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Qualitative and quantitative analysis of pork in beef food with LC-MS/MS

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Introduction

Food fraud is a problem on global scale, especially the meat fraud. Such as, horse meat was added in the beef food, and pork was used as beef. Consumers and the food industry are increasingly aware of this problem, and the specific detection of amounts of certain meat species in processed food is still problematic. Presently, ELISA and PCR methods are routinely used for the species authentication. As we know, The ELISA method often results in false positive or false negative results, and the PCR method is not so accuracy for DNA being prone to degradation. So the characteristic peptides multiple reaction monitoring (MRM) method by LC-MS/MS is a good choice for the qualitative and quantitative analysis of meat authentication.

Methods and Materials

Sample preparation:

The meat was cut into slices and minced using an electric meat grinder. Approximately 2 g of sample material (beef spiked with pork at the ratio of 5%, 10%, 20%, 40%, 60% and 80%) was weighed in a centrifuge tube (50 mL), and 10 mL of extraction buffer (7 M urea, 2 M thiourea, and 50 mM Tris-HCl, pH 8) was added. All samples were vortexed for 20 s and extracted using an Ultra Turrax T-25 (IKA, Germany) with a 10 N dispersing element. Samples were dispersed for 30 s at 8000 rpm, followed by 30 s at 9000 rpm and finally 30 s at 11000 rpm. Following extraction, samples were centrifuged for 30 min at 4 °C at 20000 rpm. After extraction, the samples were reacted with DTT and IAA, and then digested with trypsin. After digestion, the samples were pretreated with HLB SPE column to obtain the samples for analysis.

Analysis conditions

Instruments	: LC-30A+LCMS-8050
LC condition	
Column	: Shim-pack GISS (2.0 mm I.D.×150 mm L., 2.1 µm) ;
Mobile phase	: A-water+0.1% formic acid ; B-ACN+0.1% formic acid ;
Binary gradient	: 5%B (0 min)-50%B (12 min)-80%B (12.01-14 min)- 5%B (14.5 min)-Stop (18 min);
Flow rate	: 0.3 mL/min;
Column temperature	: 40 °C;
Injection volume	: 5.0 µL
MS condition	
Ion type	: ESI+;
Scan mode	: MRM;
Interface temp.	: 300 °C
DL temp.	: 250 °C
Heating block temp.	: 400 °C
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
Detector voltage	: Tuning result
Dwell time	: 10-20 ms

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Figure 1. Shimadzu LCMS-8050

Result

CE energy optimizing for characteristic peptides

According to the related report, these four peptides SALAHAVQSSR, TLAFLFAER, YDIINLR, LVVITAGAR were selected as the characteristic peptides for port, and TLALLFSGPASGEAEGGPK, EASGPINFTVFLNMFGEK, HPSDFGADAQAAMSK, ALEDQLSELK, LVIITAGARF were selected as the characteristic peptides for beef. In order to establish good quantitative method, the CE energy was optimized with the software Skyline (Figure 2 and 3). In consideration of the optimizing results and the matrix effect the characteristic peptides TLAFLFAER (m/z 534.30>853.45) and TLALLFSGPASGEAEGGPK (m/z 901.45>1290.60) were selected as the quantitative peptides for pork and beef, respectively.

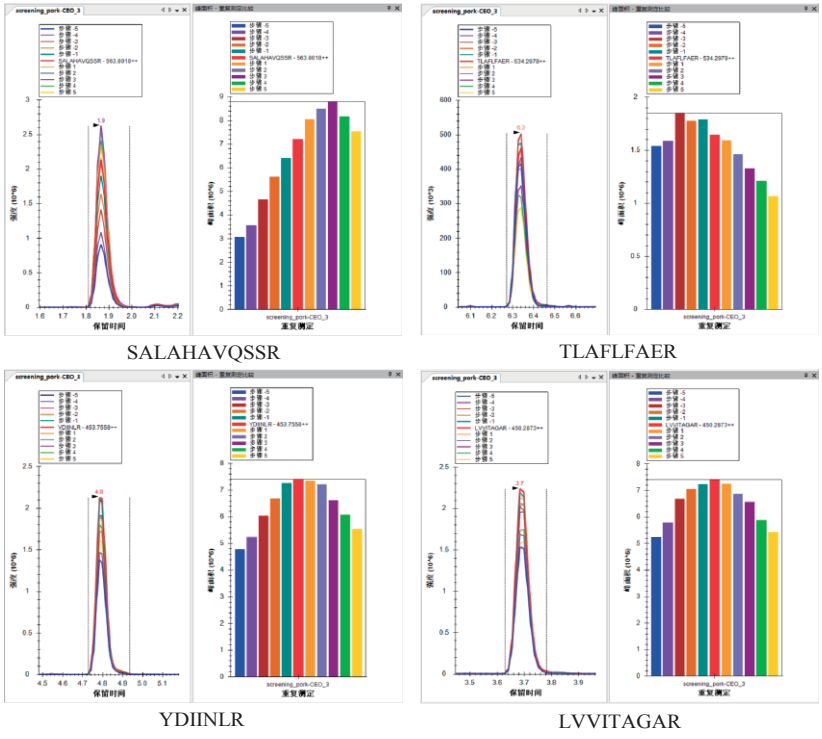

Figure 2. CE energy optimizing with Skyline (characteristic peptides for pork)

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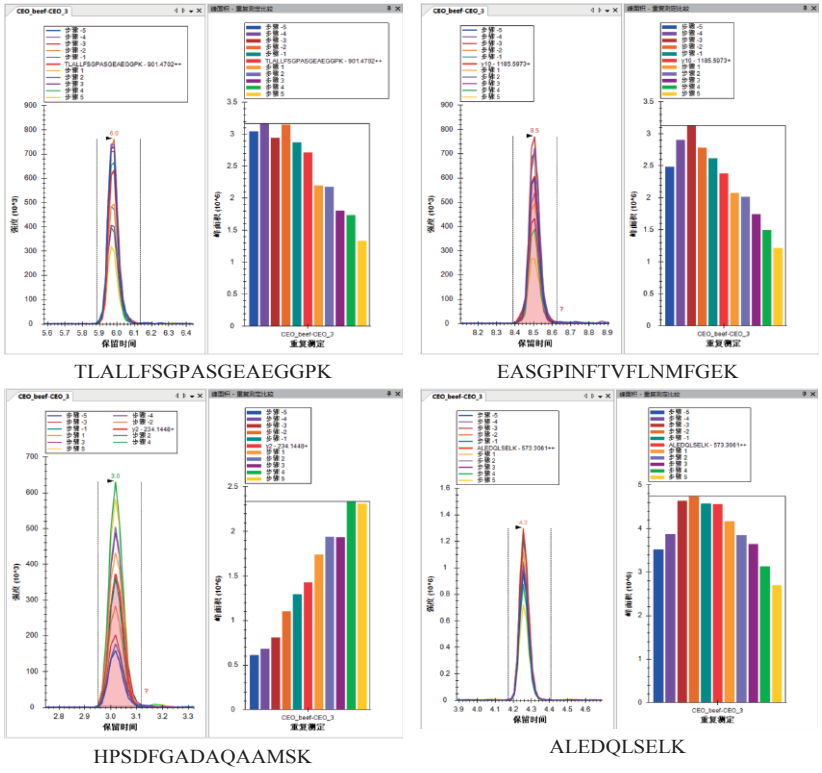

Figure 3. CE energy optimizing with Skyline (characteristic peptides for beef)

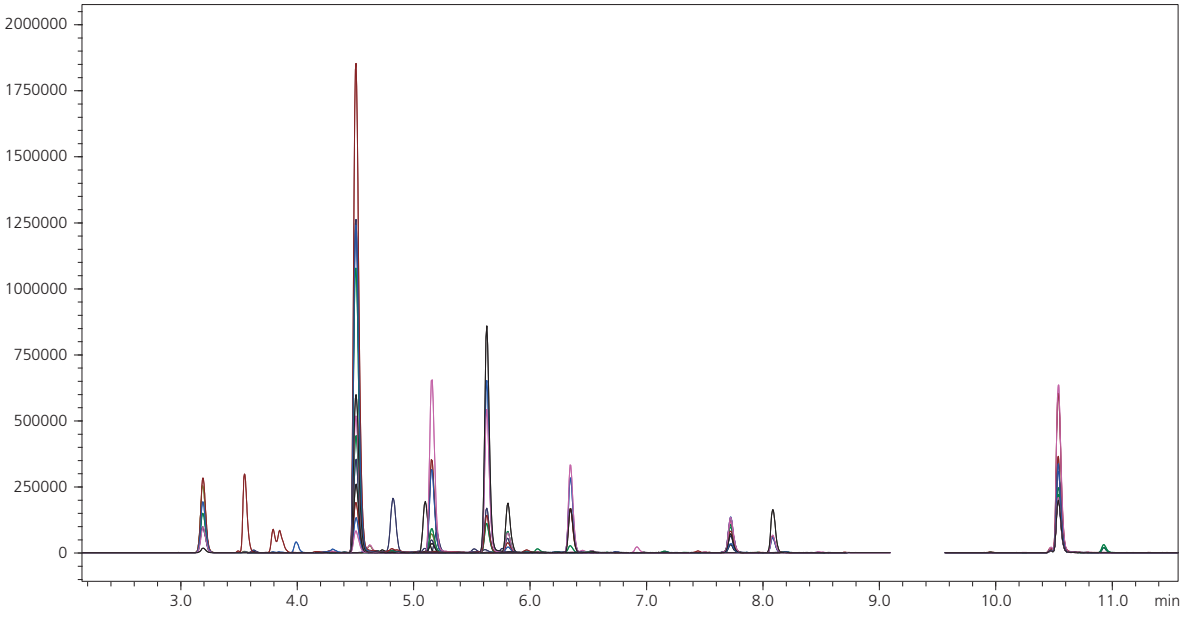

Figure 4. MRM chromatogram of beef with 40% pork in it

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Construction of calibration curve for pork and beef

Different amounts of pork and beef were weighed according to the ratio 5/95, 10/90, 20/80, 40/60, 60/40, 80/20, so the calibration curve for the pork was 5%, 10%, 20%, 40%, 60% and 80%, and for the beef was 20%, 40%, 60%, 80%, 90% and 95%. The result indicated that the accuracy for the pork calibration curve was 87.4~110.2%, and for the beef calibration curve was 95.2~104.9%, and the correlation coefficient (r value) is 0.9960 and 0.9982 for the pork and beef calibration curve, respectively.

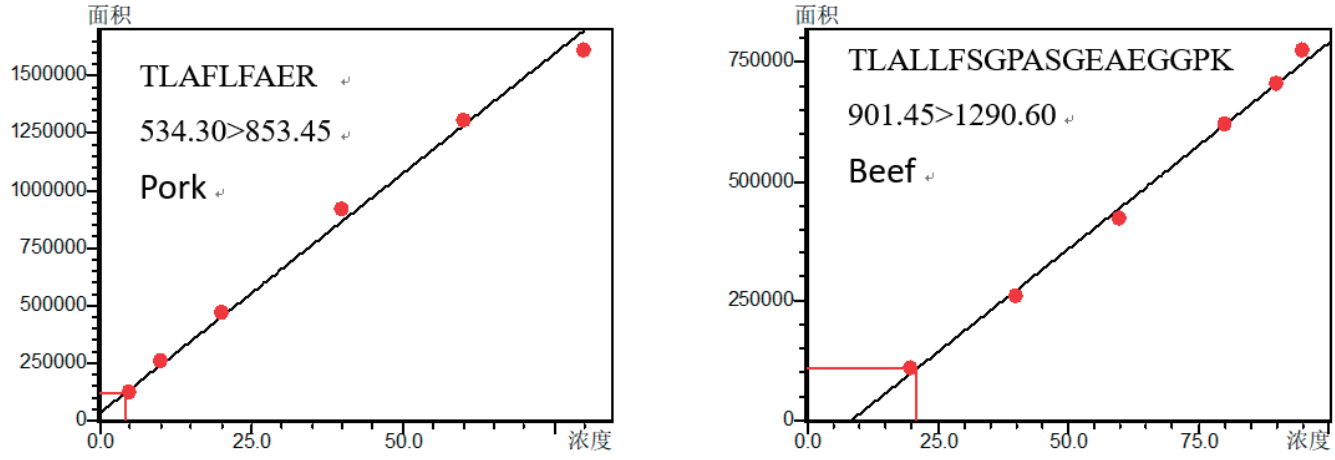

Figure 5. Calibration curve for the characteristic peptides of pork and beef

Meat	Characteristic peptides	Calibration Curve	Linearity range	r	Accuracy (%)
Pork	TLAFLFAER	Y = (9580.83)X + (32460.7)	5~80%	0.9960	87.4~110.2
Beef	TLALLFSGPASGEAEGGPK	Y = (75581.2)X + (452441)	20~95%	0.9982	95.2~104.9

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Conclusions

In this paper, 4 characteristic peptides were selected for each kind of meat. The peptides SALAHAVQSSR, TLAFLFAER, YDIINLR and LVVITAGAR were characteristic for pork, and the peptides TLALLFSGPASGEAEGGPK, EASGPINFTVFLNMFGEK, HPSDFGADAQAAMSK, ALEDQLSELK and LVIITAGAR were characteristic for beef. All these peptides were imported to the Skyline software for the ionic transition selection and CE energy optimization. The peptides TLAFLFAER and TLALLFSGPASGEAEGGPK were selected for the quantitative peptides of the pork and beef, and the quantitative transition for these two peptides were m/z 534.30>853.45 and m/z 901.45>1290.60. Different amounts of pork and beef were weighed according to the ratio 5/95, 10/90, 20/80, 40/60, 60/40, 80/20, so the calibration curve for the pork was 5%, 10%, 20%, 40%, 60% and 80%, and for the beef was 20%, 40%, 60%, 80%, 90% and 95%. The result indicated that the accuracy for the pork calibration curve was 87.4~110.2%, and for the beef calibration curve was 95.2~104.9%, and the correlation coefficient (r value) is 0.9960 and 0.9982 for the pork and beef calibration curve, respectively.

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