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Authentication of processed meat products by peptidomic analysis using rapid ambient mass spectrometry

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ABSTRACT

We present the application of a novel ambient LESA-MS method for the authentication of processed meat products. A set of 25 species and protein-specific heat stable peptide markers has been detected in processed samples manufactured from beef, pork, horse, chicken and turkey meat. We demonstrate that several peptides derived from myofibrillar and sarcoplasmic proteins are sufficiently resistant to processing to serve as specific markers of processed products. The LESA-MS technique required minimal sample preparation without fractionation and enabled the unambiguous and simultaneous identification of skeletal muscle proteins and peptides as well as other components of animal origin, including the milk protein such as casein alpha-S1, in whole meat product digests. We have identified, for the first time, six fast type II and five slow/cardiac type I MHC peptide markers in various processed meat products. The study demonstrates that complex mixtures of processed proteins/peptides can be examined effectively using this approach.

1. Introduction

Despite the existence of extensive mandatory regulations in most countries, food adulteration is still a global issue which attracts attention at international level and increases public concern regarding food quality. In 2013, the horse meat scandal revealed the weaknesses in the food safety system and contributed to a decrease of confidence in the food industry. Fraudulent practices, i.e. the presence of undeclared horse DNA in food products labelled as containing beef, were confirmed in 4.66% and 0.61% of controlled foods in 2013 and 2014, respectively (European Commission, 2014) as a result of tests in the 28 EU countries. Recent studies have revealed an even higher level of food mislabelling, for example 68% mislabelling was found in sausages, burger patties and meats collected from butcheries and retail outlets in South Africa (Cawthorn, Steinman, & Hoffman, 2013) and in seafood in the USA, a rate of 33% of investigated samples were mislabelled according to U.S. Food and Drug Administration (FDA) guidelines (Kimberly, Walker, Lowell, & Hirshfield, 2013). Similarly, the results of inspections carried out in Poland in 2011 by the Office of Competition and Consumer Protection (UOKiK) and Department of Trade Inspection revealed that 24.7% of the examined batches of luxury processed meat products, i.e. conventional, traditional and organic products sold at high prices, were adulterated/labelled incorrectly (UOKiK, 2012). Continuous monitoring of food quality and safety is now mandatory in the EU and other countries but the increasing sophistication of adulteration means that analytical methods require continuous improvement to ensure effective fraud detection. The rigorous analysis of complex and processed products requires the development of novel analytical methodology which has potential for high-throughput analysis and provides rapid, specific and reliable results.

At present, established methods for meat speciation are based on ELISA and PCR techniques, which are robust when applied to raw or moderately processed samples (Ballin, Vogensen, & Karlsson, 2009; Chen & Hsieh, 2000; Fajardo, González, Rojas, García, & Martín, 2010; Köppel, Eugster, Ruf, & Rentsch, 2012). The reported lower efficiency of these methods in highly processed samples has been linked to processing conditions, thermal denaturation and degradation of the markers compounds monitored (typically DNA or protein epitope) and problems with cross-reactivity

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between species giving unreliable results (Arslan, Irfan-Ilhak, & Calicioglu, 2006; Musto, Faraone, Cellini, & Musto, 2014; Şakalar, Abasiyanik, Bektik, & Tayyrov, 2012). The difficulty with reliable multiplex detection in a single test and contamination of DNA from other organisms also place severe limitations on analysis of complex samples. However, some proteins are quite resistant to heating (Buckley, Collins, Thomas-Oates, & Wilson, 2009; Buckley, Melton, & Montgomery, 2013; Montowska & Pospiech, 2012) and hence peptidomic analysis techniques have potential advantages when applied to authenticate processed (cooked) food.

Recently, considerable improvement in mass spectrometry (MS) instrumentation has enabled the detection of peptide markers by liquid chromatography–MS techniques (LC–MS) and this has enabled identification of specific proteins from soybean (Leitner, Castro-Rubio, Marina, & Lindner, 2006), fish (Carrera et al., 2011) and meat species (Buckley et al., 2009; Montowska & Pospiech, 2013; Sentandreu, Fraser, Halket, Patel, & Bramley, 2010; von Bargen, Brockmeyer, & Humpf, 2014). In our previous work, we evaluated ambient MS techniques for standard protein identification in mixtures and for the analysis of meat digests to discriminate between five meat species (Montowska, Rao, Alexander, Tucker, & Barrett, 2014). Subsequently, we detected heat stable peptide markers derived from meat proteins after thermal denaturation using our previously introduced ambient liquid extraction surface analysis mass spectrometry (LESA-MS) methodology (Montowska, Alexander, Tucker, & Barrett, 2014).

It is known that the primary structure of some meat proteins is relatively resistant to processing and that certain skeletal muscle proteins are both species- and tissue-specific and hence there is good potential for the use of specific muscle proteins and peptide markers for meat authentication (Buckley et al., 2009, 2013; Montowska & Pospiech, 2012; Sentandreu & Sentandreu, 2011). We consider that the ease of use and rapid nature of ambient MS has advantages for high-throughput screening of processed food and we wish to explore the potential application of our LESA-MS peptidomic approaches (Montowska, Alexander, et al., 2014; Montowska, Rao, et al., 2014) for this purpose. We suggest that the peptidomic analysis can serve as a tool not only for species identification but also for the assessment of the quality of the product. In this study we define 'product quality' as a general term linked with different authenticity issues, such as the detriment of the quality of the product by illegal change of meat to less valuable components of animal origin (e.g. meat of lower class, offal, connective tissue, blood plasma), undeclared plant or milk additives as well as a change in proportion of ingredients. Analysis of myosin isoforms due to their extensive diversity may help to trace some illegal practices in processed meat products.

Unlike highly conserved actin, myosin exhibits extensive variations in vertebrate striated muscles, which is translated into differences in fibre composition and shortening velocity. In adult mammals, pure fibres (slow type I red, and fast type white IIA, IIX, IIB) are expressed by a single myosin heavy chain (MHC) isoform (1, 2A, 2X, and 2B) whereas hybrid fibres may contain several MHC isoforms (1/2A, 2A/1, 2AX, 2XA, 2XB, and 2BX) each encoded by a separate gene (Pette & Staron, 2000). Therefore in this study, besides species identification, we wish to identify heat stable peptides unique to fast and slow type MHC isoforms. Peptidomic analysis may be a viable way to discriminate between the processed meat and non-meat components to examine the quality of the processed meat products.

In this paper, we present the application of our previously established LESA-MS methodology (Montowska, Alexander, et al., 2014; Montowska, Rao, et al., 2014) for detection and identification of heat stable beef, pork, horse and poultry peptide markers in various processed meat products. This rapid peptidomic approach aims to identify heat stable peptides without the need for purification and chromatographic separation. We also describe the application of in-solution tryptic digestion of processed meat samples followed by deposition onto a polymer surface, desorption and direct analysis by LESA-MS for protein/peptide composition of processed meat in order to compare the identified MHC isoforms and select heat stable peptides unique to fast and slow type MHCs.

2. Materials and methods

2.1. Preparation of samples

Meat products ($n = 18$) were purchased at English and Polish supermarkets or manufactured in our own pilot plant. Samples of raw sausages were cooked from chilled in an oven at 190 °C for 30 min according to the manufacturers' instructions. In-house processed sausages (3 batches) were prepared in a pilot plant of the Institute of Meat Technology in Poznan (Poland) exclusively from cured pork with the addition of spices and were coarsely minced, smoked and cooked. All samples of about 5 cm length or 5 g were cut from fresh products and kept at −80 °C until further MS analysis. Sample information and details about processing methods and meat composition are given in Table 2.

Washing, digestion and mass spectrometry analysis were performed according to the procedure described previously (Montowska, Alexander, et al., 2014). Preparation for LESA-MS analysis of samples of processed meat products involved washing procedures followed by digestion. For this purpose, thin sections of sausages (slices of 0.5 g) or 1 g of meat spreads were transferred to glass vials and washed to remove contaminants such as physiological salts, fat, and other soluble low molecular weight compounds. Sample was rinsed twice for 30 s in ethanol/water (70:30) followed by a 15 s wash in ethanol and then by a 30 s wash in methanol/water (90:10). The sample then was rinsed for 2 × 30 s in deionized water, and finally for 30 s in 100 mM of aqueous ammonium bicarbonate. Washed samples were placed to dry for 30 min in a desiccator.

2.2. In-solution digestion

Dried samples (10 mg) were rehydrated in 100 μL of water and subsequently digested in a solution containing 0.083 μg/μL of trypsin in ammonium bicarbonate at room temperature over a period of 24 h. Digested solution was then centrifuged for 10 min at 13,400 rpm, and the supernatant was diluted 10-fold with deionized water. Samples of 1 μL were spotted onto a Permanox slide, 75 × 25 mm (Nunc, Thermo Fisher Scientific, Rochester, NY, USA) and allowed to evaporate in air at room temperature prior to analysis.

2.3. LESA mass spectrometry

The LESA source was a TriVersa NanoMate (Advion, Ithaca, NY) coupled to a Thermo Fisher LTQ Velos ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) operated in positive-ion electrospray ionization mode. The NanoMate platform operated at nanoESI tip voltage of 1.6 kV, with a gas pressure of 0.4 psi and a capillary temperature of 190 °C. The same spray/extraction solvent acetonitrile/water/formic acid (50:50:1) was used in all LESA experiments. Total solvent extraction volume was 5 μL, dispensed and aspirated volumes were 3.5 and 3.2 μL, respectively. Each data set was collected from a single protein spot. Data-dependent analysis (DDA) tandem MS/MS data were collected in full scan mode with m/z range of 50–2000 divided into four segments (m/z 60–600, 550–1050, 1000–1550 and 1500–2000), 1 microscan, 100 ms max injection time, AGC mode on. DDA mode