LONGHAND DATA

Stable Isotope Reference Analysis

Project Report on Authentication of Country of Origin of Pork and Pig Meat in England & Scotland using Isotope Reference Analysis

June 2010
Final version

Authenticated brand

BPEX

BPEX Quality Standard Marks for UK Pig Meat
www.bpex.org.uk

Client

AHDB Agriculture & Horticulture DEVELOPMENT BOARD

Agriculture & Horticulture Development Board
www.ahdb.org.uk

Service Provider

LONGHAND DATA LTD

LONGHAND DATA

www.longhanddata.com

Table of Contents

Introduction

The 2009 BPEX Isotope project followed on from a series of smaller pilot phases in the period 2006-8: pilots that confirmed the underlying technology as sound. They also generated the necessary understanding and subsequent interpretation protocols for authentication of pig and pork products using stable isotope analysis. The underlying technology works because the ratios of isotopes of some elements as found in living, or once living things, is specific to the location where the food product was grown or reared. By sampling products of certain origin from producers across an area it is possible to build a reference library of isotope signatures representing that geographical area. It is then possible to test the provenance of unknown or uncertain similar food products by comparing the signatures as found in the test sample with the range of reference signatures, the question being: is the test sample from the geographical area as represented by the reference library.

Summary

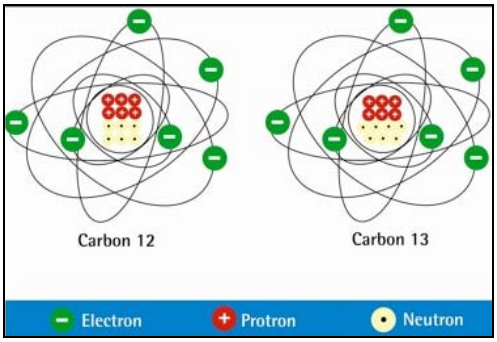
The 2009 BPEX Isotope Project delivered an isotope reference library for pig and pork products representing the pig producers of England and Scotland; that reference library is referred to below as 'the database'. The project then compared anonymously supplied samples of pig and pork products, to confirm that their provenance can be determined from comparison of test signature against reference data. In this final phase of the project 35 anonymously labelled samples (15 fresh pork, 10 bacon or gammon and 10 ham) were supplied, of which the producer address (in England) was categorically known by BPEX only for five samples. Having analysed the 5 samples and compared their signatures against the reference database, the project was successful in locating those definitely known to be from the UK, doing so with a probability above 99%.

The 2009 Project

First sampling in the commercial phase began on 31st March 2009; by 11th March 2010 a total of 228 samples had been sent for analysis to Agroisolab, Germany; of which 153 were from known English and Scottish farm locations, 63 were non-UK samples supplied by a commercial producer on behalf of BPEX, whose origin is known only at country level. In addition, 12 water samples from abattoirs cleansing bowls were analysed to calibrate the hygiene standards used during sample taking. The total of these results constitute the Isotope Signature Reference Library (the database) for pig meat in this project. Therefore, discounting the water samples, the combined UK and non-UK database comprises signatures from 216 English and Scottish locations.

1. **Project principles**

The science behind Stable Isotope Reference Analysis (SIRA) This analysis technology relies on it being possible to measure the natural but small difference that exists between the mass of isotopic forms of the same element. Mass difference arises because the number of neutrons in each atom of an element can vary whilst the number of electrons and protons remains the same; for example carbon exists in a number of isotopic forms, of which two: ^{12}C and ^{13}C have respectively 6 protons/6 neutrons and 6 protons/7 neutrons. These particular isotopes of carbon are interesting in the context of analysis of food products. ^{12}C is different to ^{13}C to the extent of just one neutron in each ^{13}C atom.



^{12}C AND ^{13}C: TWO ISOTOPES OF CARBON

1.1 **What SIRA measures**. The difference one additional neutron makes to an atom is extraordinarily small at just 1.7 x 10^{-27} kg. Despite this minuteness it is possible using a mass spectrometer to measure the abundance of each isotope in a sample under analysis. SIRA does not express results as the absolute abundance but rather the ratio of pairs of isotopes.

1.2 For example, a typical ratio for the two carbon isotopes interesting in food authentication ^{12}C and ^{13}C, could be 98.89% ^{12}C and 1.11% ^{13}C. For simplicity's sake this ratio statement is more easily expressed as a whole number; this is achieved by multiplying the ratio by 1,000 and appending the permil sign: ‰. Thus the ratio expression above is represented by the permil figure 11.22‰.

2. **SIRA Results**. A further complexity of analysis sees actual results shown as the variance between the ratio in the tested sample compared to a standard ratio as provided by the International Atomic Energy Authority (AEA). For example the AEA standard for hydrogen and oxygen is called the Standard Middle Ocean Water (SMOW). While the differences

between samples and the standard may appear small, a difference of even 1 permil is significant. A typical analysis result therefore might look like this

-40.0‰ D/H v.s. SMOW

This expression can be read as showing a -40 permil (-4 percent) variance for the ratio of Deuterium[1]/Hydrogen in the analysed sample compared to the D/H ratio in the Standard Middle Ocean Water (SMOW).

Longhand SIRA tracks six trends in the isotopic signatures of four elements.

Hydrogen D/H
Hydrogen D/H_{org}
Carbon $^{13}C/^{12}C$
Carbon $^{13}C/^{12}C_{lipid}$
Nitrogen $^{15}N/^{14}N$
Sulphur $^{33}S/^{32}S$

The isotopes of hydrogen appear twice, once as the D/H ratios found in water extracted from between muscle cells (whose origin is the drinking water provided to the animal, and thus representative of the local water supply to the farm); secondly hydrogen also appears as D/H_{org}, representing D/H ratios extracted from tissue protein (whose origin is the animal ration).

Carbon $^{13}C/^{12}C$ ratios are measured in protein and separately in lipids (fat), the distinction in labelling being indicated by adding 'lipid' to the element expression thus: $^{13}C/^{12}C_{lipid}$.

3. **Isotopes and authentication**. Isotopes of the same element have nearly the same chemical characteristics and behave identically in biology. The constituents of a pig ration: the proteins, fats, carbohydrates, trace elements and vitamins, plus water are made up of a combination of many elements, each occurring in one or more isotopic form.

3.1 **Geographical factors** The significance of this becomes interesting and commercially valuable when reasons for ratio variance are understood. In water, for example, there is a strong correlation between the isotope signature and the geographical location where the sample originates. This geographical factor is

[1] The ^{1}H isotope of Hydrogen is shown as H, whilst the ^{2}H variant, called Deuterium, is shown as D

strong enough to determine indicative origin on a country by country basis analysing water alone.

3.2 **Local factors**

3.2..1 **Nitrogen and Sulphur** Every plant as it grows draws nitrogen and sulphur from the soil. There are a unique set of circumstances for each location that defines isotope signature of that location. The isotopic signatures of nitrogen and sulphur as found in plants is defined by the supply available from the soil: the use of FYM v.s. synthetic fertilisers, previous cropping, the nature of existing soil organic material, invertebrate populations and the population density and activity of nitrogen fixing bacteria.

3.2..2 **Carbon.** The influences on carbon isotope signatures found in plants (and by extension also in the tissues of the animals that eat them) is determined by the plant type, more specifically the type of plant metabolism; for example C3 plants (that make up over 95% of terrestrial plant life: grasses, cereals, sugar beet), have a metabolism that generates higher depletion of ^{13}C than the metabolism of C4 plants (maize, sugar cane, sorghum, millet). This has significance to SIRA authentication since C4 plants have higher ^{13}C content in their isotopic signature; a characteristic that is carried forward to animals fed a high C4 content diet; in farming terms this means animals exhibiting high ^{13}C will likely have been fed a ration rich in maize content - unusual in the UK.

4. **Authentication aims.** The authentication aims of this project are to protect the BPEX Quality Pork Standard as marketed under the 'No More Porkies' campaign *(see appendices 5 & 6).*

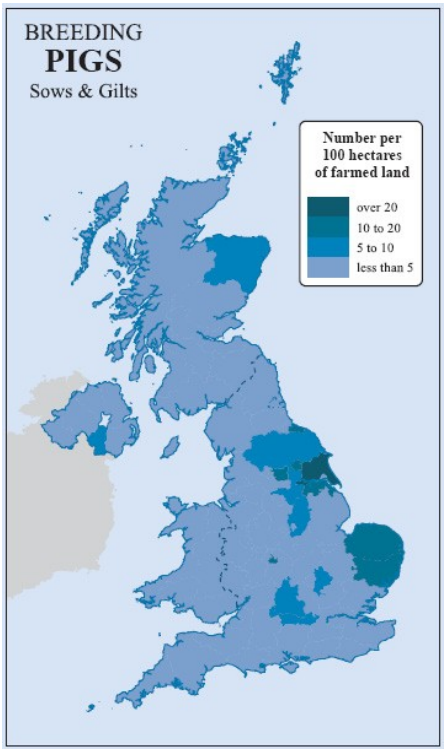
4.1 Authentication will be achieved in the SIRA project by confirming or refuting the stated (labelled) country of origin for pork and pig products on sale within the UK. To achieve this BPEX will compare SIRA signatures: one sample of known origin against one of unknown or suspect origin.

4.2 The 2009 AHDB funded BPEX project produced a database of UK SIRA signatures, with reference samples selected to be suitably representative of UK pig production, and against which test samples can be compared. The territory protected is England and Scotland. No samples were referenced from Wales or Northern Ireland.

5. **Reference Library – Design aims and objectives.** Experience from other authentication projects in continental Europe has shown that a territory can be successfully defined in a SIRA library by collecting samples geographically representative of an area, not by attempting to sample every production location in the area, desirable though that may be. A country, or region within a country, can be adequately represented by careful choice of reference locations. In considering which locations to include the following are taken into account

- Distribution of all locations to be authenticated
- Location of ground water aquifers relative to locations
- Boundary considerations: closeness of locations within and without the authenticated region.

5.1 **The BPEX SIRA Library - UK.** The authenticated regions referenced in this project are England and Scotland. Distribution of pig production is concentrated in East Anglia and North East Yorkshire in England and up the east coast in Scotland; however production is not confined to these regions alone and there are pig units in every county in England and Scotland:



DISTRIBUTION OF UK PIG PRODUCTION

In considering the geographical protocol the importance of regions of greatest concentrations of pig farms must not override the need to run an analytical fence around the whole area to be authenticated. There are 10,000 UK holdings with pigs, of which 1,700 to 2,000 holdings are responsible for 80% of production. Whilst it is not practical

within the constraints of budget to sample all 2,000 farms in the first year, it might be easily possible, if a rolling programme continues the reference build in year two and onward.

5.2 **The BPEX SIRA Library – Non-UK.** 63 samples were analysed and included in the database from five European countries; these were important samples as their variance to the UK signatures gives a good indication of the robustness of difference that will be achievable when retail products are tested against the reference data.

Non-UK countries referenced:

- Spain
- Denmark
- France
- Germany
- Holland

6. **Reference Sampling.** The precise nature of sampling carcass tissue was given careful consideration and tested by experimentation (see appendix 1). The protocol for selecting farms was arrived at in consultation with BPEX.

6.1 **Picking farms**. At the outset when no data was to hand locations for sampling were defined by the areas with highest numbers of pig farms and sampling from East Yorkshire and East Anglia dominated first weeks of activity. When a farm had been sampled the post code for that location was stored alongside the Longhand reference number in a spreadsheet used to record SIRA scores. Using the post codes, pins identifying each farm by number were inserted on a wall map to show graphical distribution of the growing library. Distribution of sampled farms was monitored carefully and as the volume of data increased care was taken to reject for analysis farms close to those already sampled – with the exception of the two eastern regions where concentrations of pig farms is high. For these areas whilst exact duplicates (same village) were avoided, the density of sampled farms was driven to match the regional density of total farms. Contrariwise, for the least dense areas of pig production the number of sampled farms was correspondingly low, making sure nevertheless that as much as possible of the whole of England and Scotland was represented by at least one sample. A wall map was supplied to BPEX with numbered pins showing the location of each sampled farm.

6.2 **Sampling method.** Working wit**h Dennis Homer,** BPEX Meat Technologist, Longhand drew up a sampling protocol whose aim was to deliver robust

confirmation of origin of each sample, accurate labelling and appropriate hygiene as a prevention of cross-contamination between carcasses. The full protocol can be seen in *Appendix 1*. In summary, the protocol demands for two to five sub-samples to be taken representing one location – each sub-sample coming from a separate carcass of the same production source. Quantities of pigs in consignments sent to slaughter on any one day vary depending on the size and type of the producer location: size of producer facility was reflected in the range of pigs per consignment at slaughter, ranging from above 200 pigs per consignment for large fattening units to as little as two animals for small farms.

6.3 **Location on the carcass.** During pilot phases in 2007 & 2008 a number of sampling methods were tested. These included blood taken from living pigs on-farm by a vet; tissue samples taken on-farm from fallen stock and docked tails. However for a variety of reasons it was concluded that on-farm sampling is not practicable, not least because prevention of cross-contamination between samples could not be reliably controlled. There was also an issue of scalability since it was calculated that within the year allocated, there was insufficient time to visit all farms for the number of samples required to build the database; whereas collecting in an abattoir could deliver samples at the rate of 15 farms per day. Abattoir collection quickly developed into a productive routine in which the five samples from each location (as defined by the slap mark) were bagged, labelled and stored in ice prior to transit for analysis.

6.4 **Water samples.** When defining and implementing the sampling protocol, effort was placed on ensuring that when tissue samples were cut and removed to sample bags, this process could be done without contamination from abattoir water. However to offset the likelihood that despite best efforts contamination does occur, samples of abattoir sterilising wash water have been taken. These samples will be used to check any tissue sample SIRA score that appears to be out of line with others of the same post code origin.

6.5 **Storage and forwarding of samples**. The sample collection team comprised a BPEX representative, Dennis Homer assisted by a Longhand staff member. Actual sample selection and cutting from the carcass was carried out by Dennis.

6.6 **Reference Archive.** For each 30 to 40 gram sample sent for analysis, a duplicate sample was been retained and is stored at -80°C in a laboratory grade freezer under Longhand management. The archive facility has additional power generation capacity available during power cuts.

6.7 **Slapmarks used on multiple pig units**. Most producer units (the actual geographical location where a pig is reared) can be correctly identified using the post code associated with a particular slapmark. However a significant minority of producers use a common slapmark across two or more locations. This means slapmarks alone cannot always be relied upon as a means of determining the post code for the location of rearing. Knowing this weakness in the system Longhand and BPEX cross-referenced additional location information available at the time of slaughter to arrive at a postcode whose accuracy could be relied upon.

7. **Results**. The full range of results was supplied separately to this report as data confidential to BPEX. However graphical representation of result distribution can be seen in Appendices 4 & 5. Interpretation of results, analysis and comments are the work of the chief food scientist at TÜV Rheinland Agroisolab, Dr Markus Boner. He and the laboratory are responsible for the technical and scientific aspects of this project.

Interpretation of results falls into two aspects:

- **UK v.s. non-UK.** Can clear distinction be seen between UK and non-UK SIRA signatures?

- **Confidence in results.**

7.1 **UK v.s. non-UK.** *APPENDIX 4* is an image that represents graphically the analysis results of the total reference samples collected and analysed in this project. The graph is an important component in the BPEX authentication programme since it confirms that significant discrimination between UK and non-UK meat can be achieved using isotope analysis. On the graph each sample signature is represented by a dot, appended by the country of origin. The analysis tool used to produce this interpretation, Principle Component Analysis (PCA), is a statistical modelling algorithm that reveals the internal structure of data in a way which best explains the variance in the data, presenting it in a visually meaningful way. On this basis it is reasonable to propose that the reference data collected in this project can be seen as sufficient to act as a reference database testing against samples taken from retail and elsewhere in the supply chain.

7.1..1 **Trends within results - Sulphur.** For ratios of sulphur isotopes trend analysis, by plotting reference location on a map shows some coherent trend detectable within the data, though weak. See APPENDIX 3 for details.

7.1..2 **Trends within results - D/H.** The same trend analysis applied to D/H ratios produces a more marked north/south trend; the results can be seen in APPENDIX 2. For D/H ratios the north/south correlation is represented by increased depleted ratios to the north. A less marked trend exists longitudinally together with some coastal effects: where enriched D/H appears in coastal regions compared to inland areas.

7.2 **Confidence in the results**. When the reference library is used to authenticate samples taken from the supply chain it will be important that there is clarity in the way results are presented. Statistical analysis will be used to determine where any particular result lies in the range that extends from "definitely UK origin" to "definitely not of UK origin". The position being expressed as a probability of a result being at a particular point along the range. In practice four ranges will be presented, shown as statistical variance from the mean. The variance, represented by the Greek letter sigma (symbol σ) will be accompanied by a statement for that range, expressed in terms of provenance to UK (or not) of the test sample being compared to the reference database.

CONFIDENCE RANGES

Results will be presented as a percentage score representing the confidence of any particular stable isotopic test signature being of the reference database.

1. A score in the range **95-100%** means the isotopic signature puts the test sample as being **definitely** from the database.

2. A score in the range **90-95%** means the isotopic signature puts the test sample as being **typical** for the database.

3. A score in the range **40-90%** means the signature puts the test sample as being **unlikely** to be from the database.

4. A score in the range **0-40%** means the signature puts the test sample as being **definitely not** of the database

8. **Comparison of 35 anonymously labelled samples against the database.**

8.1 Having completed reference sampling and preparation of the reference database 35 anonymously labelled samples were supplied by BPEX for comparison against the database. The 35 samples were made up as follows:

- 5 UK sourced fresh pork samples of certain origin, obtained by BPEX directly from abattoirs in England and Scotland
- 30 retail samples
  - 10 Fresh pork
  - 10 Bacon/ham
  - 10 gammon
- Of which
  - 19 labelled 'British' or 'from Britain'
  - 5 labelled 'UK'
  - 6 labelled from other countries, Denmark, France, Germany and Netherlands (non-UK)

8.2 **RESULTS**. Analysis and comparison of the 35 samples produced the following results:

- The five samples of UK sourced fresh pork of certain origin (known to BPEX) were correctly identified from within the 35 – all five identified as consistent with the database with 99.1-99.9% probability, thus "definitely UK".
- Four samples were determined "Definitely not from the UK database" (0.0-24.7%)
- One sample was determined as "Typical of the UK database" (92.6% probability) though labelled of French origin.
- One sample was determined as "Definitely of the UK database" (97.4% probability) though labelled as being from the Netherlands.

- Of the remaining 24 (retail) samples

  - Analysis of 14 labelled ‘British or from Britain’ indicated this to be so: “Definitely of the UK database” with 95.1-99.7% probability.

  - Analysis of three retail samples showed a probability of 91.5-93.3%: “Typical of the UK database”. Of these, one was labelled ‘from the UK’, the others either ‘British’ or ‘From Britain’.

  - Analysis of one sample labelled ‘British or from Britain’ indicated it was “Unlikely to be from the UK database”; probability 58.3%

  - Six retail samples (four labelled UK, two labelled either ‘British’ or ‘From Britain’) were determined as “Definitely not from the UK database” (probability 3.9-35.4%)

Trust in the true origin of the 5 fresh samples supplied by BPEX is certain; so that analysis result can be held to be accurate and reliable.

However trust in the results of the remaining 30 retail samples is compromised to the extent of the face value that can be attributed to the retail labels.

APPENDIX 1

BPEX ISOTOPE ANALYSIS SAMPLING PROTOCOL V3.1 APRIL 2009

1. **Quantity of reference samples.** 200 farms in first year, collected and forwarded for analysis at the rate of approx 10 to 20 samples per week.

2. **Choice of farms – first samples.** First reference sampling will be from farms delivering pigs to abattoirs in England and Scotland.

3. **Sampling team.** Staff representing the Supplier and BPEX (or a BPEX nominated meat technologist).

4. **Choice of farms – subsequent samples.** The Supplier will monitor and map the locations of farms sampled and based on the principle of geographical and hydrological separation, develop and apply a more considered selection of farms so that a UK-wide representative data set is available at the end of year one. Some farms will be re-sampled to test how isotope signatures drift over time.

5. **Sampling.** Using a disposable knife, 40g of tissue will be cut from muscle located between the hind legs, exposed when the first ventral opening cut is made to a carcass post-slaughter. This location is not prime meat but does constitute tissue comprising muscle and fat. Importantly the location is inside the carcass, not contaminated by surface material or scald water.

    5.1 To further ensure the sample is not cross contaminated, boars will be selected for sampling as cutting to remove testicles acts as a cleaning process of the slaughterer's knife to remove abattoir sterilising water that remains on the blade when a fresh knife is drawn from the sterilising bowl. In this way the ventral cut – exposing the sample area – is not contaminated. Sampling tissue from sows is appropriate either when sows only are available or where slaughterers open several carcasses between dipping knives in sterilising bowls. In these instances sampling is carried out on the second carcass with the same slap mark opened by a line worker using a knife already used on a carcass since sterilising his knife.

    5.2 Sample hygiene. Both the Supplier's and BPEX staff will wear disposable gloves. Knives will be washed (in abattoir knife cleaning units) then dried between each group of carcasses representing one farm.

5.3 All samples will be sealed in labelled plastic sample bags and frozen down – starting with abattoir-supplied ice if available. The Supplier will ensure that both analysis and archive samples will be frozen on the day of collection, or soon thereafter. Dispatch of samples by courier will be in a frozen state.

5.4 For each abattoir approx 25ml of cleaning unit water will be sampled and analysed for the purpose of crosschecking isotope carcass cleanliness.

5.5 Up to five separate carcasses will be sampled from each farmer supplied consignment of pigs supplied to the abattoir on the day of sampling. The initial 40g sample will be divided into two approx 20g samples – one for analysis, one for archiving. These samples will be analysed together to provide one reference signature for each farm.

6. **Forwarding samples for analysis.** The Supplier will courier samples to the lab each week for analysis at the rate of 10 – 20 per week. Second samples will be bagged, labelled and archived by the Supplier at -80°C.

7. **Other reference material:**

a. BPEX will supply samples of fresh pork and cured pork from countries outside the UK as deemed appropriate and practical.

b. Bacon pre/post testing: BPEX will supply 5 samples of pork from each of Sweden, Denmark and Holland, plus 5 samples of bacon cured from same source (total of 10 samples) to determine differences in isotope signatures pre and post curing.

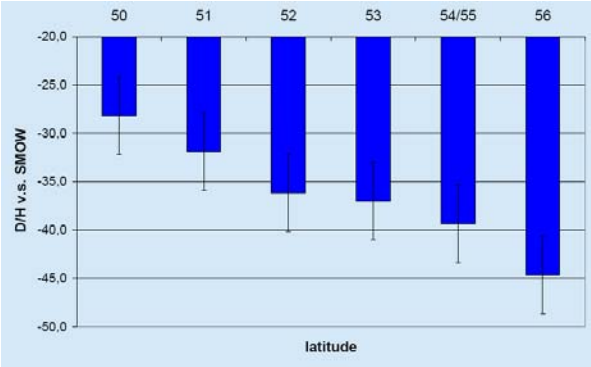
c. Assuming some multiples, total additional samples: about 30

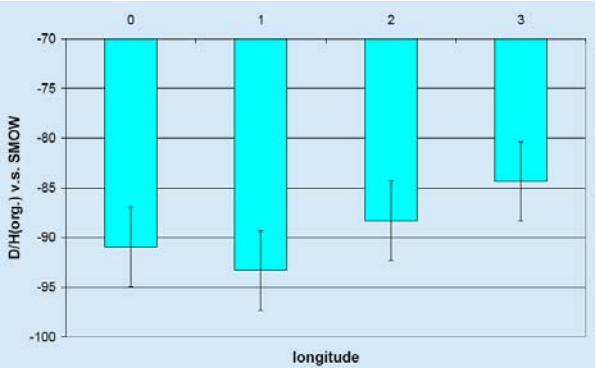
d. Sample to be frozen on same day as collected and transferred frozen to the Supplier for routing to laboratory for analysis.

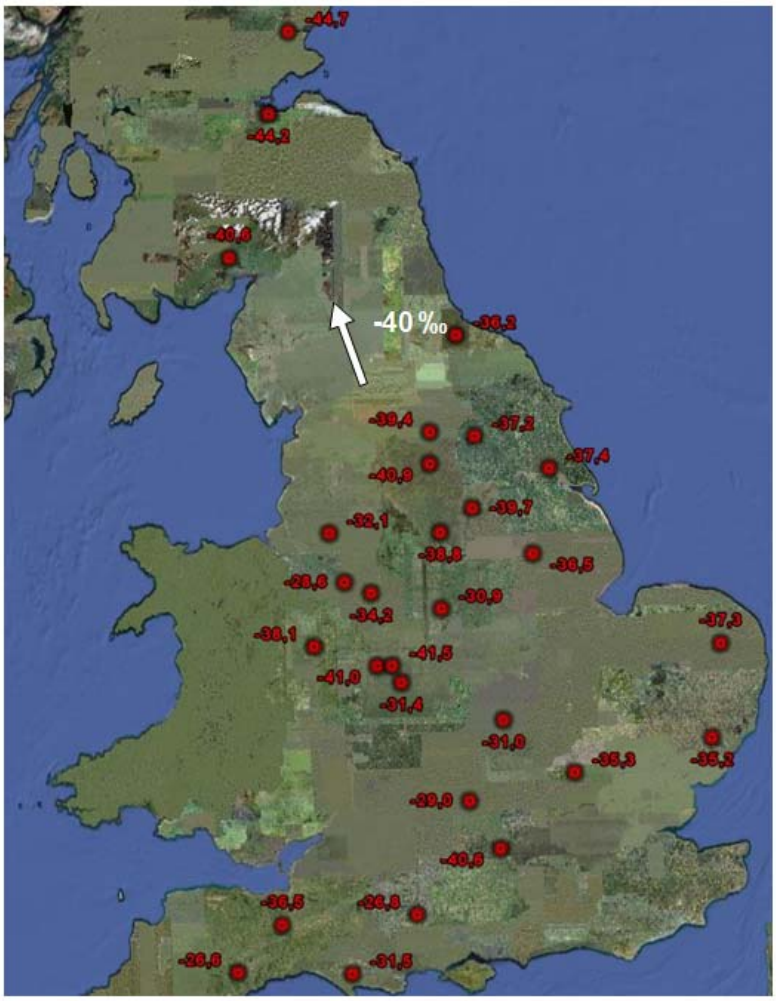
8. **Test Samples.** Testing against the database can start early on, provided non-UK reference material (Para 6.) is to hand and provided the declared provenance of test samples indicates origin is within already referenced areas. Test samples will need to be of at least 40g and accompanied by the package labels. Test samples should be couriered frozen to the Supplier for onward shipping and analysis.

APPENDIX 2

D/H V.S. SMOW LATITUDE AND LONGITUDE DRIFT





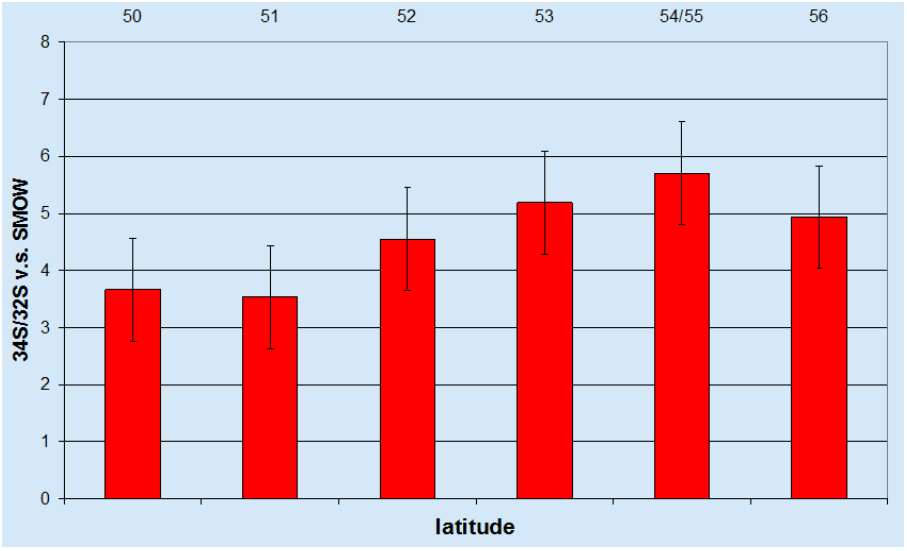


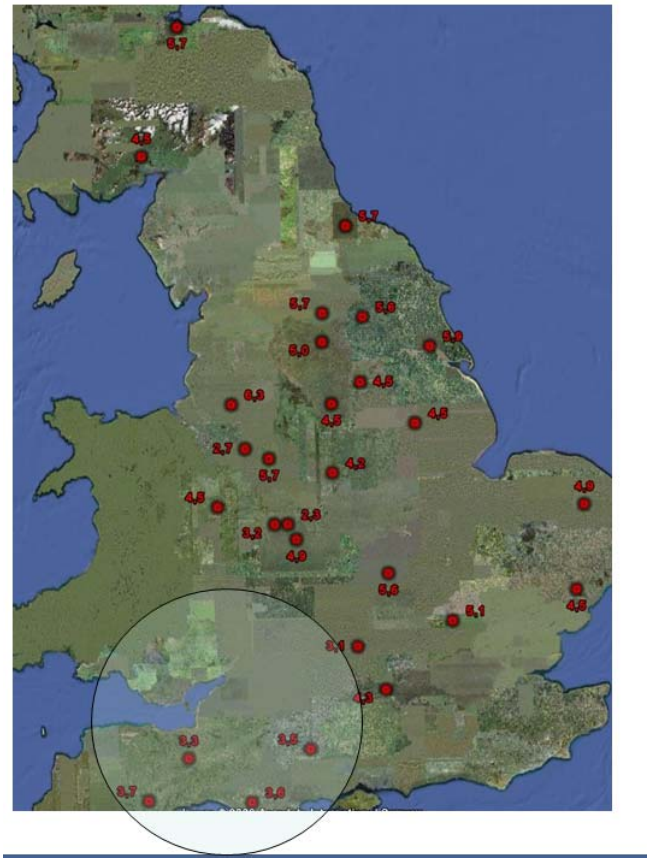
Latitude 50 is at Lands End; latitude 56 cuts across Scotland at Edinburgh. The charts above show a correlation exists with latitude between depletion drift from enriched D/H ratios in the south to depleted ratios in the north. In addition there are coastal effects (not shown here), that manifest as enriched D/H in coastal regions compared to regions further from the sea.

For longitude the correlation is less marked, though perhaps helpful for regional differentiation; more obvious longitudinal correlation may emerge as more reference is available.

APPENDIX 3

$^{34}S/^{32}S$ V.S. SMOW LATITUDE DRIFT FOR SULPHUR IN RAW PROTEIN

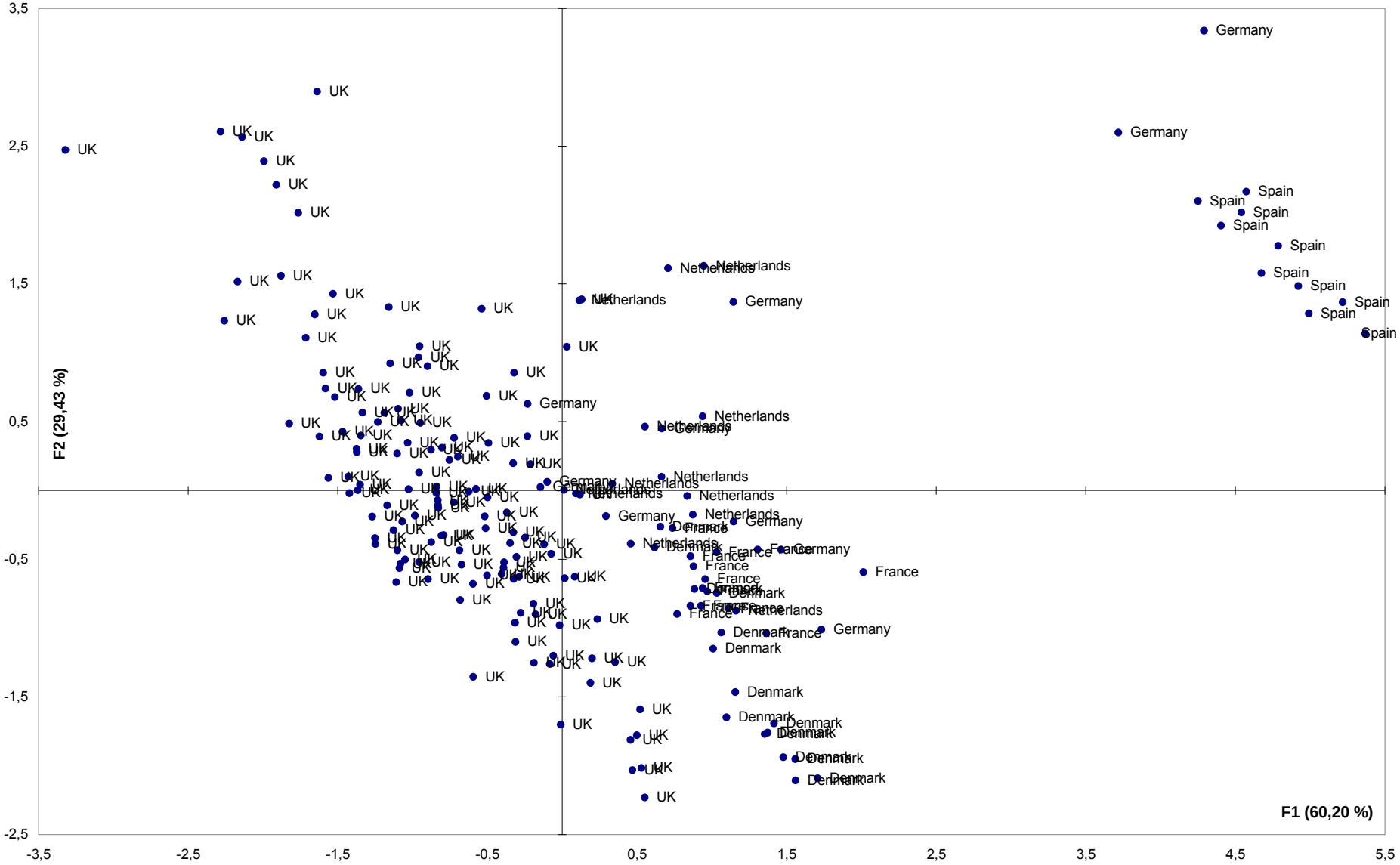




Samples from farms in the south of England, latitude 50-51, show depleted $^{34}S/^{32}S$ ratios in comparison to remaining samples. Normally the ratio is in the range 2- 6‰ and it is unusual that there is a tendency for depleted $^{34}S/^{32}S$ ratios. The reason could be a special situation of geological structure of the soil, but further sampling may provide indications that can be understood in the future.

APPENDIX 4

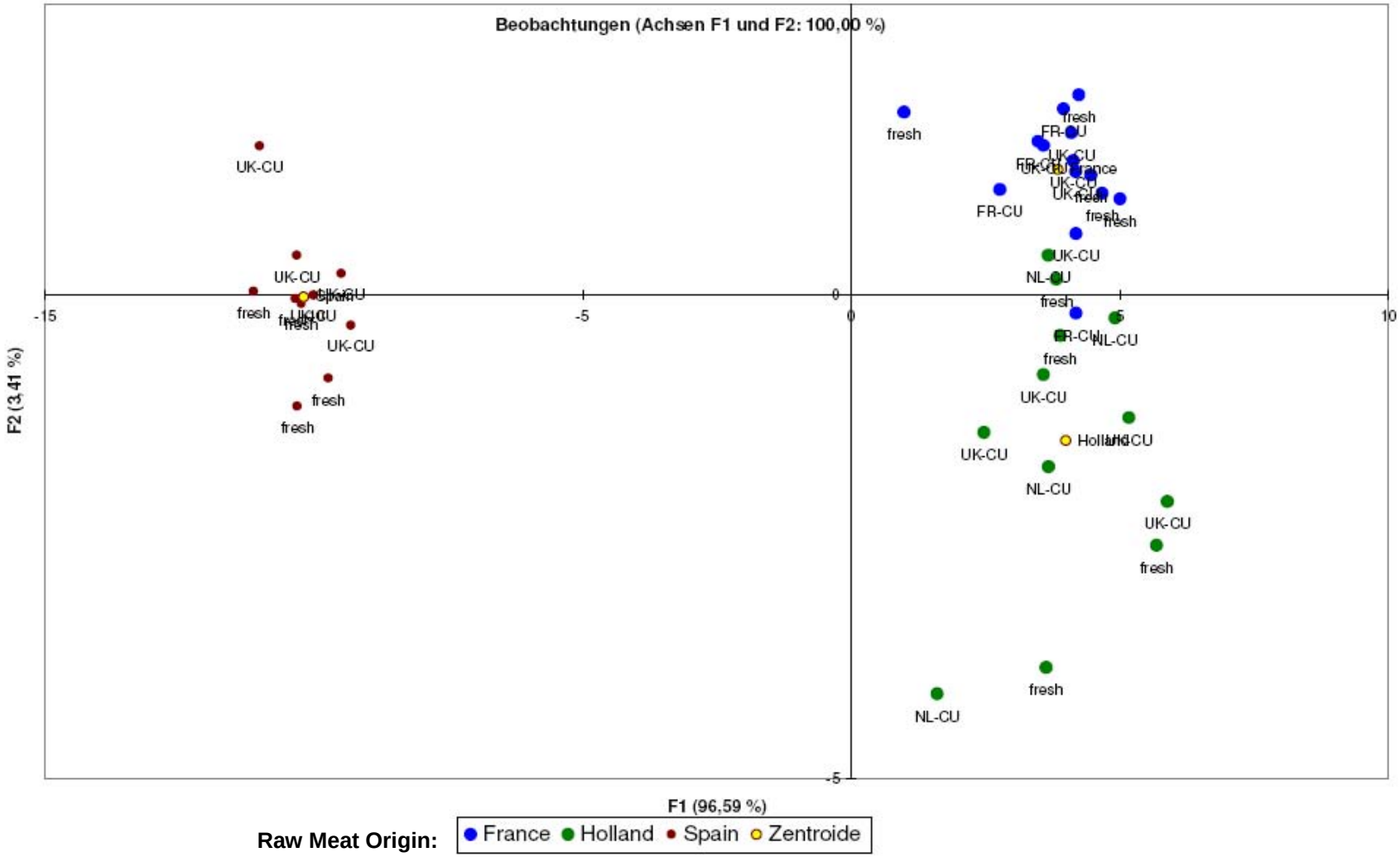
Principle Component Analysis using ^{13}C (from lipids and raw protein) and ^{34}S, ^{15}N



APPENDIX 5

Principle Component Analysis (PCA) showing isotope signatures for samples sourced from continental Europe and cured in the UK.

Illustrates ability to discriminate country of origin despite contamination during UK cure process.



APPENDIX 6

Trade press advertising campaign

APPENDIX 7

Flyer for the BPEX authentication marketing campaign 'No More Porkies'

Factual Guide to Country of Origin Labelling