CURRENT ISSUES IN FOOD AUTHENTICITY

Sophie Rollinson, Mark Woolfe, Food Standards Agency
Why Have An Authenticity Programme?

- Contributes to Agency objectives
  - to promote honest and informative labelling
  - to help consumers make informed choices
  - Protect consumers from food fraud and illegal practices
- Assists in the improvement of enforcement of food standards
- Supports food labelling and standards policy
Regulation (EC) 178/2002
General Food Law

Article 8 - Protection of consumer interests

- Food law provides basis for consumers to make informed choices about the food they consume

- Aims to prevent:
  - fraudulent or deceptive practices
  - adulteration of food
  - practices that may mislead the consumer
Uncovers the Misdescription of Food

- Not meeting the requirements for a legal name
- Substitution by cheaper but similar ingredient
- Extend food using adulterant, e.g. water, starch
- Undeclared process, e.g. irradiation, freezing
- Incorrect origin, e.g. geographic, species or method of production
- Quantitative ingredient declaration
Food Authenticity Programme

R&D Method Development

Auth Sampling Sub-Group

Auth Methodology Working Group

Food Surveys

Technology Transfer

Feasibility

Development

Evaluation
Previous
- Meat speciation survey
- Olive oil, Apple juice,
- Tuna products,
- Water in chicken - retail & catering
- Irradiated foods
  (herbal supplements & prawns)
- Maize Oil
- Added H₂O in scallops /scampi
- Potato varieties,
- Horsemeat in imported meat products e.g salami
- Basmati rice
- Fat in minced meat
- GM soya in soya ingredients
- Farmed and ‘wild’ fish
- Meat content calculations

SURVEYS

Enforcement Exercises
- Added H₂O etc. in chicken breast,
- Irradiated herbal supplements

Current
- Fish species
- Previously frozen poultry

Future
- Vegetarian foods
- Origin of Beef
- Organic foods
## COMPLETED DNA-BASED PROJECTS

<table>
<thead>
<tr>
<th>Issue</th>
<th>DNA Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice varieties</td>
<td>Microsatellites or Simple-sequence length polymorphism (SSLP-PCR)</td>
</tr>
<tr>
<td>Potato varieties</td>
<td></td>
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<tr>
<td>Origin of tea</td>
<td></td>
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<tr>
<td>Olive varieties</td>
<td></td>
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<tr>
<td>Fruit species in jams and pulps. Rice varieties</td>
<td>Single Nucleotide polymorphisms (SNPs)</td>
</tr>
<tr>
<td>Olive varieties</td>
<td></td>
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<tr>
<td>Durum wheat pasta</td>
<td>Real time PCR</td>
</tr>
<tr>
<td>Quantitative meat and fish species</td>
<td></td>
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<tr>
<td>GM soya</td>
<td></td>
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<tr>
<td>Meat species</td>
<td></td>
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<tr>
<td>Meat in vegetarian foods</td>
<td></td>
</tr>
<tr>
<td>Differentiation of meat tissues, CNS, offal</td>
<td>Methylation events - PCR</td>
</tr>
<tr>
<td>Fish species</td>
<td>Restriction fragment length polymorphism (RFLP-PCR) and single strand conformation polymorphism (SSCP-PCR)</td>
</tr>
<tr>
<td>Meat Species</td>
<td></td>
</tr>
</tbody>
</table>
TRANSFER OF DNA METHODS TO PUBLIC ANALYSTS

• Find a suitable platform to transfer DNA methods
• Encourage Public Analysts to acquire equipment
• Have programme of R&D to transfer and validate methods on chosen platform
• Run training courses in methods
• Run blind trials or challenge tests
AGILENT BIOANALYZER LAB ON A CHIP SYSTEM

12 Sample wells

30mm

Detection Point

Separation Channel
Extract DNA from fish sample

Separate fragments by capillary electrophoresis.
Identify species-specific profile

Amplify (PCR) cytb gene

Digest PCR product with enzymes
Analysis of results

Use the sizes to complete a blank chart like this. Repeat for all 3 enzymes.
# Technology Transfer to Public Analysts

<table>
<thead>
<tr>
<th>Issue</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish species</td>
<td>DNA- RFLP</td>
</tr>
<tr>
<td></td>
<td>Transfer complete – extend database</td>
</tr>
<tr>
<td>Basmati rice</td>
<td>DNA- microsatellites, Indels</td>
</tr>
<tr>
<td></td>
<td>Transfer complete</td>
</tr>
<tr>
<td>Meat species-commercial, exotic bushmeat</td>
<td>DNA- specific probes, RFLP</td>
</tr>
<tr>
<td></td>
<td>Training course in Jan 09</td>
</tr>
<tr>
<td>Durum wheat pasta</td>
<td>DNA-specific probes</td>
</tr>
<tr>
<td></td>
<td>Validation complete</td>
</tr>
<tr>
<td>Orange juice adulteration</td>
<td>DNA- heteroduplex</td>
</tr>
<tr>
<td></td>
<td>Validation in progress</td>
</tr>
</tbody>
</table>

Sequencing training course – Mar/April 09
DNA METHODS AVAILABLE IN FOOD STANDARDS ENFORCEMENT

DNA methods now available on lab-on-a-chip:
• Fish species
• Basmati rice
• Bushmeat
• Meat species incl. exotic meats

Coming soon…
• Orange juice adulteration
• Durum wheat pasta
  • Potato varieties ?
  • Wild boar ?
  • Traditional cattle and pig breeds ?
R&D Programme

Methods to verify the origin of foods geographic /production/species are an active part of the research programme

Production Origin

<table>
<thead>
<tr>
<th>Issue</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic vegetables</td>
<td>Nitrogen 15/14 isotopes</td>
</tr>
<tr>
<td>Organic meat</td>
<td>Fluorescent microscope and image analysis</td>
</tr>
<tr>
<td>Corn-fed chicken</td>
<td>Carbon 13/12 IRMS</td>
</tr>
<tr>
<td>Wild and farmed salmon, seabass and seabream</td>
<td>Fatty acid profile Carbon 13/12, oxygen 18/16, Nitrogen 15/14</td>
</tr>
</tbody>
</table>
R&D Programme

Isotopic ratios and trace elements are particularly good for determining the geographic origin of foods closely linked with their environment.

<table>
<thead>
<tr>
<th>Geographic Origin</th>
<th>Issue</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GeoBeef</td>
<td>Nitrogen, oxygen, carbon, strontium isotopes, multi-element</td>
</tr>
<tr>
<td></td>
<td>Geopoultry</td>
<td>As above</td>
</tr>
<tr>
<td>TRACE – NMW, honey, lamb, olive oil, cereals, chicken</td>
<td>Isotopes, ME, DNA, high resolution NMR</td>
<td></td>
</tr>
<tr>
<td>Wine- re-examination of existing data.</td>
<td>Isotopes, ME, polyphenols</td>
<td></td>
</tr>
</tbody>
</table>
How can we use isotopic fractionation to obtain authenticity information?

<table>
<thead>
<tr>
<th>Isotope ratio</th>
<th>Fractionation</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^2\text{H}/^1\text{H}$</td>
<td>evaporation, condensation, precipitation</td>
<td>geographical</td>
</tr>
<tr>
<td>$^{13}\text{C}/^{12}\text{C}$</td>
<td>C3 and C4 plants</td>
<td>diet (geographical proxy)</td>
</tr>
<tr>
<td>$^{15}\text{N}/^{14}\text{N}$</td>
<td>nitrification/denitrification, trophic level, marine and terrestrial plants</td>
<td>agricultural practice, diet (geographical proxy)</td>
</tr>
<tr>
<td>$^{18}\text{O}/^{16}\text{O}$</td>
<td>evaporation, condensation, precipitation</td>
<td>geographical</td>
</tr>
<tr>
<td>$^{34}\text{S}/^{32}\text{S}$</td>
<td>bacterial</td>
<td>geographical (marine)</td>
</tr>
<tr>
<td>$^{87}\text{Sr}/^{86}\text{Sr}$</td>
<td>underlying geology</td>
<td>geographical</td>
</tr>
</tbody>
</table>

These isotopic signatures are translocated through an animal to their product and can be used to trace the food origin.
Can use carbon isotopic analysis to verify production origin for corn-fed chicken.

Other cereals have less heavy isotope.
Are these ‘corn-fed’ chicken?
Have they been fed a 50% maize diet during their rearing life?

Retailer 1 free range chicken
Retailer 2 corn-fed chicken drumsticks
Retailer 3 corn-fed chicken drumsticks
Retailer 3 corn-fed chicken
As the corn content of feed increases, the $^{13}\text{C}$ heavy isotope value increases.

Other feeds, wheat and soya have lower $^{13}\text{C}$ heavy isotope value.
Blind Testing of Chicken Samples

Compared with 0% and 50% corn-fed standards – retailers 1 & 3 samples are correctly described. Retailer 2 does not meet the standard for corn-fed...
A plot of $\delta^{13}C$ and the $\delta^{15}N$ values from authentic beef defatted dry mass (DDM)

C$_3$ diet  |  C$_3$/C$_4$ diet  |  C$_4$ diet

Authentic beef FFDM $\delta^{13}C$$\%_{\text{PDB}}$

$\triangle$ England
■ Ireland
● Brazil

C$_3$ to C$_4$ switch~167 days*

Rationale for deuterium ($^2$H) and oxygen-18 ($^{18}$O) as geographic tracers

Other light isotopes can be used as indicators of geographic origin. IAEA map mean deuterium and oxygen levels in ground water globally. More heavy isotope at the equator and inland.
Trace project built up a database of isotopic ratios in soil and groundwater from over EU 600 locations.
New high resolution map of these isotopic values across Europe

655 Mineral = ground waters from Europe TRACE project

Data processed by Gabe Bowen Purdue Univ.
±50 Precipitation waters sampled by IAEA program
The Concept of Food Origin Mapping

- Link the isotopic and TE composition of the commodity to the composition of soil and climatic parameters in the production region.
- Predict the multi-isotopic and ME specification for same food from different origin by extrapolation.

![Food Origin Map of Western Europe](image-url)
Chemometric passport

Encode elemental and isotopic specifications and geographical coordinates on to food label

Compliance testing of chemical specifications by measuring parameters by certified labs and compare with label

Web interface in shop showing customer map of region of origin or location of producer (Google earth?)
## R&D Programme

<table>
<thead>
<tr>
<th>Meat</th>
<th>Issue</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibrimex, blood detection</td>
<td>LC-MS/MS/MS, immunoassay</td>
</tr>
<tr>
<td></td>
<td>Previously frozen poultry</td>
<td>HADH assay</td>
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<tr>
<td></td>
<td>QUID – individual species</td>
<td>Proteomics- LC-MS</td>
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<tr>
<td></td>
<td>Offal detection in meat</td>
<td>Proteomics- LC-MS</td>
</tr>
<tr>
<td></td>
<td>Vegetarian/Vegan Foods</td>
<td>Extraction improvement</td>
</tr>
<tr>
<td></td>
<td>- Gelatine</td>
<td>Proteomics – species</td>
</tr>
<tr>
<td></td>
<td>- Meat ingredients</td>
<td>DNA, Sterenes</td>
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<tr>
<td></td>
<td>- Animal fats in veg. fds</td>
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<tr>
<td></td>
<td>MSM/MRM</td>
<td>Light microscopy</td>
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<td>Metabolomics LC-MS/MS</td>
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</tbody>
</table>

Proteomic approach to look for species/tissue specific peptide biomarkers
Proteomic Species Quantification

In-Gel trypsin digestion of Myosin Light Chain 3 after fractionation. Identify species specific marker (quantitatively) in the peptide mass fingerprint.

100 % Pork

2288.774
2375.808
2561.818
2675.964
2759.861
2864.842

100 % Chicken

2288.692
2331.653
2500.757
2675.964
2759.861
2804.723

1 % chicken in pork

2288.875
2331.839
2483.984
2804.020

Chicken-specific biomarker
Matching Fragmentation Patterns of Peptide Markers in Gelatine to determine species origin

Gelatin standards

- Bovine gelatin B532
  - M/Z 1181.60
- Porcine gelatin P533
  - M/Z 1778.84

Plumping agent

Injection powder 1
- M/Z 1181.60

Injection powder 2
- M/Z 1778.81

Cow

Pig
Research Dissemination

• All reports now published on **foodbase** – open access repository to make the results of the Agency's science more accessible.

• Technical reports of work, surveys, SOPs – suite of authenticity SOP’s will be published

• launched on 11 September this year [www.foodbase.org.uk](http://www.foodbase.org.uk)
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