Non-declared addition of foreign proteins and protein-hydrolysates to meat products

F. Schwägele, K. Dolch, B. Kranz, C. Stader, S. Münch, S. Andrée, W. Jira, D. Brüggemann

Max Rubner-Institut (MRI), Department of Safety and Quality of Meat, Kulmbach, Germany

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There is a wide range of potential foreign protein sources which could be used in meat products to substitute meat protein. The worldwide production quantities as well as the protein contents were taken as a basis for a categorization of those foreign protein sources. Additionally, all substances that may cause allergies or intolerances were highlighted. For those substances, a lower detection limit is necessary. To circumvent health problems, a correct declaration is mandatory and, therefore, surveillance systems are necessary. Due to the high grade of processing, especially emulsion-type sausages are likely to be adulterated. Consumers are no longer able to identify such protein substitutions visually. Therefore analytical techniques are required. One possibility is the detection of the deoxyribonucleic acid (DNA) of the foreign protein sources as each species has their unique genome. The method of choice is quantitative real-time polymerase chain reaction (qPCR).

In the following, it is exemplarily shown for six cereals how to establish two triplex qPCR systems. Barley, wheat, oat and rye contain gluten and therefore they can cause allergies or intolerances. In the European Union, for gluten-free food the limit is set at 20 mg/kg. Therefore, a low detection limit (< 20 mg/kg) is necessary. Additionally, maize and rice were chosen due to their high production rate. First, the theoretical detection of the mentioned cereals can be performed by using bioinformatic tools like gene databases. However, since not all plants are fully sequenced, those gene databases are incomplete. Therefore DNA has to be isolated from those plants of interest and from closely related plants. The creation of a DNA library allows practically testing the specificity of the chosen qPCR systems. If no wrong signal is detected, the single qPCR systems can be combined to multiplex qPCR systems. And again it is checked for false positive signals. In the last step, emulsion-type sausages spiked with plant protein are produced and analyzed. It was possible to establish two triplex qPCR systems for the simultaneous detection in the ppm-range in emulsion-type sausages. The first one is for barley, oat and rye and the second one for wheat, maize and rice. In the future, both systems have to be validated and the influence of processing on detectability will be tested. Additionally, new multiplex qPCR systems will be designed for further foreign protein sources.

Another possibility to detect grain proteins in meat products is the direct detection using High Performance Liquid Chromatography (HPLC) in combination with Tandem Mass Spectrometry (MS/MS). Here, a sensitive HPLC-MS/MS-method for the simultaneous detection of the six main grain species barley, maize, oat, rice, rye and wheat was developed. After protein extraction and tryptic digestion, 3 to 4 characteristic marker peptides for each grain species were selected and measured by HPLC-MS/MS. The uniqueness of the marker peptides was checked by BLAST search using protein data bases as well as by analyzing common ingredients of meat products. For each marker peptide, three intensive mass transitions were detected. The limits of detection (LODs) of the method were in the range of about 5 to 10 mg/kg in emulsion-type sausages and consequently below the limit of 20 mg gluten/kg food, which is established in the European Union as a limit for “gluten free” food. Furthermore, good correlations between the peak areas of the marker peptides and the contents of the grain proteins in the analyzed meat products were determined.

A special case of potential food fraud is the use of substances which are already included in other ingredients of sausages. An example of this type of adulteration is the addition of blood plasma, which is always present in meat due to the occurrence of residual blood. Consequently, the qualitative detection of blood plasma is no evidence of food fraud in meat products. Another problem in this case is the fact that the content of residual blood in meat/sausages is strongly varying. Therefore, an important aim of this project is to find analytical tools to be able to proof a possible addition of blood plasma to meat products.

Protein hydrolysates, like proteins, can be added to meat or meat products. Protein hydrolysates are chemically or enzymatically hydrolyzed proteins. This results in amino acids and peptides. The degree of hydrolysis is described as the relative ratio between amino acids and peptides, i. e. the proportion of hydrolyzed peptide bonds of the protein in percent. A distinction is basically made between protein hydrolysates containing amino acids and peptides and those consisting only of amino acids. The former are usually produced by the use of proteases (enzymes), the latter usually cost-effectively by chemical hydrolysis. They generally have a higher solubility in water than the corresponding proteins and are therefore easier to use in the product. In contrast to proteins, protein hydrolysates are generally not harmful to health according to current knowledge.

Turkey meat was used to establish a method to detect the non-declared addition of protein hydrolysates since it has a high absorption capacity of water. Due to a low profit margin it is especially susceptible to manipulation. Pork gelatin hydrolysates at about 15 % by weight were added to the fresh meat. Contents of free amino acids were analyzed by cation exchange chromatography. Free amino acids patterns in fresh turkey meat containing protein hydrolysates were compared to untreated samples which were used as reference data sets. The contents of 19 of the 20 proteinogenic amino acids (L-tryptophan is acid labile) were

determined repeatedly during 99 h after slaughter as amino acid levels increase over time caused by endogenous proteases in the meat. The variation of free amino acid contents as a result of the addition of protein hydrolysates with high degrees of hydrolysis was well above the natural variations and could thus be detected reliably.

Since other protein hydrolysates with high degrees of hydrolysis (bovine casein, wheat gluten) could also be detected, it is assumed, that the analytical method developed is suitable to prove the addition of such protein hydrolysates reliably in meat. For this, however, the reference data sets need to be extended. Additionally, the analysis of the peptide pattern was carried out in order to determine the addition of protein hydrolysates with low degrees of hydrolysis. This requires an extensive sample preparation. Finally meat products need to be taken into consideration.