Rapid Evaporative Ionisation Mass Spectrometry (REIMS) for food authenticity testing
急速蒸発イオン化質量分析法（REIMS）の食品の信頼性試験への応用

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INTRODUCTION

The quality, safety and authenticity of food are of principle interest for society and are regulated by legislation. 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. For example, fraudulent adulteration of food products with meat from undeclared species is a problem on a global scale. 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. One example of fraud in the sale of seafood occurs when a less expensive species is substituted for a more expensive species. The EU fish labelling regulations stipulate that fishery products must identify which species of fish is contained within the product, ensuring that the commercial designation of fish species correctly describes the species of fish present.

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 (*e.g.* ELISA, PCR or proteomics) are time consuming, costly and typically located in a laboratory some distance from the producer and retailer.

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

METHOD

Samples of different types of meat and fish were procured from commercial sources. All samples were analyzed using an electrosurgical RF-generator and monopolar cutting electrode (i-Knife) by cutting the tissue surface.

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 clustered ionised and neutral species. It is transferred from the cutting location on the surface of the tissue 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. Molecules are ionized at the heated impactor surface and 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, 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.

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"

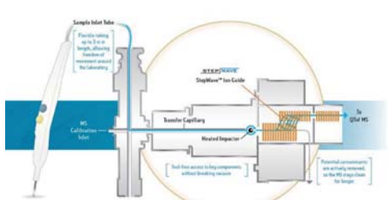

Figure 2. Schematic showing how the aerosol is sampled, how ions are formed and transferred to the QToF MS for mass measurement

Two analytical workflows are presented here that use REIMS couple with multivariate statistics:

1. Progenesis QI for the identification of significant markers for classification of different fish species
2. Prototype real-time recognition software to verify the authenticity of meat samples

The models presented here were created by the acquisition and processing of training sets but have yet to be validated or by the analysis of independent test samples.

RESULTS AND DISCUSSION

1. Fish speciation

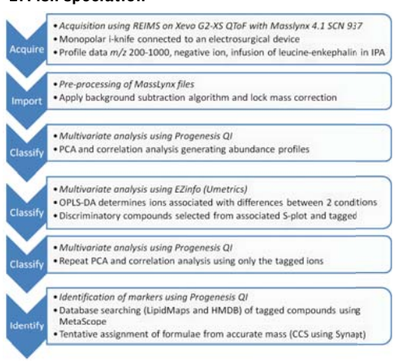

Figure 3. Workflow used for investigation of fish speciation

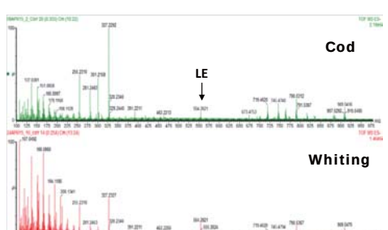

Figure 4. Mass spectra acquired from analysis of fillets of different species of white fish from the Gadidae family

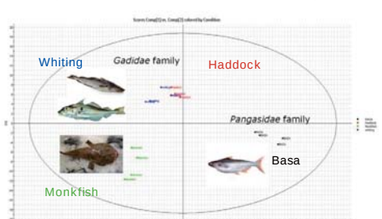

Figure 5. PCA plot using spectra from different fish species

Mass spectra contain singly-charged ions identified as being derived from fatty acids, glycerophospholipids and phosphatidic acids (Fig.4).

-The mass spectra showed a correlation between the fish species and the location on the PCA plot (Fig.4,5).
-Reducing the data set so that it only contained significant compounds improved classification (Fig.6).
-This was required for species for the same family
-The identity of marker compounds could be tentatively assigned based upon accurate mass measurements and searches of lipid databases directly from Progenesis QI
-Identification is supported by separate MS/MS experiments Investigating separation by IMS using Synapt G2-Si HDMS

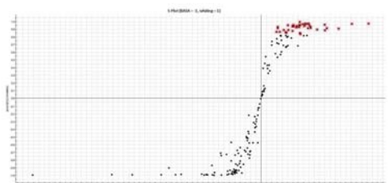

Figure 6. S-Plot (EZinfo) showing significant markers for Whiting

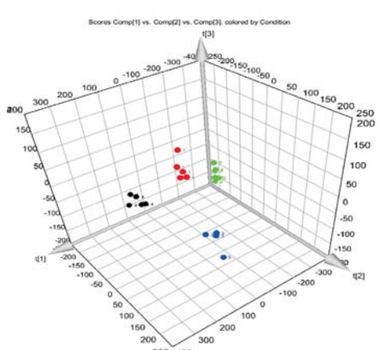

Figure 7. OPLS-DA plot showing four distinct sample groups using marker data obtained from different white fish species

2. Meat tissue speciation

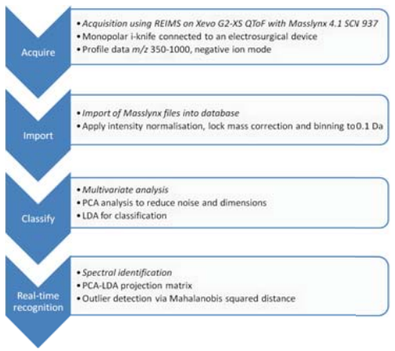

Figure 8. Workflow used for investigation of meat speciation

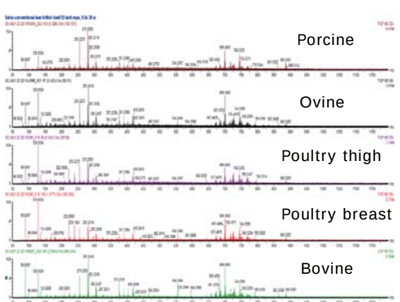

Figure 9. Mass spectra acquired from analysis of fillets of different species of meat

Classification of unknown samples against the model, created from spectra in the database does not require excessive computational operations so the process could be completed in real time using simple decision reporting tools (i.e. red light/green light) (Fig.11).

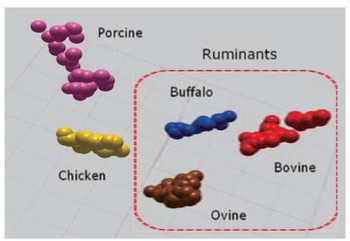

Figure 10. PCA plot using spectra from different meat species

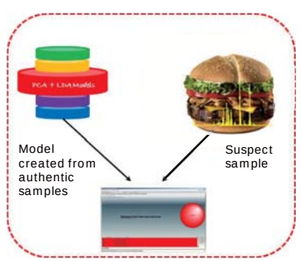

Figure 11. Schematic of real-time classification of meat species and recognition

Conclusion

- Combining REIMS with multivariate statistics provides a useful tool for the rapid analysis of animal tissues with no sample preparation required
- We have demonstrated its potential for the determination of both species and level of adulteration in fish and meat using:
- A system using Progenesis QI for classification of species from mass spectra and the identification of the significant markers
- 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

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