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# The use of NMR- and GC-MS -based metabolomics for the detection of food fraud in turkey breast meat (#654)

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## Introduction

Non targeted metabolomics approaches became important tools in bioanalytical research during the recent years. The core techniques are chromatography coupled to mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy allowing for the analysis of pattern changes of the metabolite network upon a specific treatment, for a specific condition or a particular biological system (Fig 1). NMR spectroscopy is highly quantitative, reproducible, non-destructive and noninvasive; furthermore it requires no or little sample preparation and it is possible that a high number of metabolites can be detected simultaneously in a short time period and in one measurement. Main drawback of NMR is the required sample amount that does not allow analysis of very small sample sizes or detection of very low concentrated metabolites. MS represents a highly sensitive and selective tool and enables detection of molecular masses and fragmentation patterns for chemical structure identification. The different combinations of separation, ionization and detection techniques allow a broad coverage of metabolites for profiling and quantification. Nevertheless, careful extraction is needed and reasonable selection of measurement methods to avoid degradation and ion suppression. The objective of this study was to compare the applicability of NMR and GC-MS for the detection of food fraud in turkey breast muscle as introduced by the addition of amino acid hydrolysates.

#### Methods

Turkey breast muscle was treated with different plant and animal based protein hydrolysates. The hydrolysates were produced by enzymatic digestion and by acidic hydrolysis. A non-treated and a water treated sample served as controls. For NMR and GC-MS lyophilized and grinded samples were extracted using methanol, water and chloroform. Afterwards, the dried extracts were prepared for NMR and GC-MS measurement, respectively. For NMR spectroscopy analysis, samples were re-dissolved with 540µl D<sub>2</sub>O, 25µl MilliQ water, 25µl D<sub>2</sub>O containing 0.05 wt% TSP and 10µL Maleic acid as internal standard. All samples were analyzed with a Bruker 400MHz spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). For GC-MS analysis, samples were derivatized by methoxyamination followed by silvlation with MST-FA. The GC-MS system consisted of a gas chromatograph combined with a fast-scanning quadrupole mass spectrometer (Shimadzu GCMS QP2010). For chromatographic separation a non-polar Rxi-5SilMS (Restek, Bellefont, USA) was chosen. For statistical analysis and visualization of the data the software tool SIMCA-P (version 13.0; Umetrics, Umeå, Sweden) and for quantification of the NMR signals ChenomX NMR Suite (version 8.1; ChenomX Inc., Edmonton,

# AB, Canada) were used.

Results

The principal component analysis (PCA) of all signals obtained by NMR and GC-MS, respectively, showed a clear differentiation between control samples (left side in the PCA score plots) and hydrolysate treated samples (right side in the PCA score plots). Furthermore, clear variations between the types of injected hydrolysates were also observed (right side, top to bottom in the PCA score plot) (Fig.2). Analysis of corresponding loadings revealed that proteinogenic amino acids were responsible for the strong differentiation between controls and hydrolysate treated samples, whereas byproducts play a role for differentiation of the various hydrolysate types. These byproducts are present in cheap protein sources and/or formed during the acidic hydrolysis process. Plant derived enzymatic protein hydrolysates additionally contained sugars like glucose or maltose that were converted to levulinic acid in the acidic hydrolysates. Animal derived enzymatic hydrolysates contained higher amounts of glycerol, ornithine and oxoproline. By means of GC-MS higher levels of aminomalonic acid, hydroxyproline and 3-MCPD were detected in acidic hydrolysates of animal derived proteins. The analysis via NMR showed also that the addition of hydrolysates to the turkey breast muscle affected the organic acid profile. The control sample showed higher levels of anserine, lactate and creatinine.

#### Conclusion

The study showed that non-targeted metabolite profiling represents a valuable tool for the detection of food fraud in turkey breast muscle caused by introduction of protein hydrolysates. A definite classification of the hydrolysate type was not possible when only the proteinogenic amino acids were taken into account. Presence of byproducts coming from the protein source itself or formed during the hydrolysis helped to classify the hydrolysate source.

With both techniques the same results were revealed (Fig 2). A clear advantage of NMR compared to GC-MS is a shorter measurement time so that NMR is better suited for high-throughput screenings. If quantitative data are required, NMR allows direct quantification of compounds whereas in GC-MS analysis additional calibration curves for the individual compounds would be needed. On the other hand, GC-MS is more sensitive and allows detection of low concentrated compounds. In conclusion, depending on the scientific question it has to be taken into consideration which analytical technique would be most suited.



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Notes



### Figure 2.

PCA score plots from NMR and GC-MS based on the metabolic profile of turkey breast extracts.



 $\ensuremath{\textit{Figure 1.}}$  The appearance of the metabolome is a result of various factors.

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