in other countries. The need for these analyses has been demonstrated through BBC and FSA investigations of fraudulent labelling and further disseminated in the press. The importance and broad applicability of these approaches has been further highlighted by this year's horsemeat scandal.

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

1. 2011 Census (England and Wales).

2. BBC programme http://www.bbc.co.uk/programmes/b00lrjk4#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

3. 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 4 June 2009 'New study highlights undeclared ingredients in chicken products'. http://webarchive.nationalarchives.gov.uk/20101224202640/http:food.gov.uk/news/newsarchive/2009/jun/chicken

4. Head of the Food and Feed Unit, EU Reference Laboratory.

5. Head of Chemical and Biochemical Profiling at FERA.