

Differentiation of raw meat using MALDI-TOF and PCA statistical analysis

Michael D. Nairn¹;

¹Shimadzu, Manchester, United Kingdom

1. Overview

Statistical analysis was applied to MALDI-TOF MS data acquired from meat extracts to differentiate 4 meat types (beef, lamb, pork and chicken) including simple mixtures (50%). A simple extraction and sample preparation procedure was developed. To improve alignment of the spectra and subsequent statistical analysis, an internal standard was used as a lock mass peak. Using this method, it was possible to clearly differentiate pure meat samples and simple meat mixtures (up to 50%).

2. Introduction

Food fraud can lead to consumers consuming foodstuffs that have been adulterated and may not be fit for human consumption. In 2013, the horsemeat scandal was big news in Europe, where beef products were found to contain large quantities of horse meat (up to 100%) that had entered the human food chain through criminal activity.

In 2021, the horse meat scandal resurfaced with reports horse meat being passed off as veal^{2,3}. This type of criminal activity is particularly disturbing as it illustrates a complete breakdown of traceability and quality standards in our food chain.

According to the UK Parliament's POSTNOTE 624 on food fraud⁴, one of the barriers to tackling food fraud is the cost and availability of testing. This work sets out to demonstrate the utility of MALDI-TOF for the screening of raw meats to identify the species of origin and to provide the ability to rapidly and cost-effectively confirm meat species to improve food security and prevent meat adulteration at the outset.

3. Methods

The optimised sample preparation and analysis workflow is shown in Figure 1. Sixty (60) raw meat samples (15 beef, 15 lamb, 15 chicken and 15 pork) were collected from local and online UK supermarkets. The samples collected were in a variety of forms (e.g., chicken thigh, breast, lamb leg, chop, mince etc.) and stored at -20°C until required. Each meat sample (40 mg) was homogenised in 500 µL of 0.1 % TFA for 1 minute using a motorized pestle mixer (Argos Technologies). Homogenised samples were centrifuged at 21.3k RCF for 5 mins. The supernatant was aspirated whilst avoiding any fatty layer and stored at -20°C until prepared for analysis.

The homogenates were pH checked and adjusted with 5 µL 10 % TFA to ensure the sample maintained an acidic pH (pH 1). 200 µL of sample homogenate was mixed with 200 µL ACN and any gel precipitate was removed with a pipette. Meat mixtures were prepared by mixing equal quantities of meat extracts (e.g. 10 µL pH checked chicken with 10 µL pH checked beef etc).

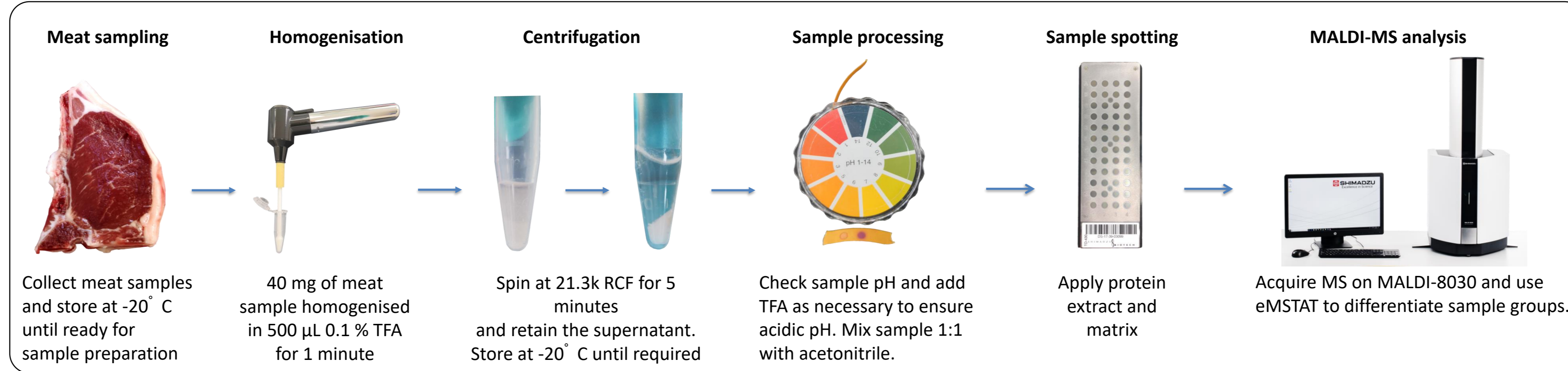


Figure 1 – Overview of the sample preparation and analysis workflow.

The matrix used was CHCA (40 mg/mL in 33/33/33 ethanol/ acetonitrile/ H₂O containing 3% TFA). Angiotensin 1 (5 pmol/ µL) was added to the matrix solution to at a concentration of 0.5% v/v to provide an internal standard for use as a lock mass during data acquisition using in *MALDI Solutions* (Shimadzu).

Meat samples and meat mixtures were spotted on *FlexiMass-DS* slides (Shimadzu). 1 µL of sample was spotted on a MALDI target and 1 µL of matrix solution added while the sample spot remained wet. Samples were then allowed to dry.

Samples were analysed in positive ion linear mode on a *MALDI-8030* benchtop MALDI-TOF mass spectrometer (Shimadzu). The Data Quality feature in *MALDI Solutions* was used during data acquisition to add only data profiles that meet specific quality criteria to the averaged spectrum.

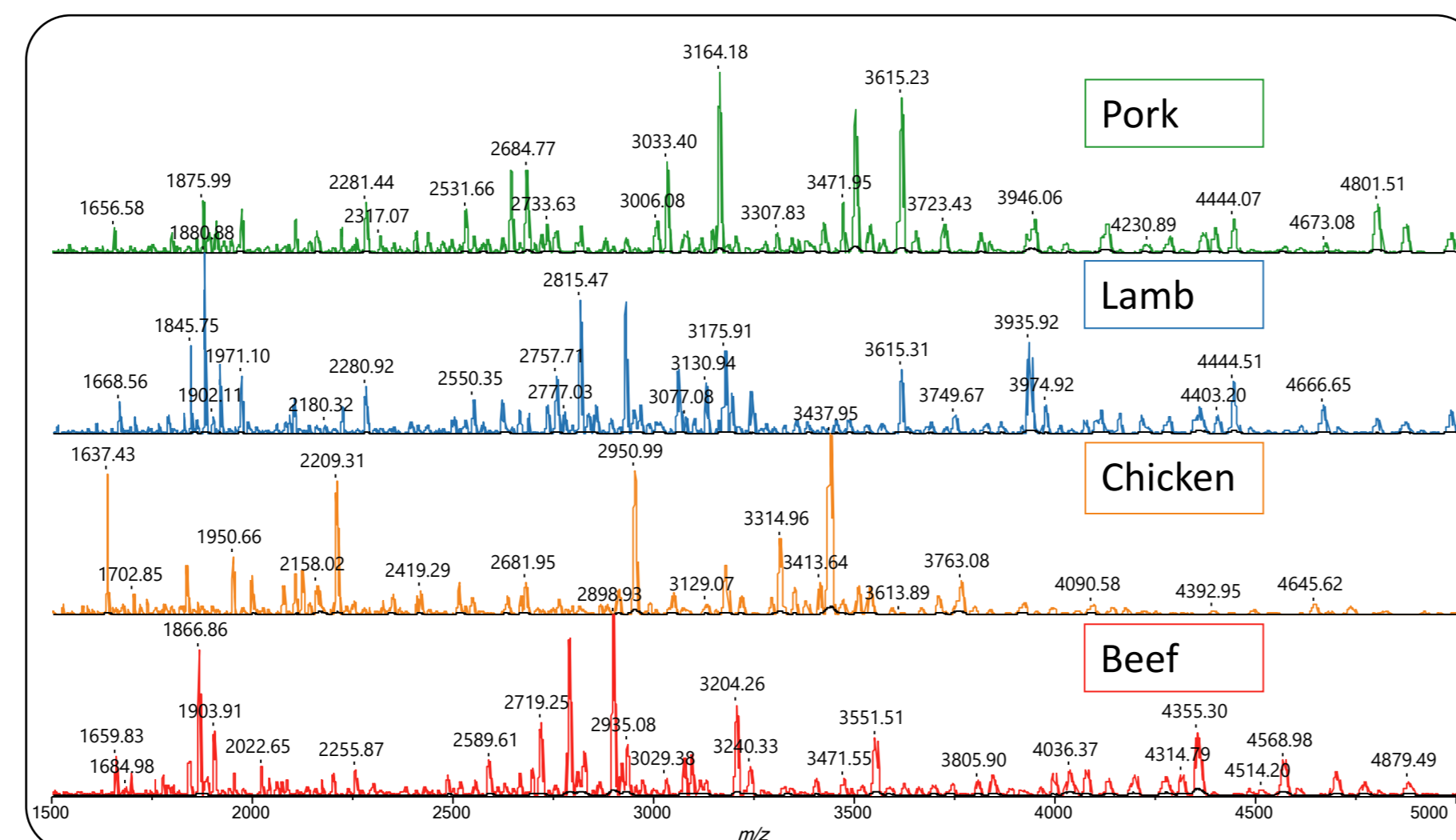


Figure 2 – Data illustrating the different mass spectra for the 4 meat types

Statistical analysis was performed in *eMSTAT Solution* (Shimadzu). The samples were optimally separated using principal component analysis (PCA) with the spectrum normalised in the range 1,500-20,000 m/z. Samples were excluded based on signal < 10 mV (excluding the internal standard) or profiles < 10.

4. Results

The results from this study show clearly that the four meats and their mixtures were differentiated using basic sample preparation methods and

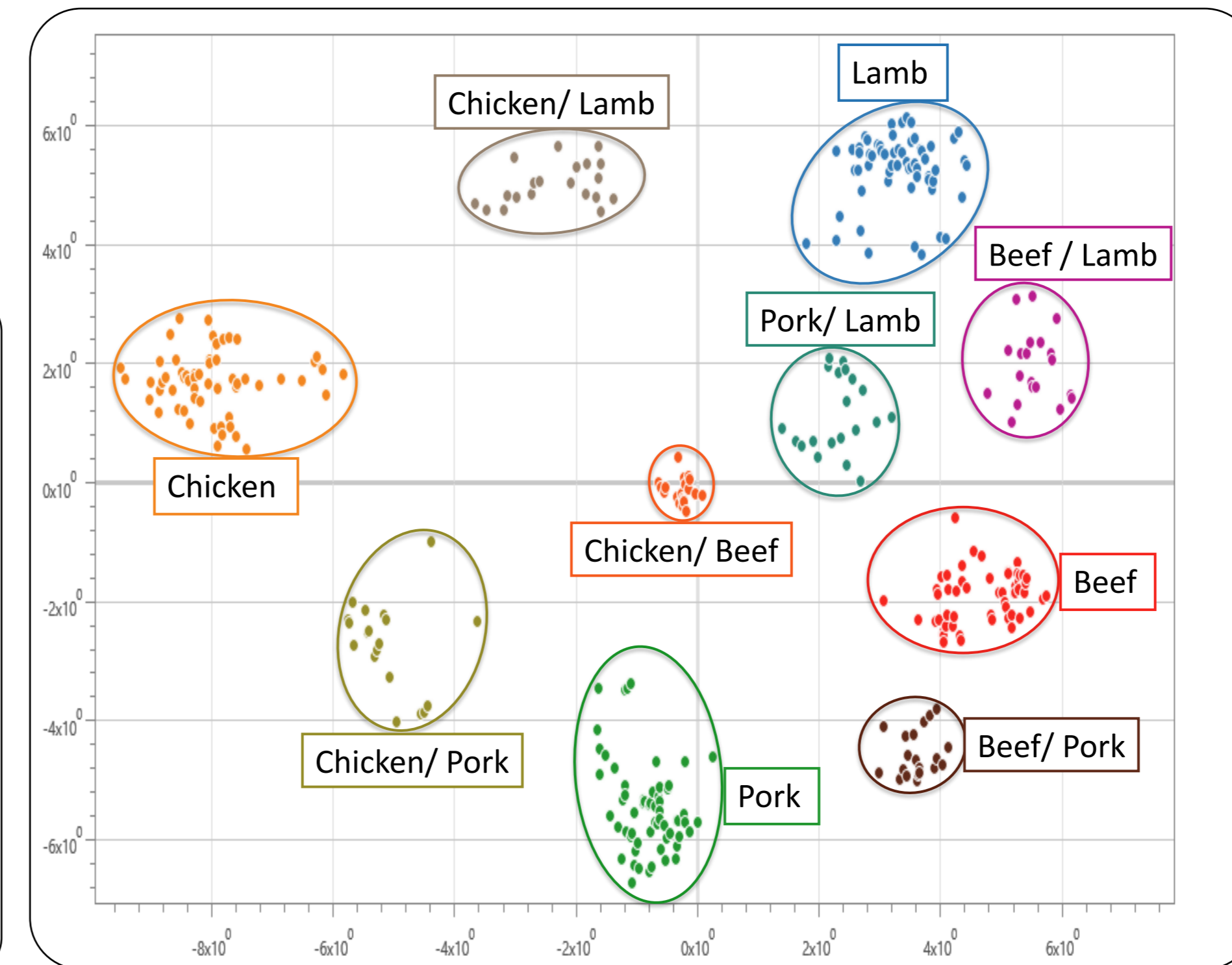


Figure 3 – Score plot of the four meat groups and 50:50 mixtures. Ovals were added manually to illustrate group boundaries.

analysis on the *MALDI-8030* followed by multivariate analysis in *eMSTAT Solution* (see Figures 2 and 3). Following data acquisition, statistical analysis was performed with principal component analysis (PCA). The score plot generated in *eMSTAT Solution* (Figure 3) shows how the four core groups can be distinguished from one another. Binary mixtures were prepared at 50:50 and 75:25 (data not shown) ratios. The 50:50 mixtures displayed clear separation between the groups but the data for the 75:25 mixtures overlapped the non-mixed samples in the case of 25% pork samples (e.g. 75% beef, 25% pork, chicken 75% pork 25% and lamb 75% pork 25%). Interrogating the data in *eMSTAT Solution* and *MALDI Solutions*, the peaks that are distinctive for pork can not be observed in the mass spectra for these samples.

5. Discussion

The aim of this work was to develop a fast method to screen meats for gross adulteration and flag up suspect samples for further analysis.

Over the course of this study, method development was performed to produce a method that would generate quality, reproducible data. The result of this development work is a method that can generate high quality mass spectra that can be used to distinguish the 4 meat types and the 50:50 mixtures tested.

The *MALDI-8030* has proven that it is well-suited for screening meat samples with minimal sample preparation. This clear discrimination paves the way for the development of a discriminant analysis model that may be used to quickly and cost-effectively check the species of meats used in food production.

Future work will investigate expanding the range of meats analysed and developing techniques to evaluate other foodstuffs.

6. References

- "Findus beef lasagne contained up to 100% horsemeat, FSA says". BBC News. 7 February 2013 <https://www.bbc.co.uk/news/uk-21375594>
- "The horse meat scandal has resurfaced" Food control. 01 September 2021 <https://affidjournal.com/en/the-horse-meat-scandal-has-resurfaced-1>
- "15 000 tonnes of illegal food and beverages off the market" Europol <https://www.europol.europa.eu/media-press/newsroom/news/15-000-tonnes-of-illegal-food-and-beverages-market>
- POSTNOTE 624 on food fraud <https://researchbriefings.files.parliament.uk/documents/POST-PN-0624/POST-PN-0624.pdf>