Meat misconduct and coffee crime: using analytical chemistry to fight food fraud

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Food fraud research – why we do it

- Mis-labelling of foods is **economic fraud on a huge scale** (**~$50 billion** annually worldwide)
- **Disadvantages honest producers/suppliers**
- **Consumer confidence** – people want to know what they are eating – nobody likes being duped & defrauded
- **Health implications** – whilst most food fraud is harmless, some incidents have killed or seriously injured people

THE SPANISH TOXIC OIL SYNDROME

Real juice, pure frau

Trading standards inspectors in Europe and North America are cracking down on

2008 Chinese milk scandal

From Wikipedia, the free encyclopedia

The **2008 Chinese milk scandal** was a food safety incident

By November 2008, China

Olive oil food fraud: pressing truths

Not everything labelled 'extra virgin' is ... ly conceived; it seems there are some very sl ... the olive oil trade, and the problem is spr

The **Pärnu methanol tragedy** (Estonian: *Pärnu metanoolitragöödia*) was

county, Estonia on September 9,[1][2] 2001 and following days that

severe disability (including blindness or brain damage) to

people.[3]

Food Fraud: 8 Hoaxes To Look Out For At T Grocery Store

Posted: 04/20/2012 12:5

Italian olive oil scandal: seven top brands 'sold virgin'

The investigation, the latest scandal to hit Italy's lucrative food sector, involves seven well-known producers accused of passin olive oil as extra-virgin

muscular changes
lar weakness
loss, scl

Pub sales cola probe: "It's not the real thing!"

PIZZA PATR ON CHEESE CHEATS

Ministry foils the instant fraud in your coffee cup

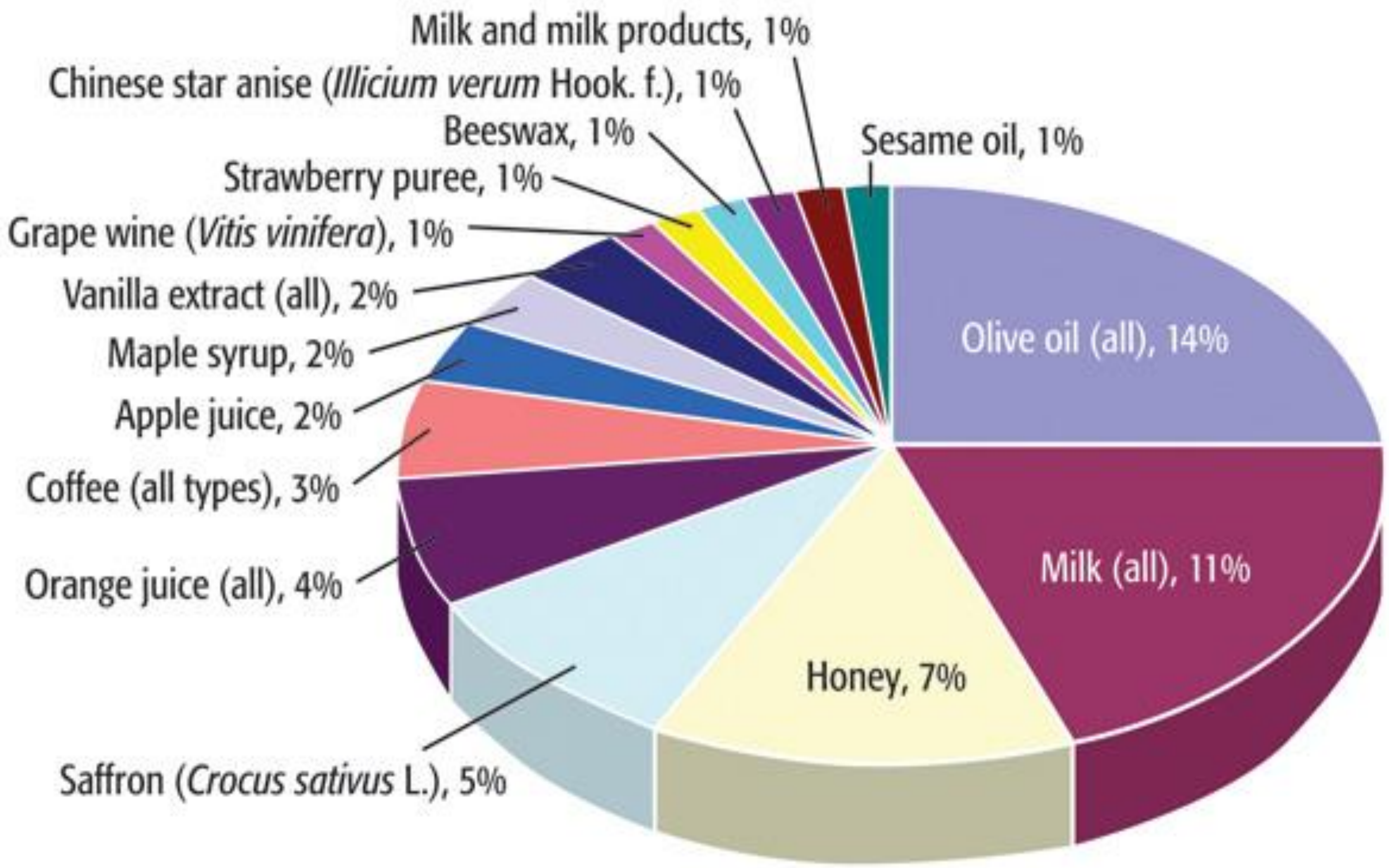
WATERED WINE FRAUD UNCOVERED BY EC

16 October 2012 Last updated at 09:04

Food fraud tackled by forensic scientists

Louise Taylor

Most prevalent food frauds – by economic value



Source: Food safety Magazine/World Customs Organization (2012)

The 2013 "Horsegate" scandal

Undeclared horsemeat was found in wide range of food products

- Partial (or even total) substitution of beef, lamb, etc with horse in ready meals, burgers,...
- Fraud was widespread across Europe – largest £££ incident ever recorded
- Occurred early in the supply chain
- Likely to have been happening for years

Routine testing of the supply chain was inadequate

- Targeted species verification – horse was not looked for
- **Too few tests – there is a need for faster, cheaper, untargeted methods**

An opportunity for Low-field NMR spectroscopy?

60Mhz permanent magnet "bench-top" spectrometers are a recent development

Compared with high-field spectrometers, these are:

- Very low capital costs (< 1/10th of high-field)
- No running costs (no cryogens, no air-con)
- Robust and easy-to-use by non-specialists

An opportunity for Low-field NMR spectroscopy?

60Mhz permanent magnet "bench-top" spectrometers are a recent development

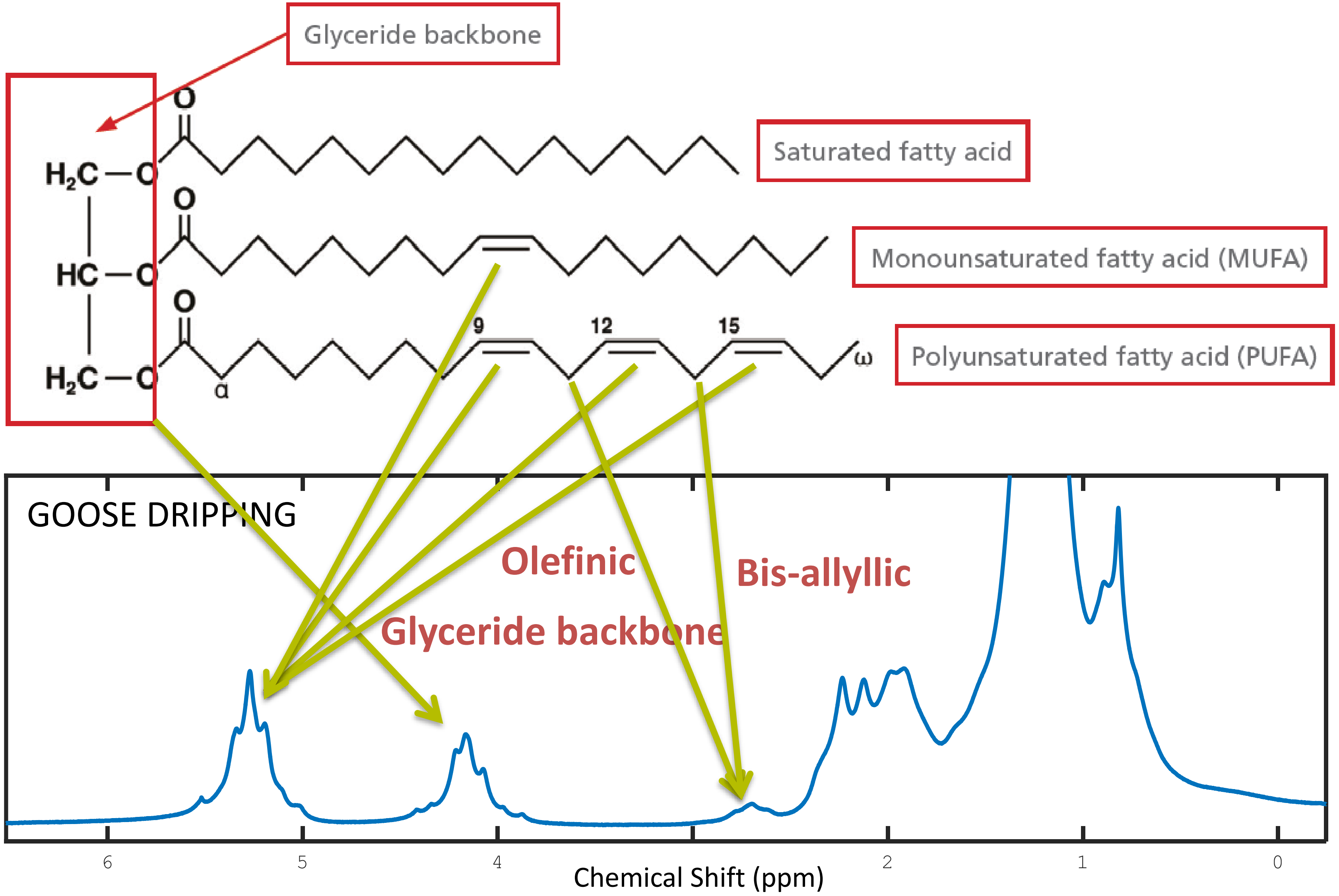
Cheap robust instrument + Modern electronics + Modern computing

= powerful combination for industrial applications

...such as **food authenticity screening**

60MHz proton NMR spectra of animal fats

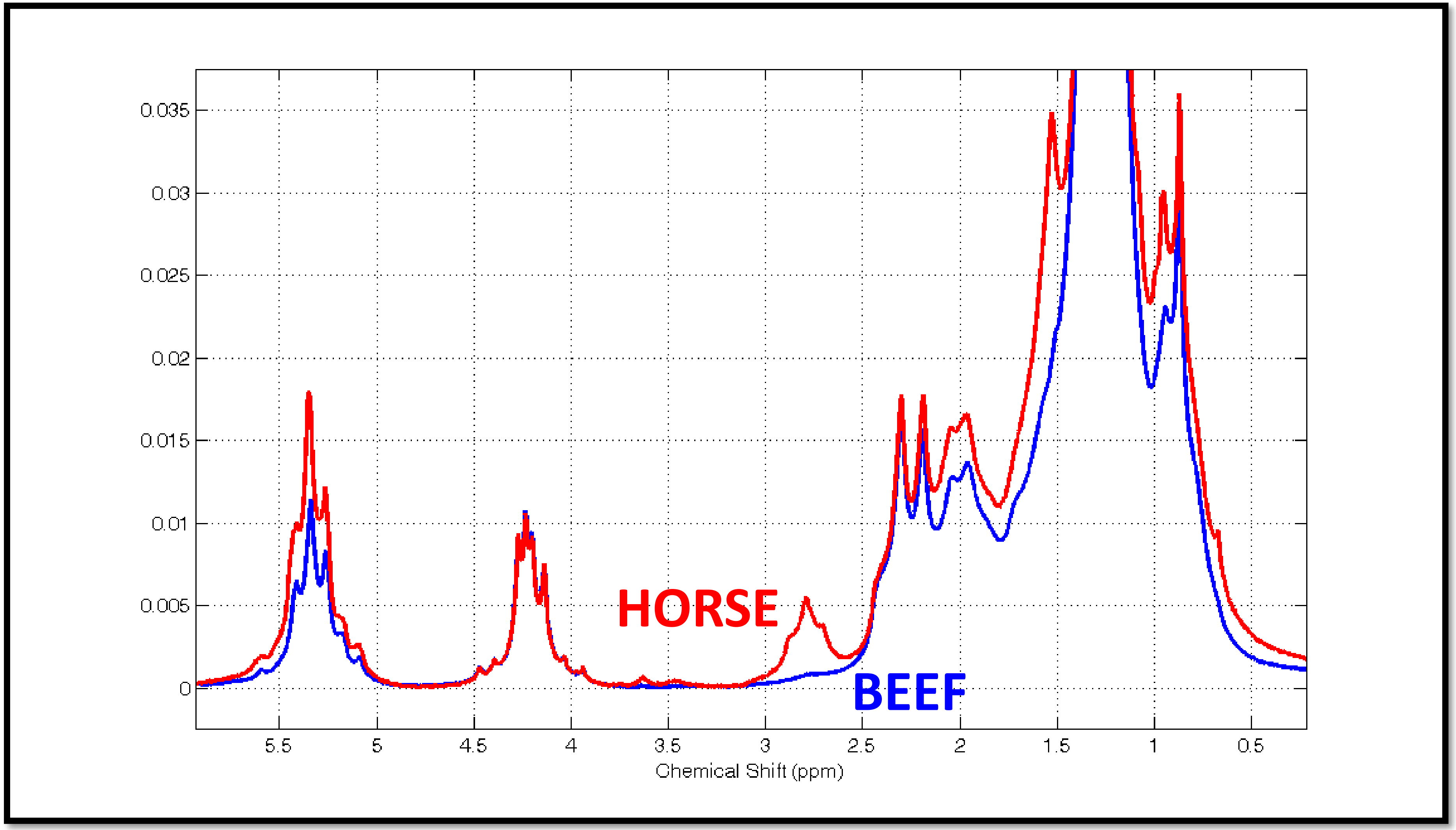
Most (>90%) of the fat in foods is present as triglycerides



Extraction and acquisition of spectra from meat

Quadram Institute

Different meats have different fatty acid profiles

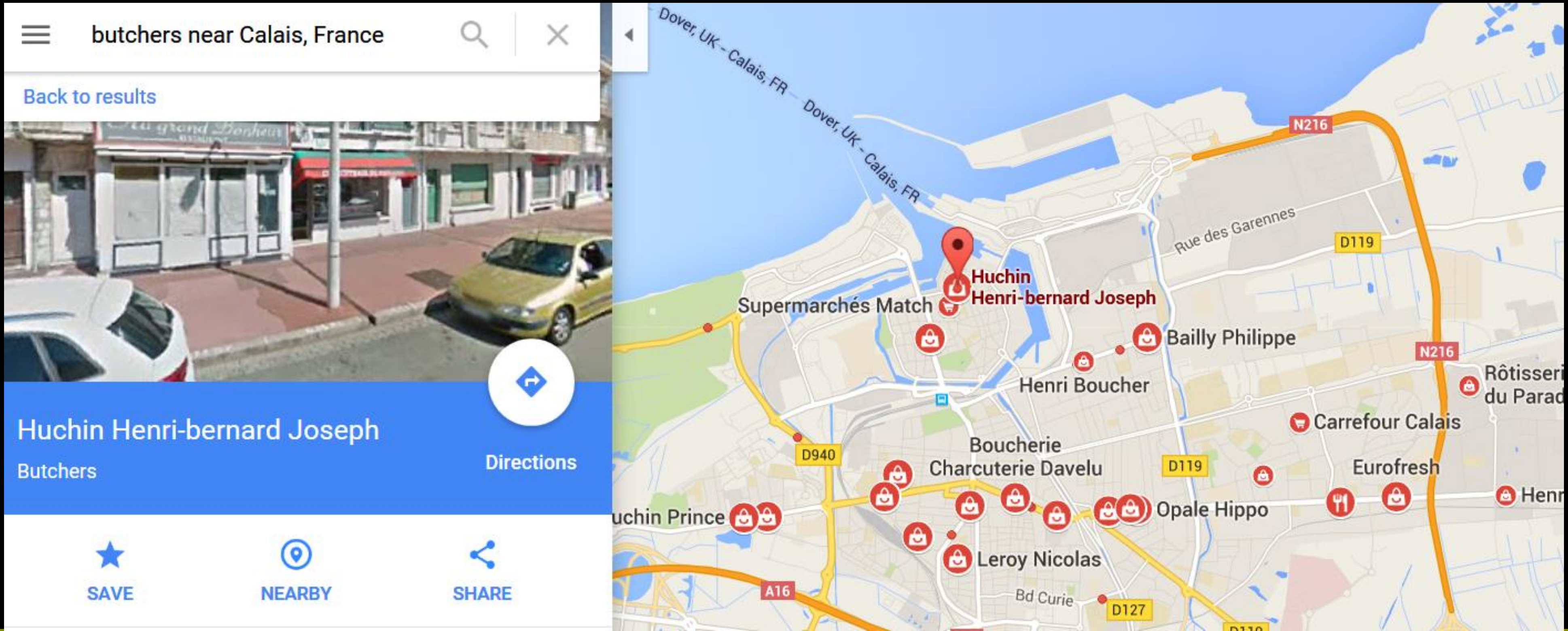


Developing a rapid authentication method for beef

Quadram Institute

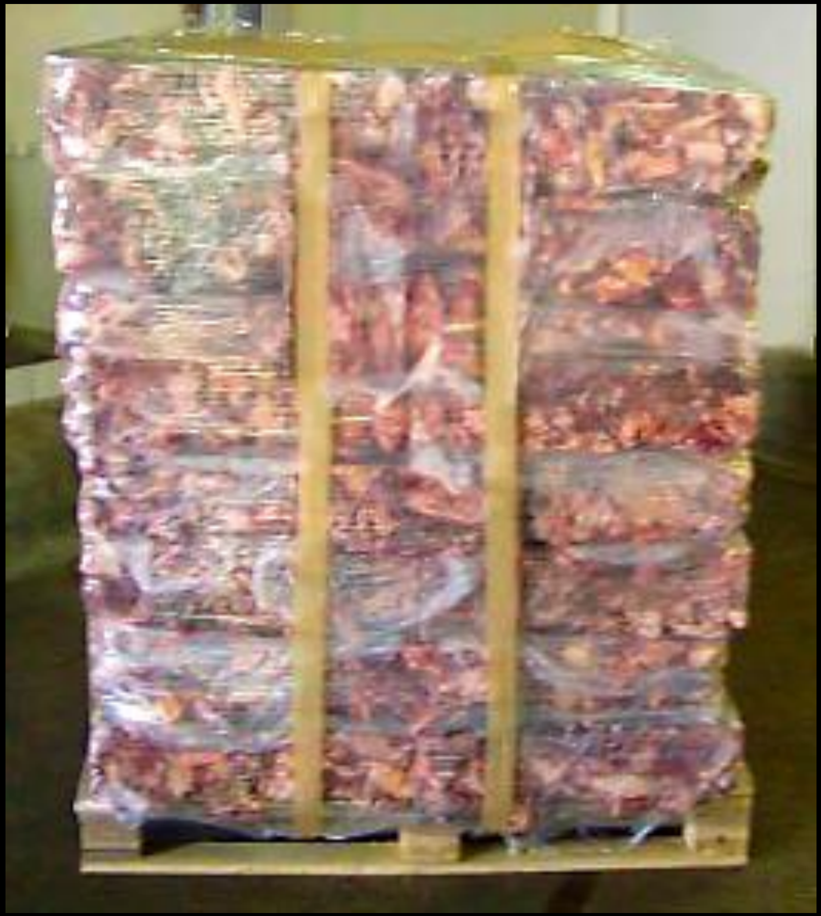
Challenges:

- Natural variability within populations
- Need lots of authentic reference samples (including potential adulterants, e.g. <u>horse</u>)

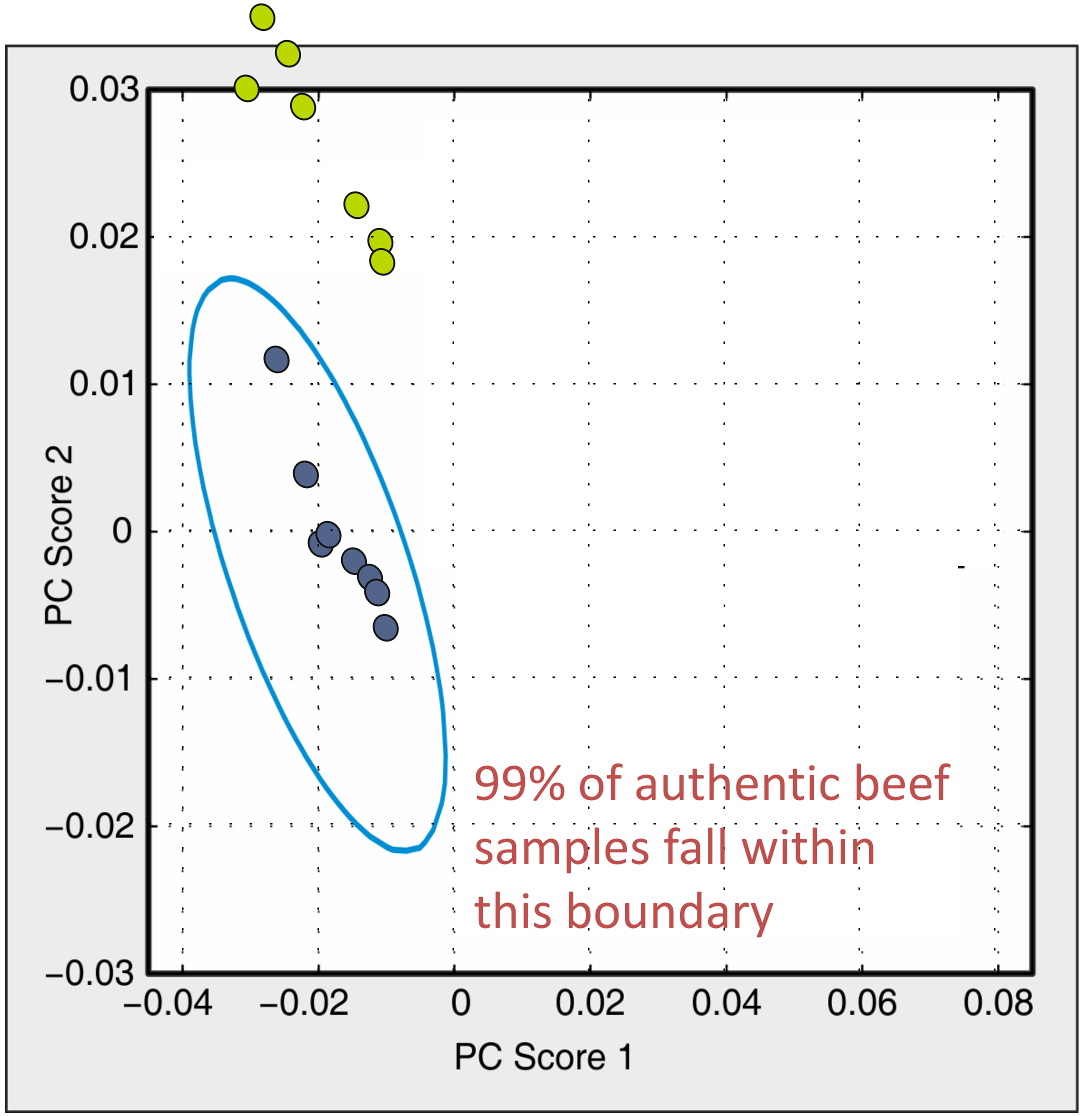


Challenges:

- Need to consider issues of representative sampling



Statistical analysis of > 500 spectra from beef and "non-beef"

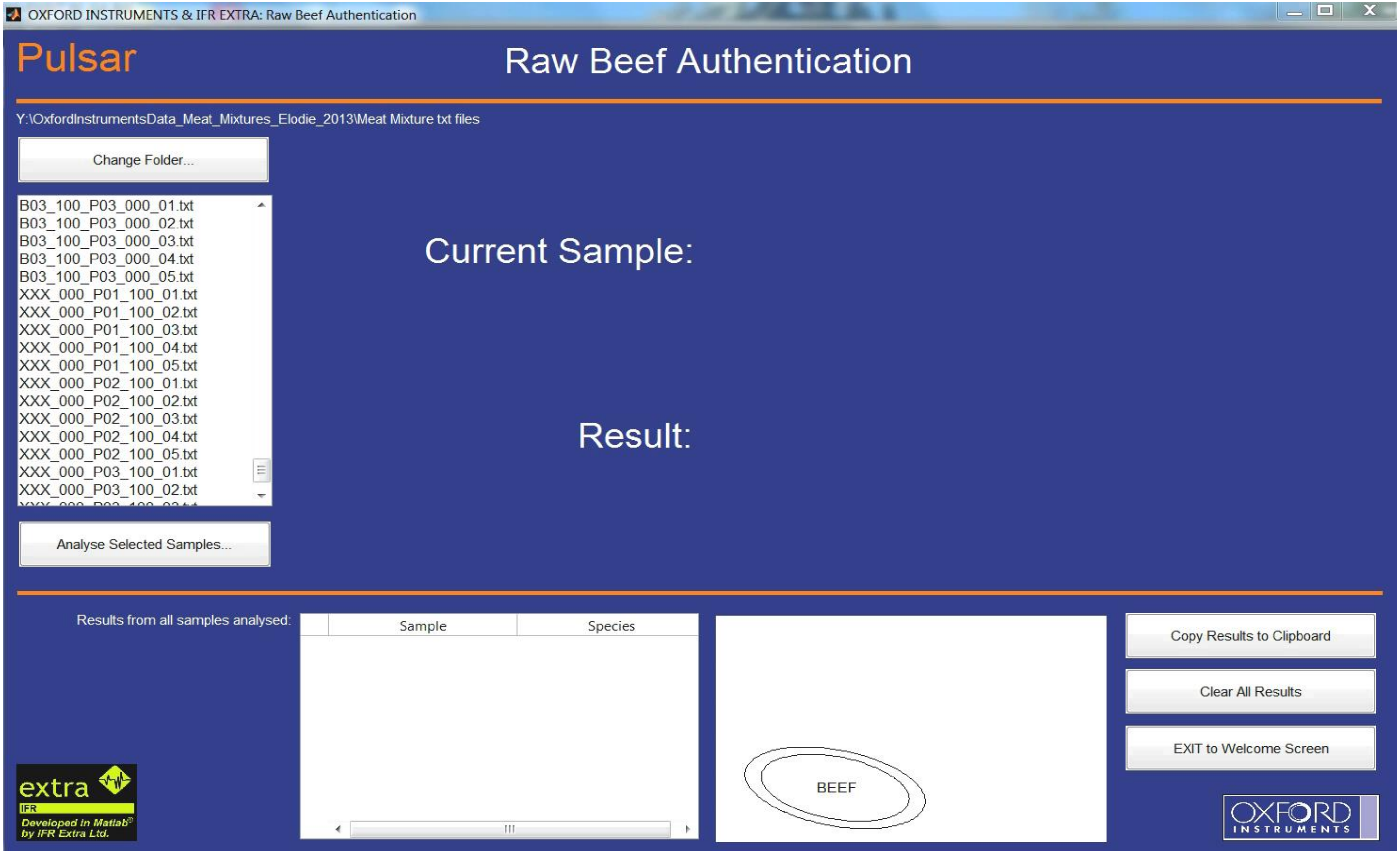


First results from commercial installation:

Test PORK Samples

Test BEEF Samples

Easy-to-use software package



Beef authentication: summary

Analytical service for beef authentication by fatty acids, offered through QIB Extra

Software package sold by Oxford Instruments

Patent:

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 March 2015 (12.03.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/033154 A1

Paper in Food Chemistry:

Food Chemistry

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Authentication of beef versus horse meat using 60 MHz ^{1}H NMR spectroscopy

W. Jakes[a, 1], A. Gerdova[b], M. Defernez[a], A.D. Watson[a], C. McCallum[a, 2], E. Limer[a, 3], I.J. Colquhoun[a], D.C. Williamson[b], E.K. Kemsley[a]

Mass Spectrometry for species identification via proteins

- Why?
  - We want methods capable of analysing “complex foods”: multiple ingredients, cooked, canned, etc
  - Protein component is stable, present in almost all foods, and quite specific to individual components

- Current work: ‘buffalo mozzarella’
  - Premium product, currently trendy, limited supply….. hence vulnerable to fraud

Mass Spectrometry for species identification via proteins

- Standard approach:
  - extract and digest the proteins in a sample
  - do proteomics and try and pick out proteins (and marker peptides) for the species of interest

→ In general, the proteins and marker peptides are chosen for the sample at hand – no particular consideration of commonality across other species

Mass Spectrometry for species identification via proteins

- Our approach: "Corresponding Proteins Corresponding Peptides" - CPCP
  - targets a protein(s) nominally "the same" across all species of interest (e.g. Alpha s1 Casein found in milks)
  - **the protein needs to possess some small but species-specific differences in the amino acid sequence**
  - the peptides carry forward these species-specific differences, giving clear species markers which can be quantified using MRM-MS

Ensuring the Integrity of the European food chain

FOOD INTEGRITY

Quadram Institute
Science · Health · Food · Innovation

GDAŃSK UNIVERSITY OF TECHNOLOGY

Universität Stuttgart

Workpackage 13:

Consumer and Brand Protection in Complex Foods

from Protein Signatures using Mass Spectrometry

Comparing sequences of the targeted protein, alpha s1 casein

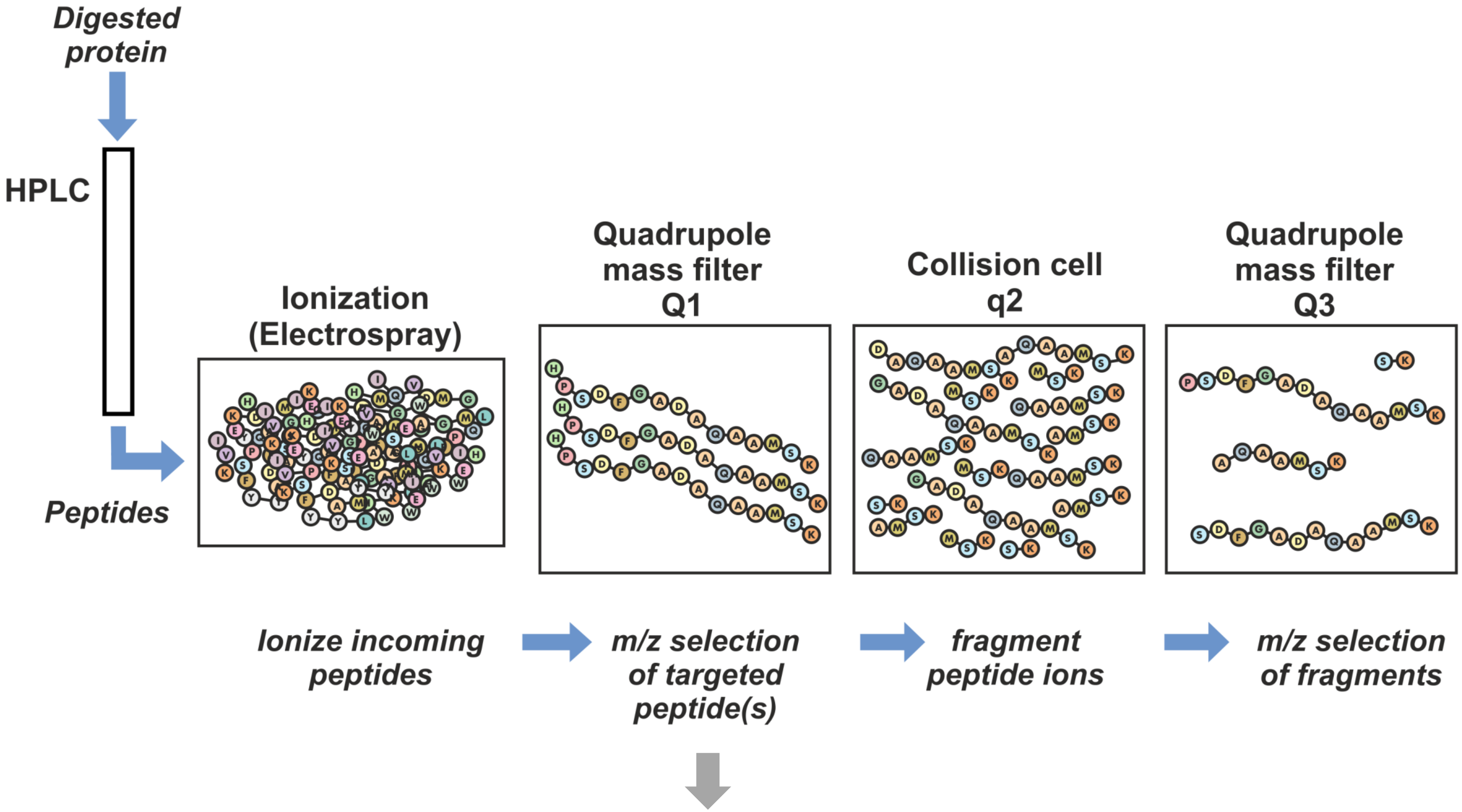
```
P02662 (bovine)  |MK•LLILTCLVAVALARPK•HPIK•HQGLPQEVLNENLLR•FFVAPFPEVFGK•EK•VNELSK•DIGSESTEDQAMEDIK•
O62823 (buffalo) |MK•LLILTCLVAVALARPK•QPIK•HQGLPQGVLNENLLR•FFVAPFPEVFGK•EK•VNELST DIGSESTEDQAMEDIK•

P02662 (bovine)  |QMEAESISSSEEIVPNSVEQK•HIQK•EDVPSER•YLGYLEQLLR•LK•K•YK•VPQLEIVPNSAEER•LHSMK•EGIHAQQK•
O62823 (buffalo) |QMEAESISSSEEIVPISVEQK•HIQK•EDVPSER•YLGYLEQLLR•LK•K•YN VPQLEIVPNLAEEQ LHSMK•EGIHAQQK•

P02662 (bovine)  |EPMIGVNQELAYFYPELFR•QFYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEK•TTMPLW
O62823 (buffalo) |EPMIGVNQELAYFYPQLFR•QFYQLDAYPSGAWYYVPLGTQYPDAPSFSDIPNPIGSENSGK•TTMPLW
```

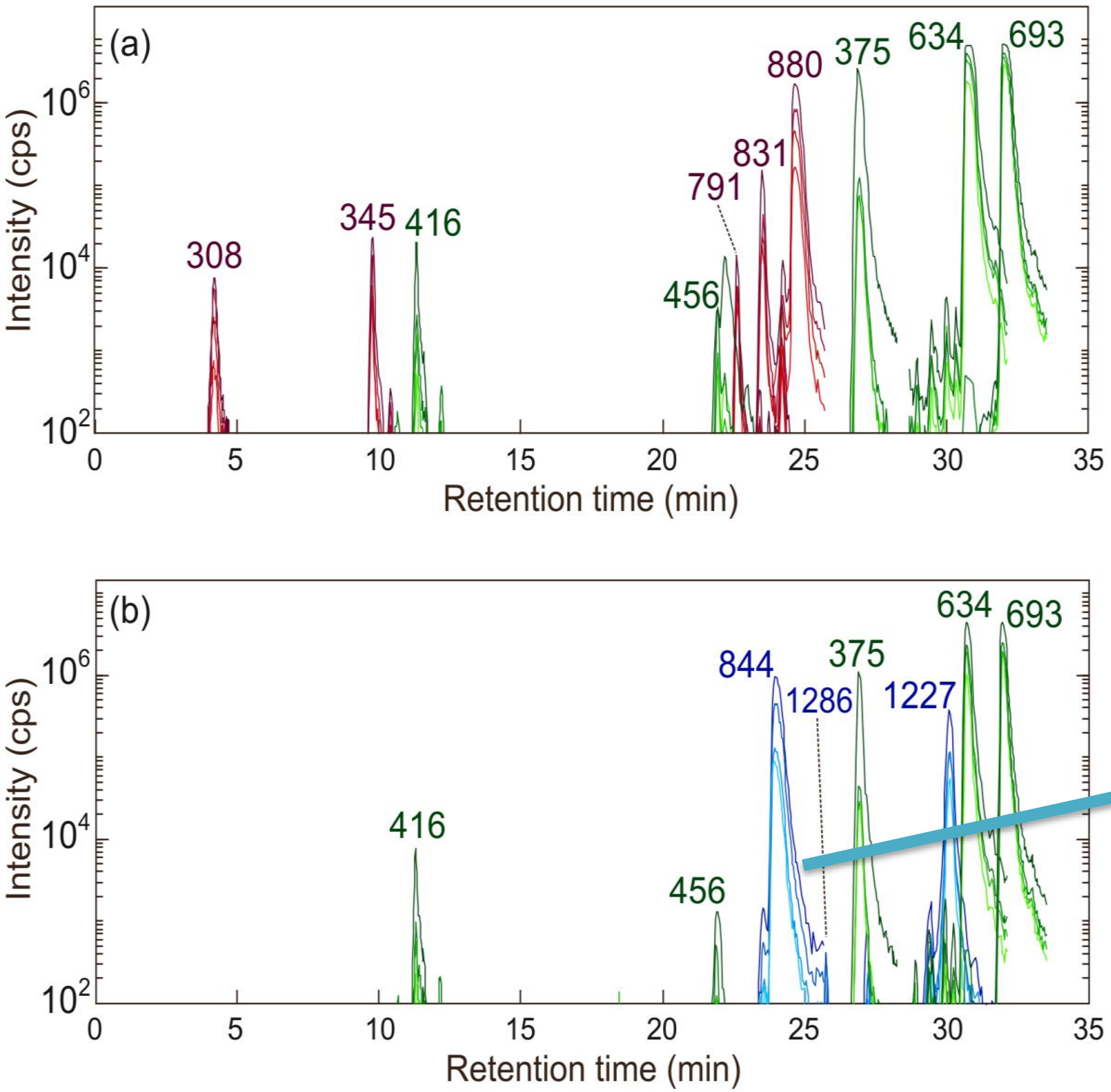
- The red and blue dots indicate cleavage sites from digestion with trypsin
- Coloured sequence strings indicate useful peptides:
  - red for bovine, blue for buffalo, with yellow highlights for sequence differences
  - green for peptides identical in both – useful for ‘calibrating’ alpha s1 casein levels

Selective / Multiple } reaction monitoring mass spectrometry (SRM-MS, MRM-MS)



e.g. precursor m/z = “844”, retention time = 23.3 min,
fragment ions 1253, 1423, 1028, 872

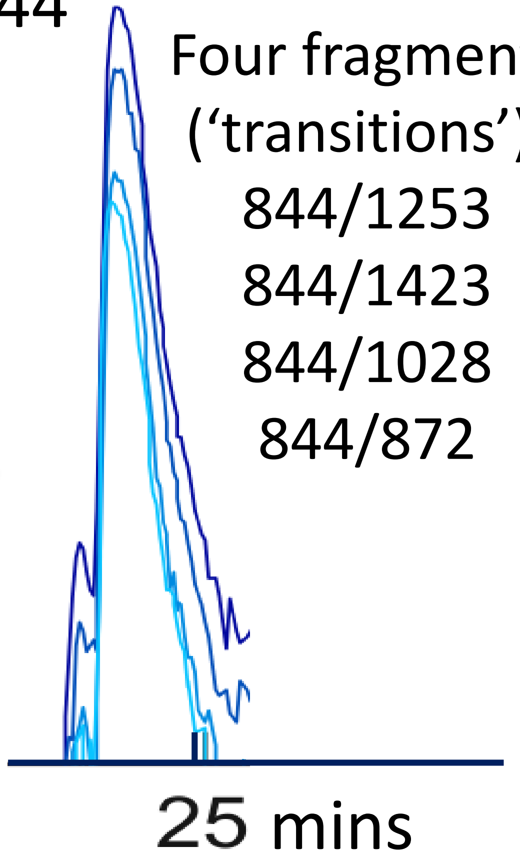
'Complete' dataset



A 'buffalo' marker peak:

m/z

844



Four fragments

('transitions'):

844/1253

844/1423

844/1028

844/872

m/z	fragments	R_t	species	Amino acid sequence
308	(365, 502, 147, 278)	4.5	Bovine	LHSMK
345	(590, 476, 234, 347)	10.0	Bovine	VNELSK
375	(205, 415, 546, 318,)	26.3	Both	TTMPLW
416	(488, 391, 587, 175)	11.4	Both	EDVPSER
456	(568, 471, 667, 304)	22.9	Both	EDVPS(Pho)ER
634	(992, 771, 658, 935)	29.8	Both	YLGYLEQLLR
693	(920, 992, 676, 1091)	31.1	Both	FFVAPFPEVFGK
791	(802, 901, 1015, 1257)	22.0	Bovine	VPQLEIVPNSAEER
831	(882, 981, 1094, 785)	22.9	Bovine	VPQLEIVPNS(Pho)AEER
844	(1253, 1423, 1028, 872)	23.3	Buffalo	HQGLPQGVLNENLLR
880	(1325, 1495, 872, 971)	24.0	Bovine	HQGLPQEVLNENLLR
1227	(1397, 278, 1496, 1609)	29.2	Buffalo	YNVPQLEIVPNLAEEQLHSMK
1286	(375, 635, 260, 504)	25.0	Buffalo	VNELS(Pho)TDIGS(Pho)ESTEDQAMEDIK

Quantifying components in mixtures

- The relative amounts of two ‘corresponding’ peptides are a proxy for the levels of alpha s1 casein, and hence levels of each species, in a binary mixture

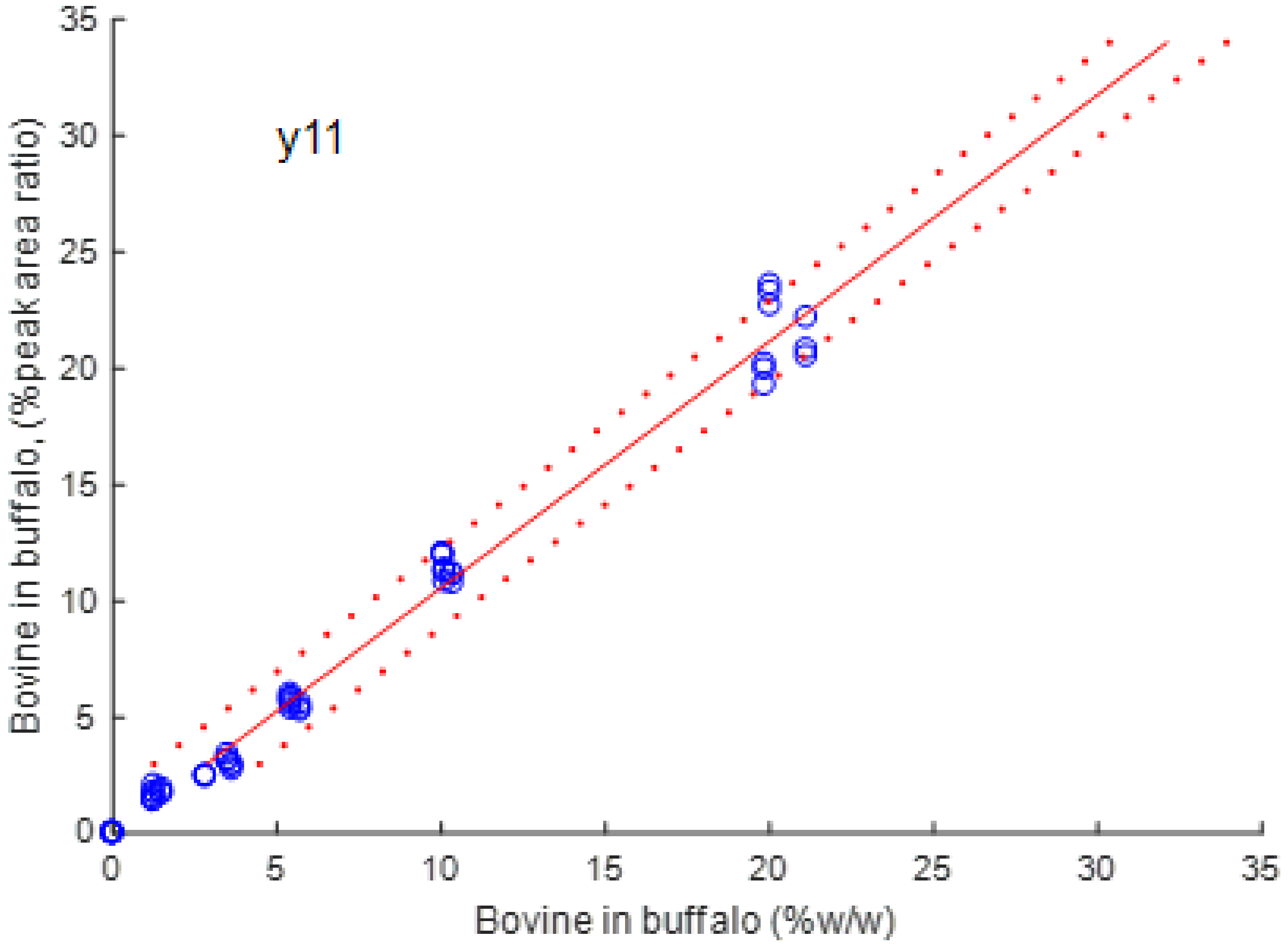
- Count fragments to measure amounts (integrated areas of peaks)

Analysis of bovine in buffalo mozzarella cheese mixtures

‘Corresponding peptide’ transitions:

Bovine 880/1325 &
Buffalo 880/1253
(called y^{11})

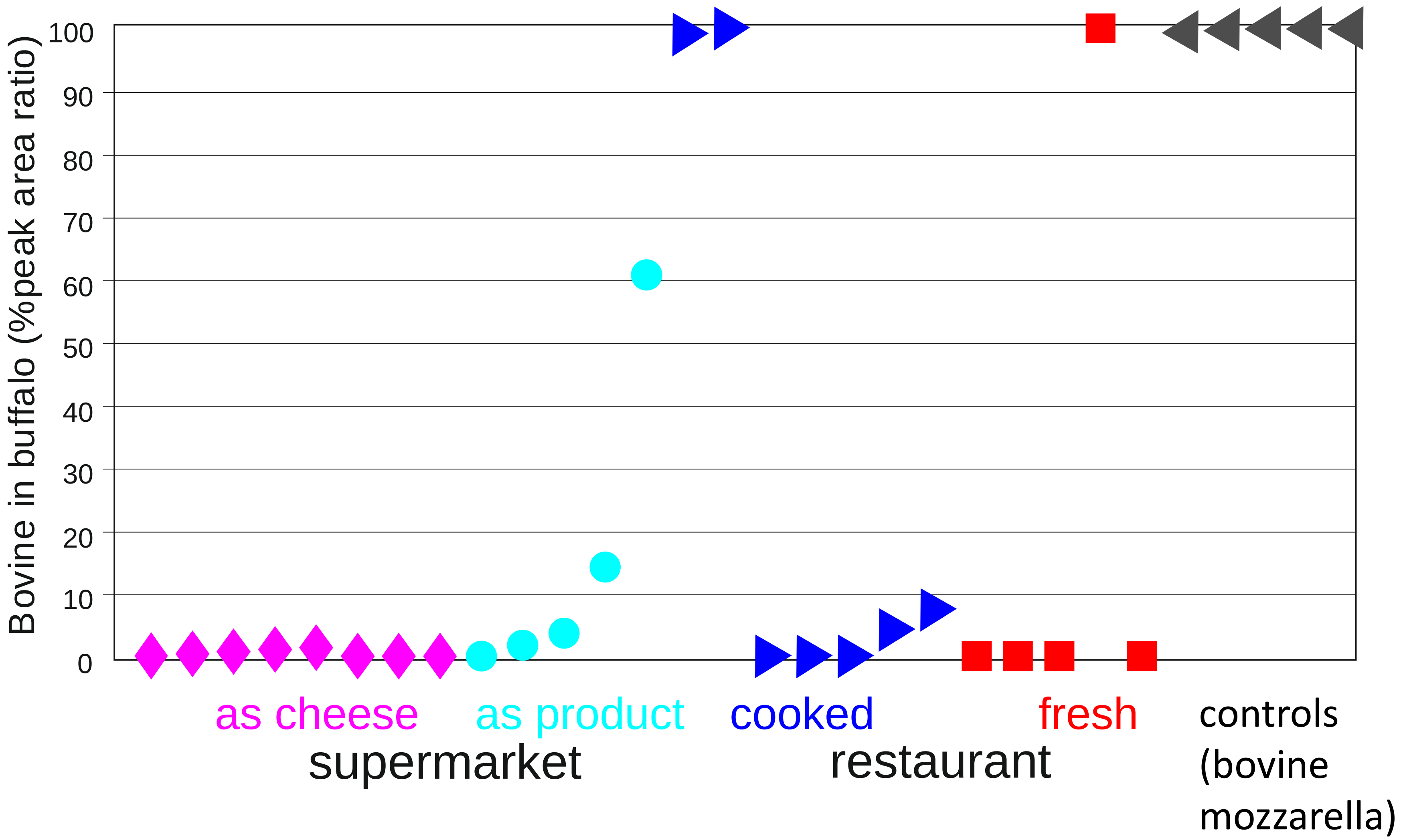
Estimated detection limit <1% w/w



Summary of MRM-MS for authentication of cheese:

- MRM-MS + CPCP method provides a route to quantitation
- Useful accuracy and precision – economic adulteration detection, not contamination levels
- Robust method with good specificity and selectivity – promising for the analysis of complex foods
- Preliminary retail/restaurant survey suggests there may be a problem in the sector!

Survey samples: labels declared as ‘buffalo mozzarella’



Low-field NMR analysis of lipophilic extracts from ground roast coffee

Can we verify the labelling claim?

- “Arabica” is used as a sign of “high quality” on expensive products

About coffee…

- 25,000,000 farmers worldwide are involved in producing coffee beans
- Over 2,250,000,000 cups of coffee are consumed every day
- Arabica has a higher sensory quality than robusta, but is lower yielding and harder to grow
- Coffee has a long supply chain, making it vulnerable to fraud

PRODUCERS (farmers, collectives) → MIDDLEMEN EXPORTERS ('coffee coyotes') → IMPORTERS (& commodity Traders) → ROASTERS (blends, Packaging) → RETAILERS (& caterers)

Economics of coffee fraud

Coffee trading prices at April 2017:

Arabica: \$3.62 per kg

Robusta:\$2.18 per kg

Worldwide annual production:

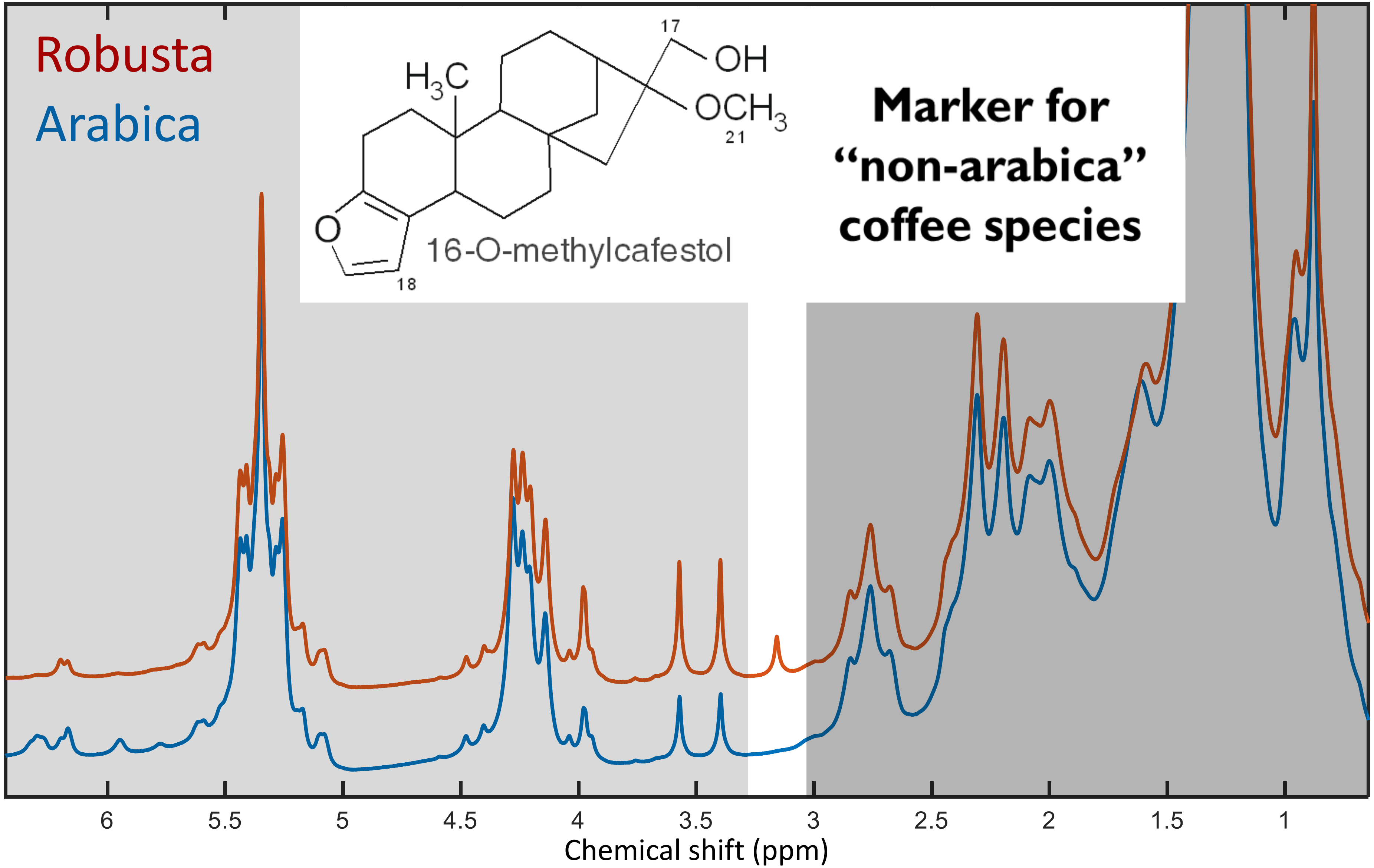
Arabica: 5.6 million tonnes (5.6 x 10^9kg)

Robusta:3.7 million tonnes (3.7 x 10^9kg)

(data source: IndexMundi)

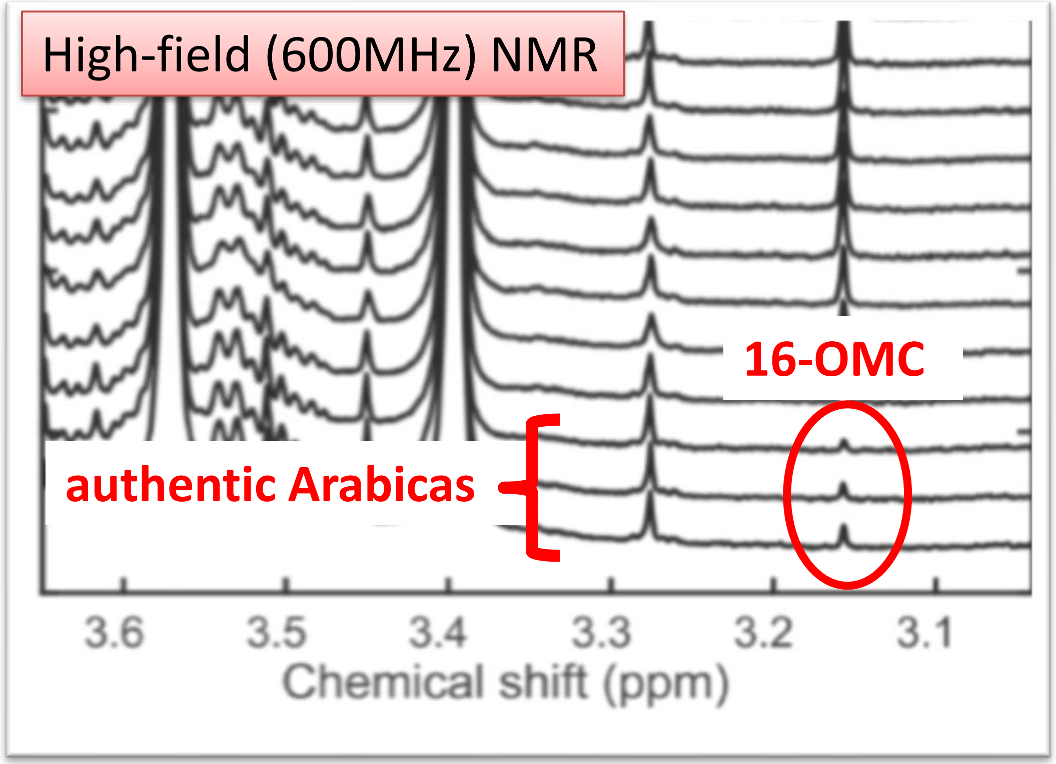
- Suppose just 5% of Arabica beans are fraudulently substituted with robusta: at trading prices, this is worth around **\$500million to the fraudsters**
- **Significantly disadvantages honest producers and traders**
- Fraud of any kind diminishes trust in the food supply chain

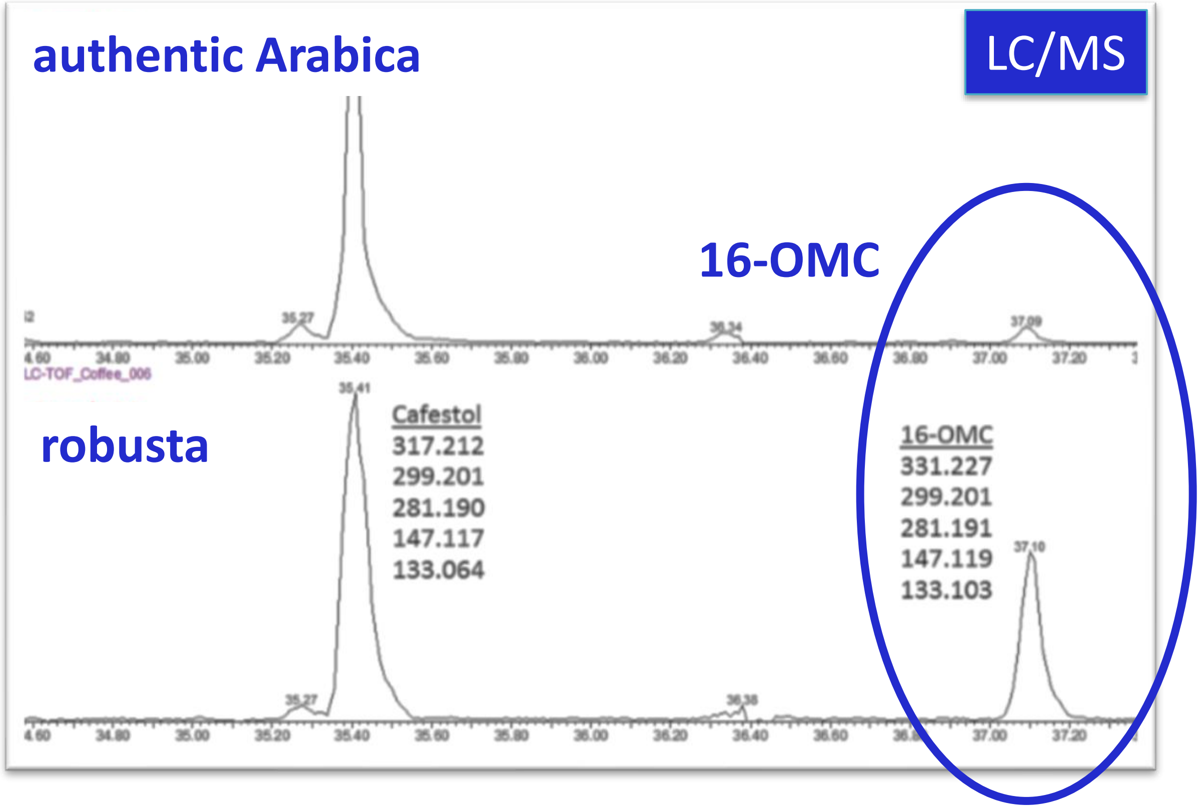
60MHz ^{1}H NMR spectra of lipophilic extracts from coffee beans



Confronting the literature opinion

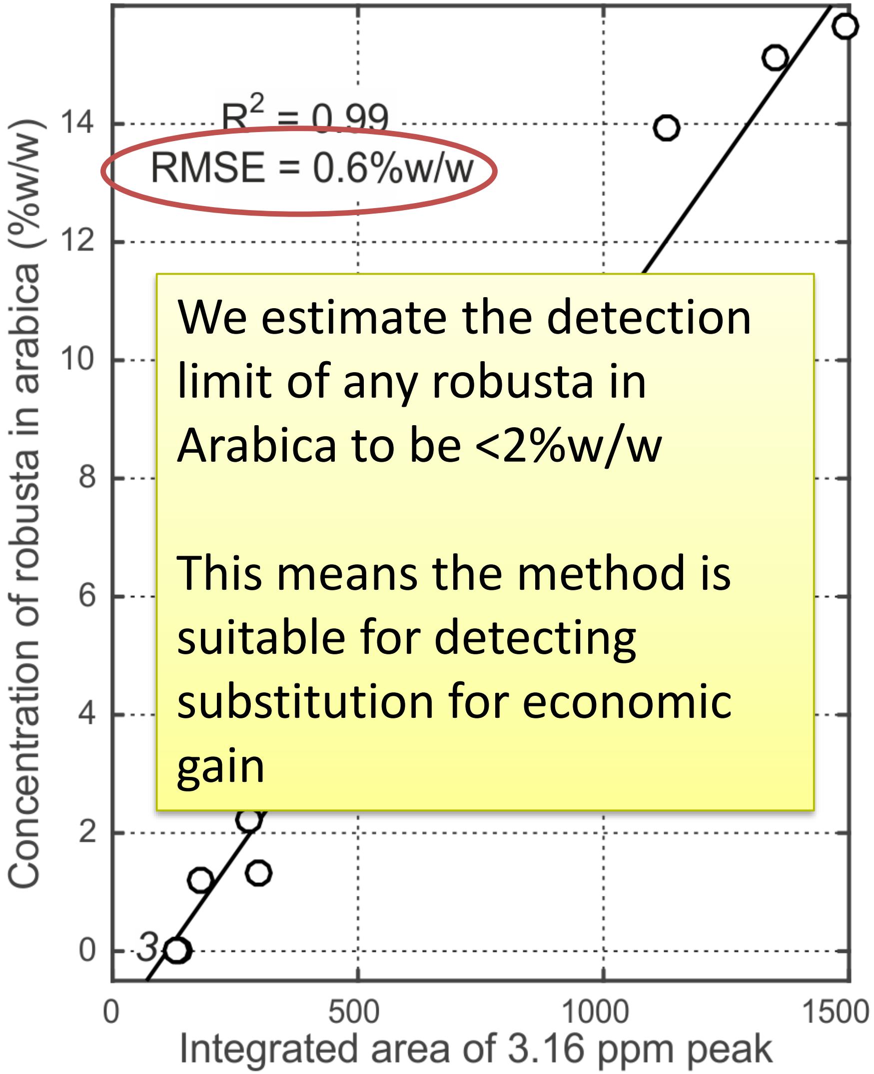
- Until 2017, the consensus was that Arabica coffee beans contain no 16-OMC at all
- Our work was the first to show that this was not true
- By NMR and LC/MS independently, we found 16-OMC in extracts from authentic Arabica beans (which had been collected at source and supplied to us by RBG (Kew Gardens)





Detection limit via serial addition of robusta to Arabica

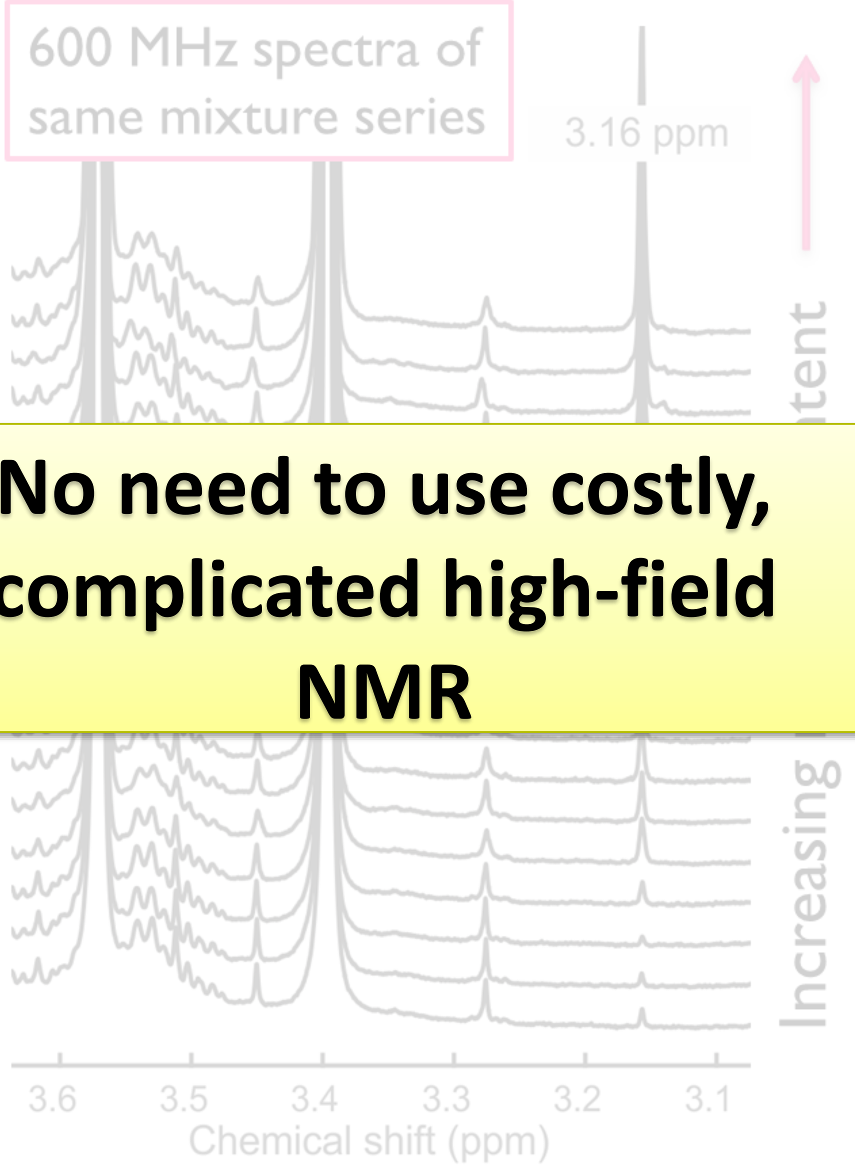
Calibration curve for robusta content of Arabica/robusta mixes



We estimate the detection limit of any robusta in Arabica to be <2%w/w

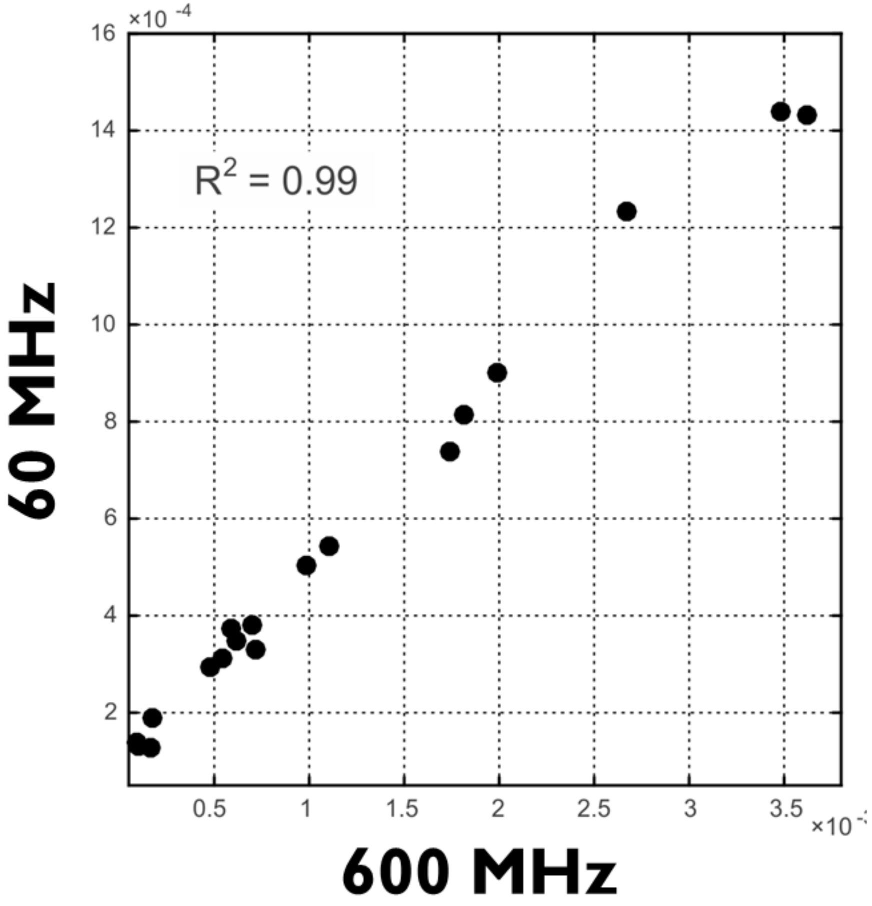
This means the method is suitable for detecting substitution for economic gain

Comparison of results with high-field (600MHz) NMR

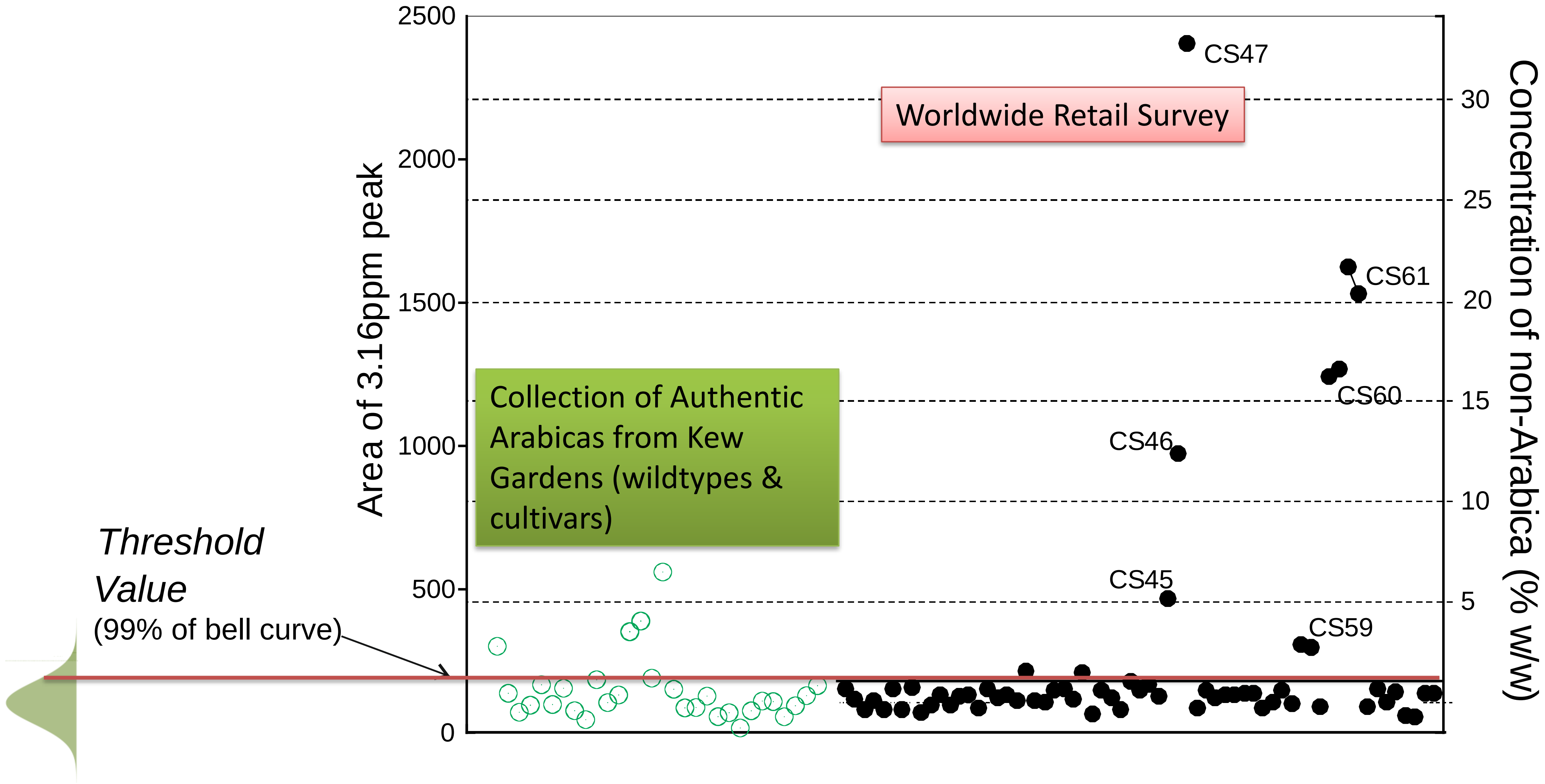


No need to use costly, complicated high-field NMR

3.16 ppm peak areas



Developing the method: establishing normal range of 16OMC/K in Arabica



Summary…

- Turnaround time: ~45mins per sample (extraction and spectral acquisition)

- ~100 coffee samples of known composition used to test protocol

- Survey of retail samples obtained worldwide suggest problems in the sector

References

ELSEVIER
Food Chemistry
Volume 248, 15 May 2018, Pages 52-60

Rapid Communication

16-O-methylcafestol is present in ground roast Arabica coffees: Implications for authenticity testing

Yvonne Gunning [a], Marianne Defernez [a], Andrew D. Watson [a], Niles Beadman [a], Ian J. Colquhoun [a], Gwénaëlle Le Gall [a], Mark Philo [a], Hollie Garwood [a], David Williamson [b], Aaron P. Davis [c], E. Kate Kemsley [a]

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Food Chemistry
Volume 175, 15 May 2015, Pages 1-9

Rapid Communication

Authentication of beef versus horse meat using 60 MHz ^{1}H NMR spectroscopy

W. Jakes [a, 1], A. Gerdova [b], M. Defernez [a], A.D. Watson [a], C. McCallum [a, 2], E. Limer [a, 3], I.J. Colquhoun [a], D.C. Williamson [b], E.K. Kemsley [a]

ELSEVIER
Food Chemistry
Volume 216, 1 February 2017, Pages 106–113

Low-field ^{1}H NMR spectroscopy for distinguishing between arabica and robusta ground roast coffees

Marianne Defernez [a], Ella Wren [a], Andrew D. Watson [a], Yvonne Gunning [a], Ian J. Colquhoun [a], Gwénaëlle Le Gall [a], David Williamson [b], E. Kate Kemsley [a]

A D Watson et al (2015) **“Meat Authentication via Multiple Reaction Monitoring Mass Spectrometry of Myoglobin Peptides”** Analytical Chemistry 87 (20) 10315–10322

Y Gunning et al (2016) **“Species determination and quantitation in mixtures using MRM mass spectrometry of peptides applied to meat authentication”** Journal of Visualised Experiments

Y Gunning et al (2019) **Quantitative authenticity testing of buffalo mozzarella via αs1-Casein using multiple reaction monitoring mass spectrometry** Food Control doi.org/10.1016/j.foodcont.2019.02.029