



## **Advances in Direct Mass Spectrometry Techniques Coupled with Chemometric Modelling for the Rapid Detection of Food Fraud**

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## Overview

- Food Fraud / Adulteration Introduction
- Vanilla Authenticity
- Belgian Butter (PDO)
- Olive Oil





## Introduction to food fraud

- A food **safety** incident is typically an unintentional act with unintentional harm
  - e.g. German *E. coli* O104:H4 outbreak in 2011
- A food **defence** incident is an intentional act with intentional harm
  - e.g. Punjab sweet poisoning with chlorfenapyr in 2016
- Food **fraud** is most commonly referred to as the intentional defrauding of food and food ingredients for economic gain
- Food fraud encompasses the terms:
  - Food **authenticity**; ensuring that food offered for sale or sold is of the nature, substance and quality expected by the purchaser
  - Economically motivated **adulteration** (EMA); intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product or reducing the cost of its production



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## Food Products at Risk for Food Fraud

- Meat
- Milk
- Olive oil
- Fish / Seafood
- Organic foods
- Cereals, grains and rice
- Honey and maple syrup
- Coffee and tea
- Select herbs and spices
- Wine
- Fruit juices



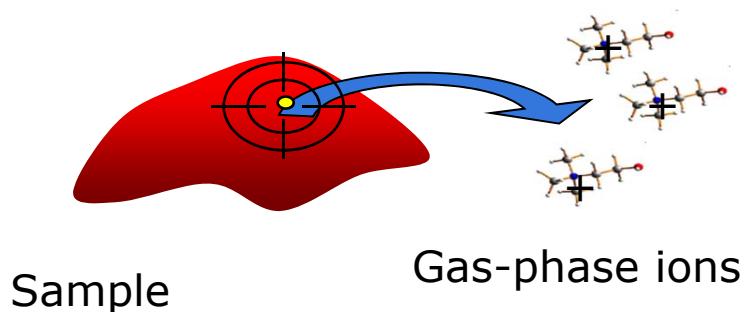


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## Mass Spectrometric Profiling

### Traditional

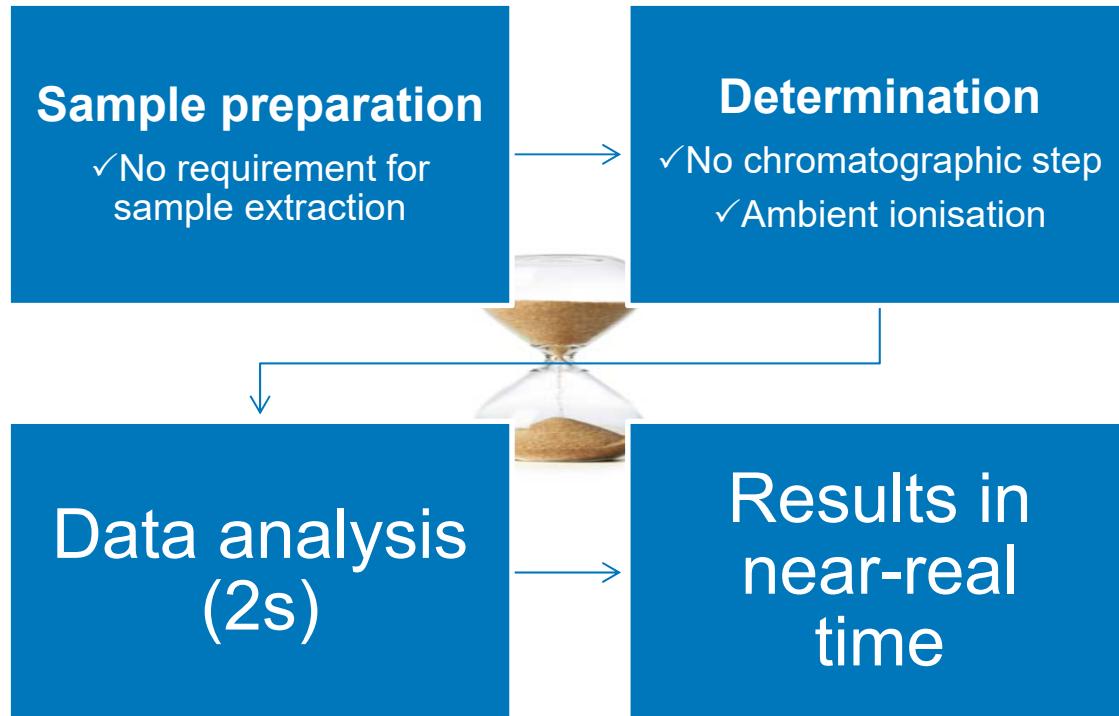
- Simple, sample preparation
- GC-MS
- LC-MS, LC-MS/MS
- Few thousand components in 5-30 min



### Direct

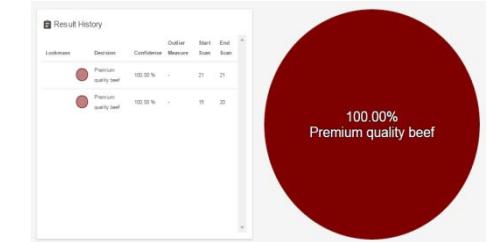
- No sample prepared
- Formation of sample originated gaseous ions
- Spatially well defined
- Few hundred components in **1-5 sec**

# Direct MS for flexible & rapid screening



✓ Point-of-control analysis

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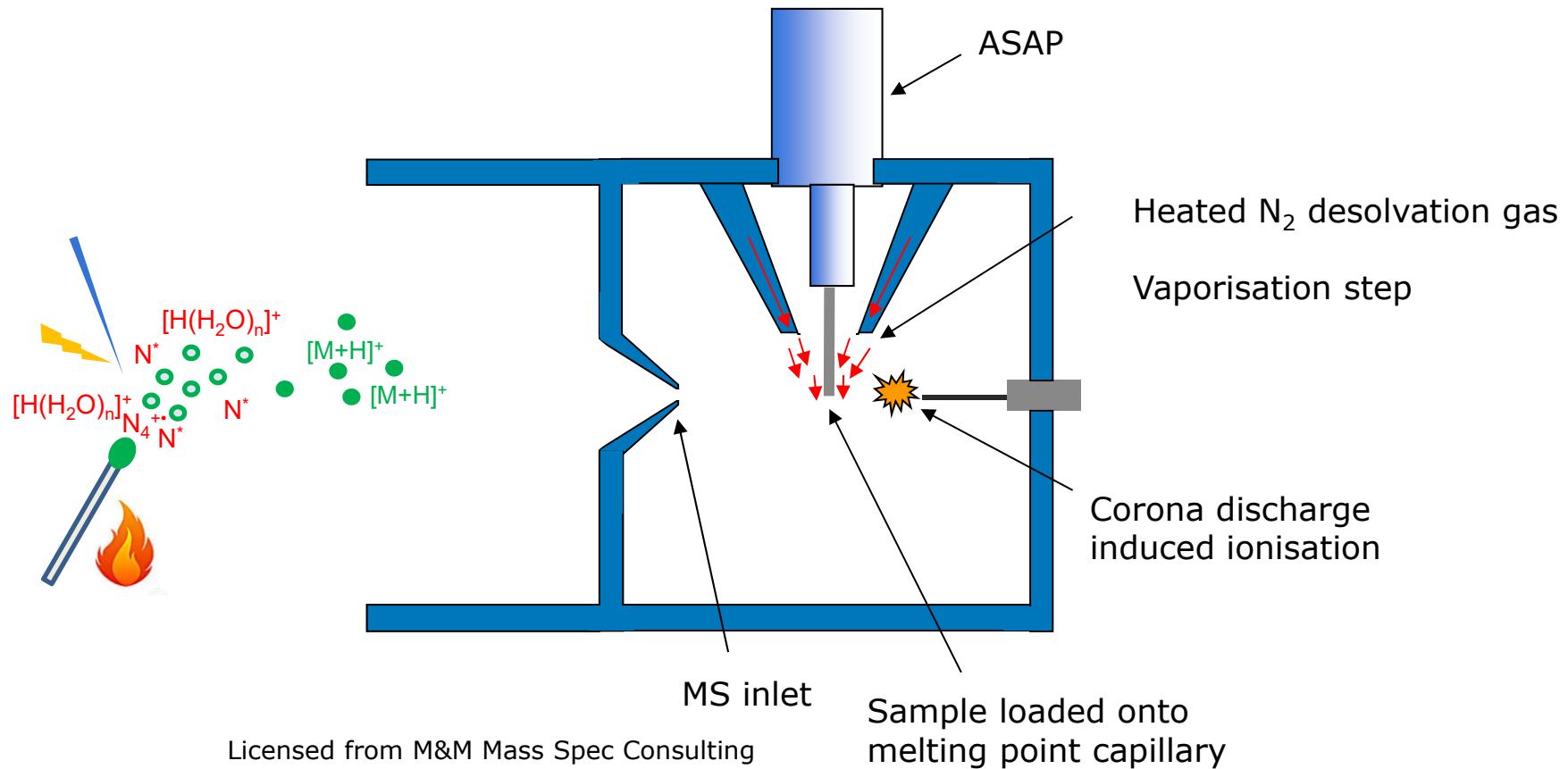
## Vanilla Authenticity Testing





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## ASAP – how does it work?



Licensed from M&M Mass Spec Consulting

# Vanilla Extract



Vanilla Orchid



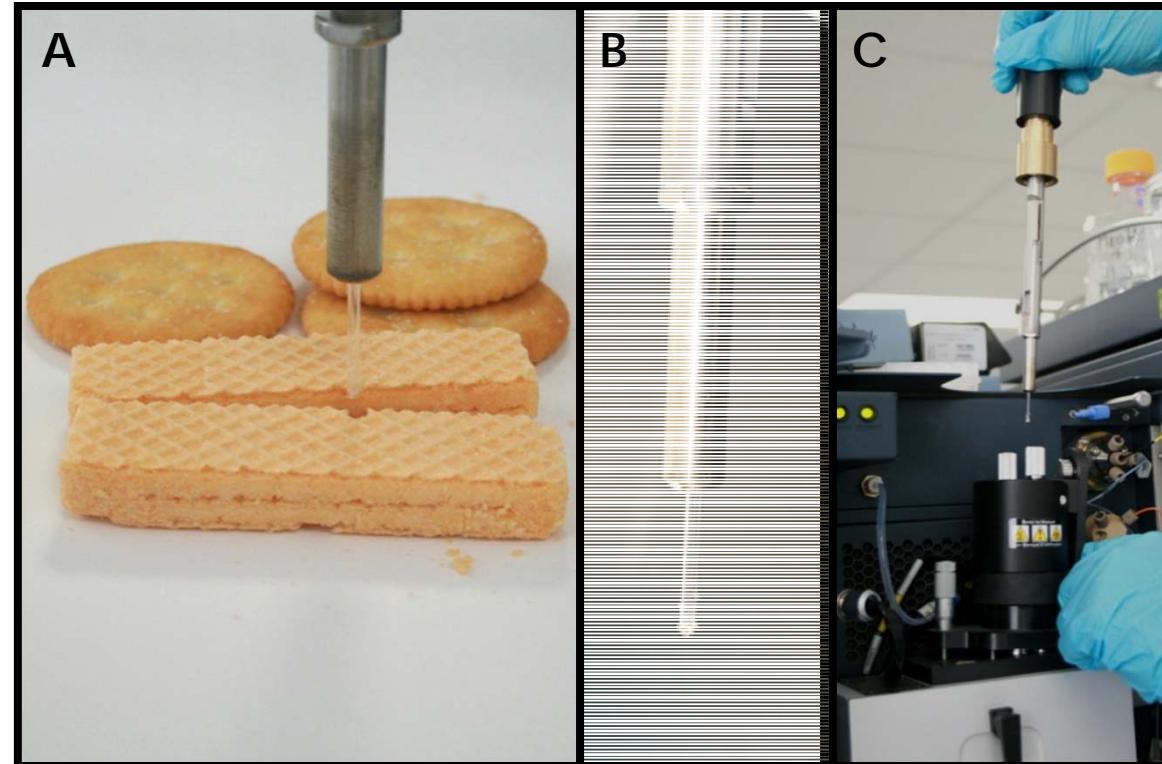
Vanilla Bean





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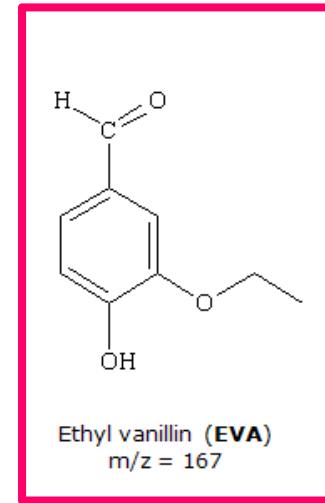
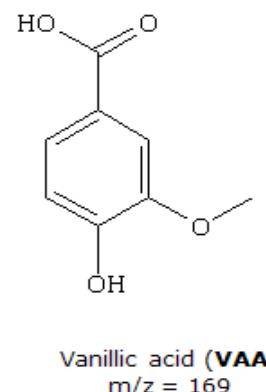
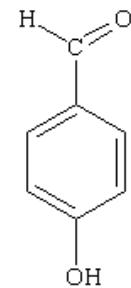
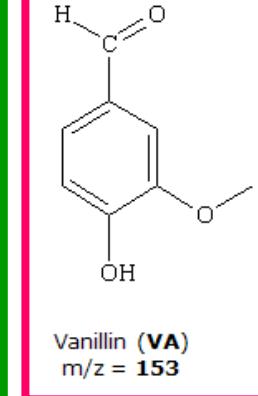
## ASAP: Sample Loading Procedures



< 5  
seconds



## Vanilla Flavor Related Compounds

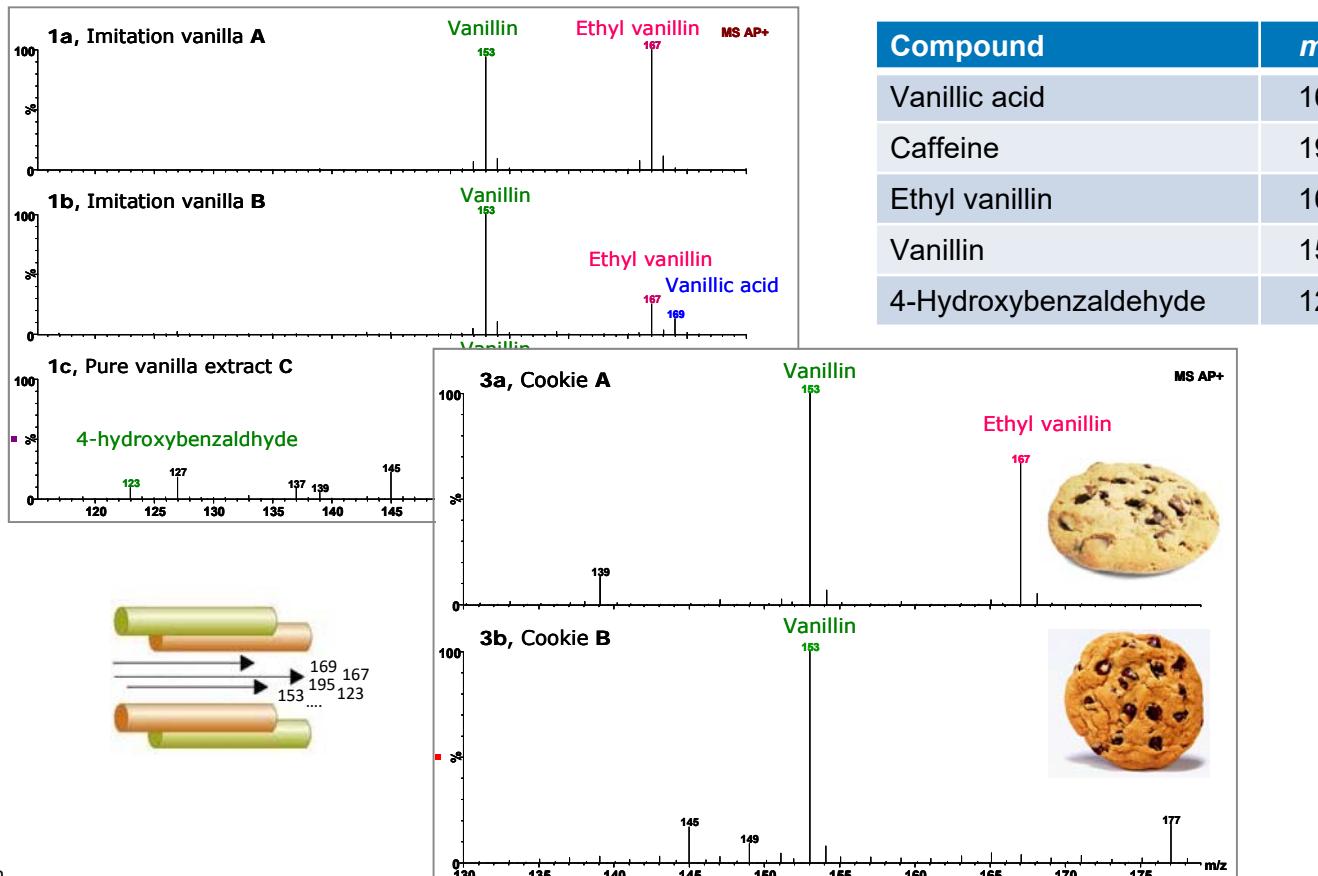


Major components of pure vanilla extracts

Synthetic flavor marker

# Ingredient Authenticity

## - Food and Beverage Products

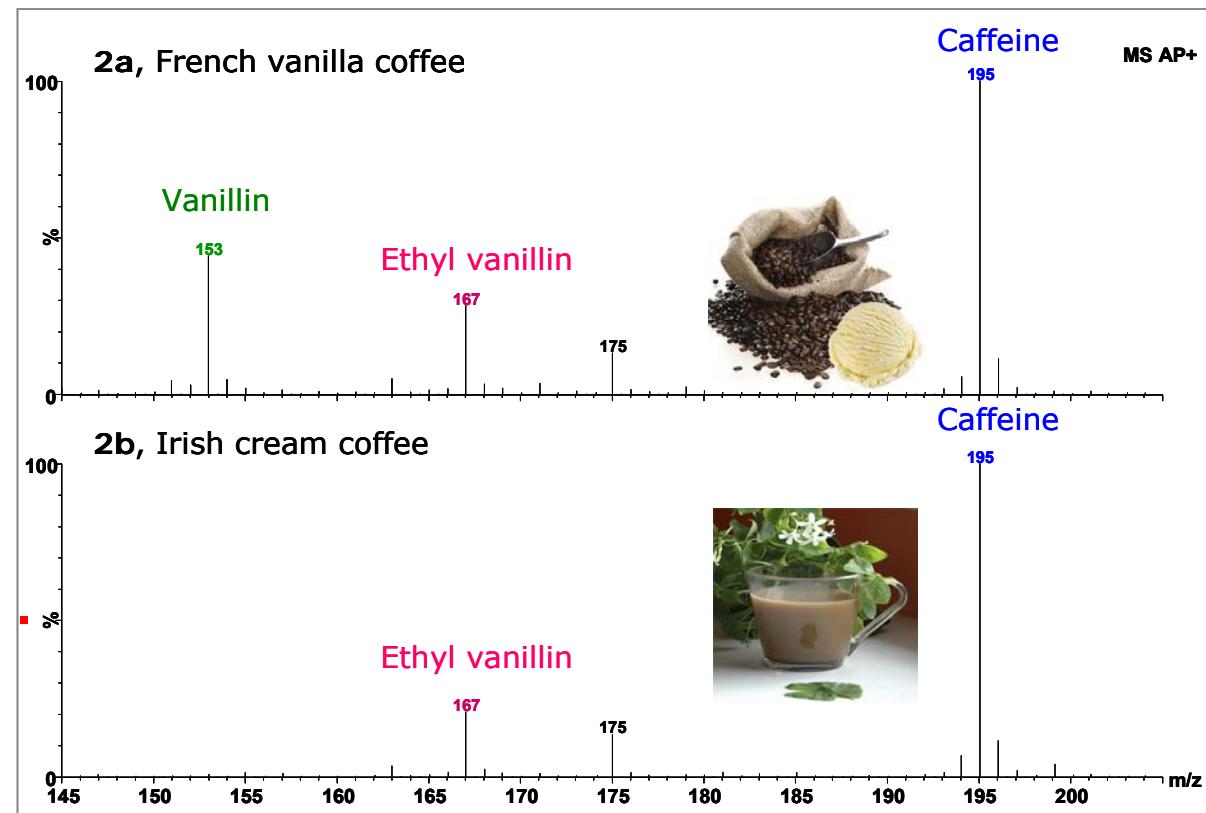


# Ingredient Authenticity

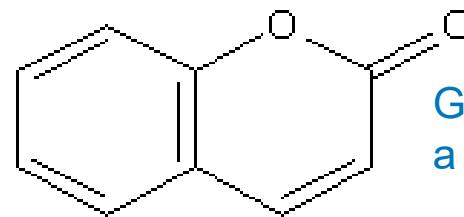
## - Food and Beverage Products



Compound	<i>m/z</i>
Vanillic acid	169
Caffeine	195
Ethyl vanillin	167
Vanillin	153



# Coumarin Screening



Gives Imitation Vanilla  
a better taste

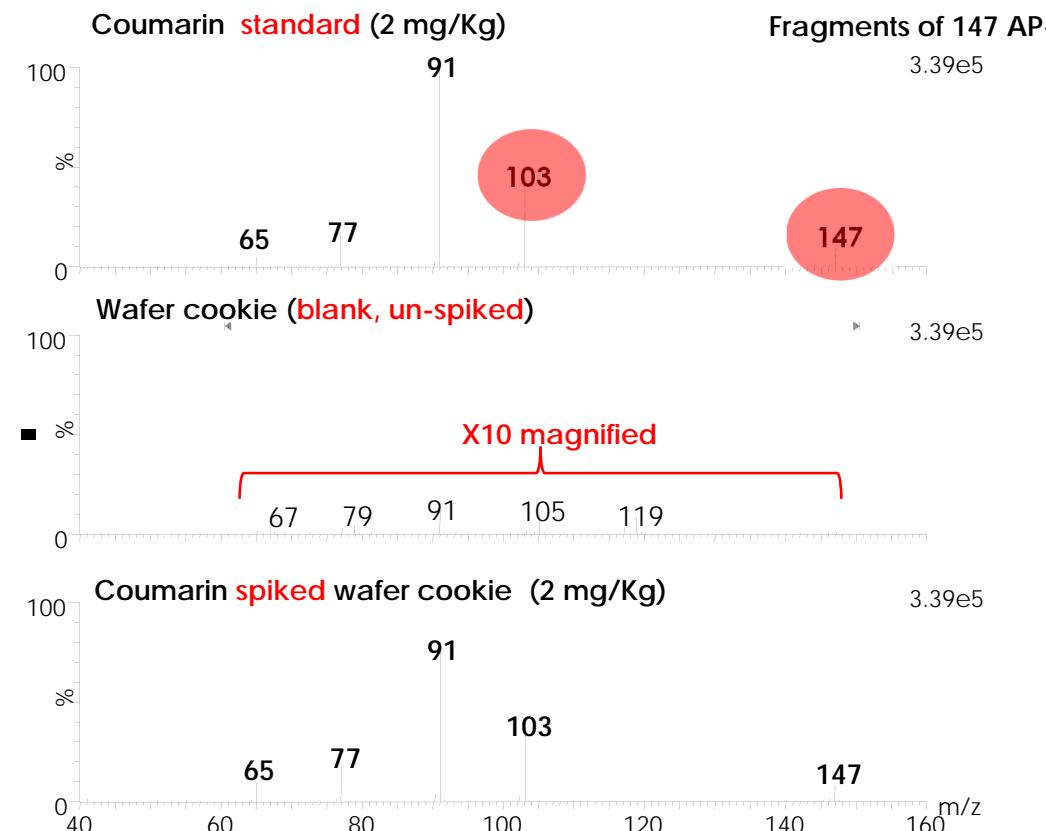
**Coumarin**  
 $m/z = 147$





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## Product Ion Spectra of Coumarin

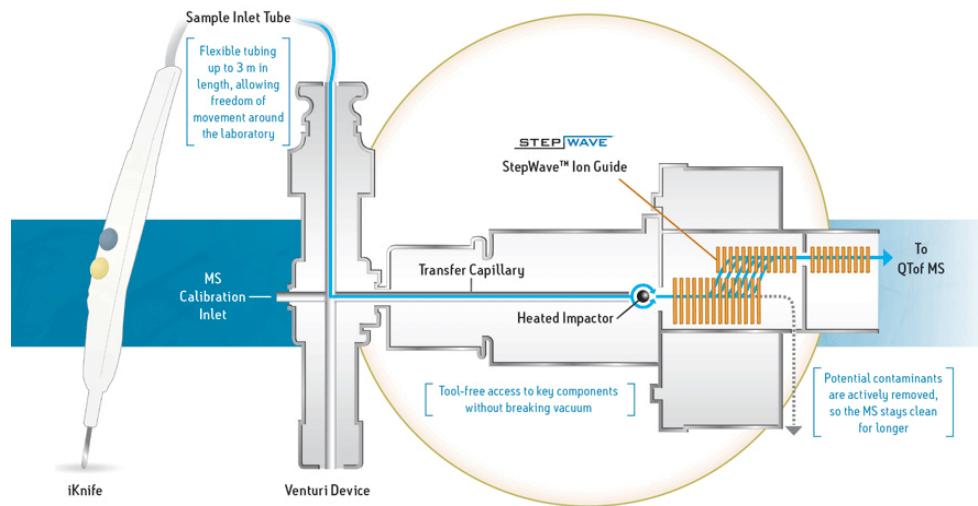




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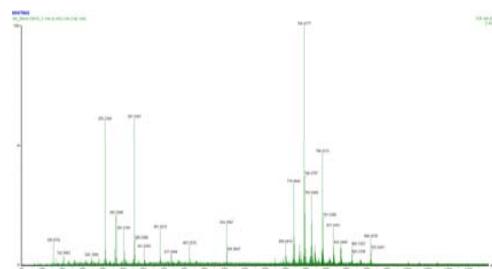
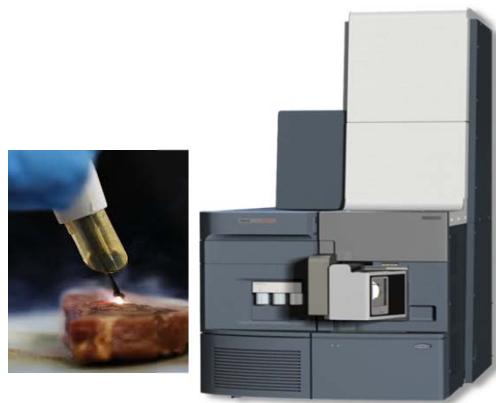
## REIMS

# Rapid Evaporative Ionization Mass Spectrometry

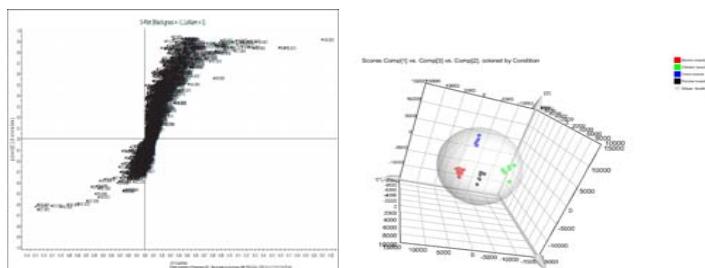
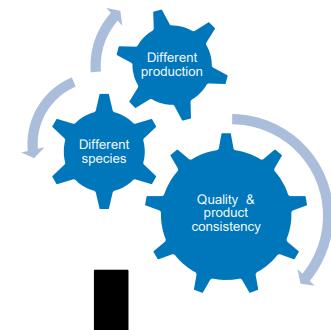




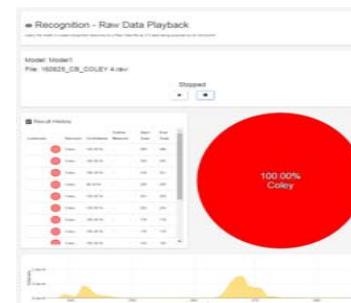
## REIMS workflow



## Model training & validation

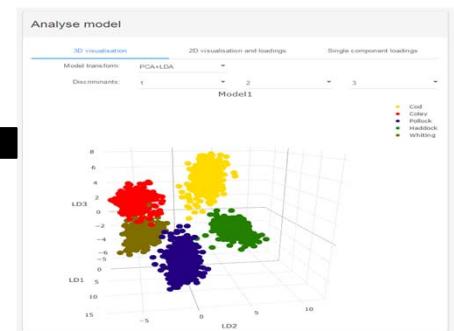


## Real time recognition



## Biomarker discovery

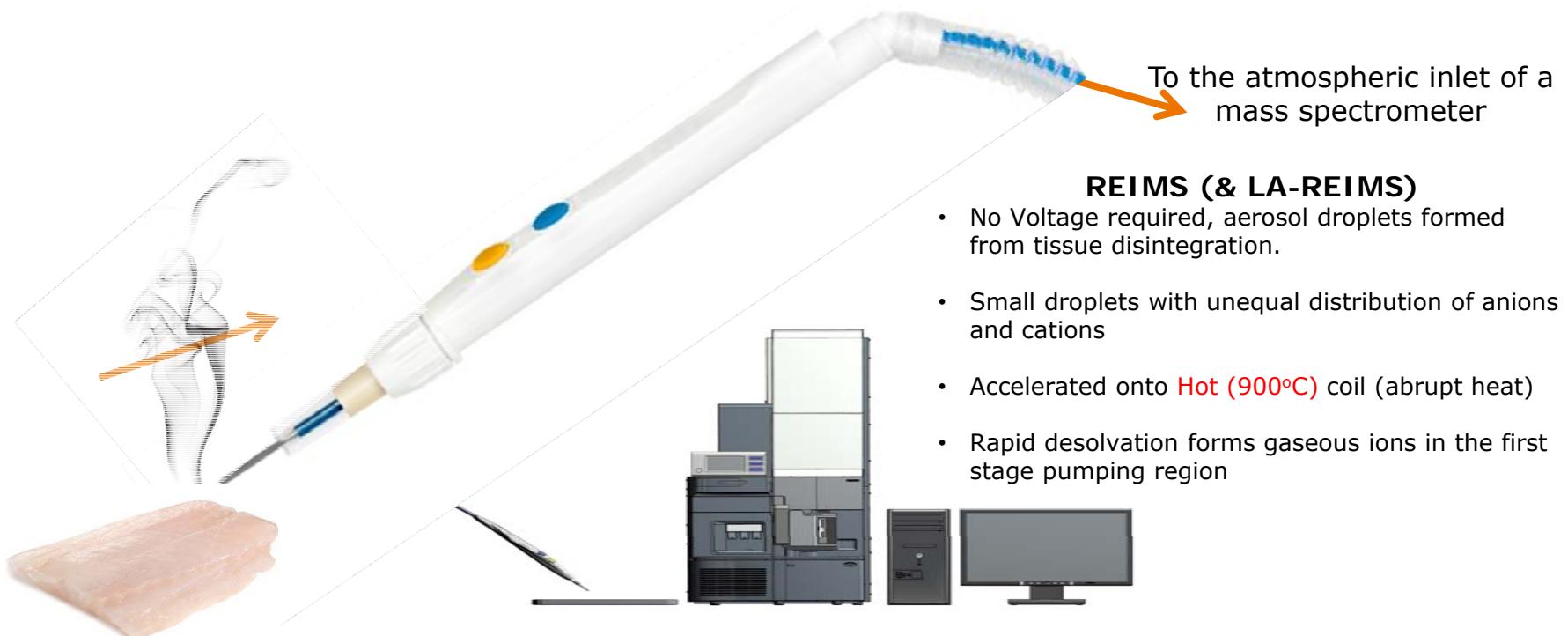
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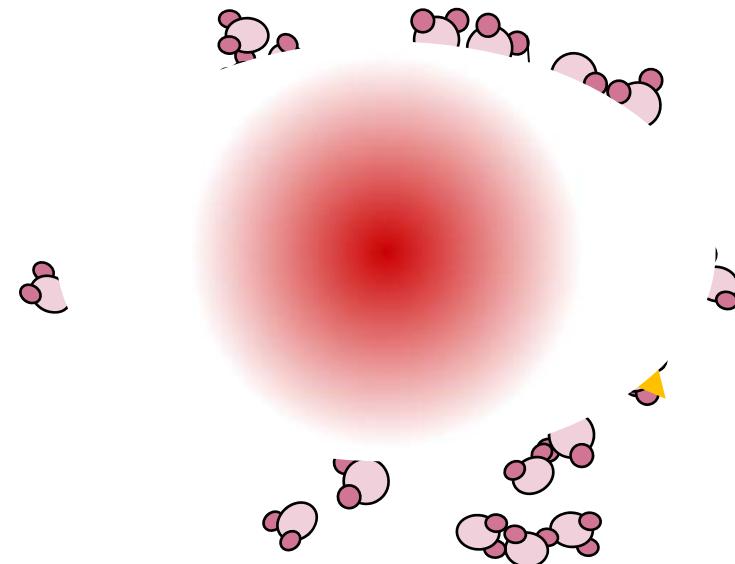
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## REIMS - How does it work?



## Ionisation Method

- An atmospheric sampling technique
  - Uses rapid heating to evaporate the sample
    - Joule heating
    - Contact heating
    - Radiative heating
    - IR LASER



- Sample the aerosol produced



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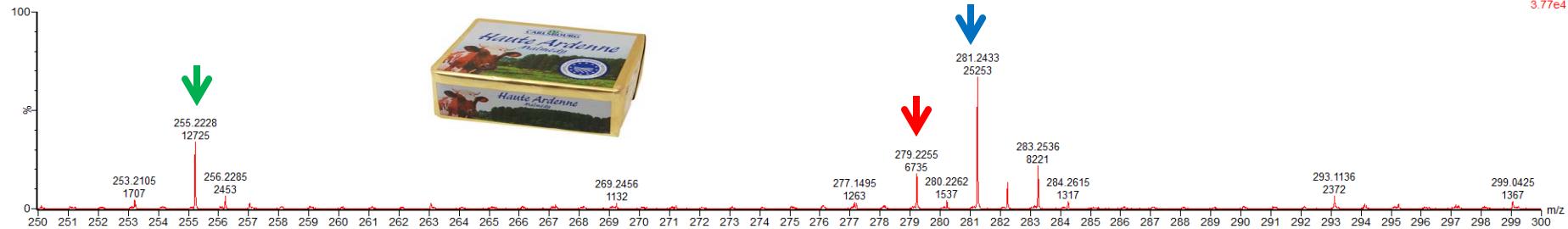
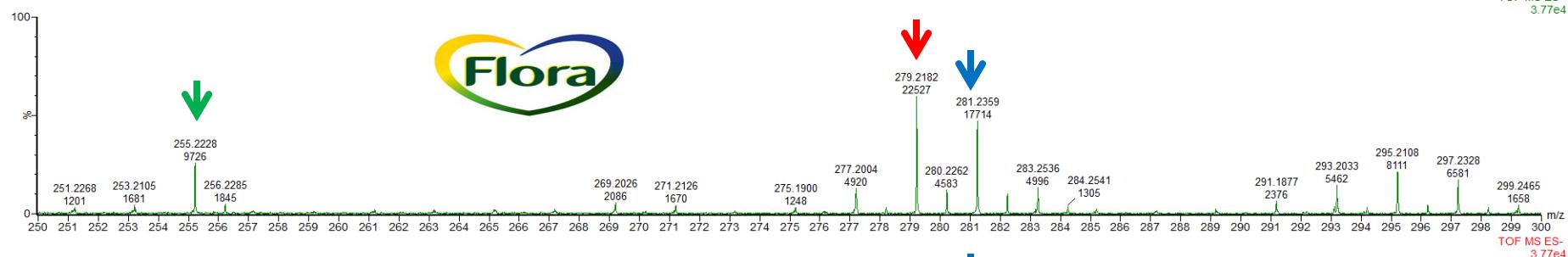
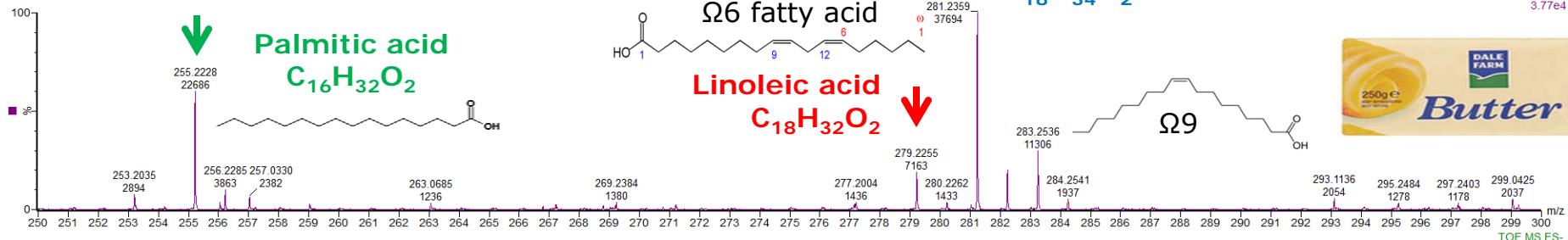
## Product authenticity

### Belgian butter – PDO status (1996)



# Fatty acids ToF MS neg ion 250–300 m/z region

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TOF MS ES-  
3.77e4

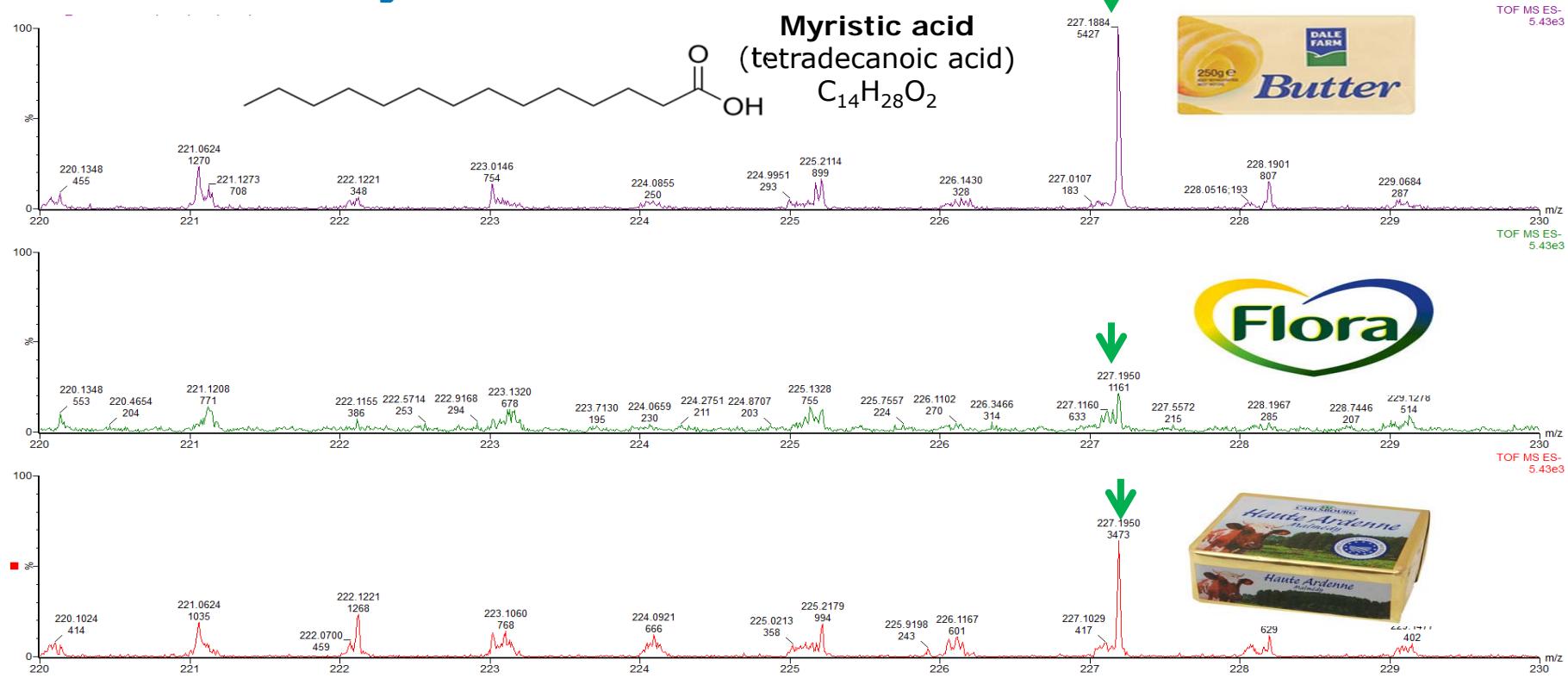




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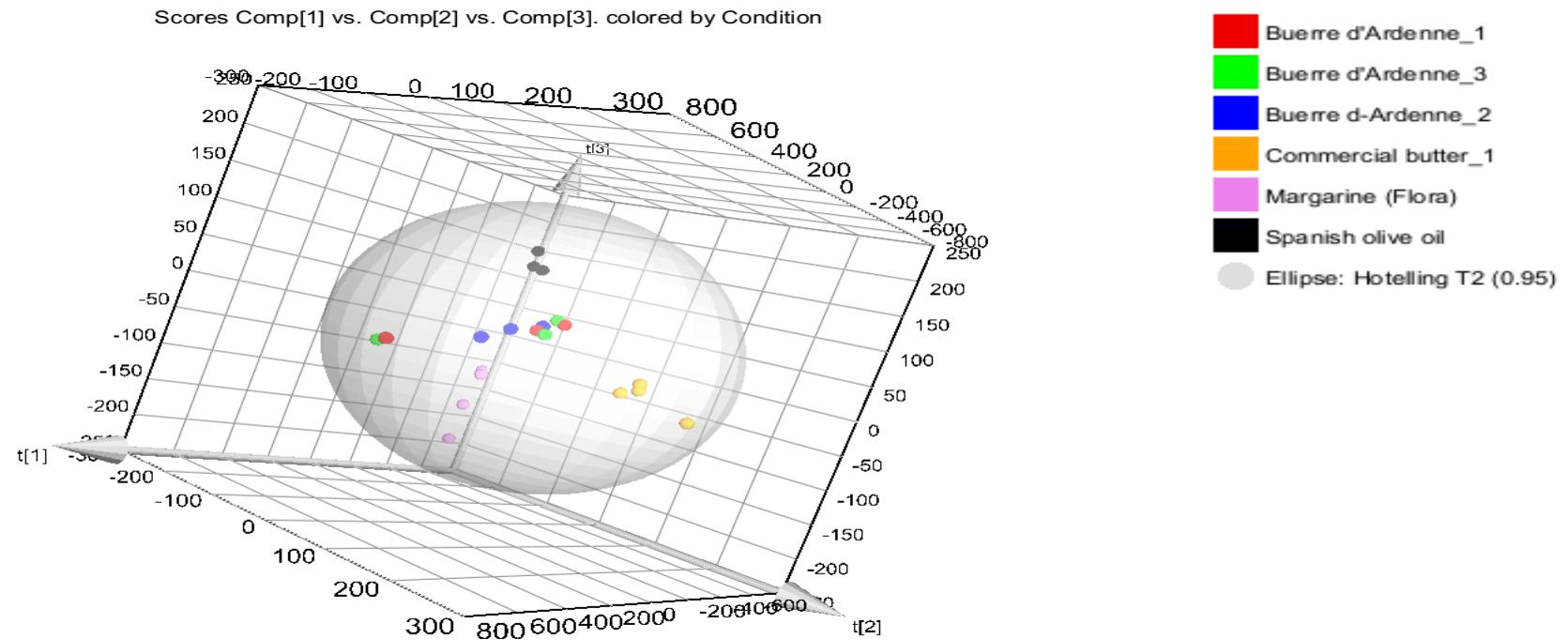
F WHAT'S POSSIBLE.<sup>TM</sup>

## Saturated fatty acid in cow's milk

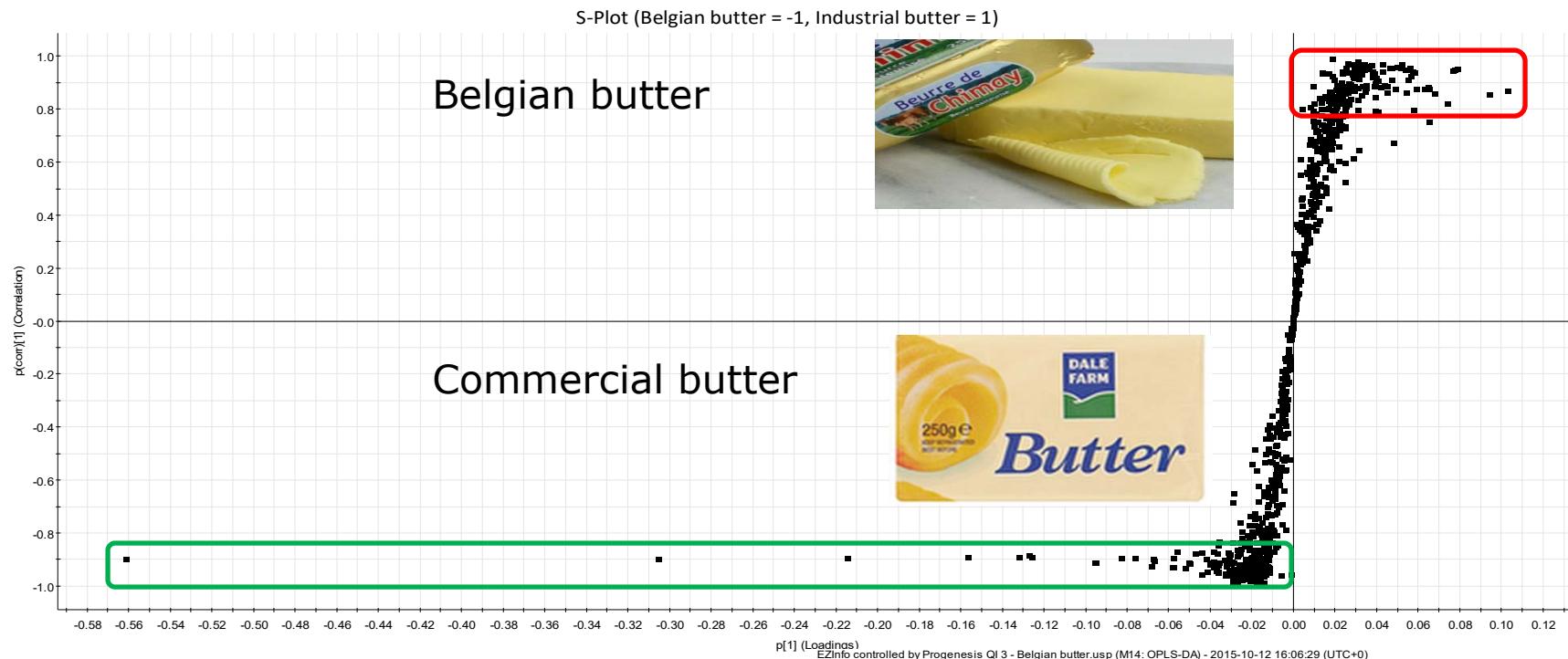


**HMDB 00806:** Myristic acid is a saturated 14-carbon fatty acid occurring in most animal and vegetable fats, particularly **butterfat** and coconut, palm, and nutmeg oils

# OPLS-DA Scores Plot



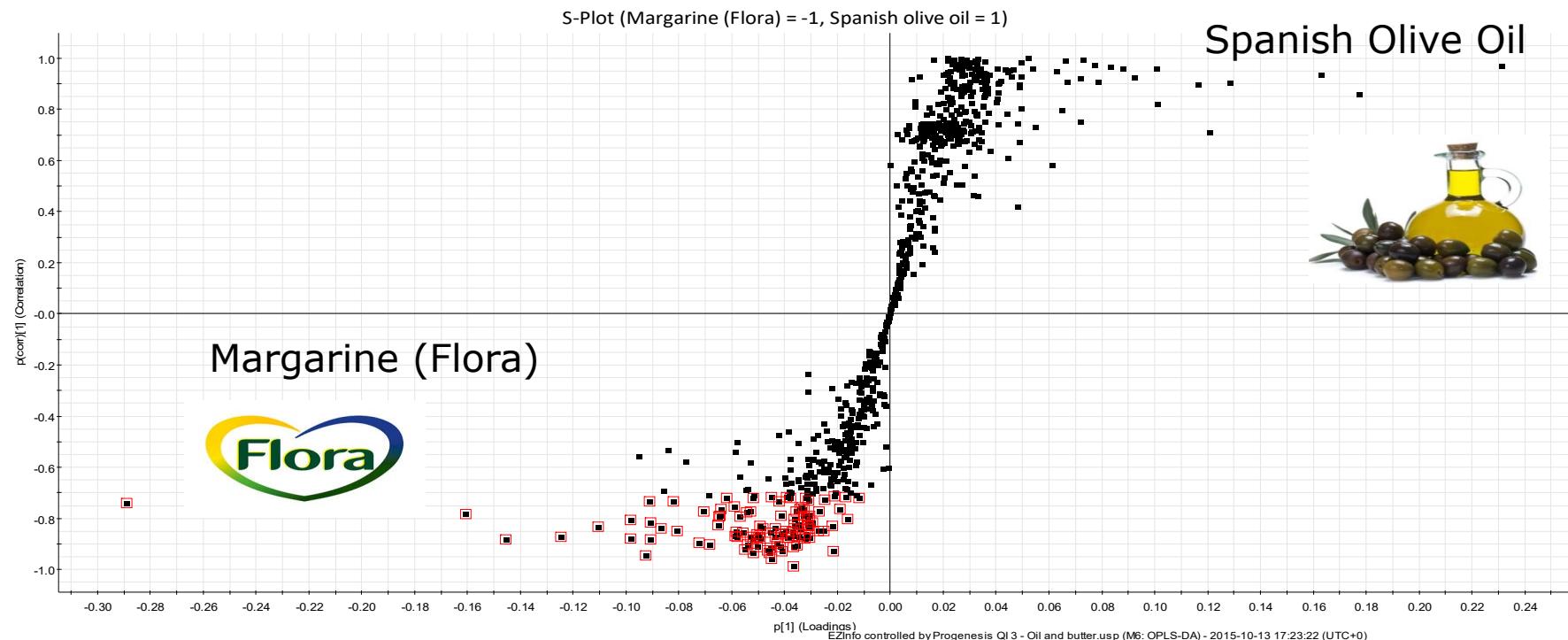
## S-Plot Selective markers



## S-Plot Selective markers



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# Real time model builder software

## PCA & LDA space

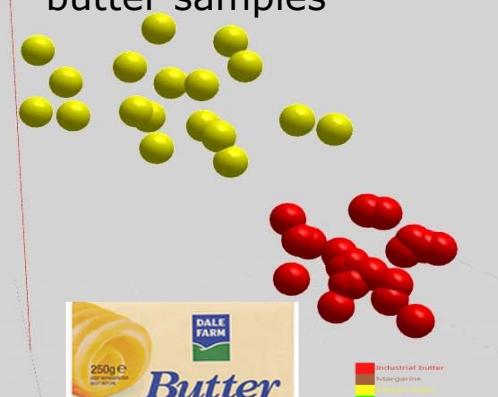


Industrial butter  
Margarine  
Belgian butter  
Olive oil

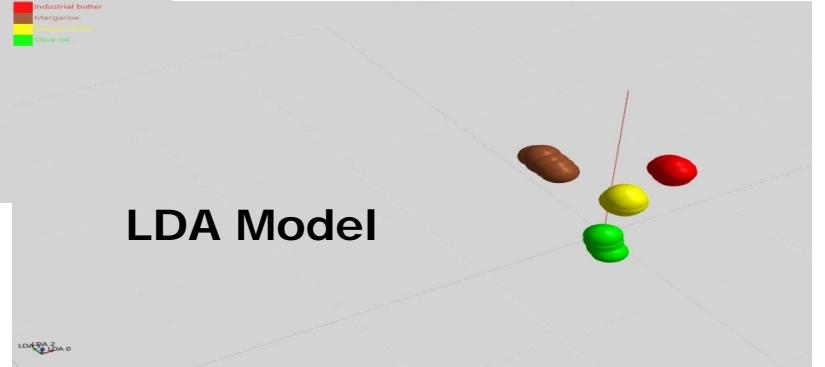
PCA Model



4 different Belgian butter samples



LDA Model



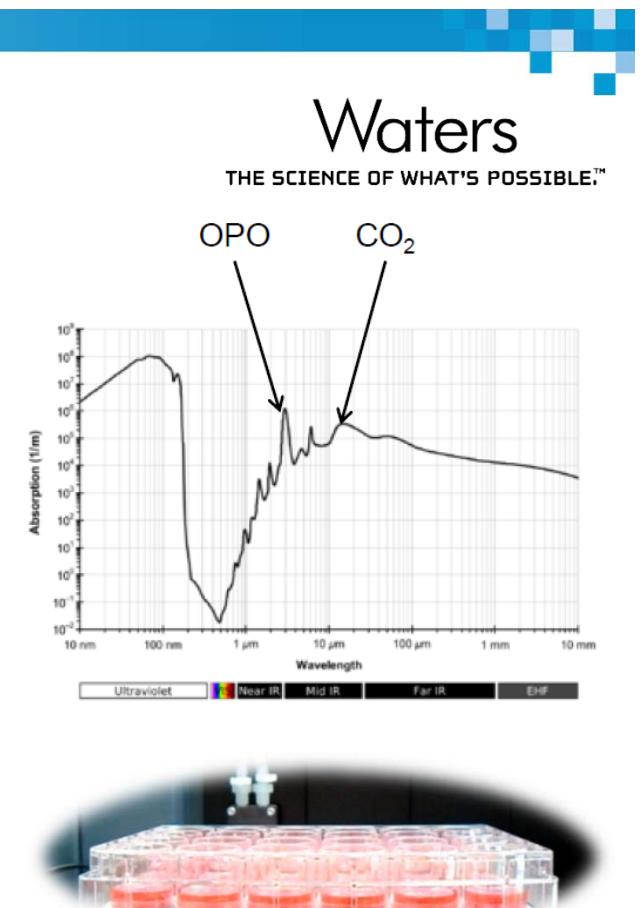
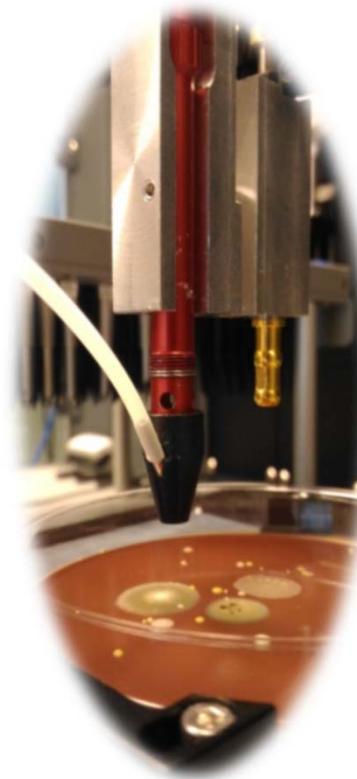


## Laser REIMS Olive Oil



## REIMS Using Lasers or LA-REIMS

- IR Laser used to vaporise Sample
  - Similar spectral response as D-REIMS
  - Currently
    - CO<sub>2</sub> ( $\lambda = 10.6 \mu\text{m}$ )
    - OPO ( $\lambda = 2 - 3 \mu\text{m}$ )
      - Higher Performance
  - Advantages
    - Non conductive samples
    - Minimal Damage
    - Non Contact
      - Reduced Contamination





## LA-REIMS Workflow



Three regional IGP/DOP EVOO samples

8 x 5 uL spots on microscope slides

Analysed using LA-REIMS platform (5 s per spot)

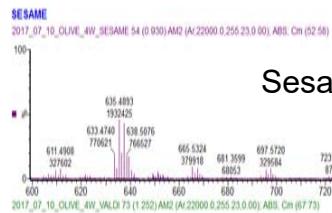
Data processing using LivID and analysis with  
PLS-DA and Random Forest

# LA-REIMS for IGP / DOP EVOO traceability

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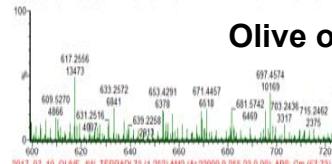
SEASAME  
2017\_07\_10\_OLEIE\_4V\_SE(SAME)54 (0.930)AM2 (A:22000 0.255 23.0 0.0) ABS. Cm (52.58)

Sesame oil



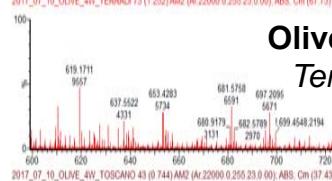
2017\_07\_10\_OLEIE\_4V\_VALDI 73 (1.252)AM2 (A:22000 0.255 23.0 0.0) ABS. Cm (67.77)

Olive oil - Valdi



2017\_07\_10\_OLEIE\_4V\_TERRADI 73 (1.252)AM2 (A:22000 0.255 23.0 0.0) ABS. Cm (67.77)

Olive oil - Terradi



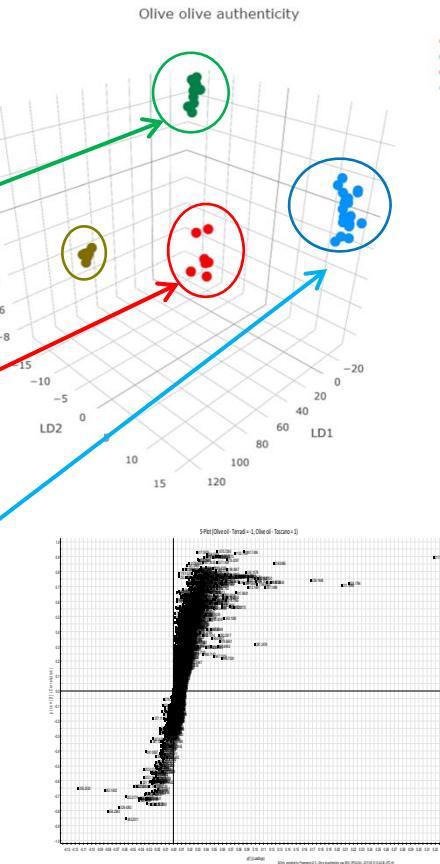
2017\_07\_10\_OLEIE\_4V\_TOSCANO 43 (0.744)AM2 (A:22000 0.255 23.0 0.0) ABS. Cm (37.43)

Olive oil - Toscano



600-1000  $m/z$

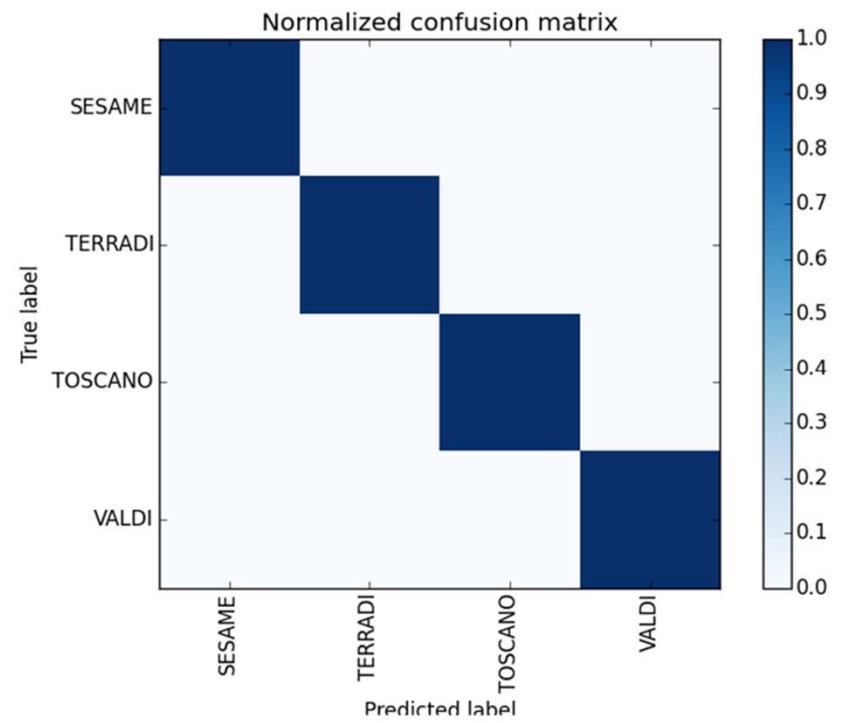
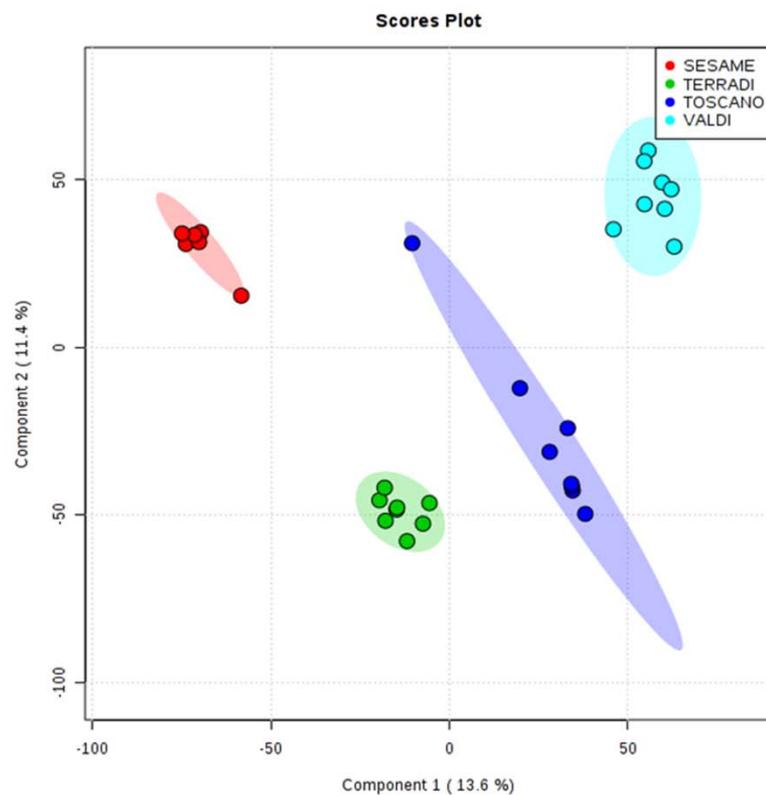
Olive olive authenticity





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## PLS-DA and Random Forest Modelling



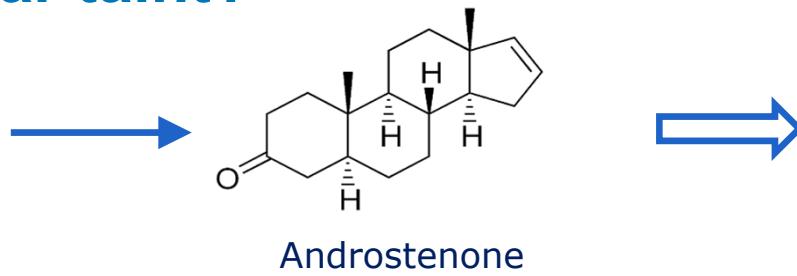
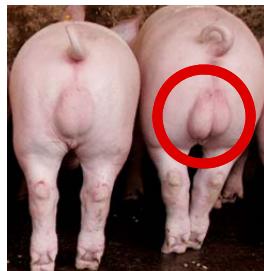
# At-Line Determination of Boar Taint Contaminated Carcasses

## Prof Lynn Vanhaecke

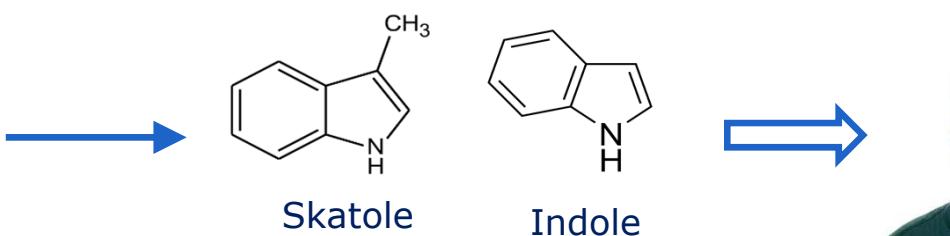
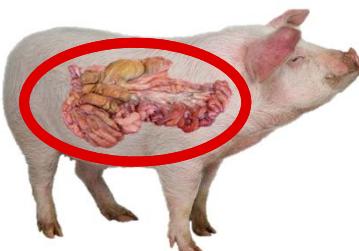




## What is boar taint?



Urinary-Sweaty odour



Fecal odour



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## Fast detection of boar taint

### Sensory approaches

#### Soldering iron method



- ☛ Applicable at slaughter line
- ☛ Holistic detection of boar taint
- ☛ Inter-individual variation
- ☛ Habituation & fatigue

### Analytical approaches



Sensor technology  
Fast GC-MS  
MS based electronic nose  
RAMAN spectroscopy

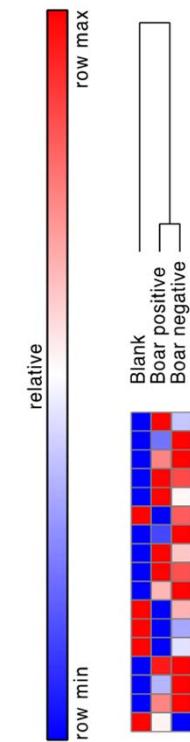
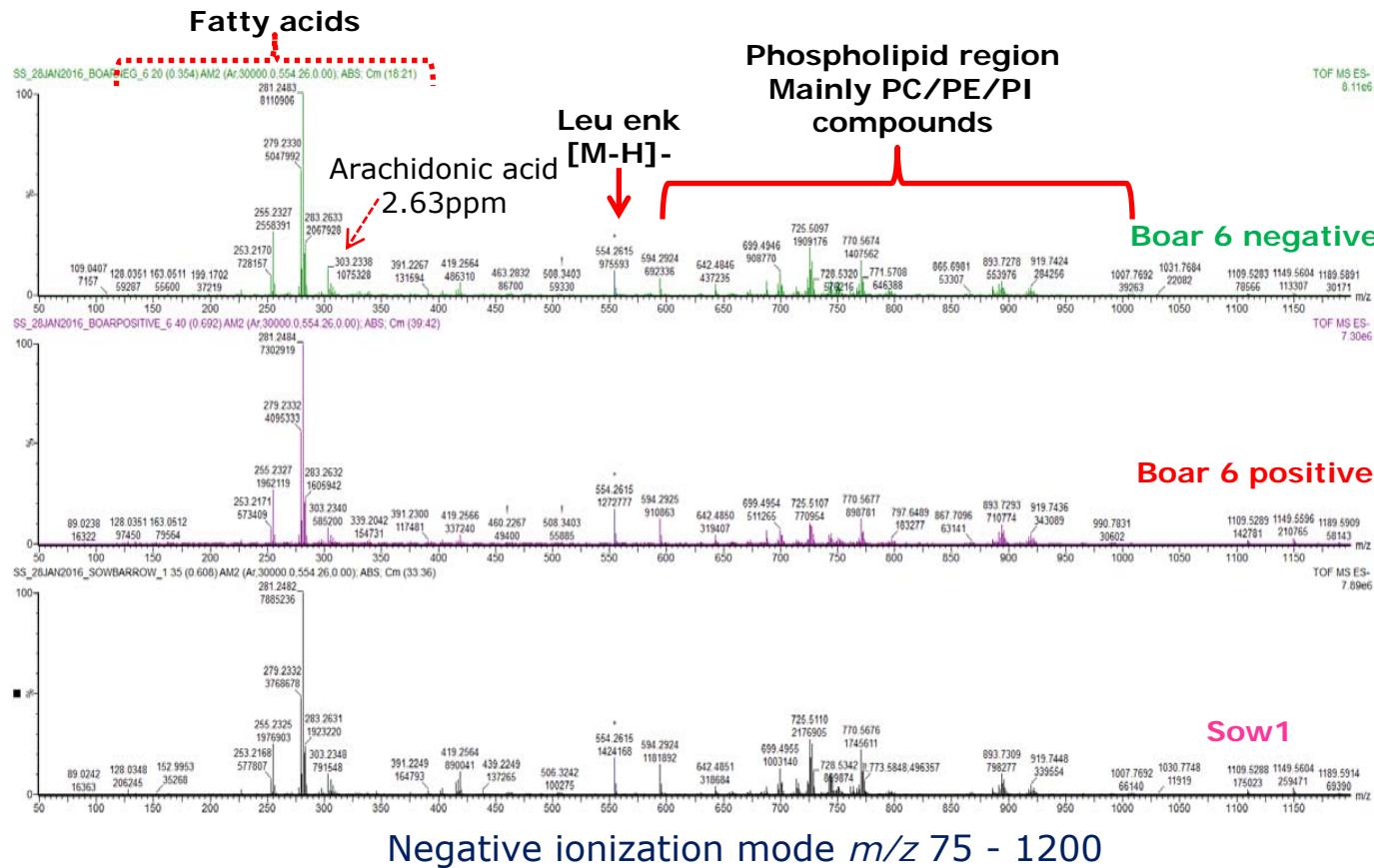
- ☛ Accurate
- ☛ Sensitivity & specificity?
- ☛ Lack testing & validation
- ☛ Speed

➡ **Applicability at slaughter line?**

# Mass spectral profiles

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# REIMS boar taint LiveID model (scale-up)



**Build & Parameters**

Boar taint combined  
Select and adjust modeling parameters, or accept the defaults, then build your model. Your model currently contains 3 classes with a total of 954 spectra.

Statistical Model Type: PCA-LDA

PCA Components: 250  
1 - 953 (total number of spectra minus 1)

Linear Discriminants: 2  
1 - 2

Outlier by: Standard Deviation (Quantile)

Outlier Threshold (Std Dev): 15  
>= 0

Binning Resolution: 0.1  
0.01 - 1.00 (to 2 d.p.)

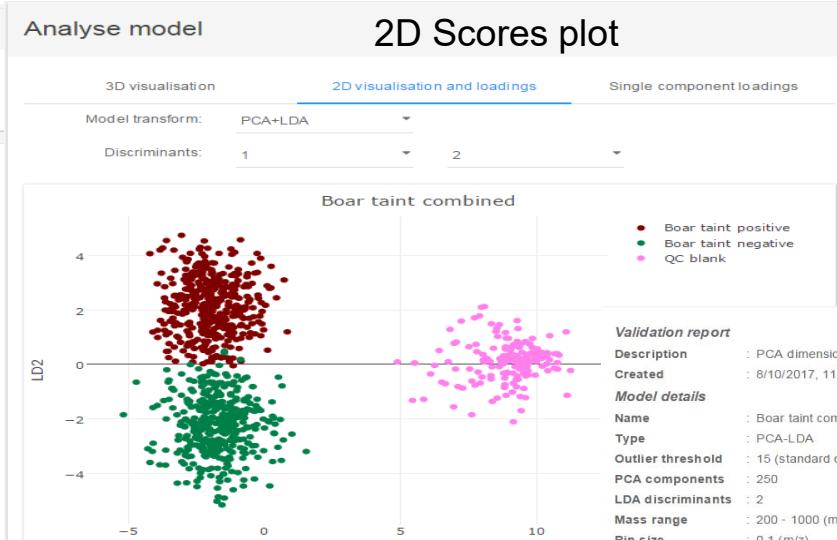
Mass Range: Start: 200  
200 - 1199

Mass Range: End: 1000  
201 - 1200

**Boar taint combined**

2 customer sample sets

- 405 Boar taint positive
- 391 Boar taint negative
- 158 QC blank



PCA 250 dimensions/  
LDA 2 dimensions



Stratified 5 fold *in silico* validation

	Spectra	Passes	Failures	Outliers	Correctness score
Total	973	911	62	0	93.63%
Boar taint positive	383	20	2	0	405
Boar taint negative	32	365	3	0	400
QC blank	2	3	163	0	168

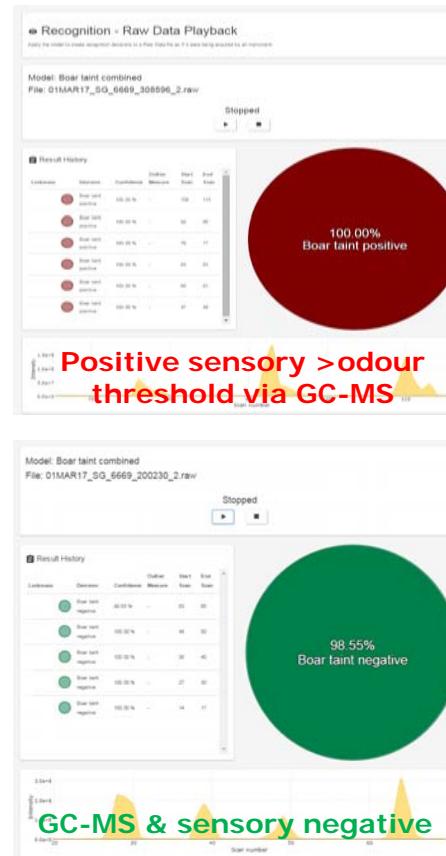
**Confusion matrix**

	Boar taint positive	Boar taint negative	QC blank	Outlier	Total
Boar taint positive	383	20	2	0	405
Boar taint negative	32	365	3	0	400
QC blank	2	3	163	0	168

# Independent validation with characterised samples from an alternative source



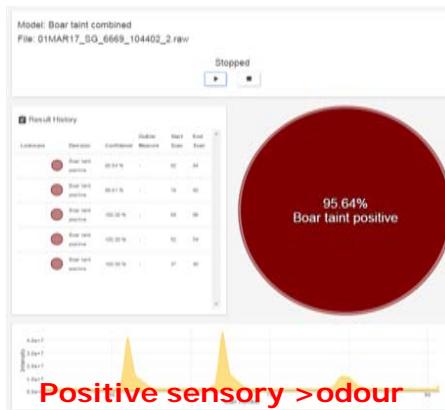
42/308834



41/308596



31/200230



36/104402



## EU requirements for fast detection method at slaughter line



- ☛ High throughput (600 carcasses/hour)
- ☛ Highy accuracy → no false negatives!
- ☛ Cost efficiency
- ☛ Simplicity
- ☛ Possibility for automation



# Detection of boar taint at-line – in the abattoir

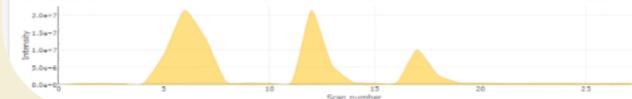


Model: REIMS\_BT\_UGhent  
File: 20170729\_SS\_UGhent\_SS10G\_UT\_RECOGNITION.raw

Stopped

Lookmass	Decision	Confidence	Otlier Measure	Start Scan	End Scan
	Boar taint	00.00 %	-	17	18
	Boar taint	02.41 %	-	12	13
	Boar taint	08.01 %	-	5	7

Boar taint negative



# How can REIMS help the food industry?

## ■ Speciality products

- Ensuring the authenticity of protected status products (PDO, PGI, TSG)
- e.g. Beurre d'Ardenne; Mortadella di Bologna; Bronte pistachio nuts; Parmigiano-Reggiano; botanic origin of honey...



## ■ Production methods

- Organic vs. conventional production?
- Capture method (line vs. trawl caught fish)
- Dietary regime and husbandry (grass fed vs. industrial feed)



## ■ Speciation

- Verification of fish and meat species identity
- e.g. raw product to highly processed foods, canned meat/fish, gelatine, stocks



## ■ Quality, composition & safety

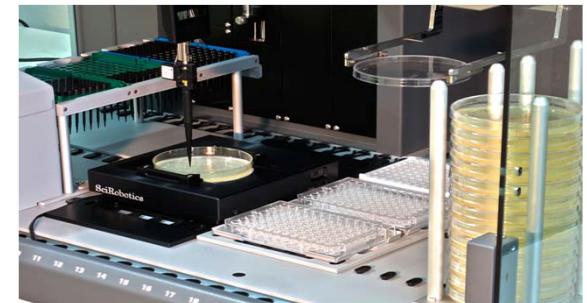
- Carcass consistency & quality
- Grading of raw product prior to processing
- Product extension, offal in processed products
- Product spoilage indicators
  - Food pathogens





## Summary & future prospects

- MS allows holistic profiling for quality & manufacturing process control
- Detection of the “unexpected” for authenticity
- Potential to identify & verify unique product markers
- Direct analysis techniques can generate results in seconds
- Simplicity of operation & potential for automation
- Point-of-control analysis... bringing the analysis to the sample





## Acknowledgements

- Prof Zoltan Takats (Imperial College, London)
- Prof Chris Elliott, Dr Olivier Chevallier, Connor Black (Queens University, Belfast, UK)
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- Prof Lynn Van Haecke, Kaat Verplanken (University of Ghent, Belgium)
- Prof Luigi Modello, Dr Francesca Rigano, (University of Messina, Sicily, Italy)
- Matthew Sharman, (FERA Science Ltd., York, UK)
- Waters Corporation
  - Kari Organtini, Sara Stead, Lauren Mullen, Renata Jandova , Steve Lai, Gareth Cleland

# Thank You



how  
where  
when  
why  
what  
whose  
who

