# RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS) FOR FOOD AUTHENTICITY TESTING

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## INTRODUCTION

**Food fraud is a collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product for economic gain. Due to their high market value, meat and fish products are often targets for species substitution and adulteration. Introduction of undeclared species into the food chain is a significant problem for consumers from an ethical or religious viewpoint, could be a serious risk for those with food allergies and undermines confidence in food chain traceability and safety. Meat and fish can pass through many different stages, spread out through many countries, before appearing at retailers as a processed product.**

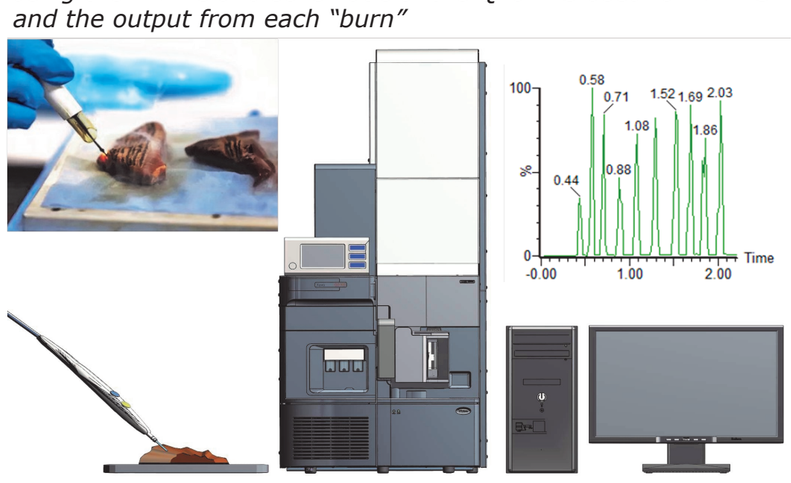
**Testing food is one of the key ways of checking whether food businesses are complying with food law. Current methods used for determination of species and adulteration are time consuming, costly and typically located in a laboratory some distance from the food chain.**

**Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is an emerging technique that allows rapid characterization of biological tissues (1,2,3). We demonstrate here that REIMS is able to differentiate meat and fish samples originating from different species, regardless of which tissue is chosen.**

## METHODS

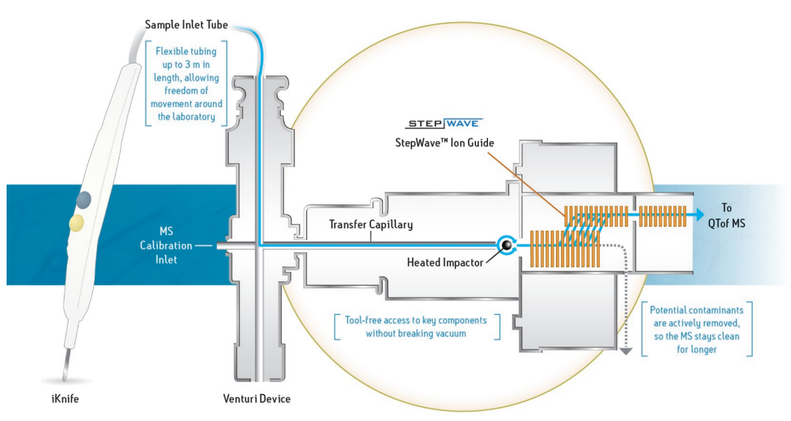
Commercial and authenticated samples of different types of meat and fish were procured and supplied by collaborators. All samples were analyzed using the i-Knife to cut the tissue surface.

*Figure 1. Photograph showing the cutting of the tissue surface using the i-Knife and schematic of the QTof MS used for REIMS and the output from each "burn"*




The i-Knife hand-held sampling device applies a high frequency electric current to the tissue via a diathermic process. This causes localized heating that cuts into the tissue. The "smoke" or aerosol produced contains gas-phase clusters of ionised and neutral species. It is transferred from the cutting location on the surface of the tissue through PTFE tubing into the transfer capillary by a Venturi air jet pump-based transfer apparatus mounted in the orthogonal position relative to the atmospheric interface of the mass spectrometer. Declustering takes place at the heated impactor surface and gas-phase ions pass into the quadrupole-time of flight mass spectrometer (Xevo G2-XS QTof). Data for each "burn" are acquired operating in negative ionization mode over a typical mass range of *m/z* 100-1000. Leucine enkephalin (LE), to be used as a lock mass, is introduced into the ion source via infusion in 2-propanol. The presence of 2-propanol has also been shown to enhance the response for lipids in REIMS in negative ion mode. Lockmass correction, adaptive background subtraction and normalization are all performed by the software post acquisition.

*Figure 2. Schematic showing how the aerosol is sampled, how ions are formed and transferred to the QTof MS*




The applications presented here use REIMS coupled with multivariate statistics and prototype software for real-time recognition. Classification of unknown samples against the model, created from spectra in the database does not require excessive computational operations so the process can be completed in real time using simple decision reporting tools (*i.e.* red light/green light).

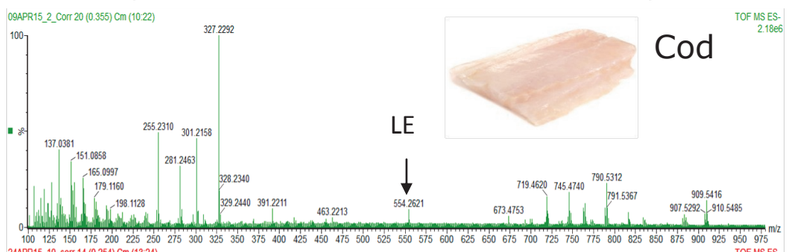
*Figure 3. Schematic showing classification and real-time recognition of a meat sample*




## RESULTS AND DISCUSSION

### 1. Fish speciation (with Queens University Belfast)

*Figure 4. Mass spectra acquired from analysis of fillets of different species of white fish from the Gadidae family*




Mass spectra from REIMS analysis of tissues contain singly-charged ions derived from fatty acids and glycerophospholipids (Pas, PEs, PCs and PIs)

*Figure 5. PCA plot of model using spectra from different fish species from commercial sources (1 sample of each) analyzed at Waters as proof of principle*

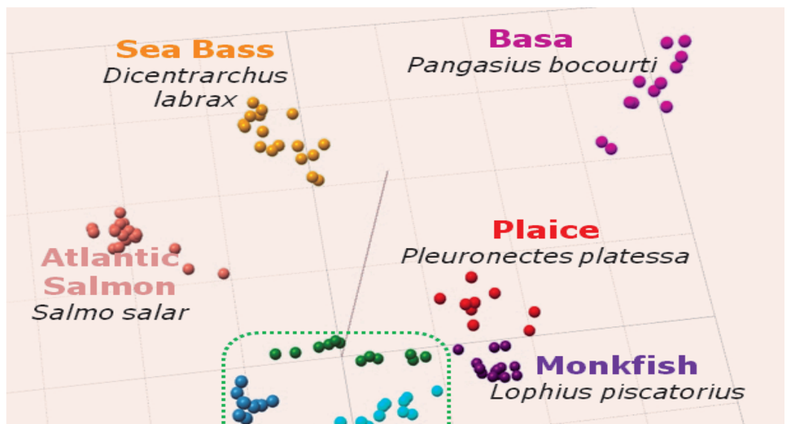



*Figure 6. PCA plot of model built using spectra from different authenticated samples of fish (>200 biological samples and >2000 spectra) analyzed at Queens University, Belfast*

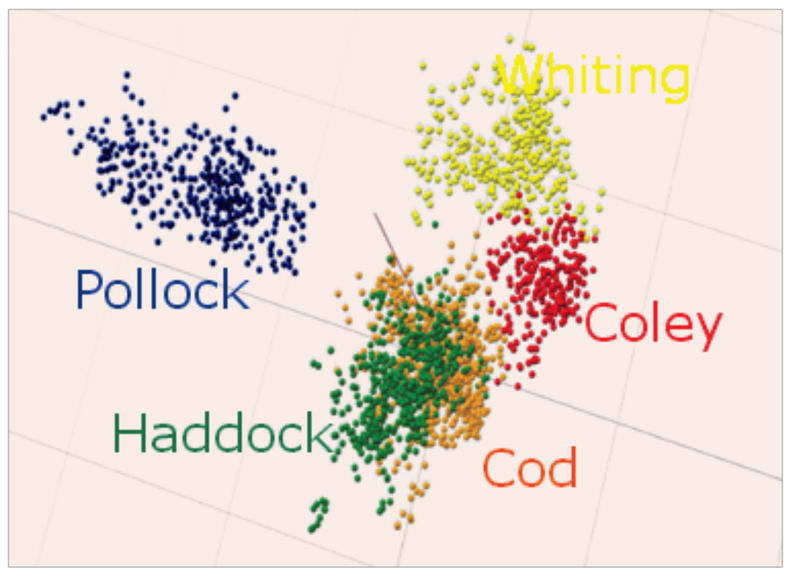



*Figure 7. Combined PCA/LDA plot of model using the same spectra from the samples of different fish species*

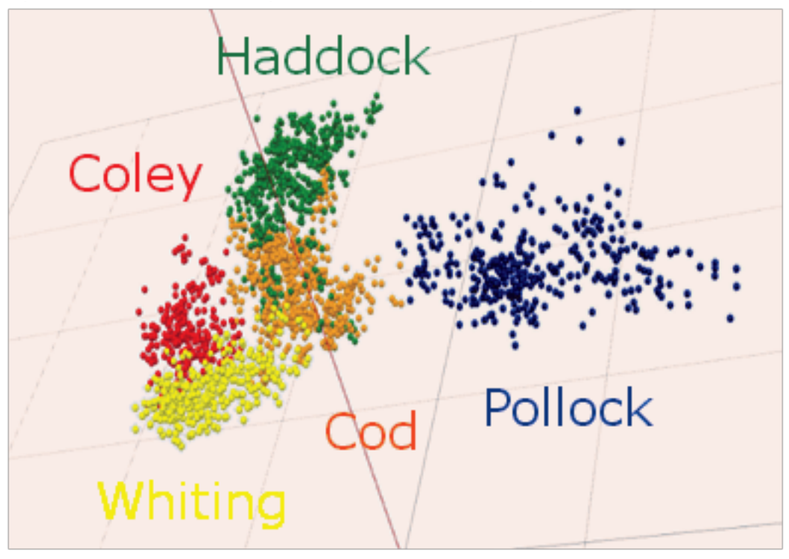



*Figure 8. Output from real time recogniser software when challenged during cross validation*

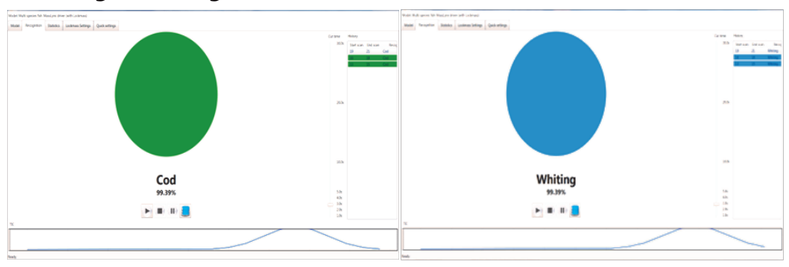



*Table 1. Results of cross validation (20 % of group left out)*

| Group | Number of spectra | Number of passes | Number of failures | Number of outliers | Correct Classification Rate |
|---|---|---|---|---|---|
| G | 2268 | 2133 | 135 | 0 | 94.05% |
| Total | 2268 | 2133 | 135 | 0 | 94.05% |

| Species | Cod | Coley | Haddock | Pollock | Whiting | Total |
|---|---|---|---|---|---|---|
| Cod | 638 | 11 | 46 | 0 | 0 | 695 |
| Coley | 1 | 260 | 0 | 0 | 7 | 268 |
| Haddock | 51 | 2 | 512 | 0 | 1 | 566 |
| Pollock | 0 | 0 | 0 | 373 | 0 | 373 |
| Whiting | 0 | 16 | 0 | 0 | 350 | 366 |

- PCA/LDA models built from the mass spectra could be used to separate samples of different fish species
- Cross-validation using five common species of white fish showed a 94% correct classification rate
- Inter-laboratory and cross platform comparisons demonstrate the robustness and validity of the database

### 2. Analysis of different meats and meat products (with Istituto Zooprofilattico Sperimentale, Bologna)

*Figure 9. Mass spectra acquired from analysis of fillets of meat*

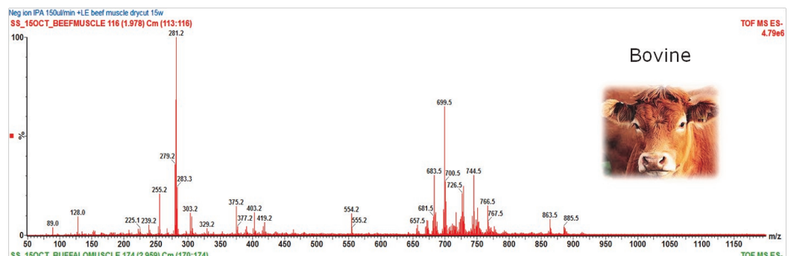



*Figure 10. PCA plot of model using spectra from muscle tissue from different animals from commercial sources (2 samples of each) analyzed at Waters, Wilmslow as proof of principle*

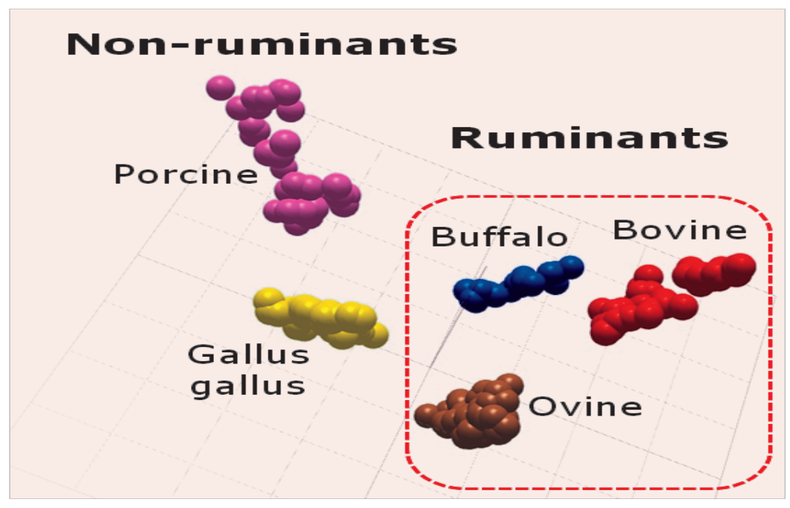



*Figure 11. LDA plot of binary model built using spectra from different Mortadella sausage samples supplied by Istituto Zooprofilattico Sperimentale, Bologna (10 samples)*

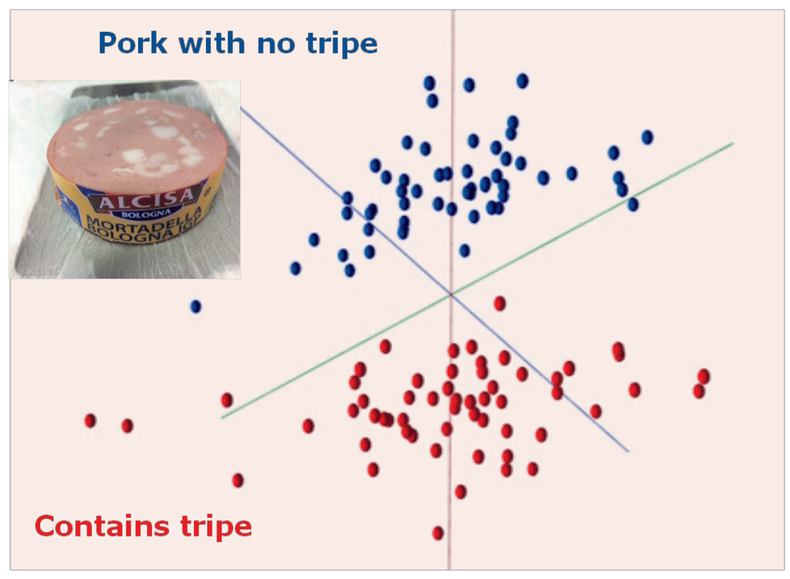



*Figure 12. Output from real time recogniser software from analysis of an independent sample labelled as containing tripe*

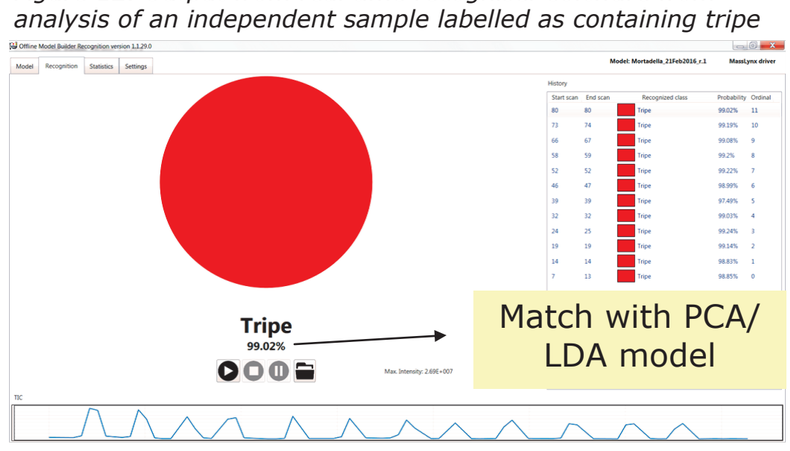



*Table 2. Results of cross validation (20% of group left out)*

Group result:

| Group | Number of spectra | Number of passes | Number of failures | Number of outliers | Correct Classification Rate |
|---|---|---|---|---|---|
| G | 114 | 95 | 0 | 19 | 100% |
| Total | 114 | 95 | 0 | 19 | 100% |

Cross-validation matrix:

| | Pork | Tripe | Outlier | Total |
|---|---|---|---|---|
| Pork | 46 | 0 | 11 | 57 |
| Tripe | 0 | 49 | 8 | 57 |

- PCA/LDA models built from the mass spectra could be used to separate sample of meat from different animal species and detect the presence of tripe in pork sausage
- Cross-validation to show whether sausage contained tripe showed a 100% correct classification rate
- Analysis of an independent sausage sample correctly detected presence of tripe

## CONCLUSION

- **Combining REIMS with multivariate statistics provides a useful tool for the rapid analysis of animal tissues with no sample preparation required**
  - **Non compliant samples may be investigated further using established techniques; *e.g.* those based upon DNA or proteomics**
- **We have demonstrated its potential for the determination of both species and level of adulteration in fish and meat using a prototype system for real-time recognition of animal tissues using a spectral database**
- **Initial objective is to provide a research tool for the development of databases but the vision is for an instrument that could be placed at the source of production or critical points along the supply chain**

**References**

1.Balog, J *et al.* (2010). Identification of biological tissues by rapid evaporative ionization mass spectrometry. *Analytical Chemistry* **82 (17)**:7343-50

2.Balog, J *et al.* (2013). Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. *Science Translational Medicine* **5(194)**:194ra93

3.Balog, J *et al.* (2016). Identification of the species of origin for meat products by Rapid Evaporative Ionization Mass Spectrometry. *J. Agric. Food Chem*. **64 (23)**: 4793–4800

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