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MALDI-TOF Mass Spectrometry Analysis MALDI-8030

Application News

Differentiation of raw meat using MALDI-TOF and principal component statistical analysis

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User Benefits

- Simple sample preparation – homogenise, add acetonitrile, adjust pH and spot.
- Low cost per sample
- Rapid analysis – MALDI acquires high quality spectra in minutes hence amenable to automated sample analysis.

■ Introduction

Food fraud can lead to consumers ingesting 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%[1]) that had entered the human food supply through criminal activity.

In 2021, the horse meat scandal resurfaced with reports of 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 supply.

According to the UK Parliament's POSTNOTE 624 on food fraud[4], one of the barriers to tackling food fraud is the cost and availability of testing.

This work sets out to demonstrate the utility of the MALDI-8030 MALDI-TOF mass spectrometer, in combination with eMSTAT™ Solution statistical analysis software, for the screening of raw meats. The identification of the species of origin and the ability to rapidly and cost-effectively profile meat species will help to improve food security and prevent meat adulteration at the outset.

■ Methods

Sixty (60) raw meat samples were collected. These samples were 15 beef (steak, ground meat), 15 lamb (chop, leg, ground meat), 15 chicken (breast, thigh) and 15 pork (loin, medallion, ground meat) from local and online UK supermarkets. The samples were stored at -20℃ 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 ℃ until prepared for analysis.

The homogenates were pH checked and adjusted with 5 µL of 10% TFA to ensure the sample maintained an acidic pH (pH < 5). 200 µL of sample homogenate was mixed with 200 µL of acetonitrile and any gel precipitate was removed with a pipette. Meat mixtures were prepared by mixing equal quantities of meat extracts (e.g. 10 µL of pH checked chicken with 10 µL of pH checked beef etc). The meat mixtures prepared were 25% meat A/ 75% meat B, 50% meat A/ 50% meat B and 75% meat A/ 25% meat B.

The matrix used was CHCA (40 mg/mL in 33/33/33 ethanol/ acetonitrile / H_2O containing 3% TFA)). Angiotensin 1 (5 pmol/ µL) was added to the matrix solution at a concentration of 0.5% v/v to provide an internal standard for use as a lock mass during data acquisition 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 met specific quality criteria to the averaged spectrum. An outline of this process can be seen in Figure 1.

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 *m/z* 1,500-20,000.

Samples were excluded based on signal < 10 mV (excluding the internal standard) or profiles < 10.

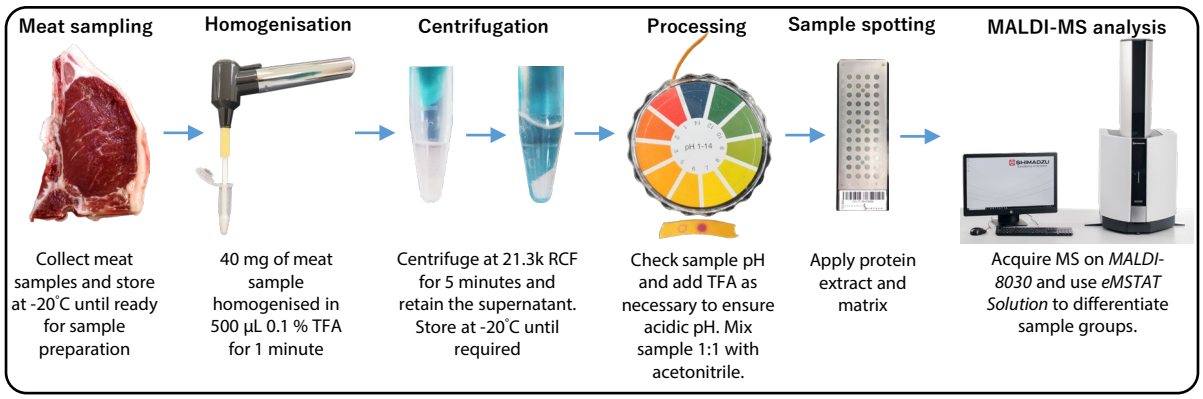

Figure 1 – Overview of the sample preparation and analysis workflow.

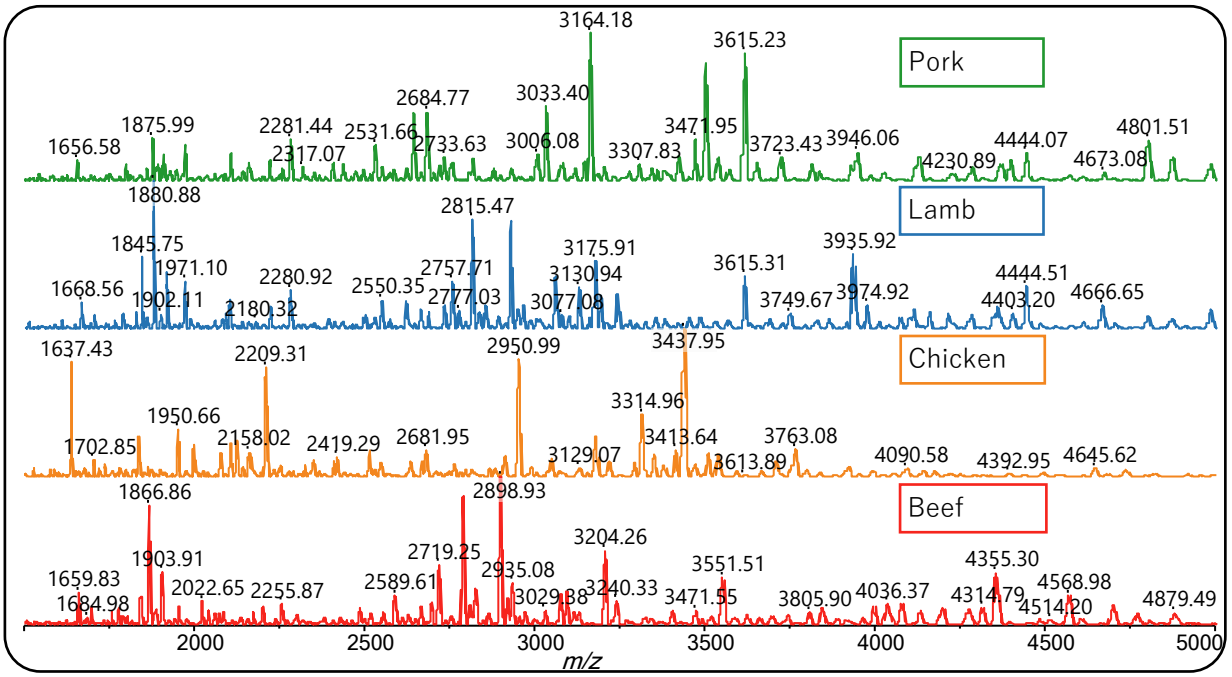

Figure 2 – Data illustrating the different mass spectra for the 4 meat types

■ Results

The results from this study show clearly that the four meats and their mixtures were differentiated using basic sample preparation methods and 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. 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, 75% chicken/ 25% pork and 75% lamb/ 25% pork). 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 25% pork samples.

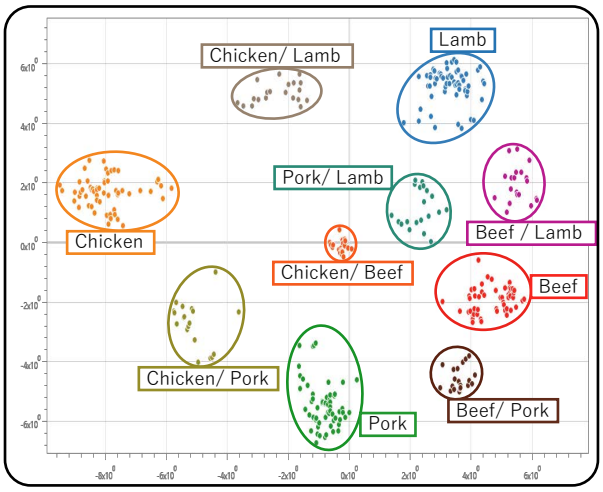

Figure 3 – Score plot of the four meat groups and 50:50 mixtures. Ovals were added manually to illustrate group boundaries.

■ 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.

Using the MALDI-8030, a method was developed 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.

■ References

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2) "The horse meat scandal has resurfaced" Food control. 01 September 2021 https://affidiajournal.com/en/the-horse-meat-scandal-has-resurfaced-1
3) "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
4) POSTNOTE 624 on food fraud https://researchbriefings.files.parliament.uk/documents/POST-PN-0624/POST-PN-0624.pdf

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