

Proteomic tools to assess meat authenticity

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Consejo Superior de Investigaciones Científicas

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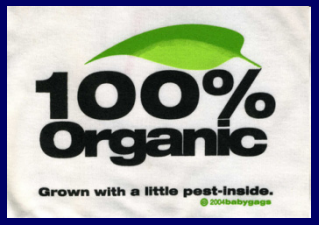
The problem of meat authentication

Clear and reliable information about food is demanded nowadays by consumers

Lifestyle affects individual's choice on food consumption



Honest and accurate food labeling is essential to assure food safety and choice

Quantitative Ingredient Declaration (QUID)

Robust and reliable methodologies are needed to assure that fraudulent or accidental mislabeling does not arise

10 Dec 2012

11 Jan 2013

15 Jan 2013

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We have immediately withdrawn from sale all products from the supplier in question, from all our stores and online.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

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…the 2013 Horse meat adulteration scandal

7 Feb 2013

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

14 Feb 2013

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)

23 May 2013

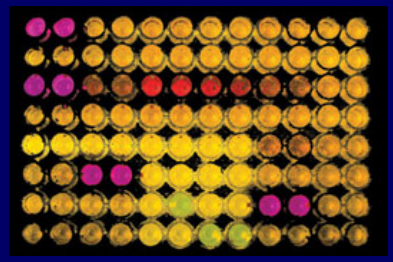
A Dutch meat wholesaler was arrested for allegedly selling 300 tonnes of horse meat as beef

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"

Methods used for the Identification of Meat Species in Foods

A) Protein detection methods (ELISA):



- Easy to use
- High sensitivity
- High throughput

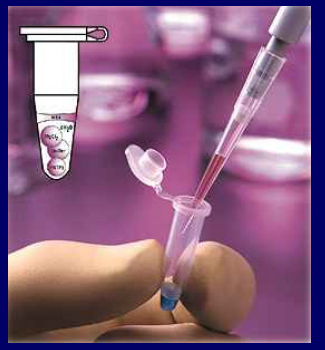
- Need for specific antibodies
- Cross-reactions → False positives
- Processing of foods can affect the immunoassay

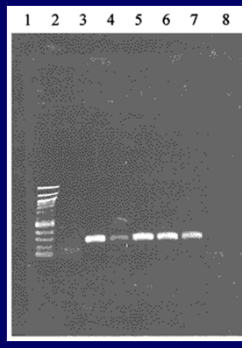
Giovannacci, I. et al. (2004). *Int. J. Food Sci. Technol.* 39, 863

Identification of Meat Species in Foods

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…

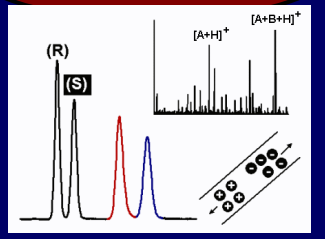
} **Low reliability**

Need to develop alternative analytical approaches for species identification

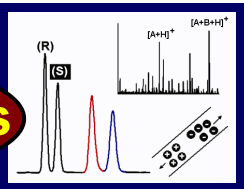
Identification of biomarker peptides

MASS SPECTROMETRY

Peptidomics



Peptidomics



Peptide biomarkers as a reliable and accurate way to reveal food composition






Study	Food source	Approach
Detection of allergenic proteins	Peanuts Wheat Milk	LC-MS/MS (Q/TOF) MALDI-TOF; LC-Q/TOF; Ion trap Edman sequencing; MALDI-TOF/TOF; LC-Ion trap
Authentication of seafood products	Fish Shrimp	MALDI-TOF; LC-Ion trap
Addition of soybean proteins	Meat products	LC-Ion trap
Addition of collagen hydrolysates	Chicken meat	LC-Ion trap
Detection of transgenic food	Soya / maize	MALDI-TOF; LC-Q/TOF
Use of banned proteins	Animal feedstuffs	MALDI-TOF; LC-Ion trap

Peptidomic approach for differentiating horse from beef meat:

Beef / Horse Meat

↓

Protein extraction
(Tris buffer, pH 8.0)

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Protein fractionation by
Liquid Isoelectric Focusing

1 2 3 4 5 6 7 8 9 10 11 12

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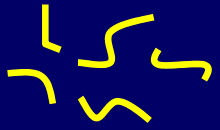
Trypsin digestion



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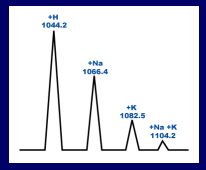
LC-ESI-MS/MS



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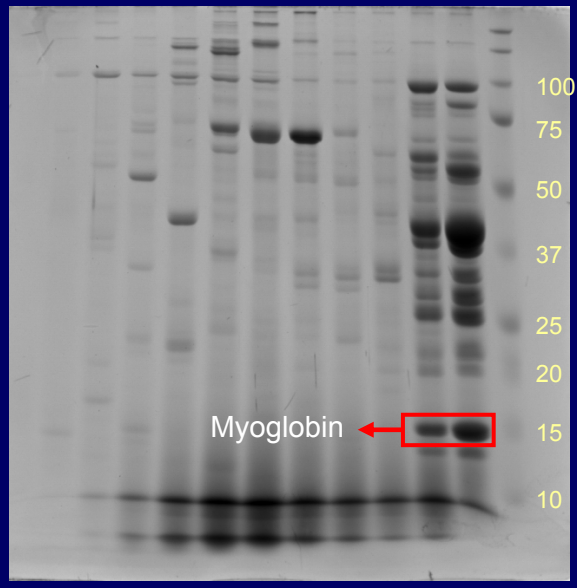
Identification of Horse and Beef peptide biomarkers

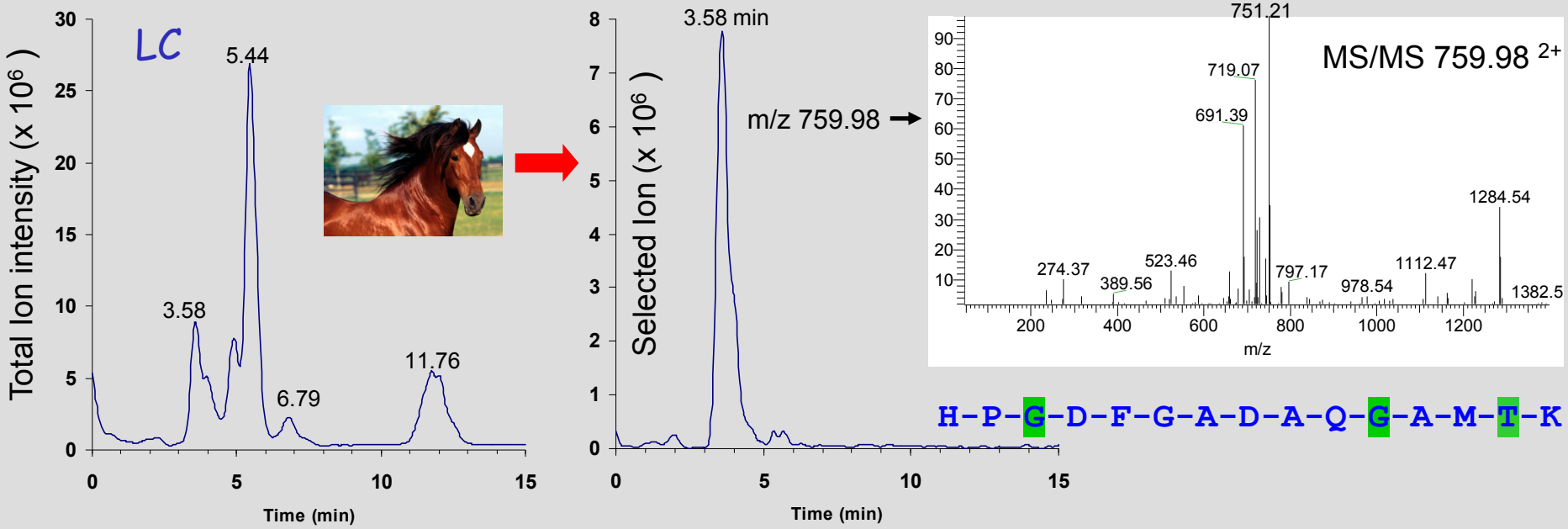


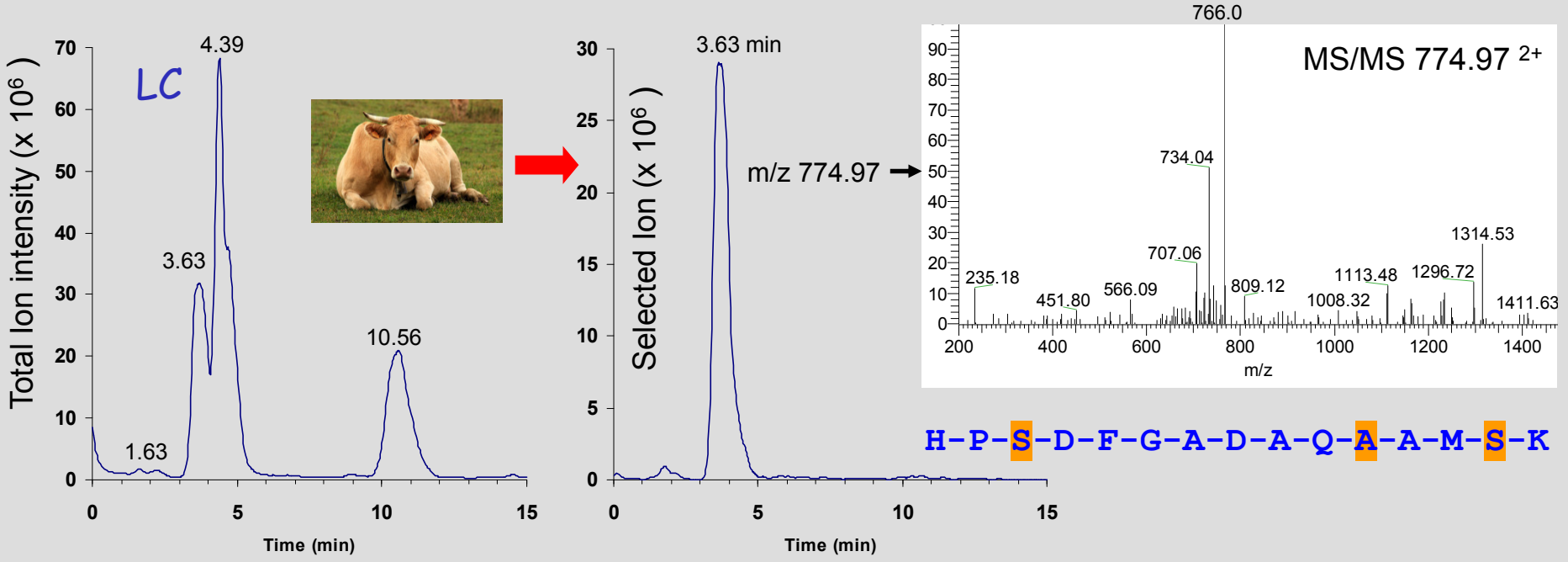
Horse vs. Beef Myoglobin

```
HORSE   MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASE 60
BOVIN   MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASE 60

HORSE   DLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKH 120
BOVIN   DLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHAKH 120

HORSE   PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG 154
BOVIN   PSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG 154
```

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:





Peptidomic approach for differentiating horse from beef meat

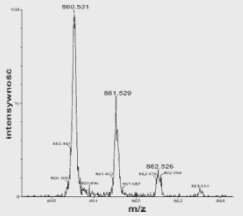
```
HORSE   MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASE 60
BOVIN   MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASE 60

HORSE   DLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKH 120
BOVIN   DLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHAKH 120

HORSE   PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG 154
BOVIN   PSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG 154
```

Identified Horse and Beef marker peptides are located in positions 120-134 of the myoglobin sequence

Peptide	Mass	Position	Sequence	Parent protein	Species
A	759.98 (2+)	120-134	HPGDFGADAQGAMTK	MYG_HORSE	*Equus caballus*
B	774.97 (2+)	120-134	HPSDFGADAQAAMSK	MYG_BOVIN	*Bos taurus*

"Proteomic tools to assess meat authenticity"

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

- Suitable for quantitative determinations (Sentandreu et al. *J. Prot. Res.* 2010)

- Possibility to use routine, user-friendly, mass spectrometry facilities

Additional information:

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Authenticity of meat products: Tools against fraud

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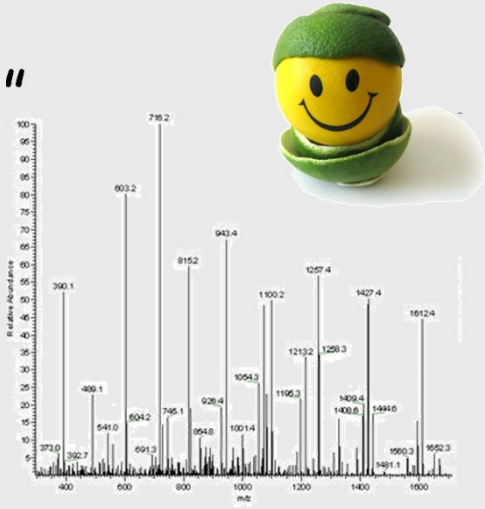
Sentandreu, MA & Sentandreu, E (2014). *Food Res. Int.* 60, 19-29

"Some things are easy to see…."

"…for others, you may need some help!"




Thank you
Valencia, Spain