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Mass spectrometry quantification of beef and pork meat in highly processed food: Application on Bolognese sauce

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ABSTRACT

Food frauds have become a very important issue in the field of food quality and safety. The risk of food adulteration is higher in highly processed food and mainly affects high added value foodstuff. The methods currently available to face this issue, PCR and ELISA, are very sensitive and specific, but they have some limitations. In the present work, tandem mass spectrometry is presented as an emerging approach to detect beef and pork meat in very complex and highly processed food matrices, such as Bolognese sauce, both in qualitative than in quantitative way. The detection is achieved using two different marker peptides, specific for beef and pork meat, both deriving from α2-collagen chain. Then, a calibration curve is set up using real sauces made by different percentages of pork and beef meat in a working range from 0 to 100%. The method here developed allows to quantify beef and pork meat in a complex product such as Bolognese sauce.

1. Introduction

Food fraud is a deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging, labeling, production information, or false or misleading statements made about a product for economic gain that could impact consumer health (Spink & Moyer, 2011). At the moment there is no definition of "food fraud" in EU legislation (http://ec.europa.eu/food/safety/official_controls/food_fraud/index_en.htm). One of the most common types of adulteration is the substitution of an ingredient with a cheaper one, that constitutes, alone, the 95% of the reported cases. The remaining incidents regard the addition of substances able to mask an inferior quality or the undeclared removal of valuable compounds. In the recent years, several scandals regarded meat derived products catalysing public opinion attention (www.foodfraud.org), in particular the partial replacement of beef with horse meat in some ready-to-eat products commercialized by famous brands. However, meat is often exposed to adulteration, the most common being: a false indication about the origin of meats and/or the animal feeding regime (for example in organic/PDO products), the substitution of the specie or the replacement of meat with fat, a missing declaration about a previous meat process (irradiation or thawing) or the possible additive presence (Ballin, 2010). Beside the legal point of view, there are also several concerns regarding quality and safety implications. For example the presence of hidden allergens, the lack of microbiological control, the possible presence of antibiotics, hormones or other food contaminants. Moreover there are also religious and lifestyle issues. Processed products are easier to be adulterated, due to their complexity and to the higher difficulty of detection (Flores-Munguia, Bermudez-Almada, & Vazquez-Moreno, 2000). In fact, ground meat cannot be recognized by the only visual inspection,

Abbreviations: CE, collision energy; DNA, deoxyribonucleic acid; ELISA, enzyme linked immunosorbent assay; ESI, electrospray ionization; EU, European union; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; LRMS, low resolution mass spectrometry; LRMSMS, low resolution tandem mass spectrometry; MRM, multiple reaction monitoring; MS, mass spectrometry; MW, molecular weight; PCR, polymerase chain reaction; PDO, protected designation of origin; RP, reverse phase; RT, real time; SIR, single ion recording; Tris, tris(hydroxymethyl)aminomethane; UHPLC, ultrahigh performance liquid chromatography.

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and the mixing with other ingredient in ready to eat foods makes the revelation even more difficult. Given the importance of food frauds, both at economical and safety level, a lot of attention has been focused on the development of analytical methods for adulteration detection (Sentandreu & Sentandreu, 2014).

Despite numerous articles regarding meat authenticity in the last ten years (182 results for "meat authenticity" in Web of Knowledge database, accessed 14/09/2016), only twelve of them took into account processed food products: Hossain et al. (2016) developed a multiplex polymerase chain reaction–restriction fragment length polymorphism assay to detect beef, buffalo and pork meat also in processed foods such as frankfurters. In this work, detection was possible down to 0.1% of adulteration. The same group developed also a short amplicon-length PCR able to detect cat meat in cooked burgers down to 0.01% (Ali et al., 2016). Multiplex PCR, besides fresh meat product, was also applied to some cooked whole muscle meat, detecting several adulteration of beef with chicken meat (Chuah et al., 2016). Cases of substitution of beef with pork meat and of lamb with beef were reported by Premanandh, Sabbagh, and Maruthamuthu (2013) using a DNA-based approach. Despite the severe processing occurring for gelatine production, a PCR method was able to detect down to 0.1% of pork gelatine in bovine gelatine (Shabani et al., 2015). Duplex PCR was also applied to assess the authenticity of donkey meat (liable of adulteration with horse and mule), with a limit of detection of 1% (Chen, Wei, Chen, Zhao, & Yang, 2015). Among game meat, PCR-based methods are available for the detection of roe deer, red deer and hare meat (Rak, Knapik, Bania, Sujkowski, & Gadzinowski, 2014) and for the detection of game birds (Rojas et al., 2009). After the horse meat scandals, a lot of efforts were put in the development of new and sensitive analytical methods for the detection of these meat species. For example, Pegels, Garcia, Martin, and Gonzalez (2015) developed a TaqMan RT-PCR to detect horse DNA also in processed products, like cured meat, sausages, burgers and pet food. As described by Stefanova, Taseva, Georgieva, Gotcheva, and Angelov (2013), DNA extraction is often a critical step, especially in processed products. In the last years, a promising technique in this field is DNA barcoding, able to detect DNA also in processed fish (Yang, Huang, Hsieh, Huang, & Chen, 2012). Among methods relying on proteomics, Claydon, Grundy, Charlton, and Romero (2015) identified several horse meat derived peptides that were resistant to food processing and that can be detected in canned corned beef and baby foods.

Nowadays, the most diffused analytical methods used by industries for the detection of food frauds (and meat in particular) can be divided essentially into two main groups: DNA-based methods and protein-based methods. DNA-based methods are constituted mainly by PCR (Natonek-Wisniewska, Krzyscin, & Piestrzynska-Kajtoch, 2013), that is sensitive and allows multi-ingredient detection, but, being specie-specific, it cannot distinguish between beef and milk or egg and chicken (and this is a problem for multi-ingredient preparations). Moreover it is an indirect method, and the absence of DNA does not necessarily mean the absence of proteins, since the thermal stability of nucleic acids and proteins is different. Protein-based methods relies essentially on immunoenzymatic assays (ELISA) (Asensio, Gonzalez, Garcia, & Martin, 2008), that are highly specific for the target ingredient and sensitive: at the same time, the presence of interfering compounds (e.g. polyphenols, etc.) can negatively affect the analysis and, moreover, denatured proteins could not be detected but still be present. This is particularly true in thermally treated products, where heating induces several modifications in proteins, such as denaturation, lysine reaction with carbonyl groups (Maillard reaction), serine and threonine dehydration, cross linkage due to the formation of isopeptides and lysinoalanine (Gerrard, 2002). All these modification lead to a strong decrease in protein solubility, besides a much harder detectability.

Thus, in the recent years several mass spectrometry methods were developed to assess meat authenticity (Sentandreu & Sentandreu, 2011). For complex food matrices, mass spectrometry can indeed give the right selectivity, sensitivity and discriminating capacity in order to identify eventual food frauds. It has been demonstrated that horse and pork meat can be detected both in raw and in processed foods using HPLC-MS/MS achieving a detection limit of 0.0024 mass fraction units: in these works MRM^3 experiments were performed on myoglobin tryptic peptides (Von Bargen, Brockmeyer, & Humpf, 2014; Von Bargen, Dojahn, Waidelich, Humpf, & Brockmeyer, 2013). Four marker peptides for processed pork meat were identified by Sarah et al. (2016), that developed MRM methods for their detection. Montowska, Alexander, Tucker, and Barrett (2015) identified, with a fast LESA-MS methodology, 25 heat stable peptides for five meat species (beef, pork, horse, chicken and turkey meat). Claydon et al. (2015) constructed a database of heat stable unique tryptic peptides for nine meat species: this method was able to detect down to 0.5% cooked and raw horse in a meat mixture. An untargeted approach was instead developed by Ohana et al. (2016), using shotgun spectral matching: specie identification was possible for 26 different mammalian and bird meats, both in raw and processed foods. However, besides detection and specie identification, a quantification cannot be carried out by the reported methodologies. In another work, raw meat from beef, horse, pork and lamb could be differentiated using myoglobin tryptic peptides reaching a limit of detection of 1%, and in this case the method was demonstrated suitable for raw materials but no food processing was taken into account (Orduna, Husby, Yang, Ghosh, & Beaundry, 2015). Beside mammalian differentiation, a mass spectrometry approach was also used for the detection of chicken in meat mixes, and a quantification was achieved using isotopically labelled peptide standards (Sentandreu, Fraser, Halket, Patel, & Bramley, 2010).

Ii is our opinion that, at the moment, the main gap in literature concerning meat speciation issue is the lack of proper reference materials, that perfectly resemble the commercial product to be analysed. Most of the published papers (with really few exceptions, Von Bargen et al., 2014) take indeed into account samples made by mixing fresh meat or even cooked meat, but not a real food product, made with different ingredients other than meat that have a strong influence on the detection capability of the method (e.g. dilution effect, matrix effect, interfering compounds). Up to now, no quantitative methods designed on a real food product are available.

The development of a quantitative method suitable for commercial products, will allows to detect not only the presence/absence of an undeclared ingredient, but also the relative quantification of a complex matrix with different species mixed together. A quantitative method is indeed a powerful tool also to monitor the entire industrial supply chain, in order to verify the compliance from the raw materials to the finished products. For example, a quantitative method is a helpful tool to discriminate between a simple contamination episode or an intentional food fraud. Moreover, in the case of multi-ingredient food products such as ground meat mixtures, the presence of beef and pork together is allowed and declared in the label but, since the price of the two commodities is different, a fraudulent shift towards the cheaper specie (even if it is not completely substituted) can be detected with this method.

In this work, we focused on the detection and accurate quantification of beef and pork meat in a complex and thermally treated food product (Bolognese sauce), not only to detect the presence of these two species in the products in which they are not declared on the label but also to verify the relative amount of the two species