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Mass spectrometric detection of the addition of porcine blood plasma to emulsion-type pork sausages (#652)

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Introduction

The adulteration of meat products by the undeclared addition of blood plasma powder is quite conceivable due to low costs, high protein contents and advantageous functional properties [1]. This applies, in particular, to pork having the highest meat production rate in the European Union. To prove this type of food fraud is rather difficult until now due to the lack of appropriate analytical methods, especially, when adding plasma to meat of the same animal species. Consequently, a rapid HPLC-MS/MS method for the detection of porcine blood plasma in emulsion-type pork sausages was developed.

Methods

Emulsion-type pork sausages with 0.5, 1, 1.5, 2 and 3 % meat substitution by plasma (16 series) and blank samples (32 series) were produced from different raw materials with two commercial plasma powders, and under two different thermal treatments (semi- and full preserves). Within each series, the pork used for the production of the sausages came from one single animal. The blood plasma proteins from the meat products were extracted and digested with trypsin in a rapid one-pot process (1 h at 55 °C). After purification by solid phase extraction, twelve species-specific marker peptides for porcine blood plasma proteins were measured by HPLC-MS/MS.

Results

On the basis of HPLC-MS/MS measurements of the tryptic digests of commercial porcine plasma powders (maXis UHR-QToF) and subsequent submission of the obtained peak lists to the MASCOT database search tool, more than thirty heat stable unique marker peptides for porcine blood plasma proteins were identified. From this group of preselected peptides, twelve peptides were selected for the HPLC-MS/MS method using the QTrap 5500. Meat and meat products always contain a certain amount of residual blood [2] and, consequently, blood proteins. This fact has to be considered with regard to a reliable detection of the addition of porcine plasma powder to meat of the same animal species. Therefore, the sole qualitative detection of plasma peptides does not allow clear conclusion about the addition of blood plasma. As a consequence, at least comparative investigations of control samples are required to detect the addition of blood plasma to meat products. In order to identify the most appropriate plasma peptides, the following five criteria were considered: (a) intensity of the peptide, (b) the relative standard deviation (RSD) of the mass transition ratio, (c) the slope and coefficient of determination (R^2) of the regression between the concentration of plasma powder and the peak area of the peptide, (d) the effect of thermal processing on the intensity of the peptide, (e) the RSD of the repeatability.

Four plasma peptides gave the best results with regard to these criteria, and were therefore considered to be the most suitable peptides. As a consequence, the peak areas of these four plasma peptides were identified to enable the best differentiation between control samples and sausages with added plasma powder. The one-sided 97.5 % confidence limits of the peak areas of the four most suitable plasma marker peptides were compared between the control samples and the batches with different concentrations of plasma powder (Table 1).

Table 1: One-sided 97.5 % confidence limits (lower limits) of the peak areas of the four most suitable plasma peptides in different batches of sausages with plasma powder (N = 25, each) in relation to the corresponding upper limits of the control samples (N = 50; normalized to 100 %; ratios > 100 % highlighted in gray).

Peak area of	Batch				
plasma peptide [%]	0.5	1	1.5	2	3
A	64	86	104	119	151
B	82	117	150	168	260
C	51	78	104	117	159
D	56	80	111	142	187

The screening method allows the detection of 1 % (peptide B) or 1.5 % (peptides A, C and D) meat substitution by porcine plasma powder in emulsion-type pork sausages by a comparative measurement of the peak areas of the four plasma peptides (error probability: 2.5 %).

Conclusion

The HPLC-MS/MS method developed allows detection of the addition of porcine plasma powder in emulsion-type sausages of the same animal species using characteristic tryptic peptides. Through the combination of protein extraction and tryptic digestion in a one-pot process, the time for sample preparation was significantly reduced. Furthermore, an LC/MS measuring time as short as 13 min was achieved by a short HPLC column and a high flowrate, which enables a high throughput. The method allows the detection of 1.0 to 1.5 % meat substitution by porcine plasma in emulsion-type pork sausages with an error probability of 2.5 %.

References

1. Kim, S., S. Jin & J. Choi. (2017). Journal of the Science of Food and Agriculture 97: 4501-4507.
2. Warriss, P.D. (1984). Veterinary Record 115: 292-295.

Notes