Recent Developments in Meat Science in Europe: Analytical Approaches for Tracking and Tracing

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With respect to the global distribution of feed, food and ingredients, the different countries in our world have never before been more interdependent with respect to their food supply. Consequently, a common approach with consistent standards based on sound science and robust controls is necessary to ensure consumers` health and to maintain consumers´ confidence.

In this regard the *General European Food Law*, Regulation (EC) 178 (2002), outlines the general principles and requirements of food law, establishes the *European Food Safety Authority (EFSA)*and provides procedures in matter of food safety, i.e. among other things the implementation of traceability systems in the food and feed supply chains in Europe. Article 18 of the regulation refers to traceability of food and feed.

Indispensable requirements for every food business are: appropriate process control, biosecurity, adequate traceability as well as good hygiene and manufacturing practices. Food safety and quality require various analytical approaches along the whole food chain, downstream (tracking) from primary production to the consumer and upstream from the consumer to primary production.

Analytical approaches for tracking and tracing in the meat area are for example the mass spectrometric analysis of organic residues and contaminants, as well as heat-induced substances. Furthermore, the quantitation of animal species by real-time PCR as well as the HPLC-MS/MS detection of allergens and microbial transglutaminase in meat products is relevant.

Within a representative survey of dioxins (PCDD/Fs), dioxin-like PCBs and marker PCBs in about 300 samples of German meat and meat products were analyzed in the years 2005/2006. The sampling plan included different types of meat (pork, poultry meat, beef and sheepmeat) and meat products (emulsified sausage, raw ham, cooked sausage and raw sausage). For sampling, the German National Nutrition Survey of the year 2004, the

actual consumer behaviour and the population of the different states in Germany were considered. The median content of WHO-PCB-TEQ in beef samples was 0.9 ng/kg fat. In poultry meat, a median content of WHO-PCB-TEQ was determined, that was less than one tenth of the action level of 1.5 ng/kg fat. In meat products, the WHO-PCB-TEQ ranged from 0.06 ng/kg fat for raw ham to 0.13 ng/kg fat for raw sausages. In meat and meat products the WHO-PCB-TEQ was dominated by PCB 118, PCB 126 and PCB 156, which combined contributed 87% (for pork) to 96% (for beef) of the WHO-PCB-TEQ. The median contents of WHO-PCDD/F-TEQ ranged from 0.09 ng/kg fat for pork and poultry meat to about 0.2 ng/kg fat for beef and were significantly below the maximum levels. In comparison to an earlier survey of about 10 years ago, significant decreases of dioxin contents were observed especially for beef and poultry meat.

The contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances (guaiacol, 4-methylguaiacol, syringol, eugenol and trans-isoeugenol) in smoked Frankfurter-type sausages were investigated, depending on the smoke generation method applied, in a total of 63 smoking experiments. The smoke was generated by smouldering with different air supplies (smouldering smoke), by leading overheated steam through wood chips (steam smoke), by friction of a log (friction smoke) and by heating plates (touch smoke). The type of smoke generator had a noticeable influence on the contents of PAH and phenolic compounds. The highest mean content of PAH4 (2.6 mg/kg) was observed for sausages when intensive smouldering smoke was applied, the lowest (0.3 mg/kg) in friction-smoked sausages. The highest mean sum content of the five phenolic compounds was observed for sausages smoked with steam smoke (45 mg/kg), whereas the contents in friction- (15 mg/kg) and touch- (18 mg/kg) smoked products were relatively low.

To trace fraud and adulteration in meat products the labelled animal species can be simultaneously detected and quantified applying real-time PCR to amplify target as well as reference DNA fragments. For canned meat products, DNA fragmentation increases according to heat intensity. Nevertheless, if the lengths of indicator and reference gene fragment are similar to those shown for goat (beta-casein gene fragment specific only for goat: 161 bp / myostatin gene fragment, unitary for all animal species: 154 bp), a reliable relative quantitation is possible, independent of the treatment of meat products. However,

the influence of the natural DNA-content of different types of tissue on quantification must always be taken into consideration.

The use of vegetable proteins in various types of meat products is common practice. In order to control food specifications, also with regard to food fraud and allergenic potential, a reliable detection of these additives is required. Therefore, a sensitive screening method for the simultaneous detection of lupine (Lupinus angustifolius), pea (Pisum sativum), and soy (Glycine maxima) in meat products applying High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) has been developed. After protein extraction and tryptic digestion, 3 to 4 marker peptides for each plant species were measured by HPLC-MS/MS. For matrix calibration, emulsion-type sausages with 0, 1, 6, 32, 160, 800, and 4000 mg/kg raw legume protein isolates/legume flour were produced. The mentioned legumes were detectable in sausages with concentrations of 6 mg/kg legume protein isolates/legume flour or greater. High correlation coefficients ($R^2 > 0.999$) between the peak areas of the mass transitions of the marker peptides and the contents of legume proteins in the meat products were obtained. The limits of detection (LODs) of the method were about 5 mg/kg meat product for pea protein, 4 mg/kg meat product for soy protein, and 2 mg/kg meat product for lupine protein. No false-positive or false-negative results were recorded. The applicability of the described method was tested by analyzing commercial meat products with and without added legume proteins.

A sensitive HPLC–MS/MS-method for the detection of microbial transglutaminase (TG) from *Streptomyces mobaraensis* in different types of restructured meat (pork, beef, chicken, and turkey) was developed using six tryptic marker peptides (8 – 11 amino acids). Meat binding experiments were performed with two technical TG mixtures with and without caseinate. After optimising extraction and tryptic digestion conditions, restructured meat and blank values (total samples: 62) were analysed in a raw and heated state. When investigating samples pre-treated with oil marinade, emulsion marinade, seasoning salt as well as breadcrumbs, only very little effects regarding the type of pre-treatment on the detectability of TG were found. Using four marker peptides, no false-positive or false-negative results were obtained. The limit of detection (LOD) was about a factor of 10 below the recommended amount of transglutaminase for raw as well as heated restructured meat.