DETERMINATION OF RESIDUAL BLOOD CONTENT FLUCTUATIONS OF PORK IN EMULSION-TYPE SAUSAGES USING UPLC-MS/MS

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I. INTRODUCTION

The use of blood plasma in meat products has many technological advantages. For example, it increases the water binding capacity, improves the texture properties and can be used as an emulsifier or phosphate substitute [1-3]. In 2016, 5.5 million tons of pork carcass were produced in Germany [4], according to a theoretical amount of 0.14 – 0.2 million tons of porcine blood. Therefore, the raw material blood plasma is cheap and advantageous to obtain. Because of the high protein content of 70 - 95% [5] in plasma powder, a substitution of meat by plasma in meat products such as emulsion-type sausages is also conceivable. For this reason, it is necessary to develop analytical methods for the detection of blood plasma in meat products. Due to the residual blood content in meat, the meat itself and the products manufactured from it contain a certain amount of blood plasma. Therefore, it is necessary to investigate the natural fluctuation of the residual blood content in pork or emulsion-type sausages made from pork.

II. MATERIALS AND METHODS

Seven different batches of emulsion-type sausages (full-canned samples) containing pork of different animals and one batch of sausages spiked with plasma powder were produced for measuring the repeatability of the developed mass spectrometric method. The blood proteins from the meat products were extracted and subsequently digested with trypsin. After purification by solid phase extraction (SPE), the resulting blood protein marker peptides were measured by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

III. RESULTS AND DISCUSSION

In the mass spectrometer, 6 marker peptides for porcine blood cell proteins and 14 marker peptides for porcine blood plasma proteins were analyzed using 3 mass transitions each. This ensured a clear assignment of the peaks to the peptides. Isotope-labelled standards of one blood cell peptide and one blood plasma peptide were used to check the performance of the UPLC-MS/MS system. A fluctuation in the residual blood content is characterized by altered peak areas of the marker peptides in the chromatogram.
The detection of a peptide was considered to be positive, if all three mass transitions showed a signal-to-noise (S/N) ratio of at least 3. The peak areas of the most intensive mass transition of the observed marker peptides for all cell and plasma proteins were summed up separately. For all peak areas of the cell and plasma protein peptides, the summed peak areas of the cell and plasma protein peptides as well as the ratio of cell to plasma protein peptides, the mean values and the standard deviations were calculated. The relative standard deviations (RSDs) are shown in Table 1. In addition, the RSDs of repeat measurements were determined (see Table 1). Thus, the fluctuation of the peak areas of the cell protein peptides (31.7%) was noticeably higher compared to the repeatability measurements (2.7%). The same applied to the peak areas of plasma protein peptides (34.6% compared to 1.6%).

Table 1 Relative standard deviations (RSDs) of peak areas of blood protein marker peptides analyzing sausages containing pork of different animals (n=7) and analyzing the same sausage (n=3)

	RSD (Fluctuation) [%] (N=7)	RSD (Repeatability) [%] (N=3)
∑Cell Proteins	31.7	2.7
∑Plasma Proteins	34.6	1.6
Single cell proteins	30.1 – 95.0	1.8 – 15.3
Single plasma proteins	25.7 – 49.0	0.3 – 27.1
∑Cell proteins/∑ Plasma proteins	8.6	2.6

By using summed peak areas of the marker peptides of plasma and cell proteins, the influence of individual fluctuations can be minimized. If not the summed peak areas, but the individual peak areas were compared to each other, the percentage fluctuation for cell protein peptides was between 30.1 and 95.0% and for plasma protein peptides between 25.7 and 49.0%. The RSDs observed in the repeat measurements were 1.8 – 15.3% for cell proteins peptides and 0.3 – 27.1% for plasma protein peptides. Using the quotient of the summed peak areas of cell protein peptides and the summed peak areas of plasma protein peptides, the RSD of the fluctuation was reduced to 8.6%. For repeat measurements, the RSD was 2.6% and therefore in the same range as observed for summed cell and plasma protein peptides. An additional advantage of using the ratios is that possibly occurring fluctuations in the sensitivity of the mass spectrometer might be balanced or at least attenuated.

IV. CONCLUSION

For the further development of mass spectrometric methods for the proof of the addition of porcine plasma to meat products it is recommended to use the ratio of blood cell protein peptides and blood plasma protein peptides.

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