APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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A Selective LC/MS/MS Method for Analysis of Pork Adulterated Meat by the QSight Triple Quad System

Introduction

In recent years, the addition of unauthorized materials into food products has been a major concern among consumers. For example, consumption of pork and/or products made from pork is strictly forbidden in Islam. Thus, it is imperative to develop a method that can identify and quantify the biomarkers specific for pork meat and pork related products.[1,2] In this study, a selective and sensitive LC/MS/MS method has been developed using three peptides specific for pork as biomarkers for fast identification and analysis of meat samples.

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Experimental

Sample Preparation

Ground meats or meat products (2 g) were dissolved in 5 mL of extraction solution.[1-4] Samples were vortexed and centrifuged for 10 min with 12000 rpm/min. After cooling to room temperature, 0.5 mL of supernatant was reduced by dithiothreitol, alkylated with iodoacetamide in a dark place, diluted with 800 µL of 25 mmol/L Tris-HCl (pH 8.0) and supplemented with sequencing grade modified trypsin. To allow for complete digestion, samples were incubated in a thermos-shaker at 37 °C under slow shaking overnight. The enzymatic activity was quenched with 20 µL of formic acid. Digested samples were desalted and concentrated using SPE cartridges. The SPE eluents containing the peptides were dried to reduce the volume and then filtered through a 0.22µm membrane filter prior to LC/MS/MS analysis.

LC Separation

The analytes were separated on a PerkinElmer QSight® LX-50 UHPLC system using a C18 column. The temperature of the column oven was set at 40 °C. The mobile phases consisted of water (A) and acetonitrile (B), both containing 0.1% formic acid. The flow rate was 0.4 mL/min and the elution gradient is shown in Table 1. The injection volume was 10 µL.

MS Conditions

The QSight LX-50 UHPLC system was coupled to a QSight 220 triple quadrupole mass spectrometer equipped with an electrospray ionization source operating in positive ion mode.

Table 1. LC eluent gradient.

Time (min)	Flow Rate (mL/min)	A (%)	B (%)
0	0.4	98	2
0.8	0.4	98	2
14	0.4	60	40
15	0.4	2	98
17.5	0.4	2	98
17.6	0.4	98	2
20	0.4	98	2

The mass spectrometer operating conditions were as follows: ElectroSpray Voltage: 5000 v, Heating Gas Temp: 400 °C, HSID Temp: 320 °C, Dry Gas: 100, Nebulizer Gas: 180. Detection of analytes by tandem mass spectrometry was conducted in multiple reaction monitoring mode (MRM). Three specific peptides (each with 2 MRM transitions) were used to analyze samples in a single injection. Data acquisition and processing was performed using PerkinElmer Simplicity™ 3Q software.

Results and Discussion

The specificity of the three selected peptides based on their unique MRM transitions in pork was tested in a raw pork matrix (Figure 1), and compared with other meat matrices, such as beef, lamb, chicken, and duck (Figure 2). The results demonstrated that the three peptides were unique and specific for pork, and could be used as biomarker for pork and pork originated products.

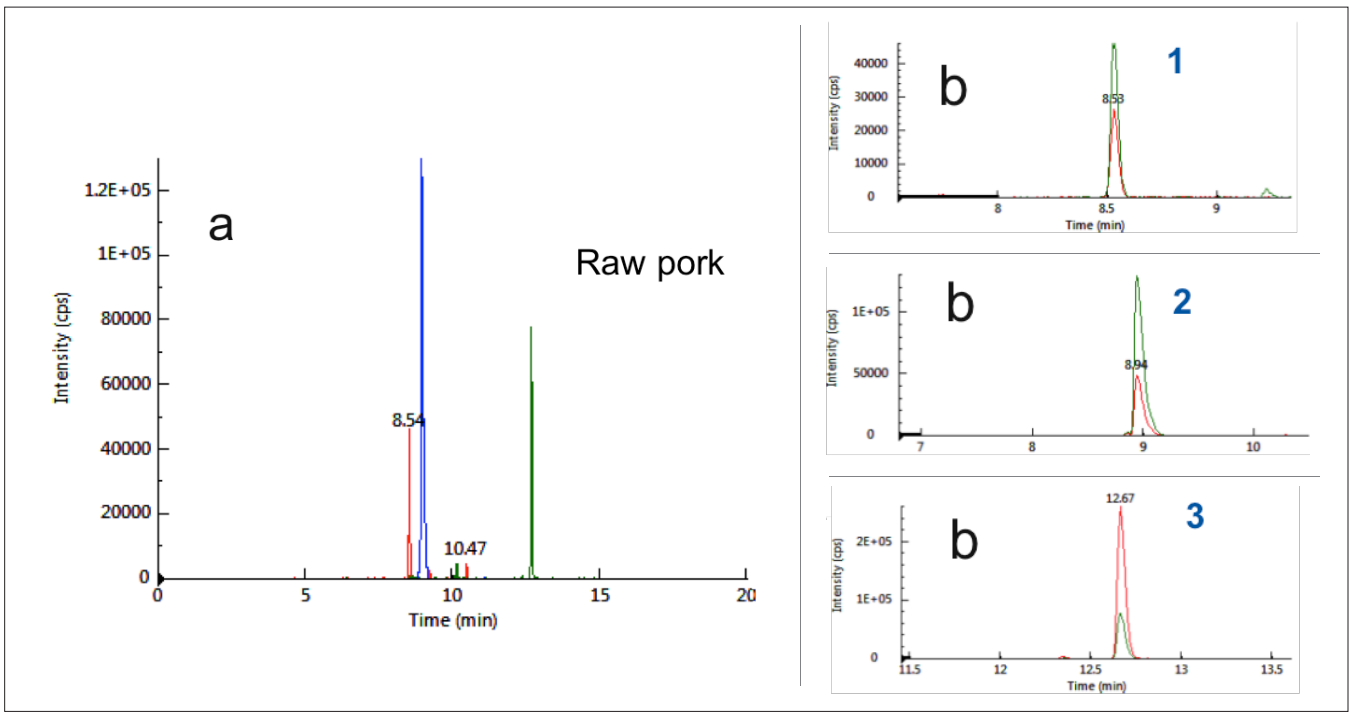

Figure 1. Typical chromatograms of raw pork as acquired by MRM mode: (a) Total ion chromatograms (b) Extract ion chromatograms. Notes: 1:Pork_Peptide_1, Rt 8.5 min; 2:Pork_Peptide_2, Rt 8.9 min; 3:Pork_Peptide_3 , Rt 12.7min.

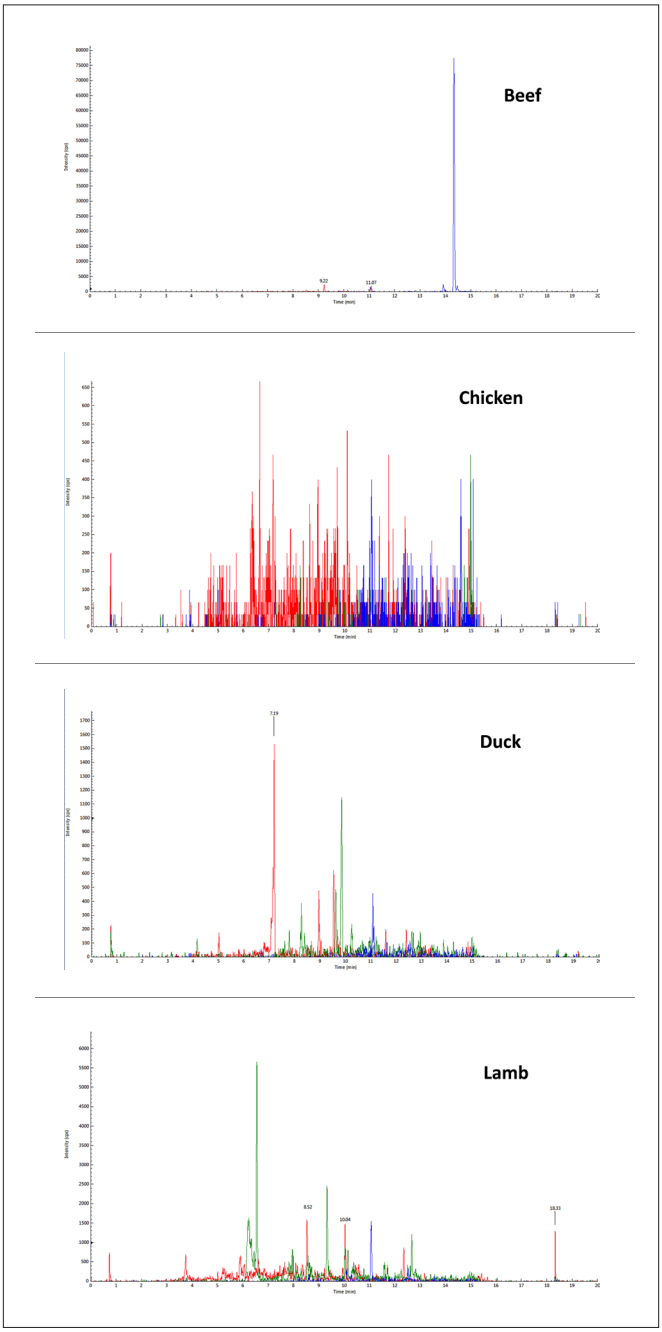

Figure 2. Typical chromatograms of other meat matrices as acquired by MRM mode: chicken, duck, lamb, beef.

The thermal stability of the biomarker peptides were evaluated by testing their MRM responses in raw and cooked pork (hard-boiled). As shown in Figure 3, each peptide was detected without significant changes in sensitivity before and after cooking, indicating their thermal stability.

Calibration curves for each of the three peptides were generated over a wide concentration range (1 to 100% w/w) with good linear response in the pork sample (Figure 4). Limits of quantification (LOQ) values for this method is 1% (w/w) spiked pork in the meat mixture.

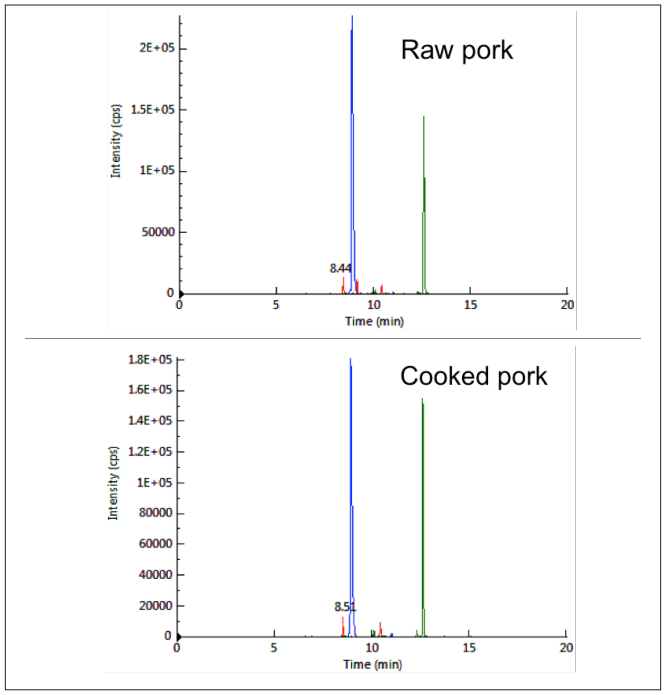

Figure 3. Typical chromatograms from LC-MRM analysis of raw (top) and cooked (bottom) pork.

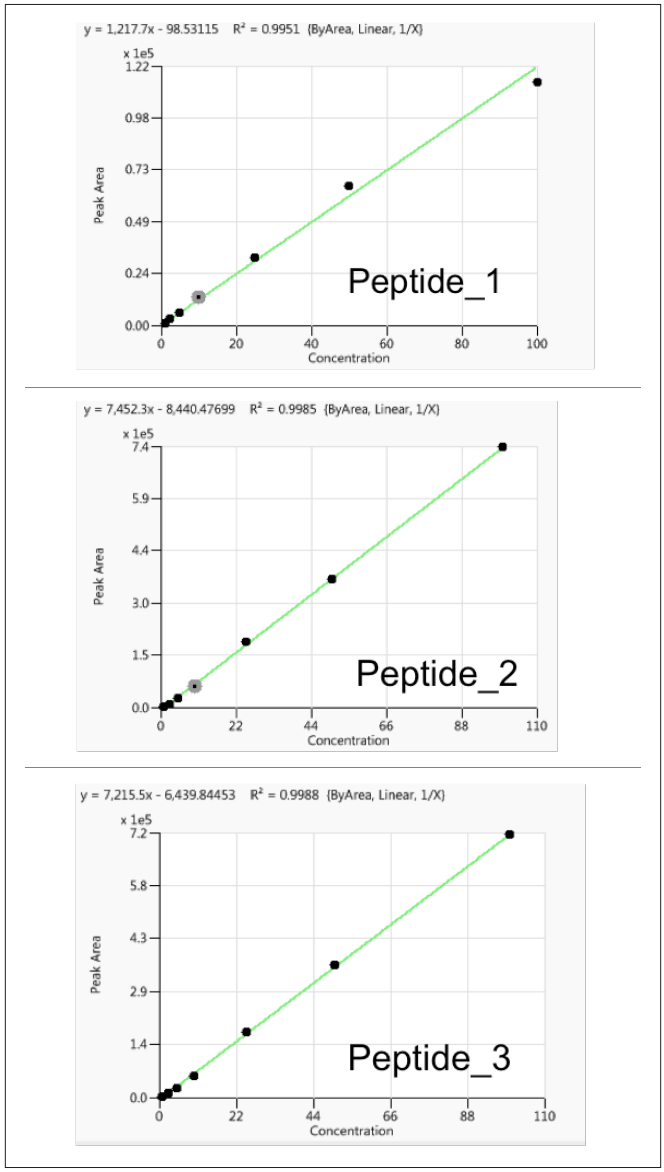

Figure 4. Calibration curves for three peptides in pork matrix (R^2>0.990).

Conclusions

A rapid, sensitive, and selective LC/MS/MS method has been developed for the analysis of three peptides in raw and cooked pork meat. The optimized sample preparation procedure is easy to follow and can be used for analyzing raw, cooked and processed meat products. This method can selectively detect peptides from pork meat species at a threshold detection limit of 1% w/w (10 mg/g) in a variety of food products and thus the method can be useful for analysis of Halal food (or pork related food products) samples.

References

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4. Yvonne G, Andrew D. W, Neil M. R, et al. J. Visu. Expert. 2016, 115, e54420, 1-7.

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