FINAL REPORT

Authentication of Country of Origin of Pork and Pig Meat using Isotope Reference Analysis

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EXECUTIVE SUMMARY

The use of stable isotope analysis for the verification of origin is based on the existence of more than one isotopic form of the same element. The different isotopes differ only in that the number of neutrons in each atom of an element can vary whilst the number of electrons and protons remains the same. This means that in most aspects the different isotopes behave in the same way - in biochemical processes in plants and animals for example. These differences in the number of neutrons, however, mean that there are small differences in mass between the isotopes. This results differential partitioning of the different isotopes as they pass through physical and biological processes. It is therefore the ratio of the different isotopic forms of an element that can give useful information related to the route by which that element was accumulated in a particular plant or animal tissue, and therefore its geographical origin.

This research project, carried out during 2009-2011, was aimed at comprehensively evaluating stable isotope analysis for the origin verification of pork and pork products, and establishing a reference database as the benchmark against which future test samples can be compared for origin verification.

The main purpose of the project was to establish a reference database and use samples of known origin (presented blind to the laboratory) to robustly test its use for origin verification. A successful outcome would be to have sufficient confidence in the results to use the database as the basis for supplementing paper-based audits.

By August 2011 a database of 380 samples was assembled - 253 from England and Scotland, 55 from Ireland (North and South) and 72 from other European countries. Analysis has been undertaken to determine the degree of confidence with which the populations from the different countries/regions represented can be separated.

Good separation can be achieved of samples from Britain against samples of other origin. Samples from Ireland are difficult to separate: (a) because it is not possible to clearly separate the isotopic signatures of Northern and Southern Ireland and; (b) because Irish pork shows a greater similarity with pork from continental Europe than does British pork, probably due to feed imports to Ireland.

Origin verification is more difficult for processed products. It has proved possible to use the approach for determination of the origin of cured pork (bacon and ham), and this can be improved by the use of correction factors for the effect of processing on the isotopic signature - to enable comparison with the reference library of fresh pork samples.

Sausages have presented difficulty because of the other ingredients in sausage recipes that confound the analysis. It is therefore recommended that this approach is only used for products that have a declared meat content of 90% or greater.

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Overall it can be concluded that isotope analysis against the known database is a very useful tool to use alongside more traditional supply chain auditing techniques to provide increased confidence to customers that the correct information is on the label.

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Authentication of Country of Origin of Pork and Pig Meat using Isotope Reference Analysis

1) INTRODUCTION

Most of the major retailers and caterers in the UK demand pork produced to meet quality standard mark (QSM) standards. To carry the QSM bearing the Union Flag pork (fresh or contained in products) must have been sourced from pigs reared and slaughtered in the United Kingdom.

Supply chains providing pork carrying the QSM are subjected to regular audits to verify that procedures are in place to ensure that the origin is genuinely the UK. Nevertheless, there is a desire to strengthen the audit process to provide greater reassurance to customers on the origin of pork. Independent sample testing that can verify the origin of pork would add robustness to the audit process and provide the opportunity to focus audit activity on any areas of the industry that did not consistently yield clear verification of the origin.

During the period 2006 to 2008 BPEX funded a number of pilot studies using stable isotope analysis as a technique for the verification of the origin of pork. These confirmed that the approach has clear potential and also established an understanding of the opportunities and potential limitations of the technology.

This research project, carried out during 2009-2011, was aimed at comprehensively evaluating stable isotope analysis for the origin verification of pork and pork products, and establishing a reference database as the benchmark against which future test samples can be compared for origin verification.

2) SCIENTIFIC BACKGROUND

The use of stable isotope analysis for the verification of origin is based on the existence of more than one isotopic form of the same element. The different isotopes differ only in that the number of neutrons in each atom of an element can vary whilst the number of electrons and protons remains the same. This means that in most aspects the different isotopes behave in the same way - in biochemical processes in plants and animals for example. These differences in the number of neutrons, however, mean that there are small differences in mass between the isotopes. This results differential partitioning of the different isotopes as they pass through physical and biological processes. It is therefore the ratio of the different isotopic forms of an element that can give useful information related to the route by which that element was accumulated in a particular plant or animal tissue, and therefore its geographical origin.

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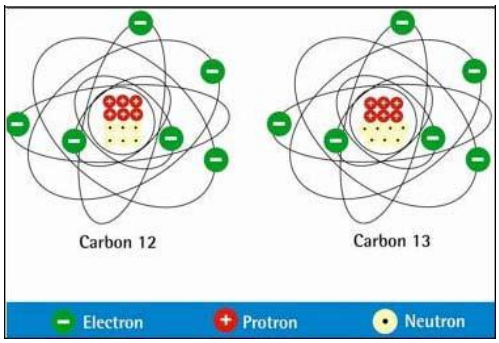
Isotopes of importance in biological systems

Clearly the isotopes that are of importance are those for the elements that are either dominant in biological tissues (eg pork meat) or that are good indicators of origin in some way. The project relied on the analysis of ratios in four elements. The main elements of interest are described below.

Carbon

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. ^{12}C is different to ^{13}C to the extent of just one additional neutron in each ^{13}C atom.

Two isotopes of carbon. Illustrating the difference of one neutron



The relative proportions of these two isotopes of carbon in meat varies with the type of plant matter from which it has been derived. 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 type of plant metabolism (photosynthetic pathway). 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 results in C4 plants having a higher ^{13}C content in their isotopic signature; a characteristic that is carried forward to animals fed a high C4 content diet.

The isotopic ratio of carbon has been measured in the protein and in lipid fractions of pork separately.

Hydrogen

Hydrogen exists as ^{1}H isotope (normally just annotated as H), and ^{2}H, called Deuterium (and shown as D). The isotopes of hydrogen are also analysed 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) and; secondly hydrogen as D/H_{org}, representing D/H ratios extracted from tissue protein (whose origin is the animal ration).

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In water there is a strong correlation between the isotope signature and the geographical location where the sample originates (eg proximity to the sea). This geographical factor can be a strong indicator of origin on a country basis.

Nitrogen ($^{15}N/^{14}N$) and Sulphur ($^{33}S/^{32}S$)

Every plant as it grows draws nitrogen and sulphur from the soil. There are a unique set of circumstances for each location that defines the 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. Differences can arise based on the use of farmyard manure vs. synthetic fertilisers, previous cropping, the nature of existing soil organic material, invertebrate populations and the population density and activity of nitrogen fixing bacteria.

Expression of isotope ratio results

The difference in mass one additional neutron makes to an atom is extraordinarily small at just 1.7×10^{-27} kg. Despite this it is possible using a mass spectrometer to measure the abundance of each isotope in a sample under analysis. This is then expressed as the ratio of pairs of isotopes.

For example, a typical ratio for the two carbon isotopes interesting in food authentication, could be 98.89% ^{12}C and 1.11% ^{13}C. For ease of presentation this ratio is expressed as the number of ^{13}C atoms per 1000 ^{12}C atoms (permil, ‰). In this example 11.22‰.

In some cases it is recognised practice to express results 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 Mean 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 vs. SMOW

This shows a -40 permil (-4 percent) variance for the ratio of Deuterium/Hydrogen in the analysed sample compared to the D/H ratio in the Standard Mean Ocean Water (SMOW).

Application to origin verification

The different causes of variation in isotope ratios in living (or once living) tissues means that in combination they can provide a signature specific to the location where the food product was grown or reared. By sampling products of known origin from producers across an area it is possible to build a reference library of isotope signatures representing that geographical area. It

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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.

Supplementary analyses

In some cases isotope signatures alone are not sufficient to uniquely identify the origin of a sample. It can therefore be useful to supplement them with additional information. The most widely used additional analysis is quantification of trace elements (such as strontium).

3) OVERVIEW OF PROJECT AIMS

The main purpose of the project was to establish a reference database and use samples of known origin (presented blind to the laboratory) to robustly test its use for origin verification. A successful outcome would be to have sufficient confidence in the results to use the database as the basis for supplementing paper-based audits. The first phase of the project therefore was to assemble and analyse samples representing the majority of pig production in England and Scotland (funded by BPEX and QMS), and test known samples against it to verify its efficacy.

Subsequent to the main database being established, agreement was reached with the Northern Ireland Meat Exporters Association to sample Northern Irish production to allow the whole of the UK pig production to be covered by the database. The opportunity was also taken to sample pigs from Southern Ireland slaughtered at the same facilities (i.e. exported live from Southern Ireland to Northern Ireland for slaughter). Analysis has been undertaken to determine the degree of confidence with which the populations from the different countries represented can be separated.

Early indications, from the pilot study and a limited number of known samples, were that cured pork could be verified against the fresh pork database without major difficulties. As the project progressed this became less clear. In addition, some retail samples of pork which, when traced through an audit process was proved to be almost certainly of UK origin, yielded intermediate probabilities for UK origin. Some additional trial work was therefore undertaken to examine the effects of curing and retail packing on the isotope signatures.

Sausages present a particular set of challenges to the use analytical techniques for origin verification. They comprise a mixture of ingredients, many of which will contribute elements with a different isotopic signature to that of the meat component, eg any vegetable protein present, added water herbs and spices. Some specific analysis of sausages has therefore been undertaken to understand the potential of the technique for verifying the origin of the pork is sausages.

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In parallel with the main scientific research, two field trails have been undertaken to evaluate the practical application of the approach in genuinely unknown samples.

4) METHODS

Reference sample collection

In order to have confidence in the database and ensure that there was minimal contamination with water in processing or cross contamination between sources, a strict sampling protocol was used for collection of reference samples in the abattoir. The protocol can be seen in Appendix 1.

Isotope analysis

The stable isotopes of the bioelements were measured with a special mass-spectrometer known as IRMS (isotopic ratio mass spectrometers). One of the most important differences between IRMS and normal organic mass spectrometers is the number of collectors responsible for the detection of the different isotopes. Normally only one collector is sufficient for an organic measurement. In the field of stable isotope applications three collectors are necessary to measure the masses simultaneously, resulting in highly reproducible values.

Furthermore the isotopic ratio mass spectrometers only detect the isotopic ratios of light gases as hydrogen, carbon dioxide, nitrogen and sulphur dioxide. Therefore it is necessary to use different types of element analysers and high temperature combustion units to pre-treat tissue water and organic material and generate these light gases.

Trace element analysis

Trace elements were measured using AAS (atomic adsorption spectroscopy).

The stable isotopes deliver a range of information to characterise the original meat. Nevertheless, further differentiation is available through the concentration of the rare elements such as strontium. Normally these rare elements are insufficient for verifying origin, but in combination with the stable isotopes they could deliver helpful information.

Normally the rare elements are of limited value on fresh meat, because they can be changed easily through processing (e.g. water addition).

Statistics

For the verification of the origin of food products such as pork it is necessary to combine various stable isotopes (and the trace element information where applicable) to optimise the differentiation. The use of multiple parameters

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require multivariate statistics for the evaluation of the sample. Two different statistical tools were used - principle component analysis (PCA) and canonical discriminate analysis (DA).

PCA is a helpful statistical tool to reduce the numbers of parameters in combining the parameters in vectors. Therefore it is possible to visualize many parameters in two dimension figures.

DA is a statistical tool which generates the optimised differentiation vector of groups out of the various parameters, for example the pork from England in comparison to pork from Denmark. Use of the discriminate vectors make it possible to describe the differences of one group from another as a differentiation probability. For the testing of unknown samples, this approach allows the sample to be assigned to or excluded from a group with a probability.

5) ESTABLISHING AND TESTING THE DATABASE

Samples taken to populate the database

During the period 31 March 2009 to 11 March 2010 a total of 216 pork samples of known origin were analysed:

- 153 from known English and Scottish farm locations
- 63 non-UK samples supplied by a commercial producer with verified origin representing the following major pig-producing countries of the EU:
    - Spain
    - Denmark
    - France
    - Germany
    - Holland

These 216 samples constitute the starting point for the isotope signature reference library (the database) for pig meat in this project. It was recognised from the start of the project that this database would be supplemented with additional samples to increase its representation of the pig population of interest in parallel with its future use.

At this stage the regions referenced in this project were 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 As far as possible, samples in the database represent a spread across GB (excluding Wales where pig production is minimal) while having higher representation of regions with the greatest concentrations of pig farms

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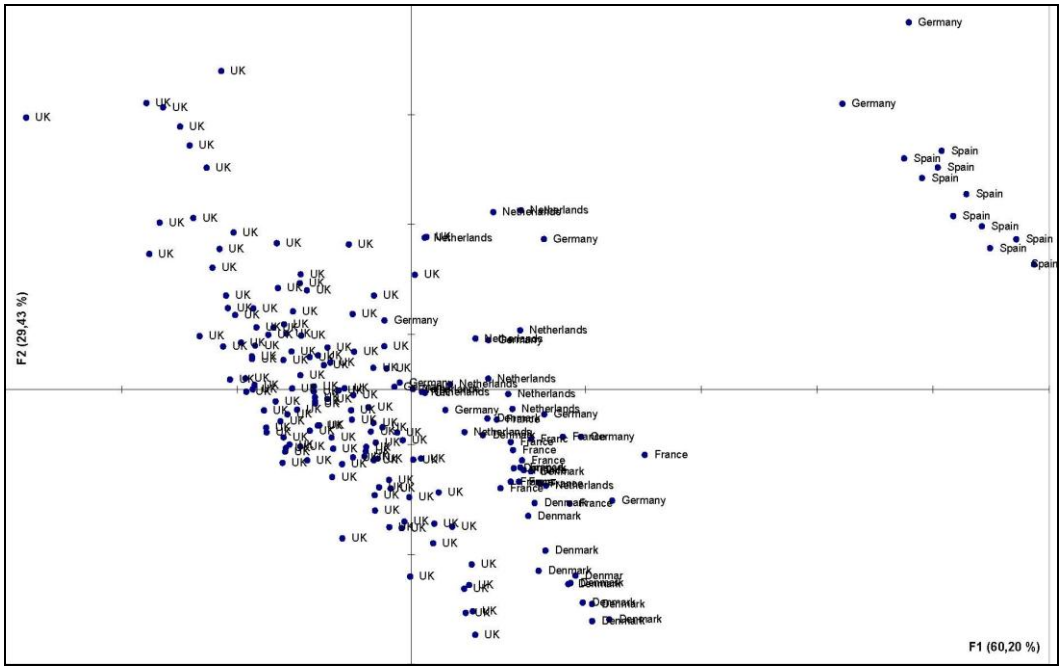
Water samples

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, twelve samples of abattoir sterilising wash water were taken. These samples can be used to check any tissue sample isotope profile that appears to be out of line with others of the same post code origin.

Separation of samples by origin

The actual isotope analyses are not presented here as, in themselves they are not particularly useful. The results were analysed to examine whether the samples could be separated on the basis of their country of origin. The results are illustrated graphically in Figures 1 and 2 using principle components analysis (PCA). In PCA the data are combined to explain the maximum possible amount of variation the smallest number of components. This allows visual presentation of the results. It does not necessarily represent the most efficient multivariate analysis for the separation of samples and others such as canonical discriminate analysis are used. The PCA plot in Figure 1 shows that significant discrimination between UK and non-UK meat can be achieved using isotope analysis. 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.

Figure 1. Principle Component Analysis using ^{13}C (from lipids and raw protein) ^{34}S and ^{15}N



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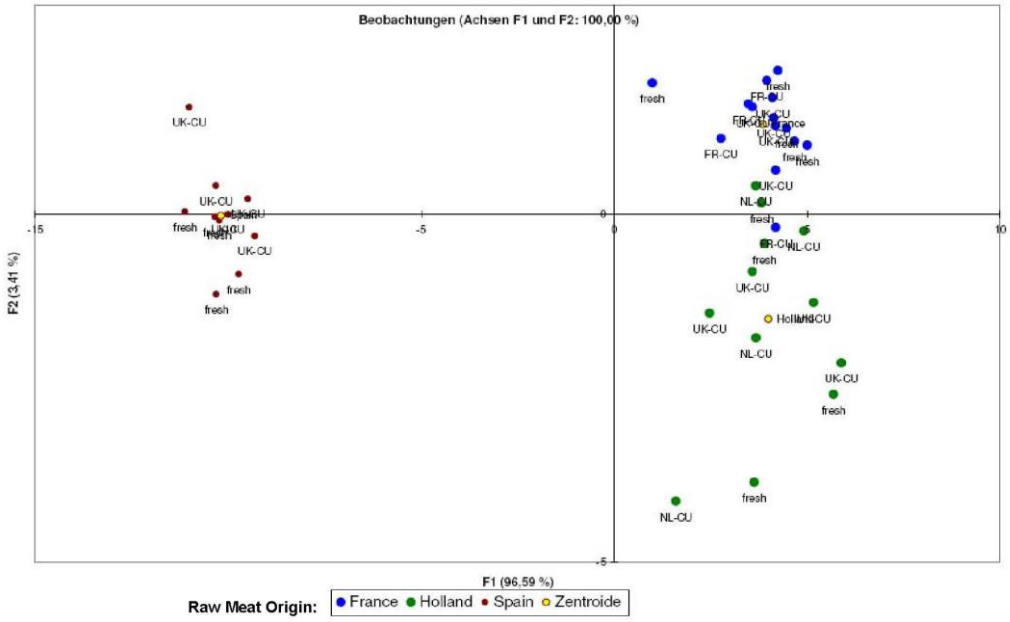
During sampling from other EU countries fresh pork, pork cured in the country of origin and pork cured in the UK were all included. This provided the opportunity to evaluate whether curing would shift the isotopic signature to an extent that rendered the reference database unsuitable for cured products. Figure 2 shows a PCA plot of the samples of pork sourced from France, The Netherlands and Spain. It can be seen that the cured pork, whether cured in the country of origin or in the UK, clusters with the fresh pork of the same origin. This illustrates the ability to discriminate the country of origin of the meat despite the introduction of water and other agents during the curing process.

Figure 2. Principle Component Analysis (PCA) showing isotope signatures for samples sourced from continental Europe and cured in the UK.



Samples taken to test the database

As part of Field Trial 1 (see section 10) five samples of known origin were sourced from abattoirs in England/Scotland and sent to the laboratory "blind" to test against the database. These were not identified separately from the other 30 samples collected in the field trial and therefore the laboratory had no prior knowledge that these were expected to be of British origin.

The results are shown below:

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Table 1. Results of blind test samples of known origin tested against the database.

Post code area	Probability of UK origin
LN4	99.9
DE65	99.9
IP28	98.2
CA8	99.1
DG11	99.7

The five samples of UK sourced fresh pork of certain origin were thus correctly identified from within the 35 and clearly consistent with the database, all with over with 99% probability of being of UK origin. This gives considerable confidence in the value of the reference database and the use of the technique.

6) ADDITION OF IRISH SAMPLES TO DATABASE

Phase 1 Irish sampling

Samples Collected

In August 2010, 44 samples were collected from an abattoir in Northern Ireland, according to the sampling protocol. From these the following were selected for analysis to be added to the reference database:

- 23 samples from pigs from Northern Ireland
- 15 from pigs from Republic of Ireland

Geographical separation of Irish samples

Multivariate analysis was undertaken to examine the ability to separate the populations from England, Scotland, Northern Ireland and the Republic of Ireland. The results are summarised in table 2 below.

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Table 2. Separation of samples from different countries of the British Isles.

True origin	Predicted origin				Total	% correct
	England	Scotland	Northern Ireland	Southern Ireland		
England	99	2	1	1	103	96.1
Scotland	4	23	0	0	27	85.2
N. Ireland	4	1	14	4	23	60.9
S. Ireland	1	0	6	8	15	53.3
Total	108	26	21	13	168	85.7

This shows samples from England can be correctly allocated to England in 96.1% of cases, and of those incorrectly classified, only 1 sample is predicted to be from Southern Ireland (ie 99% of samples would be correctly identified as from the UK if they where actually of English origin). For samples originating in Scotland, the correct allocation was 85.2% but all would have been predicted to be from the UK (Scotland or England).

Samples from Ireland present the biggest challenge in terms of separating them by origin, with the greatest difficulty being separating those North and South of the Irish border. Further analysis against the other countries in the database showed that the Irish samples are closer in their isotopic signatures to continental European countries than those from the rest of the British Isles.

Following analysis of these results a second phase of sampling in Ireland was undertaken to strengthen the database and enable better separation of samples.

Phase 2 Irish sampling

Samples Collected

In December 2010/January 2011 further sampling of Irish pigs was undertaken and the following selected for analysis: 14 samples from Northern Ireland and 3 samples from Southern Ireland. These were selected to give the widest possible geographical distribution across Ireland (in combination with the existing samples).

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This resulted in a total of:

- 37 samples from Northern Ireland
- 18 samples from Southern Ireland

Geographical separation of Irish samples

A full analysis of the reference data set (including the additional samples from Ireland) was undertaken in August 2011 and is described in section 7 below.

7) PERFORMANCE OF DATABASE AT COMPLETION OF PROJECT – including strontium analysis (August 2011)

Description of reference database

At the completion of the research project the reference database comprised the following samples:

Denmark	26
France	14
GB (England and Scotland)	253
Northern Ireland	37
Southern Ireland	18
Netherlands/Germany	22
Spain	10
Total	380

The following isotopes have been used for differentiation:

- D/H (hydrogen; tissue water, raw protein)
- 13C/12C (raw protein, lipids)
- 15N/14N (nitrogen; raw protein)
- 34S/32S (sulphur; raw protein)
- 18O/16O (tissue water).

Differentiation of samples

With the current reference database it is possible to differentiate samples from of Great Britain (England and Scotland) with a high probability (in excess of 99%) as shown in table 3.

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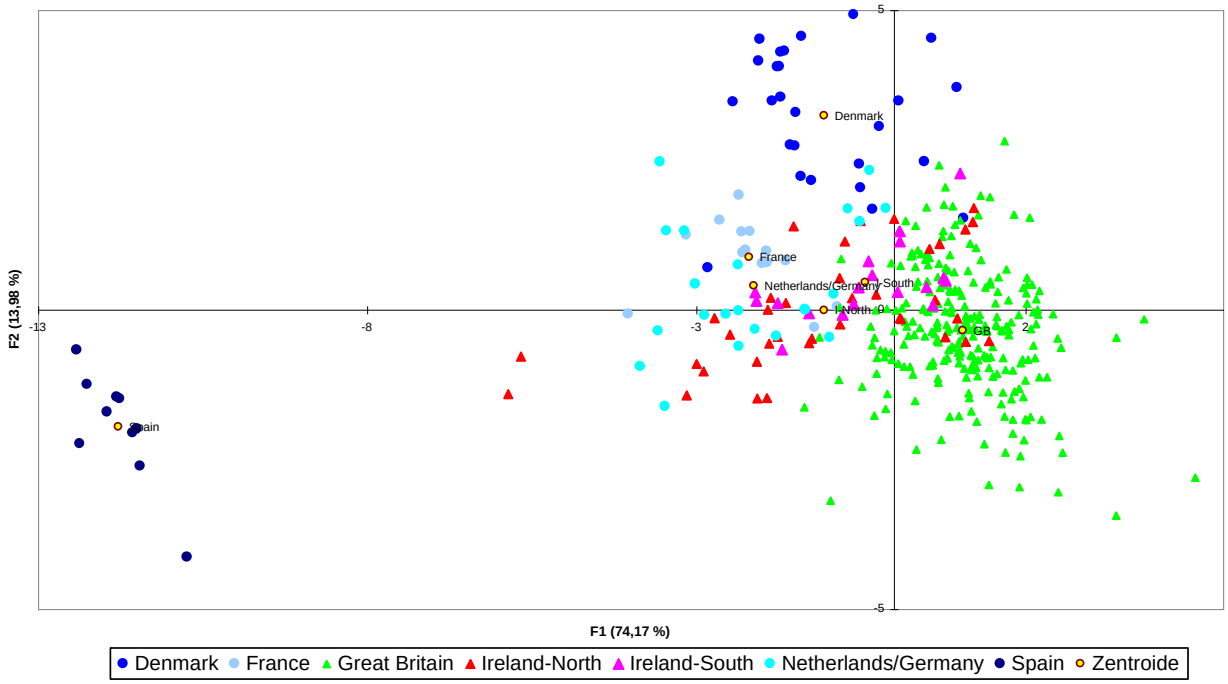
Table 3. Separation of samples from different countries in the database.

True Origin	Predicted origin							Total	% correct
	DK	FR	GB	NI	IE	NL/DE	ES		
Denmark	23	0	2	0	0	1	0	26	88.5
France	0	13	0	1	0	0	0	14	92.9
GB (England and Scotland)	0	0	251	1	1	0	0	253	99.2
Northern Ireland	0	1	11	20	1	4	0	37	54.1
Southern Ireland	0	0	3	8	7	0	0	18	38.9
Netherlands/ Germany	1	1	2	2	0	16	0	22	72.7
Spain	0	0	0	0	0	0	10	10	100.0
Total	24	15	269	32	9	21	10	380	89.5

Figure 3. Principle Component Analysis (PCA) separation of isotope signatures for samples sourced from different countries.



However, the combination of Great Britain with reference samples from Northern Ireland to define a reference set for the United Kingdom is a complicating factor. The isotopic signatures from Northern Ireland have significant overlap with Southern Ireland and with the Netherlands and France. That is due to the fact that the isotopic parameters in the pork derived from animal feed (sulphur, nitrogen and carbon) are often similar to those in Europe. This is most likely to be as a result of feed is imports into Northern Ireland.

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On the other hand, some of the reference samples (11 samples) from Northern Ireland show high similarity to the reference samples from Great Britain. That is due to the fact that the isotopic pattern of the ground water in Ireland and Great Britain is more or less similar resulting in a similar isotopic pattern in pork. In consequence the regional parameters of hydrogen and oxygen are helpful to define a UK cluster, but the feed parameter of pork from North Ireland could deliver a wrong indication to continental European pork.

The practical consequence of this for the use of isotopes for origin verification is to make the primary test for origin against reference samples from Great Britain. A second step could be to check samples with a possible origin of Northern Ireland samples with stables isotopes in combination with an active marking system (elemental stable isotopic marking with water or with marked phosphate in the feed) or with genetic testing.

Strontium analysis

As a possible approach to improve the separation of Northern Ireland samples from non-UK samples, the concentration of strontium was measured for 108 samples from the reference library.

The strontium concentration in pork would contribute to the confirmation of a sample from Great Britain, as the samples normally show very low strontium concentration (see Table 4). Strontium concentration resulted in a greater degree of separation between England, Scotland and Ireland (as a whole) but does not provide better separation between Northern and Southern Ireland, as they show very similar strontium concentrations.

The concentration of strontium could be helpful in the differentiation of Northern Ireland samples from European samples, as the European samples have a strong tendency to high strontium concentrations.

Table 4. Strontium concentrations of pork samples from different countries represented in the reference database.

	no.	Average ppm	Standard deviation	Median	Lower quartile	Upper quartile
England	23	0.08	0.06	0.07	0.06	0.09
Scotland	10	0.08	0.04	0.07	0.06	0.08
Northern Ireland	23	0.14	0.08	0.11	0.09	0.16
Southern Ireland	15	0.13	0.05	0.11	0.10	0.19
Netherlands/ Germany	14	0.25	0.12	0.26	0.17	0.33
Spain	8	0.32	0.14	0.31	0.21	0.44
Denmark	6	0.31	0.06	0.31	0.26	0.36
France	9	0.39	0.11	0.40	0.28	0.45

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Effects of production systems

There are particular production systems where the nature of the diets may be expected to influence the isotope signatures and therefore the ability to correctly allocate samples to origin.

Liquid feeding is one such example. A small number of samples from liquid-fed pigs in the database have tested against the rest of the database and their origin was correctly identified. So there is no indication that wet-feeding has a negative influence on the performance of the technique. As the database is used this can be verified with further samples.

There are insufficient samples available that are of known organic origin to test the effect at present, however it is highly unlikely that there is any negative influence, because normally organic farming is only expressed in higher 15N ratios.

8) SAUSAGES

In parallel with Field trial 1 (see section 10 below) a number of samples were analysed for a commercial supply chain. This included some sausage samples. The results suggested that the technique may need refining for analysis of sausages. A study was therefore established to determine the performance of the test on sausages containing meat of known origin.

Sample Preparation

Sausages were prepared in a small scale facility (factory “test kitchen”) to commercial recipes using meat of known sourcing:

- Sausages made to standard recipe using pork 100% derived from British origin
- Sausages made to standard recipe using pork 100% derived from Danish origin
- Sausages made to standard recipe using pork 50% derived from British origin and 50% derived from Danish origin

From these three batches of sausages the following samples were analysed blind by the laboratory:

1. Four sausages made with 100% Danish pork
2. Four sausage made with 100% British pork
3. Four sausages made with 50% Danish and 50% British pork
4. Four sausages made using 100% Danish pork (from the same batch as 1. above)

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Results of sausage analysis

Table 5. Results of blind sausage test

Sample	Actual origin	Predicted origin	% probability of UK origin	Correct?
1	Danish	non-UK	6	✓
2	British	UK	92	✓
3	Mixed	UK	86	×
4	Danish	non-UK	6	✓

The laboratory commented that the signatures of samples 1 and 4 were consistent with the samples being of Danish origin (with no prior knowledge of the sourcing of meat for this study).

The results of this test are reassuring for sausages made from meat of a single origin. It is clear that pork of mixed origin can result in a difficulty in correctly identifying the origin of the meat.

It is likely that the additional ingredients in the sausage contribute to the isotope signature and therefore interfere with the correct identification of the origin.

To enable better understanding of the effect of other ingredients on the results for sausages, samples where taken of four commercial batches of sausages and sent to the laboratory together with samples of all the ingredients used to make them. The sausage types were as follows:

- Economy sausage (40% meat)
- Standard British pork sausage
- Organic pork sausage with leek
- Free range Hampshire breed sausage

The laboratory measured samples from two of these batches, which proved sufficient to draw conclusions on the analysis of sausages.

For the evaluation of product samples such as sausages the laboratory normally only uses the D/H ratios of the lipids and raw protein, 13C/12C ratios of the raw protein and the isotopic ratios of nitrogen and sulphur. The D/H ratio of the tissue water is deliberately excluded because the tissue water could be influenced by water added in the recipe. That could be demonstrated clearly in both the analysed sausages. Both end products show the same D/H ratios as the processing water, which was quite distinct from that of the raw meat in the recipe.

Similarly, the rusk used in the recipes had a marked effect on the D/H ratios of both products. The D/H ratio in the finished product was increased in comparison with the meat base. This would have no effect on the overall conclusions if the amount of rusk added was low (<10 %), but in these recipes, in common with many British sausages, the content of rusk exceeds that. Indeed the proportion of rusk was sufficient to also have a slight effect

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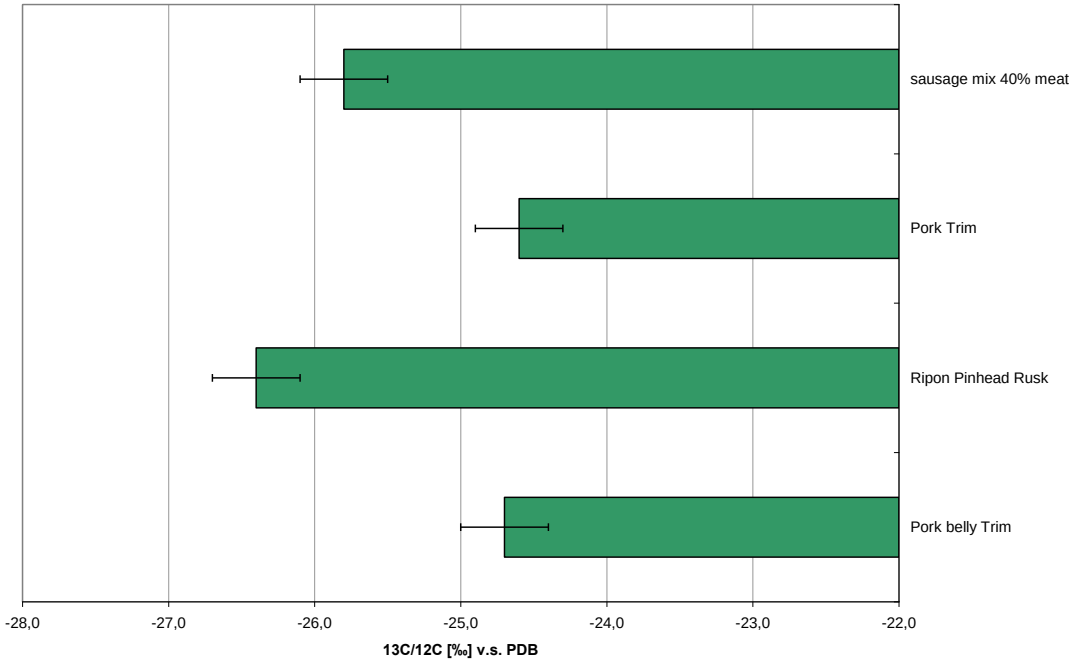
on the 13C/12C ratios of both products. Both end products show 13C/12C ratios which are between the meat and the pinhead rusk.

There was a slight influence detectable for $^{15}N/^{14}N$, but that was considered negligible. As expected, the important ratios of the lipids ($^{13}C/^{12}C$, D/H) and sulphur ($^{34}S/^{32}S$) were not affected.

It was concluded that to use the technique for origin verification of the meat content of sausages without unacceptable errors, only products meat content of about 90 % or more should be analysed.

Figure 4. $^{13}C/^{12}C$ isotope ratios of the raw protein from sausages and their ingredients

Sausage 1



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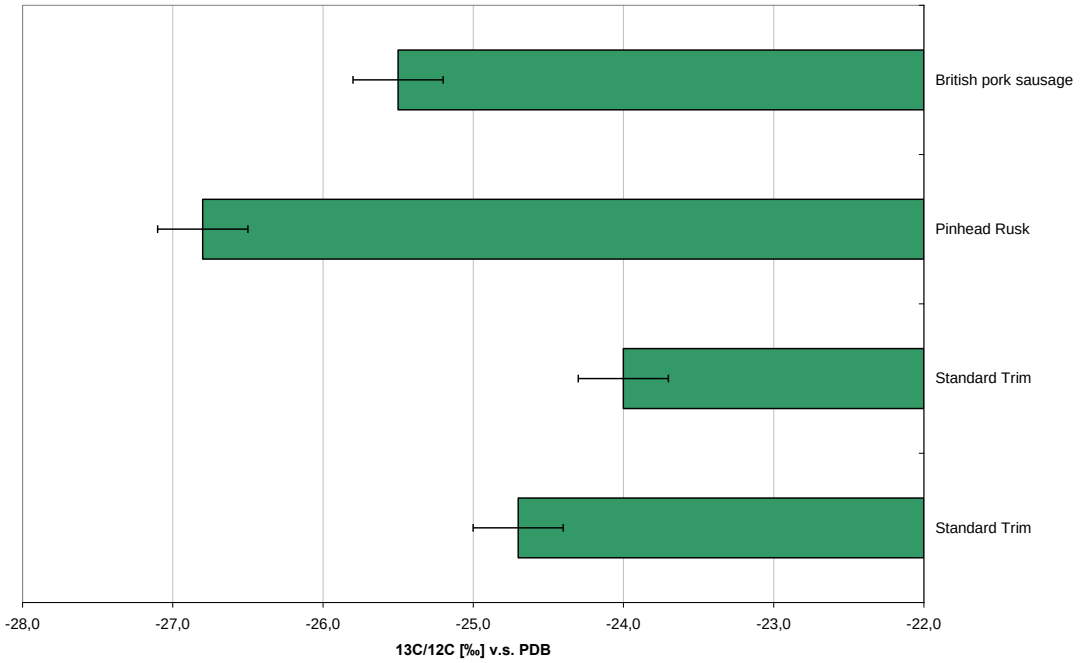
Sausage 2



9) EFFECT OF CURING AND PACKAGING

During the course of the field trials retail-sourced samples were submitted to the laboratory for which the origin was expected to be in line with the label information. A number resulted in less convincing probabilities for British origin that would have been expected and this raised some concern that something in the processing or retail supply chain might be influencing the isotope analyses.

A small experiment was, therefore, set up to investigate the effect of curing and retail packaging (in modified atmosphere gas packs) on the resulting isotope analysis.

Samples were taken from four carcases at different stages of processing for fresh pork, bacon and ham as shown in the diagram below:

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Figure 5. Process flow with indication of sampling points for investigation of interference with isotope testing

Slaughter				
Carcases into Chillers – (usual factory sampling point)**				
Overnight in chiller				
Primal cut				
Pack into dolavs/hung on "Christmas trees"				
Fresh Pork	**Bacon**		**Cooked Ham (standard)**	**Cooked Ham (roast)**
Chiller	Chiller		Chiller	Chiller
Retail Pack	Cure		Brine	Brine
5d in Chiller **	Tumble		Tumble	Tumble
	Smoke **	Unsmoked **	Cook	Cook (roast)
	Temper (freeze)	Temper	Temper	Temper
	Slice	Slice	Slice	Slice
	Retail pack	Retail pack	Retail Pack	Retail Pack
	5d in Chiller **	5d in Chiller **	5d in Chiller **	5d in Chiller **

** = sample point
Repeated four times (ie four carcases).

The results showed clear effects of processing and packing on the isotopic signatures. The average effects are summarised in Table 6 and detailed results are shown in Appendix 2. It can be seen that the carbon ratio is basically unaffected by processing. The raw protein $^{15}N/^{14}N$ ratio is increased by a similar proportion regardless of the process conditions. The effect on tissue water D/H, raw protein D/H and raw protein $^{34}S/^{32}S$ ratios are, however, dependent on the process. It should be noted that there was considerable between-pig variation in the effects of processing on these parameters.

Table 6. Average percentage difference in isotope results of retail packs from abattoir sample

	Tissue Water D/H%	Raw Protein D/H org%	Raw Protein $^{13}C/^{12}C$	Lipids $^{13}C/^{12}C$	Raw Protein $^{15}N/^{14}N$	Raw Protein $^{34}S/^{32}S$
Fresh Pork (retail packed)	-5%	-6%	0%	0%	-18%	+3%
Smoked Bacon	-18%	-6%	0%	0%	-15%	+2%
Unsmoked Bacon	-15%	-8%	0%	0%	-15%	+11%
Plain Ham	-8%	-6%	+1%	0%	-15%	+3%
Roast Ham	-9%	-4%	+1%	0%	-17%	+6%

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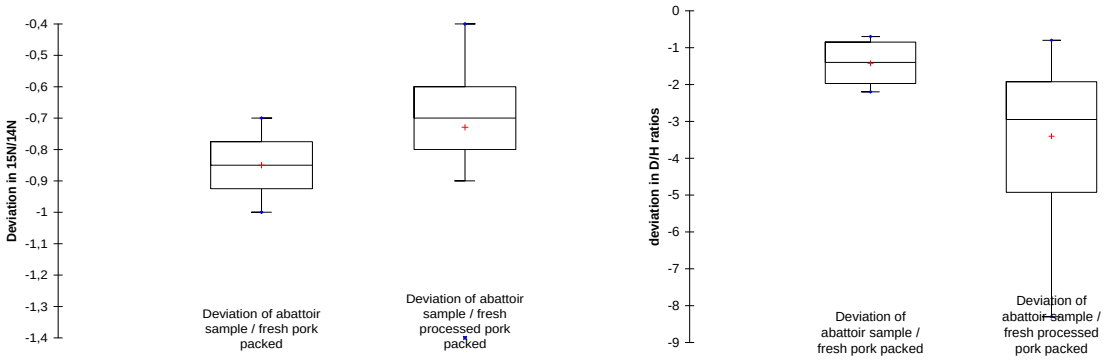
It was concluded that adjusting the D/H ratios by +4 and the 15N/14N ration by +0.75 would enable a direct comparison of retail samples (fresh or cured) with the database of abattoir samples with a reduced error in classification. This was tested with known British samples and was indeed found to increase the probability that they were a match to the British database.

Figure 6. Effect of processing or packaging on pork isotope signatures



10)FIELD TRIALS

Field Trial 1 (November 2010)

Having completed reference sampling and preparation of the reference database, but prior to the inclusion of samples from Ireland, 35 samples sourced at retail but presented blind to the laboratory, were supplied for comparison against the database. The 35 samples were made up as follows:

- 5 UK sourced fresh pork samples of known origin, obtained 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

The five samples of UK sourced fresh pork of certain origin were correctly identified from within the 35 – all five were identified as consistent with the database with 99.1-99.9% probability, thus “definitely UK”.

Of the six samples labelled as originating in other countries:

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- Four samples were deemed “Definitely not from the UK database” (0.0-24.7% probability of being from the database)
- 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 labelled as of UK or British origin:

- 14 were found to be “Definitely of the UK database” with 95.1-99.7% probability
- Three showed a probability of 91.5-93.3% and are therefore considered “Typical of the UK database”. Of these, one was labelled ‘from the UK’, the others either ‘British’ or ‘From Britain’.
- One sample labelled gave a results with a lower confidence that it was matched with 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” (probabilities in the range 3.9-35.4%)

The results of these 30 retail samples indicate some doubt in the validity of the retail labels and these were explored through the supply chain. In several cases part of the explanation appeared to be that they were of Northern Irish origin and therefore highlighted the need for Northern Irish samples to be incorporated into the reference database.

Field Trial 2 (July 2011)

In July 2011 a further field trial was conducted with 33 samples sliced as follows:

- 30 retail samples to consist of 10 each of pork, bacon and ham
    - Packs clearly marked as British
    - Coverage across retail “tiers”
    - Own label product from six major retailers
- 3 control samples
    - 3 pork loin samples of known provenance

The three control samples were clearly identified as being of British origin, again verifying the technique for known samples.

The results for the 30 retail samples are shown below:

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Category	No.
Consistent with the database for GB (England and Scotland) (>75% probability)	15
Consistent with database for the UK (>75% probability)	5
Consistent with the database for the British Isles (>75% probability)	1
Consistent with the database for the British Isles but with lower probability (<75% but > 50% probability)	5
Inconsistent with database for the British Isles (<50% probability)	4

Clearly the last category (4 samples) would be those of most concern and in future use of the database it is likely that these would be prioritised for further investigation.

11)CONCLUSIONS

It has been clearly demonstrated that stable isotope analysis can be used to verify the origin of fresh pork, although there are some limitations to the technology. Samples from Ireland are difficult to separate: (a) because it is not possible to clearly separate the isotopic signatures of Northern and Southern Ireland and; (b) because Irish pork shows a greater similarity with pork from continental Europe than does British pork, probably due to feed imports to Ireland.

Origin verification is more difficult for processed products. It has proved possible to use the approach for determination of the origin of cured pork (bacon and ham), and this can be improved by the use of correction factors for the effect of processing on the isotopic signature - to enable comparison with the reference library of fresh pork samples.

Sausages have presented difficulty because of the other ingredients in sausage recipes that confound the analysis. It is therefore recommended that this approach is only used for products that have a declared meat content of 90% or greater.

Overall it can be concluded that isotope analysis against the known database is a very useful tool to use alongside more traditional supply chain auditing techniques to provide increased confidence to customers that the correct information is on the label.

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APPENDIX 1 - SAMPLING PROTOCOL FOR REFERENCE SAMPLES

To ensure that water used in the abattoir does not contaminate reference samples a strict sampling protocol was followed.

1. As a first choice, boars were selected for sampling as cutting to remove the testicles acts as a “cleaning” process of the knife to remove abattoir hot 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.

2. Sows (or gilts) were sampled where sows only are available or where slaughterers open several carcases between dipping knives in sterilising bowls. In these instances sampling was carried out on the second (or later) carcase with the same slap mark opened by a line worker using a knife already used on a carcase since sterilising his knife.

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

4. Personnel handling the sample used disposable gloves. Knives were washed (in abattoir knife cleaning units) then dried between each group of carcases representing one farm.

5. All samples were sealed in labelled plastic sample bags and frozen on the day of collection, or soon thereafter. Samples were dispatched by courier in a frozen state.

6. For each abattoir approximately 25ml of cleaning unit water was sampled and analysed for the purpose of crosschecking for contamination of carcases.

7. Up to five separate carcases were sampled from each farmer-supplied consignment of pigs supplied to the abattoir on the day of sampling. The initial 40g sample was divided into two samples of approximately 20g: one for analysis, and one for archiving. The samples from carcases within a consignment were grouped for analysis to provide one reference signature for each farm.

8. The samples were sent by courier to the laboratory in Germany. Second samples will be bagged, labelled and archived by the Supplier at -80°C.

9. Samples of fresh pork and cured pork from countries outside the UK were sourced in the most appropriate and practical way possible while ensuring their country of origin was known absolutely.

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APPENDIX 2 – EFFECT OF PROCESSING AND PACKAGING ON ISOTOPE ANALYSES

								classification UK		
Sample	*Declaration*	*Tissue Water D/H%*	*Raw Protein D/H org%*	*Raw Protein 13C/12C*	*Lipids 13C/12C*	*Raw Protein 15N/14N*	*Raw Protein 34S/32S*	***Base***	***changed: 15N/14N: +0,7***	***changed: 15N/14N: +0,7; D/H: +4***
Pig 1	Abattoir Sample	-33.7	-85.5	-24.7	-28.3	4.9	3.4	98	98	98
Pig 1	Fresh Pork Retail Packed	-34.4	-93.5	-24.6	-28.3	4.2	3.5	96	99	99
Pig 2	Abattoir Sample	-32.8	-90.9	-24.6	-28.9	4.7	4.1	99	99	99
Pig 2	Fresh Pork Retail Packed	-33.7	-94	-24.7	-29	3.8	4.3	95	99	99
Pig 3	Abattoir Sample	-32.7	-84.2	-24.2	-27.7	4.8	4.3	87	87	88
Pig 3	Fresh Pork Retail Packed	-34.6	-87.1	-24.2	-27.5	4	4.4	67	79	79
Pig 4	Abattoir Sample	-28.5	-82.5	-24.7	-28	4.7	3.5	96	96	95
Pig 4	Fresh Pork Retail Packed	-30.7	-87.5	-24.6	-27.8	3.7	3.6	81	93	93
Pig 1	Abattoir Sample	-33.7	-85.5	-24.7	-28.3	4.9	3.4	98	98	98
Pig 1	Smoked bacon post tumble	-35.4	-93.8	-24.9	-28.1	4.2	3.4	94	98	98
Pig 1	Smoked Bacon retail pack	-39.1	-94.7	-24.8	-28.2	4.3	3.4	95	98	99
Pig 2	Abattoir Sample	-32.8	-90.9	-24.6	-28.9	4.7	4.1	99	99	99
Pig 2	Smoked bacon post tumble	-34.2	-91.8	-24.1	-27.5	4	4.8	71	84	89
Pig 2	Smoked Bacon retail pack	-36.4	-92.1	-24.2	-27.5	4	4.9	66	79	88
Pig 3	Abattoir Sample	-32.7	-84.2	-24.2	-27.7	4.8	4.3	87	87	88
Pig 3	Smoked bacon post tumble	-33.8	-87.3	-24.9	-28.7	3.9	3.4	95	99	99
Pig 3	Smoked Bacon retail pack	-37.6	-89.9	-24.6	-28.9	3.9	3.7	97	99	99
Pig 4	Abattoir Sample	-28.5	-82.5	-24.7	-28	4.7	3.5	96	96	95
Pig 4	Smoked bacon post tumble	-29.3	-87.3	-24.7	-27.8	4	3.6	86	95	92
Pig 4	Smoked Bacon retail pack	-36.8	-87.3	-24.8	-27.9	4	3.6	87	94	96

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								classification UK		
Sample	*Declaration*	*Tissue Water D/H%*	*Raw Protein D/H org%*	*Raw Protein 13C/12C*	*Lipids 13C/12C*	*Raw Protein 15N/14N*	*Raw Protein 34S/32S*	***Base***	***changed: 15N/14N: +0,7***	***changed: 15N/14N: +0,7; D/H: +4***
Pig 1	Abattoir Sample	-33.7	-85.5	-24.7	-28.3	4.9	3.4	98	98	98
Pig 1	Unsmoked Bacon post tumble	-36	-93.7	-24.8	-28.2	4.5	3.5	97	99	99
Pig 1	Unsmoked Bacon Retail Pack	-37.8	-97.3	-24.6	-28.3	4	4.2	93	97	98
Pig 2	Abattoir Sample	-32.8	-90.9	-24.6	-28.9	4.7	4.1	99	99	99
Pig 2	Unsmoked Bacon post tumble	-38.6	-93.1	-24.8	-28.4	4	4.2	93	97	98
Pig 2	Unsmoked Bacon Retail Pack	-38.4	-95.1	-24.9	-29.1	4.1	4.4	98	99	99
Pig 3	Abattoir Sample	-32.7	-84.2	-24.2	-27.7	4.8	4.3	87	87	88
Pig 3	Unsmoked Bacon post tumble	-36.2	-85.1	-24.1	-27.5	4.2	4.1	55	68	86
Pig 3	Unsmoked Bacon Retail Pack	-36	-88.2	-24.1	-27.6	4.2	4.3	68	80	91
Pig 4	Abattoir Sample	-28.5	-82.5	-24.7	-28	4.7	3.5	96	96	95
Pig 4	Unsmoked Bacon post tumble	-30.6	-86.6	-24.7	-27.9	3.8	3.6	84	95	93
Pig 4	Unsmoked Bacon Retail Pack	-34.3	-88.3	-24.7	-27.7	3.9	4	81	92	91
Pig 1	Abattoir Sample	-33.7	-85.5	-24.7	-28.3	4.9	3.4	98	98	98
Pig 1	Plain retail packed ham	-35.4	-93.6	-24.5	-28.4	4.1	3.6	96	98	99
Pig 2	Abattoir Sample	-32.8	-90.9	-24.6	-28.9	4.7	4.1	99	99	99
Pig 2	Plain retail packed ham	-35.4	-93.4	-24.5	-28.5	4.1	4.1	96	99	99
Pig 3	Abattoir Sample	-32.7		-24.2	-27.7	4.8	4.3	87	87	88
Pig 3	Plain retail packed ham	-34.7		-24	-27.6	4.2	4.3	36	48	73
Pig 4	Abattoir Sample	-28.5	-82.5	-24.7	-28	4.7	3.5	96	96	95
Pig 4	Plain retail packed ham	-32.7	-86.5	-24.5	-27.9	3.9	3.7	87	95	95

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								classification UK		
Sample	*Declaration*	*Tissue Water D/H%*	*Raw Protein D/H org%*	*Raw Protein 13C/12C*	*Lipids 13C/12C*	*Raw Protein 15N/14N*	*Raw Protein 34S/32S*	***Base***	***changed: 15N/14N: +0,7***	***changed: 15N/14N: +0,7; D/H: +4***
Pig 1	Abattoir Sample	-33.7	-85.5	-24.7	-28.3	4.9	3.4	98	98	98
Pig 1	Roast Retail Packed Ham	-36	-96.7	-24.4	-28.4	3.5	3.9	88	96	96
Pig 2	Abattoir Sample	-32.8	-90.9	-24.6	-28.9	4.7	4.1	99	99	99
Pig 2	Roast Retail Packed Ham	-34.4	-92.1	-24.7	-28.7	4.2	4.1	97	99	99
Pig 3	Abattoir Sample	-32.7	-84.2	-24.2	-27.7	4.8	4.3	87	87	88
Pig 3	Roast Retail Packed Ham	-35.3	-80.5	-24	-27.4	4.1	3.9	38	51	75
Pig 4	Abattoir Sample	-28.5	-82.5	-24.7	-28	4.7	3.5	96	96	95
Pig 4	Roast Retail Packed Ham	-33.5	-87.5	-24.6	-28	4	4.1	89	96	96

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