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Original Research Article

Determination of the animal origin of meat and gelatin by MALDI-TOF-MS

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ABSTRACT

Recent scandals in the agro-food industry highlight the necessity of increasing meat and food traceability to determine animal origin. This article presents two matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) methods that do not require complicated sample preparation. The first method concerns the determination of the origin of meat (pork, beef, horse, veal and chicken) from raw and processed meat used in culinary preparations. Samples were prepared in order to extract proteins, then analyzed with a linear MALDI-TOF-MS system. Mass spectra were computed by cluster analysis using Bruker Biotyper software. Samples representing different meat origins were efficiently separated into distinct mass spectra clusters. The second method concerns the determination of the origin of gelatin (pork or beef) in food preparations and galenic formulations. Here, gelatin was digested into peptides, themselves analyzed using a reflectron MALDI-TOF-MS system. Mass spectra enabled us to detect and distinguish pork from bovine gelatin, based on specific peak patterns. We were able to detect down to 1% of gelatin in spiked candies and detect down to 20% of pork gelatin in beef gelatin. Both techniques have potential for the systematic and routine traceability of meat and collagen.

1. Introduction

Meat traceability has become an important issue for consumers, driven by a number of high-profile food industry mishaps, such as bovine spongiform encephalopathy, scrapie (Prusiner, 1991; Hope, 2013) and *Swine vesicular disease* (Brocchi et al., 1997; Pharo and Cobb, 2011). To avoid human health risks, many countries require tracking animals from birth to slaughter. Nevertheless, this traceability does not prevent fraud. Indeed, in a recent incident in Europe, beef had been replaced by horsemeat without indication for the consumer (O'Mahony, 2013). This fraud was detected in many culinary preparations made of minced beef, such as lasagna (Stanciu et al., 2013), and caused indignation among the many consumers who had purchased the fraudulent products. Furthermore, this issue has strong ethical and religious consequences because consumers are not able to make a deliberate choice and are becoming skeptical of the sanitary quality of meat. One reason for this trickery is the slaughter of horses that are no longer welcome on the roads in Romania and the consequent collapse of the price of horsemeat. Other scandals recently came to light in Malaysia, the first concerning chocolate (Aljazeera, 2014) and the second a pharmaceutical preparation (Al Kanz, 2013), both containing pork.

These crises have highlighted the necessity to increase the traceability of meat and particularly to identify the origin of raw and processed meat. Multiple techniques are already available to determine the species of meat (Ballin, 2010; Rogberg-Munoz et al., 2013), such as protein electrophoresis (Hoyem and Thorson, 1970),

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immunological techniques (Whittaker et al., 1982), chromatography (Ashoor et al., 1988), infrared spectroscopy (Cozzolino and Murray, 2004), and, finally, molecular methods that are the most widely used techniques in this field (Köppel et al., 2009; Martin et al., 2009; Wang et al., 2010). Molecular methods are very sensitive, specific and capable of quantitatively detecting compound mixtures from different species. Unfortunately, these sophisticated techniques are expensive, time consuming and require specialized staff. Furthermore, they are not adapted to determine the origin of gelatin, which does not contain nucleic acids. Gelatin is a derived protein prepared with collagen extracted from bone, tendon and loose connective tissue (Eyre and Muir, 1975; Mariod and Adam, 2013). Gelatin from pigs is relatively inexpensive, easily available and present in many food preparations such as candies and desserts. The origin of gelatin presents a serious problem because Islam and Judaism prohibit the consumption of any food or medication containing ingredients coming from pig sources. As a consequence, a reliable technique for the detection of pork gelatin is necessary in order to protect and reassure consumers against intentional or non-intentional food fraud (Fadzlillah et al., 2011).

Protein profiling by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is now increasingly common for the routine identification of microorganisms in clinical microbiology (Seng et al., 2010). This revolutionary, reliable and cost-effective technique is simple and faster than conventional phenotypic and molecular methods for the identification of human pathogens. Because MALDI-TOF-MS is an easy, efficient and low-cost routine technique for species identification, we have developed various methods in our laboratory for the rapid routine identification of bacteria (Ayyadurai et al., 2010; El Khechine et al., 2011; Fournier et al., 2009; Seng et al., 2009, 2013), antibiotic-resistant bacteria (Kempf et al., 2012), Archaea (Dridi et al., 2012), ancient mammals from dental pulp (Tran et al., 2011), mosquitoes and ticks (Yssouf et al., 2013a,b). To date, the use of mass spectrometry as a technique for the determination of the animal origin of meat (Taylor et al., 1993; Sentandreu et al., 2010; Sentandreu and Sentandreu, 2011) and gelatin (Buckley et al., 2009; Cheng et al., 2012; Tan and Lock, 2014; Zhang et al., 2009) has been limited. Furthermore, the latest uses of this technique are not cost-effective, rapid or user-friendly because they require high performance mass spectrometers coupled to chromatography.

This article presents two methods based on MALDI-TOF-MS analyses. The first method concerns the determination of the origin of meat (pork, beef, horse, veal and chicken) from raw and processed meats used in complex culinary preparations. The second method concerns the determination of the origin of gelatin (pork or beef) in food preparations and galenic formulations. These techniques have the potential for systematic and routine traceability of meat and collagen.

2. Materials and methods

2.1. Reagents

HPLC-grade water (VWR, Fontenay-sous-Bois, France), ethanol and acetonitrile (Sigma Aldrich, Lyon, France) were used as solvents. Formic acid (reagent grade $\geq$ 95%), trifluoroacetic acid (TFA; HPLC grade $\geq$ 99%), α-Cyano-4-hydroxycinnamic acid (CHCA) MALDI matrix (purity $\geq$ 98%), ammonium carbonate (reagent grade $\geq$ 99%) were obtained from Sigma Aldrich (Lyon, France). The gelatin standards were from porcine and bovine skin (Sigma Aldrich, Lyon, France). The sequencing-grade trypsin was porcine-modified (Promega, Charbonnières, France).

2.2. Samples

Meat pieces were purchased from local butchers: pig trotters, pork filet mignon, pork sausages, garlic pork sausage, pork saveloy, lamb saddle, knuckle of lamb, horsemeat steak, roast horsemeat, horsemeat flank, minced beef, turkey breast, turkey legs, chicken breast and chicken legs. Culinary preparations were purchased in supermarkets and fast food restaurants: beef stuffed tomatoes, beef shepherd's pie, minced beef, pork ham (Casino, Saint-Etienne, France), beef shepherd's pie (Fleury-Michon, La-Roche-sur-Yon, France), minced beef (Charal, Cholet, France), minced beef (Bigard, Quimperle, France), beef steak and chicken nuggets (McDonald's, Guyancourt, France; Quick, Berchem, France), pork sausage (Knacki Herta, Nestle, Marne la Vallee, France). Halal meat preparations were poultry sausage, turkey ham and dices of turkey (Wassila halal, Casino, Saint-Etienne, France). Candies containing gelatin were Crocodile candies (Haribo, Marseille, France), strawberry candies, marshmallows and teddy bear candies (Casino, Saint-Etienne, France). Candies without gelatin were cream desserts (Danette, Danone, Saint-Ouen, France) and halal candies (Wassila halal, Casino, Saint-Etienne, France). Galenic formulations were collected from common drugs: Progesterone capsule, Omeprazole coated tablets, phloroglucinol two-piece hard capsules and Alfacalcidol single-piece soft gel capsules (Central pharmacy, Hôpital Nord, Marseille).

2.3. Sample preparation

2.3.1. Meat samples

A 3 mm^3 piece of meat (3–5 mg) culinary preparation was added to a polypropylene tube and washed twice with water. After centrifugation, the pellet was mixed with 500 μl of 70% formic acid and 500 μl of acetonitrile and ground with glass beads (acid washed, Sigma Aldrich, Lyon, France) in a Fast Prep®-24 Instrument (MP Biomedicals, Illkirch-Graffenstaden, France). The tube was centrifuged for 2 min at 13000 × *g* and 1 μl of the supernatant was spotted onto a steel MALDI target plate (Bruker Daltonics, Wissembourg, France) in quadruplicate, then dried at room temperature as described previously (Kaufmann et al., 2012). Then 1 μl of a CHCA matrix suspension (saturated α-cyano-4-hydroxycinnamic acid, 50% acetonitrile, 10% trifluoroacetic acid and water) was directly spotted onto each sample to allow co-crystallization.

2.3.2. Gelatin standards

A total of 200 mg of porcine or bovine gelatin standards was mixed with 200 μl of water to obtain a gelatinous mixture. The gelatin standards were mixed with 200 μl of 50 mM NH_4HCO_3 and heated for 1 h at 80 °C. Once the sample was returned to room temperature, 200 μl of 12.5 ng/mL of sequencing-grade modified porcine trypsin was added to the previous mixture (Shevchenko et al., 1996) and incubated for 2 h at 37 °C. Then1 μL of peptides were co-crystallized onto the MALDI target with 1 μL of matrix solution (3 mg/mL of α-cyano-4-hydroxycinnamic acid in 33% acetone, 66% ethanol and 0.1% TFA).

2.3.3. Gelatin samples

Pieces of 3 mm^3 (3–5 mg) were directly cut off of samples and then placed in a polypropylene tube. Each sample unit (i.e., pills, caps, candies), was washed with 1 mL of HPLC-grade water. The sample was mixed with 200 μl of 50 mM NH_4HCO_3 and heated for 1 h at 80 °C. Once the sample returned to room temperature, 200 μl of 12.5 ng/mL of sequencing-grade modified porcine trypsin was added to the previous mixture (Shevchenko et al., 1996) and incubated for two hours at 37 °C. Next 1 μL of peptides were co-crystallized onto the MALDI target with 1 μL of matrix