Proteomic tools to assess meat authenticity

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Clear and reliable information about food is demanded nowadays by consumers. Honest and accurate food labeling is essential to assure food safety and choice. Robust and reliable methodologies are needed to assure that fraudulent or accidental mislabeling does not arise. Quantitative Ingredient Declaration (QUID)
The 2013 Horse meat adulteration scandal:

10 Dec 2012

The FSAI sent samples to Eurofins laboratory (Germany), confirming these results. Only one out of 10 burger samples gave relevant amounts (29%) of horse DNA. The product was manufactured by Silvercrest on behalf of Tesco.

11 Jan 2013

First positive test for horse DNA found in beef meat confirmed by FSAI (Food Safety Authority of Ireland). Origin: 2 processing plants in Ireland and 1 plant in UK.

15 Jan 2013

FSAI announced horse DNA found in beef burgers in several Irish and British supermarkets. The implicated products were removed immediately.

We apologise

You have probably read or heard that we have had a serious problem with three frozen beef burger products that we sell in stores in the UK and Ireland.

The Food Safety Authority of Ireland (FSAI) has told us that a number of products they have recently tasted from one of our suppliers contained horsemeat.

While the FSAI has said that the products pose no risk to public health, we appreciate that, like us, our customers will find this absolutely unacceptable.

The products in our stores were Tesco Everyday Value 8 x Frozen Beef Burgers (397g), Tesco 4 x Frozen Beef Quarter Pounders (454g) and a branded product, Flamehouse Frozen Chargrilled Quarter Pounders.

We have immediately withdrawn from sale all products from the supplier in question, from all our stores and online.

If you have any of these products at home, you can take them back to any of our stores at any time and get a full refund. You will not need a receipt and you can bring back just the packaging.

We and our supplier have let you down and we apologise.

If you have any concerns, you can find out how to contact us at the bottom of this page, or go to any of our Customer Service Desks in store, or ask to speak to your Store Manager.

So here's our promise. We will find out exactly what happened and, when we do, we'll come back and tell you.

And we will work harder than ever with all our suppliers to make sure this never happens again.

TESCO
Revealed that Findus™ beef lasagne (UK, France, Sweden) and shepherd’s pie and moussaka (France) contained horse meat without proper declaration

The French government determine the origin of the fraud, blaming on the company Spanghero™. Their license was suspended for fraudulent labelling of meat

In UK, 3 men were arrested following searches by the FSA (UK Food Standards Agency)

A Dutch meat wholesaler was arrested for allegedly selling 300 tonnes of horse meat as beef

The Telegraph 14 February 2013:

“Long business supply chain are corruptible and can hide a multitude of crimes if no one checks for fraud or criminal activity”
A) Protein detection methods (ELISA):

- Easy to use
- High sensitivity
- High throughput

- Need for specific antibodies
- Cross-reactions → False positives
- Processing of foods can affect the immunoassay

B) **Methods based on DNA analysis (PCR):**
- High discrimination power (species-specific)
- High sensitivity

**Limitations on processed foods:**
- Difficulties on DNA extraction
- DNA degradation: pH, heat, hydrolytic enzymes…

}\{ **Low reliability**

Need to develop *alternative analytical approaches* for species identification

**Identification of biomarker peptides**

**Peptidomics**

**Identification of Meat Species in Foods**

**Identification of Meat Species in Foods**
Peptide biomarkers as a reliable and accurate way to reveal food composition

<table>
<thead>
<tr>
<th>Study</th>
<th>Food source</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of allergenic proteins</td>
<td>Peanuts</td>
<td>LC-MS/MS (Q/TOF)</td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>MALDI-TOF; LC-Q/TOF; Ion trap</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Edman sequencing; MALDI-TOF/TOF; LC-Ion trap</td>
</tr>
<tr>
<td>Authentication of seafood products</td>
<td>Fish</td>
<td>MALDI-TOF; LC-Ion trap</td>
</tr>
<tr>
<td></td>
<td>Shrimp</td>
<td></td>
</tr>
<tr>
<td>Addition of soybean proteins</td>
<td>Meat products</td>
<td>LC-Ion trap</td>
</tr>
<tr>
<td>Addition of collagen hydrolysates</td>
<td>Chicken meat</td>
<td>LC-Ion trap</td>
</tr>
<tr>
<td>Detection of transgenic food</td>
<td>Soya / maize</td>
<td>MALDI-TOF; LC-Q/TOF</td>
</tr>
<tr>
<td>Use of banned proteins</td>
<td>Animal feedstuffs</td>
<td>MALDI-TOF; LC-Ion trap</td>
</tr>
</tbody>
</table>
Peptidomic approach for differentiating horse from beef meat:

Beef / Horse Meat

Protein extraction
(Tris buffer, pH 8.0)

Protein fractionation by Liquid Isoelectric Focusing

Trypsin digestion

LC-ESI-MS/MS

Identification of Horse and Beef peptide biomarkers
## Horse vs. Beef Myoglobin

<table>
<thead>
<tr>
<th>Animal</th>
<th>Amino Acid Sequence</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>HORSE</td>
<td>MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASE 60</td>
<td>DLKKHGTVVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHAKH 120</td>
</tr>
<tr>
<td>BOVIN</td>
<td>MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASE 60</td>
<td>DLKKHGTVVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHAKH 120</td>
</tr>
</tbody>
</table>

Differences in the myoglobin amino acid sequence allow for searching of **specific peptide biomarkers**, characteristic of each animal species.
LC-MS/MS analysis of **Horse and Beef myoglobin trypsin digests**:

**Horse**

- Total Ion intensity (x 10^6): 3.58 min
- m/z 759.98
- Selected Ion (x 10^6): 759.98

**Beef**

- Total Ion intensity (x 10^6): 3.63 min
- m/z 774.97
- Selected Ion (x 10^6): 774.97

MS/MS 759.98 2+:


MS/MS 774.97 2+:

Peptidomic approach for differentiating horse from beef meat

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Mass</th>
<th>Position</th>
<th>Sequence</th>
<th>Parent protein</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>759.98 (2+)</td>
<td>120-134</td>
<td>HPDFGADAQAMTK</td>
<td>MYG_HORSE</td>
<td>Equus caballus</td>
</tr>
<tr>
<td>B</td>
<td>774.97 (2+)</td>
<td>120-134</td>
<td>HPDFGADAQAAMSK</td>
<td>MYG_BOVIN</td>
<td>Bos taurus</td>
</tr>
</tbody>
</table>
CONCLUSIONS

Current Proteomic technologies represents an interesting and promising alternative to existing methodologies already in use to assess meat authenticity.

- High resolving power → Discrimination made at sequence level

- More robustness with respect to current limitations of existing methods:
  • Analysis of both fresh and highly processed foods
  • Development of standardized extraction procedures


- Possibility to use routine, user-friendly, mass spectrometry facilities
Authenticity of meat products: Tools against fraud

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“Some things are easy to see....”

“...for others, you may need some help!”
Thank you

Valencia, Spain