71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

DETECTION OF ADULTERATED BEEF PATTIES BY MEANS OF MALDI-ToF-MS FINGERPRINTING

Manuela Peukert[1*], Rene Wittmann[1], Dagmar A. Brüggemann[1]

[1] Max Rubner-Institute, Department of Safety and Quality of Meat, Kulmbach, Germany.

[2] Institution 2 Name, City, Germany

*Corresponding author email: manuela.peukert@mri.bund.de

I. INTRODUCTION

The authenticity of meat products is a critical aspect of food safety, dietary preferences, and religious practices. A significant issue in the meat industry is the adulteration of beef products, particularly beef patties, with foreign species such as pork or offals. Such adulteration is often driven by economic motives, as cheaper meats like pork may be used to partially substitute more expensive beef meet, thereby reducing production costs. However, such practices violate food labeling regulations, undermine consumer trust, and can lead to serious health risks if harmful substances or pathogens from the adulterant are introduced.

Detecting food fraud is a crucial challenge for analytical chemistry, primarily due to the complex nature of the meat matrix. Additionally, the composition of meat undergoes substantial changes depending on storage conditions and processing, which affect analytical results, while the nature of the manipulation is often unknown. In this regard, DNA analyses can be useful if the substitution involves a different animal species. Targeted analytical methods, which focus on detecting one or a few biomarkers (proteins or metabolites), are usually laborious in method development and require prior knowledge or hypotheses. In contrast, the general conceptualization of non-targeted analytical approaches are unique fingerprints of reference samples to which an unknown sample is compared to. Those fingerprints might be useful, when reference materials remain chemically stable under various conditions. However, in cases of meat, the molecular composition strongly depends on genetic backgrounds, gender, feeding, age, housing and conditions of storage and processing, which would make the build-up of reference databases for particular meat products extremely elaborate. Therefore, the development and selection of a detection method strongly depends on the purpose and application. Since test samples are typically unknown and may contain adulterants of unknown origin, cost-effective rapid screening methods would be helpful. These could initially identify suspicious samples, which would then be subjected to targeted analysis in a second step. Among the OMICS technologies, simple MALDI-ToF-MS devices could be used for this purpose, as they can capture peptide and protein profiles in food. In this context, a protein fingerprint database for common food fish has been developed [1], allowing for the differentiation of 54 fish species at least at genus level, and depending on the genetic similarity, also at species level. Additionally, MALDI-ToF-MS fingerprinting has been proven useful for distinguishing animal species such as pork, beef, horse, veal, and chicken. However, detecting adulteration at low substitution concentrations remains challenging. For instance, the adulteration of beef gelatin with 20% pork gelatin has so far been detectable using MALDI-ToF-MS [2].

As part of the ‘Watson’ project, we are testing the MALDI-ToF-MS methodology as a rapid screening technique to identify suspicious beef patties adulterated with pork or offal. The aim is to evaluate this fast and cost-effective detection method in terms of its sensitivity and selectivity, and to compare the advantages and disadvantages of MALDI-ToF-MS fingerprinting and DNA metabarcoding.

II. MATERIALS AND METHODS

First, pork and beef meat were purchased from different markets and used for method adaption. For preparation of reference samples pork and beef shoulders (without shoulder blade and mock tenderloin) were obtained from a commercial slaughter house two days post mortem (pork) and six days post mortem (beef). Minced meat was prepared consisting of 100% beef to 100% pork in 10% steps as reference calibration material. In order to assess also storage dependent variances of the meat, 250 g portions were MAP packed under 70% oxygen and 30% carbon dioxide atmosphere, and stored for 5 and 10 days at 2°C. Additional concentrations were then prepared in the lab using a blender. Preparation of minced meat was performed in 6 independent replicates, representing different animals and slaughter days. Extraction of peptides and small proteins was performed using a 0.01% HCl solution in a ratio of 2:9 (w/v). Homogenized samples were centrifuged and the supernatant taken for a treatment with 15% sulfosalicylic acid, added 1:6 (v:v), in order to precipitate large proteins. After 30 minutes incubation at 4°C, samples were again centrifuged and 1 ml supernatant applied to solid phase extraction using HLB prime columns (1cc, 30 mg, Waters, Eschborn, Germany). Eluates were gained with 10:90 (v/v) methnol:acetonitrile and finaly stored at -

80°C until MALDI-ToF-MS analysis. For measurements MALDI targets were prepared by spotting 1 µl sample and 1 µl matrix (0.01 g/ml α-cyano-4-hydroxycinnamic acid in 2.5% trifluoroacetic acid and 50% acetonitrile. Mass spectra were determined with a Bruker microflex LT instrument (Bruker Daltonik, Bremen, Germany) operated in linear positive mode. Mass range was set to 800-10,000 m/z). For instrument calibration a mixture of polyethylene glycol (PEG 600, 2000, 4000, 6000 and 8000) was used. Data preprocessing was performed in R and consisted of baseline subtraction, smoothing, peak binning and log transformation (adapted from Gibb 2012 [3]). For statistical analysis we used the JMP software (SAS Institute Inc., Cary/NC, USA).

III. RESULTS AND DISCUSSION

One major challenge in detecting adulteration in meat products is the variation in meat constituents caused by storage-dependent degradation and oxidation processes. These changes are clearly reflected in MALDI-ToF-MS fingerprints of polypeptides (Figure 1A, PC1), indicating the complex dynamic of peptide profiles resulting from both storage and processing steps. Additionally, animal-to-animal variability contributes significantly to the observed differences in these profiles (Figure 1A and 1B).

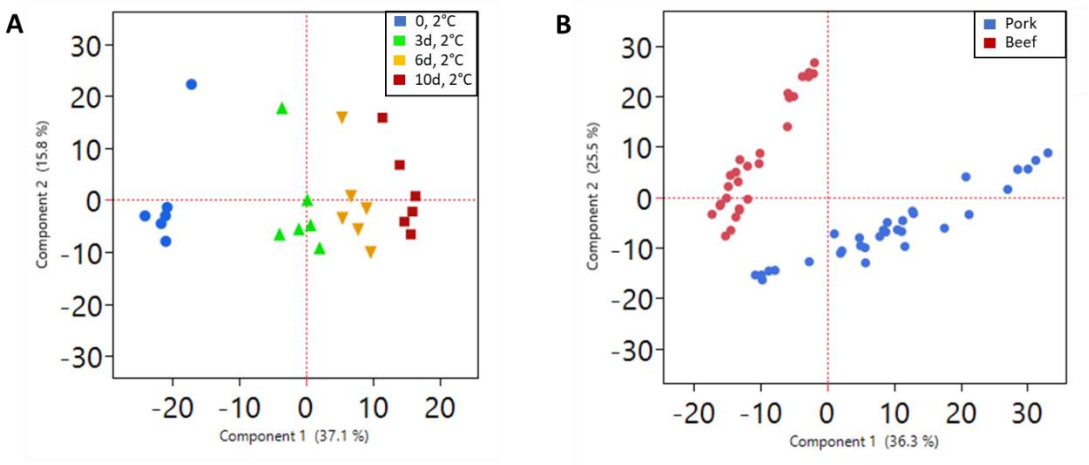


Figure 1. Principal component analysis of MALDI-ToF-MS spectra. A: Minced meat from six pig shoulders stored at 2°C for 0, 3, 6 and 10 days. B: Comparison of meat from pork and beef

Initial tests comparing pork and beef meat revealed that the majority of peaks are shared between the two species, making the detection of low-level substitutions particularly difficult. In contrast to beef, pork exhibited only a few species-specific peaks, which were also found to be sensitive to storage conditions.

IV. CONCLUSION

MALDI-ToF-MS is a valuable tool for the rapid generation of polypeptide fingerprints, and species-specific profiles can be clearly distinguished. However, the similar muscle protein composition of red meats, combined with strong storage-dependent dynamics, makes the detection of pork substitution at very low concentrations unfeasible. Ongoing research is focused on determining detection thresholds to improve the identification of suspicious samples.

ACKNOWLEDGEMENTS

This project is finally supported by the EUROPEAN RESEARCH EXECUTIVE AGENCY (REA) - Project 101084265 — WATSON.

REFERENCES

1. Stahl, A. and Schröder, U. (2017) Development of a MALDI–TOF MS-Based Protein Fingerprint Database of Common Food Fish Allowing Fast and Reliable Identification of Fraud and Substitution. Journal of Agricultural and Food Chemistry 65 (34): 7519-7527.
2. Flaudrops, C. et al. (2015) Determination of the animal origin of meat and gelatin by MALDI-TOF-MS. Journal of Food Composition and Analysis, 15: 104-122.
3. Gibb, S. and Strimmer, K. (2012) MALDIquant: a versatile R package for the analysis of mass spectrometry data. Bioinformatics 28 (17): 2270–2271