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Meat Authentication: A New HPLC–MS/MS Based Method for the Fast and Sensitive Detection of Horse and Pork in Highly Processed Food

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Supporting Information

ABSTRACT: Fraudulent blending of food products with meat from undeclared species is a problem on a global scale, as exemplified by the European horse meat scandal in 2013. Routinely used methods such as ELISA and PCR can suffer from limited sensitivity or specificity when processed food samples are analyzed. In this study, we have developed an optimized method for the detection of horse and pork in different processed food matrices using MRM and MRM^3 detection of species-specific tryptic marker peptides. Identified marker peptides were sufficiently stable to resist thermal processing of different meat products and thus allow the sensitive and specific detection of pork or horse in processed food down to 0.24% in a beef matrix system. In addition, we were able to establish a rapid 2-min extraction protocol for the efficient protein extraction from processed food using high molar urea and thiourea buffers. Together, we present here the specific and sensitive detection of horse and pork meat in different processed food matrices using MRM-based detection of marker peptides. Notably, prefractionation of proteins using 2D-PAGE or off-gel fractionation is not necessary. The presented method is therefore easily applicable in analytical routine laboratories without dedicated proteomics background.

KEYWORDS: *meat authentication, mass spectrometry, MRM, MRM^3, halal, tryptic digest, marker peptides*

INTRODUCTION

Food fraud is a problem on global scale, as exemplified by the European "horse meat scandal" in 2013. The fraudulent blending of undeclared meat into food products not only affects consumer rights from the economic point of view. In addition, it might be a significant problem for people with ethical or religious concerns regarding the consumption of species such as horse or pork. For these consumers, products containing only trace amounts of pork or horse meat, introduced into the product by accidental contamination, are problematic.

Consumers and the food industry are increasingly aware of this problem, and the specific detection of trace amounts of certain meat species in processed food is still problematic. Presently, ELISA and PCR methods are routinely used for the species authentication. Enzyme linked immuno sorbent assays (ELISA) can be used as a fast qualitative analysis systems on the production site (test-stripe based) or as a quantitative method.[1,2] In general, ELISA can also be applied for processed samples and, depending on processing and sample composition, shows LOD down to 0.5%.[3] However, specificity of ELISA in processed and highly processed samples can be critical, resulting in false positive or false negative results. In addition, multiplexing (detection and quantification of more than one species per experiment) is still problematic with ELISA.

The second method is the polymerase chain reaction (PCR), which is widely distributed in routine laboratories for meat speciation.[4] Recently published PCR methods show excellent sensitivity in unprocessed products. As an example, Soares et al. were able to detect 0.1% of raw pork in poultry.[5] One of the major drawbacks of PCR is the sensitivity of DNA to food processing. Though some authors showed that the use of small mitochondrial DNA templates can increase the robustness of PCR in processed foods,[6] others showed that the DNA template is degraded even by mild heating.[7] In addition, even mitochondrial DNA can be prone to degradation.[8−11] One advantage of PCR compared to ELISA is the possibility to develop multiplex methods. Köppel and colleagues described a multiplex PCR method with a LOD of 1% that is applicable also in mildly boiled products such as sausages.[12]

Mass spectrometry (MS)-based proteomics methods only quite recently entered the field of meat authentication. In general, specific proteins or peptides (generated after tryptic digest) are detected in MS methods. Notably, the primary structure of proteins is, in general, quite stable against processing.[13] Furthermore, a limited degree of protein degradation is often less critical for the detection of species-specific peptides than DNA shearing for the PCR detection, as fragmentation events within the relatively short peptide sequences are less likely compared to degradation of the longer DNA templates.

Initially, laborious gel-based separation of food proteomes was performed followed by protein identification using MALDI-TOF-MS.[14−16] Ongoing improvements in MS instrumentation now also allow for targeted proteomics approaches and the detection of species-specific peptides using multiple reaction monitoring (MRM). We have previously published the first MRM-based method for the detection of raw horse and pork in raw beef and chicken

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Table 1. Self-Made Samples As Described in Materials and Methods[a]

sample	type	processing type	species	declaration	state at purchase	source
S_1	frikadeller	frying	beef	roast from the hip	raw - chilled	supermarket Münster
S_2	meatballs	cooking				
S_3	meatloaf	baking				
S_4	frikadeller	frying	pork	roast from the topside	raw - chilled	supermarket Münster
S_5	meatballs	cooking				
S_6	meatloaf	baking				
S_7	frikadeller	frying	horse	roast	raw - chilled	farmer's market Münster
S_8	meatballs	cooking				
S_9	meatloaf	baking				

[a]All samples were screened with the described method.

using species-specific marker peptides.[17] Based on this method, we present here an optimized approach for the detection of specific horse meat and pork marker peptides in different processed food samples.

MATERIALS AND METHODS

Chemicals. LC-MS grade methanol (MeOH) and acetonitrile (ACN) were purchased from Sigma-Aldrich. Formic acid (FA) was purchased from Grüssing (Filsum, Germany). Water (H_2O) was prepared on a Milli-Q Gradient A10 system by Millipore (Schwalbach, Germany) and used for all buffers and other solutions including HPLC eluents.

Commercially Available Samples. All samples were purchased from German or Dutch supermarkets, butcher shops and farmer's markets, and directly stored at −20 °C in Corning (Amsterdam, Netherlands) 50-mL plastic centrifuge tubes. For self-made samples, pure beef, pork, and horse meat were bought as whole pieces to avoid contamination. Sample details are given in Table 1 for in-house prepared samples and in Supporting Information (SI) Table 1 for commercially available samples.

Preparation of In-House Processed Samples. The meat was cut into slices and minced using an electric meat grinder. The minced meat was formed to meat loafs, and frikadeller, a common European product similar to a fried hamburger patty, or meatballs. The meat loaf was placed in a porcelain tray and heated in a conventional kitchen oven to 200 °C until a core temperature of 100 °C was reached. Frikadeller were fried in a Teflon-coated pan until a brown crust typical for this dish was formed. The meatball was cooked in gently boiling water until a core temperature of 100 °C was reached.

For all in-house processed meat products no ingredients other than meat were used.

Products were cooled to room temperature and were precrushed in a freezing bag. The precrushed pieces were frozen in liquid nitrogen until the liquid nitrogen stopped boiling. Frozen pieces were ground at 8000 rpm for about 5 min in a Fritsch Pulverisette 14 rotor mill (Idar-Oberstein, Germany) with a 2-mm sieve. The powdered samples were stored at −20 °C in 50-mL plastic centrifuge tubes.

Preparation of Sample Mixtures. Samples containing more than one meat species were either prepared by weighing different amounts of ground pork or horse meat in ground beef followed by extraction of the mixture or by mixing desalted peptide solutions of relevant species (for details see Results and Discussion).

Powdered samples that contained at least 1.0 mg of pork or horse meat as contamination were prepared directly by weighing the different meat species directly in a 50-mL Corning tube on an analytical scale and were further prepared as described under sections "Extraction", "Digest", and "Desalting".

Samples that contained below 1.0 mg pork or horse meat (absolute amounts) were prepared by mixing desalted peptide solutions of respective species. In this case, pure beef (matrix) or either pork or horse meat (contamination) were processed as described below. A defined amount of the resulting, desalted pure beef peptide solution was spiked with either pure, desalted pork or horse meat peptide solution.

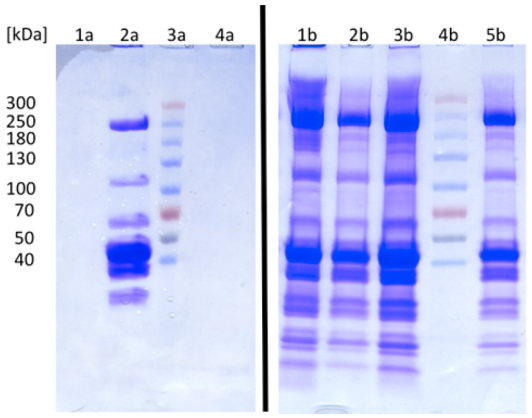

Figure 1. SDS-PAGE Gel showing the previously used extraction method (0.3 M KCl, 0.15 M KH_2PO_4, and 0.15 M K_2HPO_4) for meat samples (left gel, lanes marked #a) and the newly developed extraction method (6 M urea, 1 M thiourea, and 50 mM Tris-HCl) for processed samples (right gel, lanes marked #b). Loading scheme (in brackets volume of sample extract); all samples were made from pure pork; 1a: meatball (8 μL); 2a: raw (8 μL); 3a: marker; 4a: frikadeller (4 μL); 1b: raw (4 μL); 2b: meatball (2 μL); 3b: meatball (4 μL); 4b: marker; 5b: frikadeller (2 μL).

Preparation of Commercial Processed Samples. Salamis, sausages, meatballs, canned meat, and Frikandeln (Dutch convenience product similar to sausages) were diced to approximately 2 cm length with a kitchen knife.

Pasta sauce was removed from products by washing them with water on a lab sieve. The residue consisting of small meat and vegetable pieces was homogenized using an Ultra Turrax T-25 basic (ika, Staufen, Germany) with a 10 N dispersing element.

In the case of frozen lasagna and chilled pasta, Bolognese noodles and sauce were removed and the residual samples were also homogenized.

Extraction. Approximately 1 g of sample material was weighed in 50-mL plastic centrifuge tube, and 10 mL of extraction buffer (6 M urea, 1 M thiourea, and 50 mM TRIS-HCl (all Carl Roth, Karlsruhe, Germany) adjusted to pH 8) were added. For samples with a low overall meat content in the sample, this amount was slightly increased to approximately 1.4 g.

All samples were vortexed for 20 s and extracted using an Ultra Turrax T-25 (ika, Staufen, Germany) with a 10 N dispersing element. Samples with a higher content of connective tissue, e.g. salami, were dispersed for 30 s at 9500 rpm, followed by 40 s at 13 500 rpm and finally 40 s at 20 500 rpm. For all other sample types, the last step at 20 500 rpm was omitted. Finally, all samples were vortexed again for 20 s.

Following extraction, samples were centrifuged for 60 min at 4 °C at 12 000*g*. A 100-μL aliquot of the supernatant was transferred into a 1.5-mL Eppendorf reaction tube (Hamburg, Germany)

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Precast 4−20% gradient gels (Mini-Protean TGX, Bio-Rad, München, Germany) were used to asses extraction efficiency. Running

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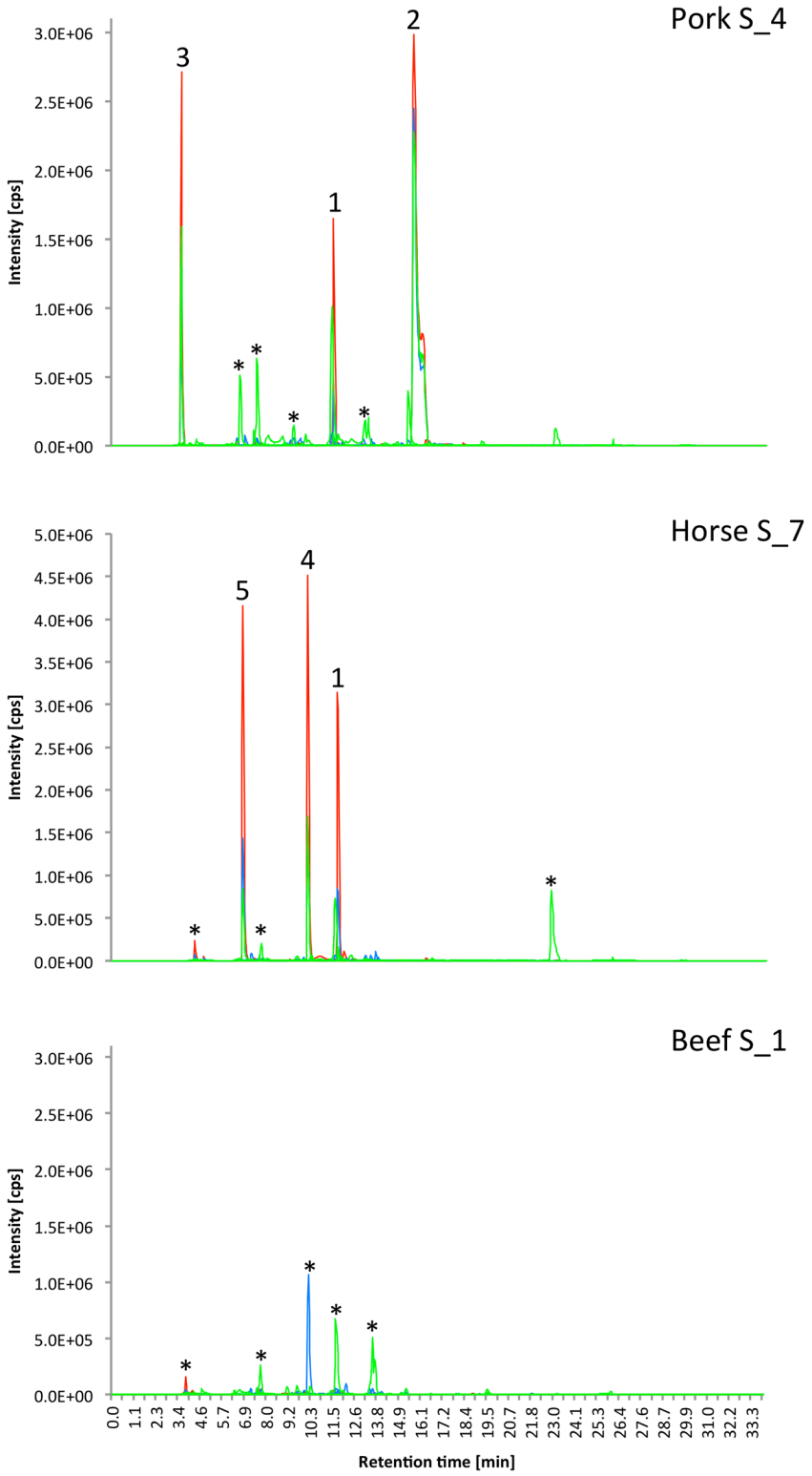

Figure 2. HPLC-MS/MS chromatograms of frikadeller samples made from pure pork, beef, or horse meat. To reduce complexity, only the three most intense MRM traces are shown for every peptide. The most intense transition is given in red, 2nd most intense is in blue, and 3rd most intense is in green. Unspecific signals are marked with asterisk. Markers are numbered according to Table 2 (for MS and HPLC parameters see SI Table 2).

buffer and sample buffer were prepared according to Lämmli.[18] SDS, Glycin, and TRIS-HCl were purchased from Roth in Bufferan quality. The protein molecular weight standard used was the Spectra Multicolor High Range Protein Ladder (Thermo Fisher Scientific, Rockford, IL, USA)

Briefly, samples were diluted with water and sample buffer, denatured at 95 °C for 5 min. Following sample application, SDS-gels were focused for 20 min at 100 V and then run at 140 V until the bromophenol blue front reached the end of the gel. Gels were fixed and stained using Coomassie brilliant blue dye for 25 min and destained. Gel pictures were taken on a standard flatbed scanner for personal computers.

Digest. An aliquot of the extract of the processed meat samples was subjected to tryptic digest following a standard protocol.[14] For each

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Table 2. Characteristic Marker Peptides for Horse and Pork[a]

marker	species	name	uniprot accession	sequence	AA position
1	pig/horse	troponin T/uncharacterized protein	Q75NG7/F6 × 010	YDIINLR	239−245/185−191
2	pig	myosin-4	Q9TV62	TLAFLFAER	619−627
3	pig	myosin-1 and -4	Q9TV61/Q9TV62	SALAHAVQSSR	1331−1341/1329−1339
4	horse	myosin-2	Q8MJV1	EFEIGNLQSK	1086−1095
5	horse	myosin-1	Q8MJV0	LVNDLTGQR	1272−1280

[a]Markers 1, 2, and 4 are the most sensitive and are displayed in the subsequent figures. AA position: Position of the biomarker peptide in the sequence of the identified protein.

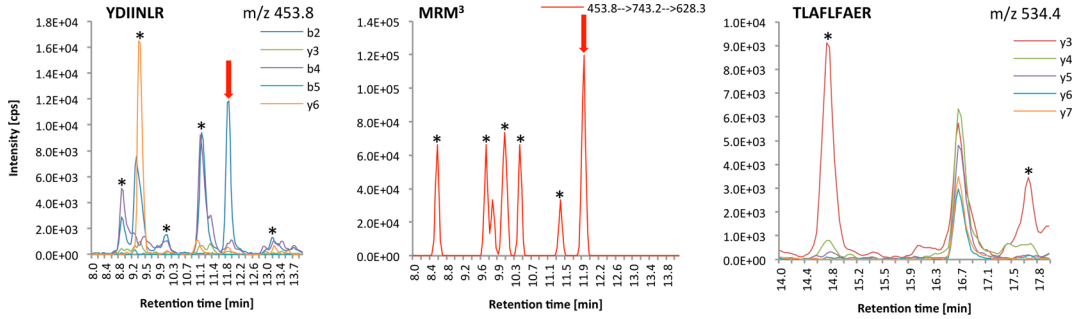

Figure 3. HPLC-MS/MS and MRM^3 chromatograms of a beef frikadeller spiked with 0.51% of pork frikadeller (mixed peptide solutions). Shown are the horse/pork-specific marker 1 (YDIINLR) and the pork-specific marker 2 (TLAFLFAER). For marker 1, four MRM transitions are detectable, but the s/n for y6 is only 3. However, the MRM^3 signal was detectable, showing a huge improvement for the sensitivity. For marker 2, all MRM transitions, but with a low overall intensity defining the LOD for this marker, were detectable. Unspecific signals have been marked with asterisks.

sample, an aliquot of 100 μL containing about 1.5 mg of protein was reduced, alkylated, diluted 1:10 with H_2O, and supplemented with 20 μg of Promega Sequencing grade modified trypsin (Promega, Mannheim). To allow complete digest, samples were incubated in a thermo shaker at 37 °C under slow shaking for at least 12 h.

Desalting. Digested samples were diluted 1:2 with H_2O and desalted using Phenomenex (Aschaffenburg, Germany) Strata-X 33u polymeric reversed phase cartridges filled with 30 mg/mL RP material. Cartridges were washed and activated using 1 mL of MeOH followed by equilibration with 1 mL of 1% FA according to the manual. The samples were loaded onto the cartridge and washed with 1 mL of 5% MeOH/1% FA. Finally, peptides were eluted with 1 mL of ACN/H_2O (90/10, 0.1% FA) and the eluate was collected in Eppendorf reaction tubes prefilled with 6 μL of dimethyl sulfoxide (DMSO) purchased from Roth (Karlsruhe, Germany).

Preparation for HPLC. After desalting, the solvent was removed under vacuum at 39 °C using an S-Concentrator BA-VC-300H purchased from H. Saur Laborbedarf (Reutlingen, Germany). DMSO is not removed during this procedure, which prevents peptides from sticking to the vessel surface and enhances peptide recovery. The samples were redissolved in ACN/H_2O (3/97; 0.1% FA) and analyzed.

MS/MS. All samples were analyzed using a QTrap 5500 LC-MS/MS system (AB SCIEX, Darmstadt, Germany) with a VWR Hitachi HPLC (Pump L-2160U; autosampler L-2200U and column oven L-2300) and a Phenomenex Kinetex 2.6 u C18 100 Å (100 × 2.10 mm) column (Phenomenex, Aschaffenburg, Germany). A Turbo V ESI source was used. For details on HPLC gradient, source, and MS parameters see SI Table 2.

Data were evaluated using Analyst Software Version 1.5.2.

To survey retention time stability and relative intensity of MRM transitions, control samples of respective species were routinely analyzed accordingly.

A more detailed and basic description of the MS experiments is given directly after SI Table 2.

RESULTS AND DISCUSSION

One of the most critical steps for the analysis of processed samples in targeted proteomics is efficient sample extraction. In our previous publication, we applied mild extraction conditions to reduce complexity of the extracted proteome and to focus on the readily soluble and highly abundant proteins of the myofibrillar and sarcoplasmic fraction in raw sample material.[17] Although target proteins in raw meat are soluble in low molar salt buffers (0.3 M KCl, 0.15 M KH_2PO_4, and 0.15 M K_2HPO_4)(Figure 1, lane 2a), heat treatment such as frying or boiling strongly reduced solubility for different meat products and led to lack of solubility of target proteins (Figure 1, lanes 1a and 4a). To overcome this problem, 11 different buffer compositions in combination with different extraction protocols such as vigorous shaking, vortexing, or the use of Ultra Turrax were assessed concerning efficiency of extraction and comparability with downstream sample preparation (data not shown) and best results were observed with 6 M urea, 1 M thiourea, and 50 mM Tris-HCl (see Materials and Methods for details). This extraction buffer gives excellent results for raw and for different processed samples as can be seen from the right gel in Figure 1 (lanes 1b−5b). In addition, the extraction time was strongly reduced to only approximately 2 min and a simple 10-fold dilution of the extract is sufficient to reduce urea and thiourea concentrations to allow tryptic digest.

It has, however, to be kept in mind that sample complexity is increased after processing and harsher extraction. We therefore assessed specificity and sensitivity of biomarker peptides under the modified conditions using pure, in-house processed beef, pork, and horse meat. Figure 2 and Table 2 give an overview of the five previously described[17] most important pork and horse meat biomarker peptides, demonstrating excellent specificity of the modified method. As expected, the specific horse and pork markers were not detectable in the beef sample. Though unspecific single MRM transitions occur, the specific marker peptides are only observed in the according samples and show retention time match, as well as matching MRM signal ratios and the full spectrum of MRM transitions. (See SI Table 3 for MRM Transitions and MS conditions). Similar results were observed for all in-house processed sample types (data not

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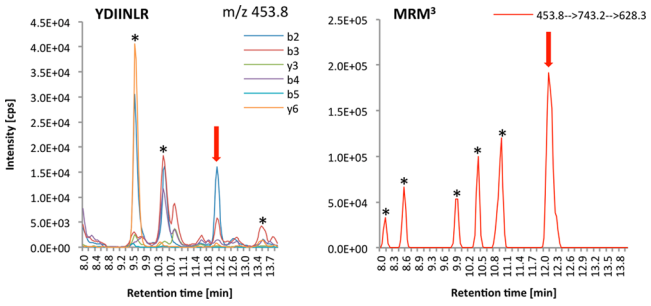

Figure 4. HPLC-MS/MS and MRM^3 chromatograms of a beef meatball spiked with 0.25% of horse meat ball (mixed peptide solutions). Shown is the most sensitive marker, the horse/pork-specific marker 1 (YDIINLR) and its corresponding MRM^3 experiment. Compared to Figure 3 it is obvious that the meatball matrix is less complex resulting in better overall intensities at lower concentration. Unspecific signals have been marked with asterisks.

shown), indicating that heat processing in general does not interfere with critical steps of our method (extraction, tryptic digest, MS analysis).

We next analyzed the sensitivity of the modified method using in-house processed samples containing trace amounts of pork and horse meat, respectively. At present, no official thresholds levels are in place for undeclared meat species. We therefore used the threshold level of 0.9% for genetically modified organisms (GMO) issued in Regulation (EC) 1830/2003 of the European Union[19] as orientation for "technically unavoidable" contaminations and the minimal performance level of our method.

Figure 3 shows results for a fried frikadeller containing 0.51% pork in beef. Marker 1 (YDIINLR) is still detectable with four MRM transitions. In addition, MRM^3 experiments provide a significant improvement of the signal-to-noise ratio and further improve specificity. In addition, all 5 MRM transitions for marker 2 (TLAFLFAER) were detected, demonstrating the specific detection of pork in this sample. However, we noted the increase in unspecific signals (as marked by asterisk) in MRM and MRM^3 experiments for highly processed samples. Fried meat products had a deep brown surface due to pronounced Maillard reaction and respective peptide solutions showed a yellow to brown color indicative for complex modification of the protein matrix.

As expected, for milder processing techniques such as boiling sensitivity was increased compared to fried samples. Figure 4 shows the results for boiled meatballs at a concentration of 0.24% horse in beef matrix. This concentration was achieved by mixing peptide solution of pure horse and beef samples. At this concentration, only marker 1 (YDIINLR) was detectable in MRM and MRM^3 experiments. Again, four MRM transitions were detectable and MRM^3 improved s/n and specificity (Figure 4). Similarly, for respective samples containing 0.24% of pork, we identified marker 1 as most sensitive marker but not the other pork-specific peptides (data not shown).

Measurement of Commercially Available Processed Samples. We next assessed the applicability of the detection method on a broad range of commercially available processed products obtained from supermarkets and a local farmers market (see SI Table 1 for details). All samples were prepared and analyzed with the described method.

Figure 5 shows the results for frikadeller (sample C_12 in SI Table 1) that was labeled to contain only pork meat. All MRM transitions from all specific peptides (see SI Table 3 for details) expected for pork were found in this sample and none of the marker peptide markers for horse, demonstrating excellent specificity for this processed sample.

An example of a more complex food matrix is given in Figure 6 (sauce Bolognese, sample C_11, see details in SI Table 1). The total meat content of the sample was declared with 3% pork and 7% beef. Detection of YDIINLR and TLAFLFAER (markers 1 and 2) confirmed the pork meat content of the product. As expected, no horse-specific markers (EFEIGNLQSK and LVNDLTGQR) were detected in this product (Figure 6). An overview of the results of the 23 different commercial samples

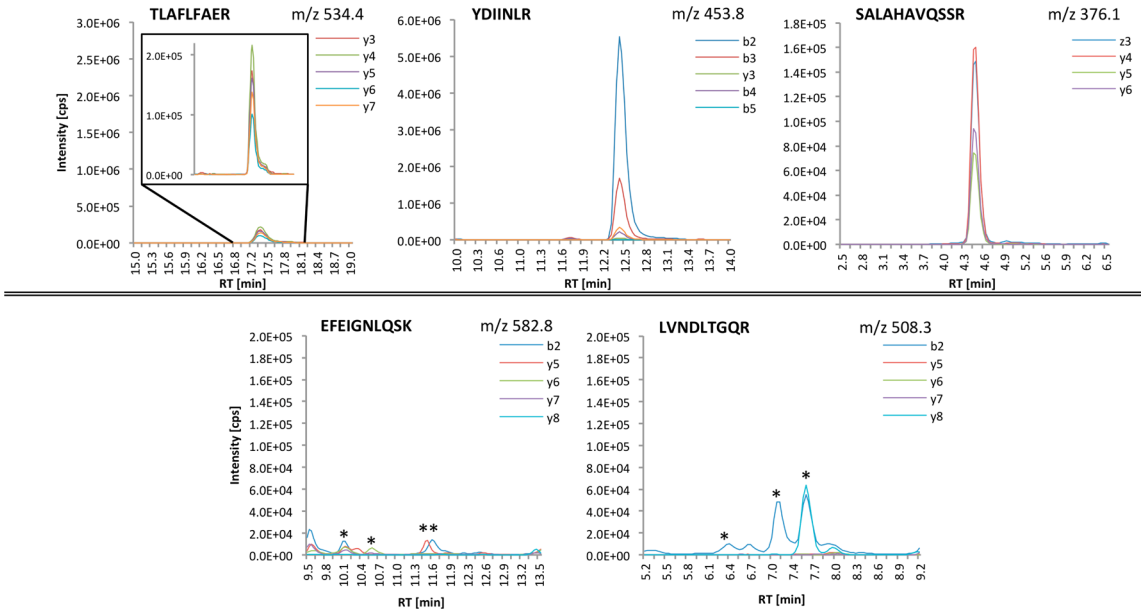

Figure 5. HPLC-MS/MS chromatograms of a commercial frikadeller (C_12) declared as pure pork. Shown are the horse/pork-specific marker 1 (YDIINLR), the pork-specific markers 2 (TLAFLFAER) and 3 (SALAHAVQSSR), and the horse-specific markers 4 (EFEIGNLQSK) and 5 (LVNDLTGQR). It is obvious that the sample C_12 contains pork and no horse was detected. Unspecific signals have been marked with asterisks.

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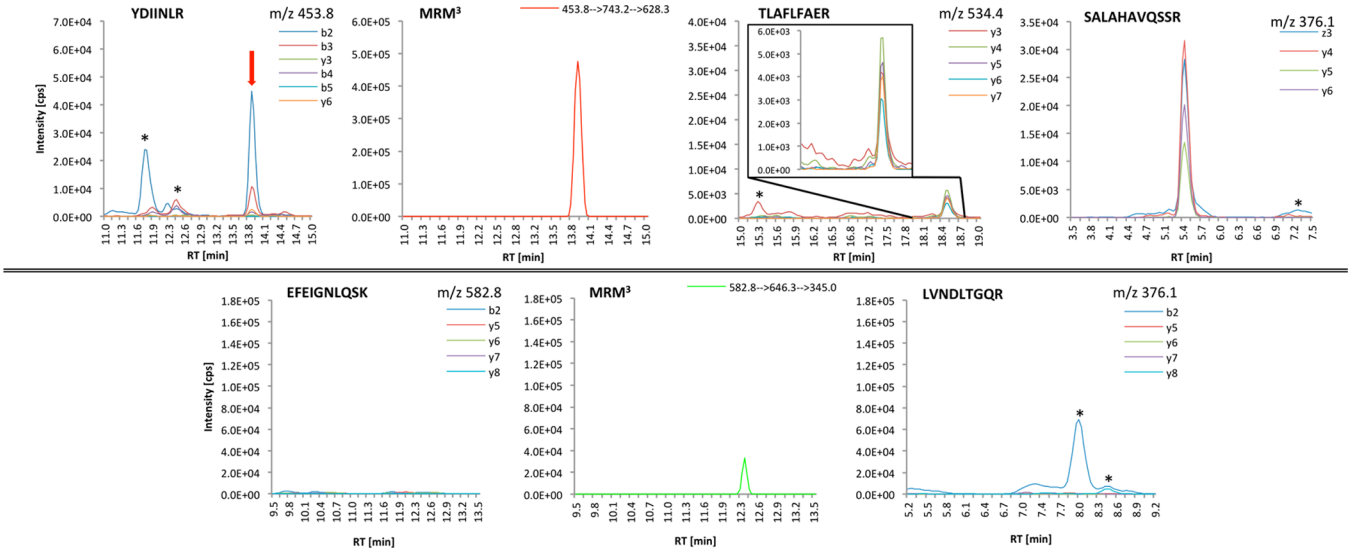

Figure 6. HPLC-MS/MS and MRM^3 chromatograms of a sauce Bolognese (C_11) declared as a mixture of beef and pork. Shown are the horse/pork-specific marker 1 (YDIINLR) with its corresponding MRM^3 experiment, the pork-specific markers 2 (TLAFLFAER) and 3 (SALAHAVQSSR), and the horse-specific markers 4 (EFEIGNLQSK) with its corresponding MRM^3 experiment and 5 (LVNDLTGQR). Unspecific signals have been marked with asterisks.

Table 3. Screening Results of Commercial Samples[a]

			pork		pork/horse		horse			
sample	declaration	species declared	SALAHAVQSSR	TLAFLFAER	YDIINLR	MRM^3	EFEIGNLQSK	MRM^3	LVNDLTGQR	declaration confirmed
C_1	corned beef A	beef	n.d.	n.d.	match	match	match	match	match	no
C_2	corned beef B	beef	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	yes
C_3	corned beef C	beef	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	yes
C_4	mini salami A	beef and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_5	pork in jus	pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_6	salami A	beef	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	yes
C_7	salami B	deer and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_8	salami C	pork and wild boar	match	match	match	match	n.d.	n.d.	n.d.	yes
C_9	mini salami B	pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_10	Bolognese sauce A	beef and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_11	Bolognese sauce B	beef and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_12	frikadeller	pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_13	hamburger patty	beef	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	yes
C_14	spaghetti Bolognese (sauce and noodles)	beef and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_15	lasagnese Bolognese	beef and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_16	meatball in sauce	beef and pork	match	match	match	match	n.d.	n.d.	n.d.	yes
C_17	mini salami C	horse	n.d.	n.d.	match	match	match	match	match	yes
C_18	frikandeln A	pork	match	match	match	match	n.d.		n.d.	yes
C_19	frikandeln B	pork and chicken	match	match	match	match	n.d.	n.d.	n.d.	yes
C_20	frikandeln C	pork and chicken	match	match	match	match	n.d.	n.d.	n.d.	yes
C_21	frikandel D	pork and chicken	n.d.	3 MRM	match	match	n.d.	n.d.	n.d.	(yes)
C_22	frankfurter sausage	horse	n.d.	n.d.	match	match	match	match	match	yes
C_23	salami D	horse	n.d.	n.d.	match	match	match	match	match	yes

[a]Match = MRM transitions were detectable at expected retention time with good intensities; n.d. = no MRM transitions were detectable.

analyzed in this study is given in Table 3 (sample details found in SI Table 1). Results of species identification and labeling was consistent for all analyzed samples except one corned beef sample (C_1, see Table 3). This sample was labeled to contain only beef material, but we detected horse-specific peptide markers using MRM and MRM^3 experiments, indicating unlabeled amounts of horse meat in this sample (Figure 7, upper chromatograms). We confirmed this result by the analysis

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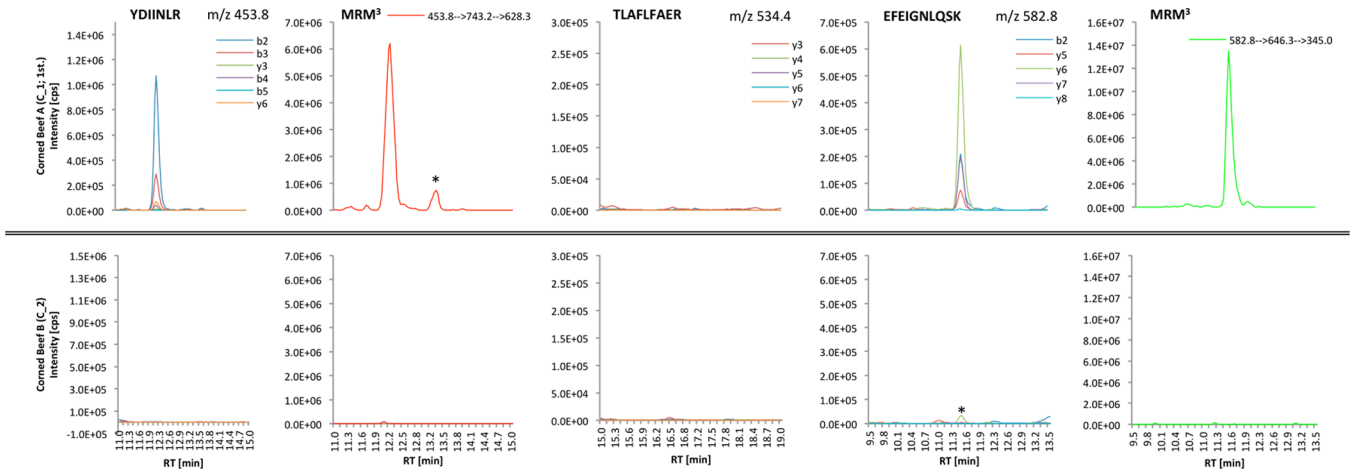

Figure 7. HPLC-MS/MS and MRM^3 chromatograms of corned beef (C_1) declared as a pure beef. Shown are the horse/pork-specific marker 1 (YDIINLR) with its corresponding MRM^3 experiment, the pork-specific marker 2 (TLAFLFAER), and the horse-specific marker 4 (EFEIGNLQSK) with its corresponding MRM^3 experiment. It is obvious that sample C_1 contains horse meat because marker 1 and marker 4 both with corresponding MRM^3 experiments were detectable. In sample C_2 (lower chromatograms), as declared, no horse or pork was detectable. Unspecific signals have been marked with asterisks.

of a biological replicate and analyzed two additional corned beef samples to rule out matrix effects of this sample material. As an example, results for corned beef sample C_2 are given in Figure 7 (lower chromatogram). Neither pork nor horse meat marker peptides were detectable, indicating that this sample contained only beef meat and that detection of horse in sample C_1 was not due to unspecific matrix effects (Figure 7). This was further underlined by use of droplet digital PCR to confirm the detection of horse meat in sample C_1 with a second analytical technique.

Taken together, we present in this study an optimized method for the specific detection of horse and pork meat in processed and highly processed food samples. Using MRM and the novel MRM^3 detection of specific marker peptides, we were able to detect down to 0.24% horse or pork in a beef meat matrix. When necessary, a further reduction of sensitivity (approximately factor 3−4) can be achieved by the application of microLC systems instead of high flow LC as shown previously.[18] We were able to demonstrate that the identified marker peptides are sufficiently stable to resist thermal processing of different types of meat products and thus allow specific and sensitive detection also in processed food. The optimization of protein extraction had major impact on method performance, as demonstrated by the systematic evaluation of different extraction protocols. Optimal results were achieved by use of high molar urea and thiourea buffer systems. In addition, streamlining of extraction and sample preparation allowed us to drastically reduce extraction time to 20 s up to 2 min, depending on sample type. It is one of the huge advantages of this method that no special proteomics infrastructure such as 2D-PAGE or offgel-fractionation is necessary for prefractionation of samples and that separation and detection is performed on conventional C18 RP-columns and triple quadrupole instumentation. The protocol can therefore easily be implemented in routine analysis of laboratories without specific proteomics knowledge.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional tables and experimental details as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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