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Simultaneous determination of heat stable peptides for eight animal and plant species in meat products using UPLC-MS/MS method

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ABSTRACT

Food adulteration and fraud is driven by economic interests; it is thus necessary to establish a high-through method that allows quantitative identification of familiar animal and plant proteins for global use. In this study, a sensitive mass spectrometric approach for the detection of eight species, including pork, beef, lamb, chicken, duck, soy, peanut, and pea, is presented and the heat stability and specificity of screened peptides are verified. To improve screening efficiency of specific peptides, several key data searching parameters, including peptides, sequence lengths, sequence coverage, and unique peptides, are investigated. Using this approach, it is possible to detect a 0.5% contamination of any of the eight species. The method is proven to have high sensitivity, specificity, repeatability, and a low quantitative detection limit with respect to adulteration of diverse types of meat products.

1. Introduction

In recent years, there has been increasingly fierce competition throughout the food industry in relation to globalization and the complexity involved in the food supply chain. In this respect, divisions in the food industry have become extremely subtle, as has food adulteration and fraud driven by economic interests, and such issues are a global phenomenon and problem (Nakyinsige, Man, & Sazili, 2012). Food-species identification has traditionally relied on morphological or anatomical analysis. However, this is a difficult task in the case of closely-related species, especially for those products that have been subjected to processing (Gallardo, Ortea, & Carrera, 2013; Ortea, O'Connor, & Maquet, 2016).

Adulteration occurs throughout the entire meat production process. Ballin (Ballin, Vogensen, & Karlsson, 2009) classified meat or meat products into four categories: meat source, meat components of the alternative, changes in the meat processing process, and addition of non-meat ingredients. The most common adulteration cases (Spink & Moyer, 2011) involve the substitution of high-price meat with low-price meat (for example, the use of chicken or pork instead of beef) and the substitution of animal protein with plant protein (for example, soy instead of pork). However, only appropriate detection methods can accurately determine the various forms of adulteration involved in each adulteration case.

Most methods used to identify meat are only able to determine one type of adulteration. However, investigators are usually confronted with complex samples and it is not actually possible to ascertain whether the product contains plant or animal protein. It is thus becoming increasingly necessary to establish a high-through method that allows accurate identification of the familiar animal and plant proteins always used in adulteration and substitution.

Different approaches have been used to determine meat authentication, such as spectroscopy, polymerase chain reaction (PCR) (Daane et al., 2011; Kesmen, Gulluce, Sahin, & Yetim, 2009; Kesmen, Yetiman, Sahin, & Yetim, 2012; Navarro, Serrano-Heras, Castano, & Solera, 2015), proteomics, enzyme-linked immunosorbent assay (ELISA) (Asensio, González, García, & Martín, 2008; Kotoura et al., 2012; Liu, Chen, Dorsey, & Hsieh, 2006; Zvereva et al., 2015) and trace element analysis (Drivelos & Georgiou, 2012; Marcinkowska & Barałkiewicz, 2016); of these, PCR (based on nucleic acid) is the most commonly used method. DNA-based species identification schemes have gained wider acceptance and reliability because of the superior stability and

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universality of DNA in all tissues and cells. However, PCR results are usually affected by many factors such as poor trace quantitative analysis (Murray, Butler, Hardcare, & Timmerman-Vaughan, 2007), sampling pollution (Woolfe & Primrose, 2004), and DNA degradation (Pinto et al., 2015) in the food processing process, which makes it difficult to repeat results or to determine whether the adulterant component has been added or whether it is simply present as a pollutant.

The proteomics method is based on specific-species or specific-component peptides (Bargen, Dojahn, Waidelich, Humpf, & Brockmeyer, 2013; Hoffmann, Münch, Schwägele, Neusüß, & Jira, 2016; Leitner, Castro-Rubio, Marina, & Lindner, 2006; Sentandreu, Fraser, Halket, Patel, & Bramley, 2010), which are the primary structure of proteins and are more stable during thermal processing than DNA (Buckley, Melton, & Montgomery, 2013). Therefore, proteomics has an unparalleled advantage, especially in relation to the deep processing of meat products and quantitative measurements. Christoph von Bargen (Bargen, Brockmeyer, & Humpf, 2014) presented the specific and sensitive multiple reaction monitoring (MRM) method for detecting mixed horse and pork meat in different processed food matrices. In addition, Magdalena Montowska (Montowska, Alexander, Tucker, & Barrett, 2014; Montowska, Alexander, Tucker, & Barrett, 2015; Montowska & Pospiech, 2013) developed an ambient LESA-MS and DESA-MS methodology that can be used to select and identify heat-stable and species-specific peptide markers in the analysis of five types of thermally treated meat species.

A number of specific or high abundance proteins have been selected as the object of studies. For example, Nicola Giaretta (Giaretta, Giuseppe, Lippert, Parente, & Di Maro, 2013) proposed myoglobin as a marker used to separate and identify edible animal species. In addition, Gap-Don Kim (Kim, Seo, Yum, Jeong, & Yang, 2017) confirmed that troponin I (TnI), enolase 3, l-lactate dehydrogenase (LDH), and triose-phosphate isomerase (TPI) are useful markers to discriminate mammals from poultry, due to their differing electrophoretic mobilities.

In the present study, a new proteomics method is introduced involving multiple species, including pork, beef, chicken, lamb, duck, soy, peanut, and pea. By optimizing the data processing process and narrowing the screening range of the target proteins, specific peptides can be screened more rapidly. In addition, heat-stable specific-peptides can be screened under different heating conditions and verified using actual samples. Results have shown that this method is accurate, sensitive, simple, and that it can be used to identify different adulteration methods.

2. Materials and methods

2.1. Materials

The following materials were used: HPLC grade methanol, formic acid (FA), acetic acid, and acetonitrile (ACN) (Merck Darmstadt: Germany); sequencing grade modified biochemical level trypsin and dithiothreitol (DTT) (Promega: Mannheim, USA); biochemical level iodoacetamide (IAA) and trifluoroacetic acid (TFA) (Sigma-Aldrich: St. Louis, USA); analytical grade urea, thiourea, Tris, and hydrochloric acid (Sinopharm Chemical Reagent Co., Ltd.: Shanghai, China).

2.2. Sample preparation

In this study, five different types of raw meat (pork, beef, lamb, chicken, and duck) were purchased from commercial slaughterhouses, and commercially available samples were obtained from local supermarkets. The meat was ground after removing of connective tissues and visible fat. All meat was then frozen at −18 °C. Peanut powder, pea powder, and soybean meal were all purchased from a commercial farm and preserved at room temperature.

2.3. Heat treatment of samples

Five kinds of heat treatment methods were used in the experiment: (1) water bath heating at 78 ± 0.3 °C for 30 min, (2) boiling at 100 ± 0.3 °C for 30 min, (3) high temperature sterilizing at 121 ± 0.1 °C for 30 min, (4) frying in boiling oil until golden brown, and (5) baking at 200 ± 0.3 °C for 30 min. For meat samples, 10 g muscle tissue was cutted into a proper block and then heat-treated using the above five methods. After completion of all treatments, samples were removed and immediately chilled in crushed ice, and then pulverized by a homogenizer. For plant samples, except method (4), the other four heat treatment methods were applied after adding 75 mL water to 10 g of powder. After completion of heat treatment, samples were immediately cooled in an ice-water bath to reduce protein degradation.

2.4. Extraction of proteins

Approximately 2 g of each ground sample was weighed in a plastic centrifuge tube, and 10 mL extraction buffer solution containing 7 M urea, 2 M thiourea, and 50 mM Tris-HCl (pH 8.0) was added. Meat samples were homogenized in an ice-water bath environment, while plant samples were treated with ultrasound at the power of 200 W for 30 min in an ice-water bath environment. All samples were then centrifuged at 15,000*g* for 20 min at 4 °C.

2.5. Trypsin digestion

A 100 μL aliquot of the supernatant was transferred into a 4 mL plastic reaction tube, and 20 μL 30 mM DTT/H_2O was added to reduce the disulfide bonds of protein. The reaction was performed at 56 °C for 1 h. After samples were cooled to room temperature, the alkylation step was initiated by adding 23 μL 100 mM IAA/H_2O for 30 min at room temperature in the dark. For each sample, an aliquot of 100 μL containing about 1.5 mg of protein was diluted 1:10 with Tris-HCl (25 mM, pH 8.0), and supplemented with 20 μg trypsin dissolved in 0.1‰ acetic acid. Finally, to complete the reaction, samples were incubated at 37 °C in a thermos-shaker (Zhicheng Inc.: Shanghai, China) at the speed of 100 rpm for at least 12 h.

2.6. Desalting

Samples were adjusted to pH < 2 using 0.1% TFA to terminate the reaction and then desalted with HLB cartridges (Waters, USA), which were activated using 3 mL acetonitrile (ACN) and equilibrated with 3 mL ACN/H_2O (50/50, v/v) and 3 mL 0.1% TFA. The samples were then loaded onto cartridges and washed with 3 mL 0.1% TFA, 2 mL 0.5% acetic acid. Finally, 2 mL ACN/0.5% acetic acid (60/40, v/v) was used to elute peptides.

2.7. QE analysis

The use of Thermo Scientific Q Exactive (QE) analysis to find and verify specific peptides of each species is the focus of this study. Separation of peptides was performed with a Thermo Scientific EASY-nLC 1000 nanoflow LC and the MS analyses were conducted using Thermo Scientific Q Exactive HF coupled to a Nanospray Flex ion source. The samples were introduced into LC using an auto-sampler (5 μL, maintained at 4 °C) in a two-mixture mobile phase: mobile phase A was 0.1% FA/H_2O, and B was 0.1% ACN/H_2O. The initial condition of 3% B was then increased linearly to attain 8% B for 2 min, then to 22% for 46 min, followed by 40% for 5 min, and then 80% for 2 min. The final value was then maintained for a further 4 min and the gradient was then established to provide a linear drop from 80% to 3% for 2 min, and maintained for 4 min. The flow rate was 0.2 mL min^{-1}.

The QE mass parameters were as follows: spray voltage, 2100 V;