Identification of animal species from meat using MALDI-TOF MS

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Introduction

Consumer protection against incorrectly labeled foods is an important goal of official food control. The declaration of the animal species is especially crucial for meat. Until now mostly molecular and immunological methods have been used for the analytical confirmation.

Mass spectrometry (MS) [1,2,3] offers a new approach to the species identification of animals. MALDI-TOF MS combines a matrix-assisted laser desorption/ionisation (MALDI) with a time of flight (TOF) analyzer and MS (Fig. 1). Hereby, ionizable large biopolymers, such as proteins from microorganisms, fish or meat, can be analyzed softly. The prevalence of this technology is rapidly increasing and MALDI-TOF MS is especially applied for the differentiation of bacteria.

The identification of an unknown sample is achieved by comparing the resulting mass spectrum with the references in a database. The database therefore is central for success. For identification of animal species from meat, however, no commercial database is available.

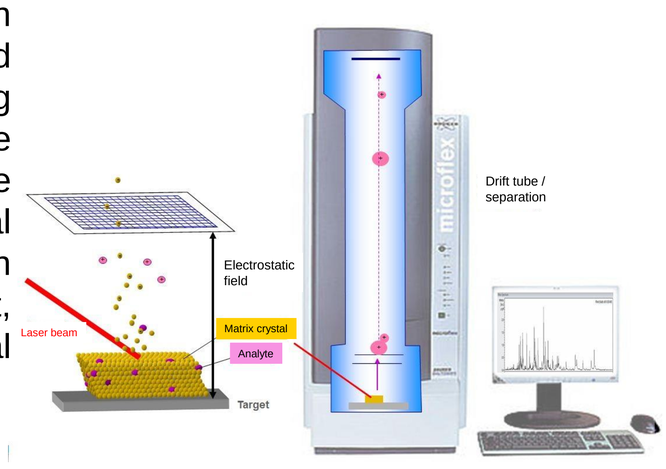

Fig. 1: MALDI-TOF MS

Reference Samples/ Sample Preparation

As a reference for creating the database, raw muscle meat from livestock, zoo, pet and wild animals was provided by our veterinary post mortem pathology.

Based on Post & Dikler 2010 [1] a sample preparation protocol optimized for meat was developed:

In 1.5 ml safe-lock tubes, around 5 mg musculature were crushed with a pestle and 5 mg zirconia/silica beads 0.1 mm (BioSpec), in a mixture of 50% acetonitrile, 47.5% H_2O and 2.5% trifluoroacetic acid.

15 s extraction on a laboratory mixer, centrifugation for 120 s at 14000 rpm.

1 µl supernatant was transferred onto a steel target, dried at room temperature (ca. 60 s), overlaid with 1 µl matrix-solution (α-Cyano-4-hydroxycinnamic acid, HCCA, Bruker).
The HCCA was dried and gently crystallized at room temperature (ca. 120 s).

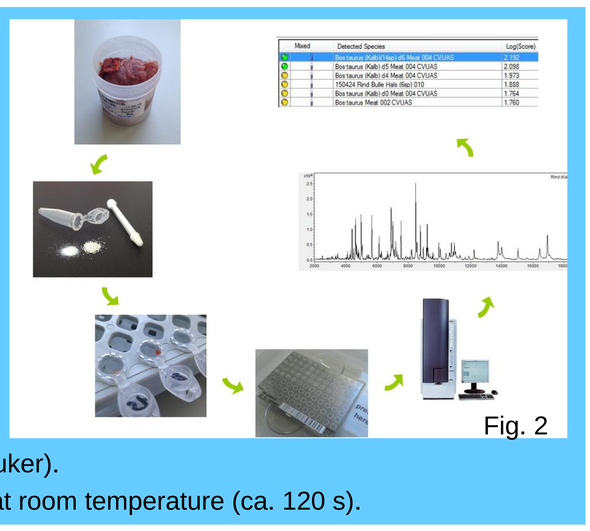

Fig. 2

Processing the Spectra / Creation of the Database

The loaded target can be directly measured in the MALDI-TOF MS (Biotyper LT-microflex, Bruker Daltonik, Bremen, Germany) (Fig. 2). The raw spectra collected in the range of 2-20 kDa are reduced to MSPs (Main Spectra Projections) (Biotyper 3.0, Bruker) (Fig. 3). These can be included as a reference in an own database. The resulting MSPs of different animal species vary considerably (Fig. 4).

The result for a sample is given as a score-value by the Biotyper system, which reflects the correspondence of the sample MSPs with the database entries.

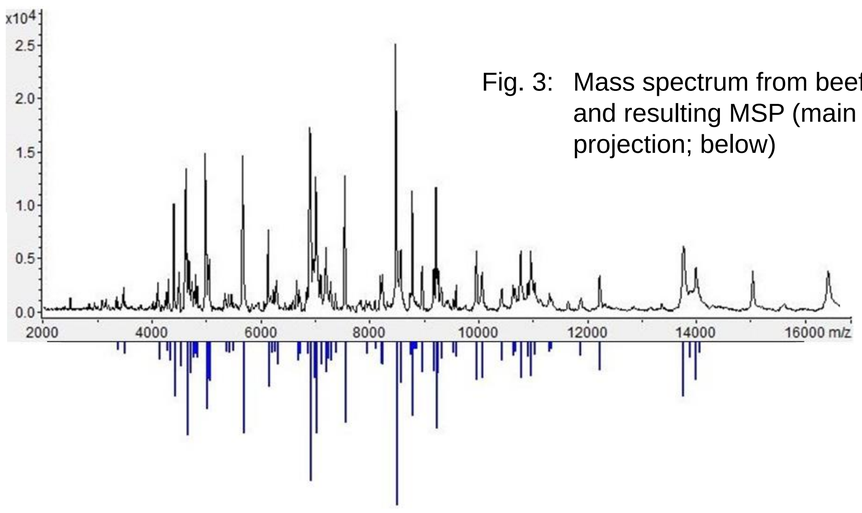

Fig. 3: Mass spectrum from beef (above) and resulting MSP (main spectra projection; below)

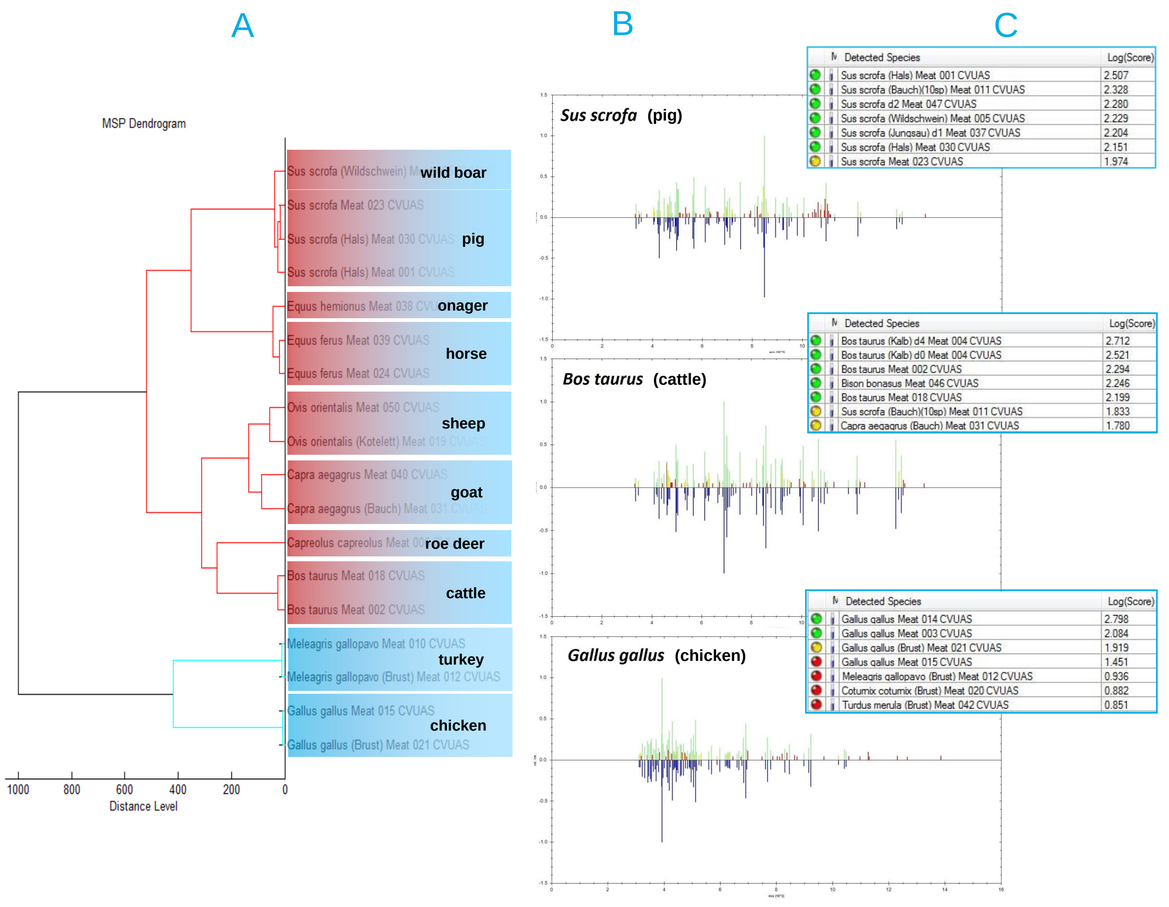

Fig. 4: A Dendrogram of MSPs from meat of different animal species
B Comparison of MSPs. Above: sample; below: reference-MSP from the database
C Hitlist of samples, depicting the identity with database entries, by descending score value

Meat Database

So far, 51 independent data entries of 29 different animal species were added to the CVUAS-database. These generated MSPs can be transferred to other equipment within the same system. A selection of existing database entries with additional information to the current status is listed on *www.maldi-up.ua-bw.de* [4].

Validation

For the validation of the system the specificity and selectivity of each parameter were determined using independent reference samples consisting of muscle meat. As an example the summary of the results for the parameters *Sus scrofa* (pig), *Bos taurus* (cattle), and *Gallus gallus* (chicken) are depicted (Fig. 5).

	Validation set	
Biotyper result	*Sus scrofa* n = 29	*# Sus scrofa* n = 36
Sus scrofa	93,1% (27)	-
# Sus scrofa	-	97,2% (35)
questionable*	6,9% (2)	2,8% (1)

	Validation set	
Biotyper result	*Bos taurus* n = 9	*# Bos taurus* n = 56
Bos taurus	100% (9)	-
# Bos taurus	-	98,1% (53)
questionable*	-	5,4% (3)

	Validation set	
Biotyper result	*Gallus gallus* n = 7	*# Gallus gallus* n = 58
Gallus gallus	85,7% (6)	-
# Gallus gallus	-	96,6% (56)
questionable*	14,3% (1)	3,4% (2)

Fig. 5: Result of validation: left pig, right cattle and chicken.
top left → sensitivity; middle right → specificity; # = „not“

Conclusion / Future Prospects

A transferable method, fit for the routine identification of a first selection of animal species from raw muscle meat using MALDI-TOF MS, is established.

It consists of an optimized sample preparation, own database entries and the first parameter validations.

The speed (<1h), ease of use and low cost of consumables already enable the screening of animal species.

Further additions to the database (animal species, technological processing steps) and the continued formal validation of parameters will increase the acceptance of this method.

This work has been carried in full by P. Stoll during his internship in his 3rd year of studies of biotechnology at the University of Applied Sciences Esslingen.
This is a translation of a poster presented on the 44. Deutschen Lebensmittelchemikertag, 14.09.-16.09.2015 Karlsruhe, Germany.

References

[1] Post A, Dikler S (2010) FACSS 2010, 583
[2] Stephan R *et al.* (2014) Food Control 46: 6–9
[3] Flaudrops C *et al.* (2015), J. Food Comp. Anal. 41: 104-112
[4] http://www.maldi-up.ua-bw.de

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