

# Meat Vulnerabilities to Economic Food Adulteration Require New Analytical Solutions

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**Abstract:** Meat has been identified as one of the food categories at most risk of food fraud. Meat species substitution has been in the spotlight with the European horse meat scandal in 2013. Analysis of cases reported on the web shows that incidents of meat substitution are still recurring worldwide. Altogether these cases highlight significant weaknesses in the supply chain transparency and traceability of raw meat materials. This has triggered recent progress from the food industry to apply new software tools enabling the mapping of meat supply chains. Nevertheless, a meat vulnerability assessment showed that meat and derivatives are highly susceptible to many fraudulent malpractices. Therefore, more effective measures are needed to manage the risk and new analytical solutions are required to increase the deterrence of meat adulteration and rapid detection of fraud. DNA-based methods have evolved rapidly as shown with the application of the new LCD array and Next Generation Sequencing (NGS) in order to detect broad meat species adulteration. Moreover, new technologies such as NGS together with the Rapid Evaporative Ionization Mass Spectrometry (REIMS) are emerging as a really promising association of analytical approaches for rapid detection of several malpractices.

**Keywords:** Economic food adulteration · Food fraud · Meat · Next Generation Sequencing (NGS) · Rapid Evaporative Ionization Mass Spectrometry (REIMS)



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## Introduction

Economically-motivated adulteration (EMA) has been defined as “the intentional fraudulent addition of non authentic substances or substitution of authentic ingredients for economic gain of the seller (often simply defined as food fraud within the European Union)”.[1,2] Recent food fraud crises such as the addition of illegal Sudan dyes in chili and paprika (2005), the enhancement of protein level indirectly *via* the nitrogen-rich compound melamine in wheat gluten (2007) and then in infant formulas (2008), as well as the European horsemeat scandal in 2013 have had significant consequences.[3] Beside their high impact on food consumer trust, these scandals highlight that the scientific community underestimated the food fraud risks. In particular, weaknesses in supply chain transparency and raw material traceability have been observed which were further increased by the rapid globalization of the food trade. Moreover, no systematic analysis of the potential vulnerabilities of food raw materials has been performed to identify and prioritize major risk and help to develop adequate mitigation plans. These recent scandals were a wake up call as a reminder that food fraud is a criminal act.[4] Although food fraud is not in principle intended to harm consumers, it may have dramatic food safety implications due to fraudster ignorance of adulterant toxicity or interest purely in the economic gain.

Meat is one of the largest agricultural products worldwide and is still often an important component of the diet. Bovine, pig, ovine and poultry meat production was evaluated in 2012 at more than 300 million tons worldwide.[5,6] However, consumers are highly concerned about the type of meat they are buying. Their choice is often based on a specific human lifestyle (*e.g.* organic food, fair trade) or religion, cultural barriers (*e.g.* halal, kosher) and potential health concerns (*e.g.* veterinary drugs) and with specific meat labelling. Despite extensive mandatory label information, the current regulations are not sufficient to prevent food adulteration. From food fraud reported in the scientific literature or diverse media (newspapers, blogs, NGOs, authorities, ...) of incidents extracted using a commercially available tool (Digimind),[7] meat has been identified as one of the most adulterated food categories (Fig. 1). Both raw and processed meat raw materials have been the target of food fraud in several regions of the world. Although primary cuts of raw meat can, in principle, easily be differentiated by a visual check (*e.g.* chuck, brisket, sirloin, shank), other parameters or meat pieces are of potential adulteration concern (*e.g.* fresh versus thawed, wild versus farmed, specific breed *etc.*).[8,9] Furthermore, as meat vulnerability is affected by the level of processing, it is more difficult to recognize the origin of minced meat or meat powders. The food and beverage manufacturing industry uses signifi-

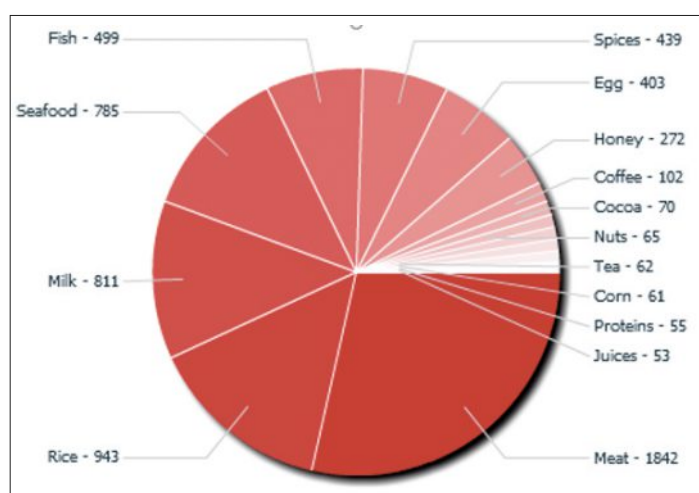
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Fig. 1. Report of food fraud (EMA) news reported in the media (out of ~600'000 web sources) over 1 year (February 2017–February 2018) using a commercially available screening tool (Digimind) showed that the meat category is still of major concern.



cant meat processing with almost half of their meat products consumed as sausages, burgers and pies.<sup>[10]</sup> The vulnerability of processed meat was highlighted in 2013 with the European scandal involving major substitution of beef by cheaper horse meat which spread from the UK and Ireland to several other countries.<sup>[11]</sup> In this context, many meat and poultry species commonly consumed by humans are quite expensive on the market and numerous less valuable meat species can easily be used as substitutes. Recent studies have revealed significant meat mislabeling in various parts of the world such as up to 35% from online specialty meat distributors in USA, 25% of luxury processed meat in Poland and nearly 70% mislabeling of sausages, burger patties and meat collected from butchers and retail outlets in South Africa.<sup>[12–14]</sup> These numbers reveal significant weaknesses in the implementation of food fraud prevention systems in the meat supply chain.

### Several Vulnerabilities are Inherent to Raw Meat Materials and Meat Products

An assessment of the specific food supply chain (*e.g.* meat) to identify the potential vulnerabilities is the initial step of food fraud prevention.<sup>[8]</sup>

#### Substitution of Species Origin

Looking at the history of raw material fraud and analysis of previous cases can provide important insight into understanding the root cause. In this context, it is important to note that a horse meat scandal was already reported in the UK in 1948 and that additional meat substitution involving horse meat was identified well before the European scandal in 2013.<sup>[15]</sup> In 1981, the United States of America (USA) imported Australian beef which was found to be adulterated with horse and kangaroo meat and in 2000 horse meat was detected in Mexican

beef burgers.<sup>[16,17]</sup> These adulteration cases confirm that a systematic analysis of past vulnerabilities is a necessary step to implement appropriate management measures. However, today the risk goes far beyond previously identified meat species substitution. Recent analysis of cases reported on the web show more surprising and extreme cases where giraffe, fox and rats have been used as substitutes.<sup>[18,19]</sup> Therefore, further analytical methodology development is required to address this major meat supply chain vulnerability that will be described further below.

Beyond substitution of species origin, many other potential, more sophisticated fraudulent malpractices that are inherent to the nature of raw and processed meat have been identified. They can be classified according to the types of nomenclature normally used for intentional food adulteration (substitution, dilution, addition, unapproved enhancement or concealment of substances for economic gain).<sup>[20]</sup>

#### Substitution of Meat Tissues

Meat offal consumption is in decline, creating a challenge for the meat industry to find a market for such material. In this context, tissue substitution using cheap raw materials such as offal (liver, kidney *etc.*) or collagen as substitute or in addition to muscle meat has been identified as a significant vulnerability for processed meat.

#### Substitution of Premium Meat with Labels or Meat based on Specific Criteria

Several raw meat materials are based on specific criteria such as geographic origin, breed, organic production, wild versus farmed meat and are often associated with specific labels. All these premium meat pieces are highly vulnerable to potential substitution with lower quality raw meat materials. In the same context, specific regulations on raw meat materials such as slaughter age (*e.g.* lamb versus mutton), fresh versus thawed are of concern regard-

ing meat adulteration. Moreover, regulations may vary according to countries (*e.g.* slaughter age) increasing the risk.

#### Enhancement of Meat Protein Level

Enhancement of specific parameters which are used as main criteria of raw material payment are critical. The protein content is a major parameter of payment for meat powders. Therefore, the risk of addition of cheap protein concentrate from plant origin (*e.g.* soya, pea, wheat) or from insect origin is significant. Moreover, hydrolyzed proteins produced from cheap raw meat materials such as residues of the leather industry or animal leftovers (*e.g.* feathers, horns) are of similar concern as cheap plant proteins. The amount of nitrogen (N) is normally applied to determine the protein content in foods, in this context the addition of nitrogen-rich compounds such as melamine can easily be added to artificially increase the protein content. The concern is particularly significant as the list of nitrogen-rich compounds (N-rich) identified recently is impressive and most of these molecules are easily available on the market and of significant economic interest. Therefore, this calls for new analytical methods or new solutions which will be discussed in the analytical section.

#### Addition of Adulterants to Increase Weight

Addition of foreign proteins as described above or vegetable fat can also be used to compensate for the addition of bulking agents in meat powders. Several illegal components such as cellulose, starch and others can be added to increase raw meat material weight. These adulterants may be difficult to detect if the proximates (protein, fat content) have been maintained at normal ranges. Moreover, dyes may have been added (if needed) to mask color changes. Increased weight by simple water addition (*e.g.* chicken breast or frozen meat) can easily be detected by an analysis of the protein/water ratio. However, the addition of specific water binding agents such polyphosphates and cheap proteins as described above (soy protein extract or nitrogen-rich compounds) will leave the ratio close to the natural one, thus requiring more sophisticated analytical methods of detection.

#### Concealment using Additives

Several additives can be added legally at low concentration (below 1%) to processed meat to improve its functionality and/or sensory properties.<sup>[6]</sup> For example, nitrite can be added for curing color, ascorbic acid for speeding up the curing reaction and other substances such as gelatin, blood plasma and others for purposes such as anti-fat separating and

extenders. However, economic gain can easily be achieved if excess amounts are used or no indication is given on the labels or if their use deviates from the original purpose. For example, smoke aromas can be used fraudulently in place of natural meat smoking. Illegal preservatives (boric acid, formaldehyde) can be added to increase the shelf life of raw meat materials and illegal dyes and synthetic aromas can be used to change meat appearance and resemble fresh meat. Some of these adulterants have been reported in the recent Brazilian beef scandal (2017) to mask the addition and odor of added minced pig heads in raw beef material. Another concern is the use of enzymes (*e.g.* transglutaminase) that may be applied legally in some countries as ‘glue meat’ to help structure processed meat (*e.g.* burgers). However, economic adulteration has been identified when they are used to prepare specific meat portions using low quality meat pieces as substitutes of premium meat cuts (*e.g.* steak).

The initial vulnerability assessment step is performed per species and group of raw meat materials (*e.g.* offal, minced-ground, powders-dehydrated and others). This information is completed by an evaluation of the potential safety concern of identified meat adulterants for humans (*e.g.* specific nitrogen compounds, illegal dyes, additives such preservatives). This step will help to assess different mitigation measures but also to prioritize the development of new analytical methods of detection.

### New Analytical Solutions to Detect Meat Adulteration

Cases of meat substitution are still identified regularly worldwide. Another major scandal of beef adulteration was reported in Brazil in 2017 (see Fig. 2, A and B, and section above on concealment using additives). Moreover, a survey performed recently on sausages on the Canadian market showed that 20% of collected samples were adulterated with foreign meat species including horsemeat.<sup>[21]</sup> Therefore, major focus will be made on meat species adulteration which further requires the development of new technologies for rapid and broad detection of species origin.

### Species Identification by DNA Technologies

For meat species identification, DNA-based technologies are considered as the most appropriate, and were applied daily during the European horsemeat crisis.<sup>[22]</sup> Different DNA-based technologies have been reported to identify meat species, generally all starting with the Polymerase Chain Reaction (PCR).

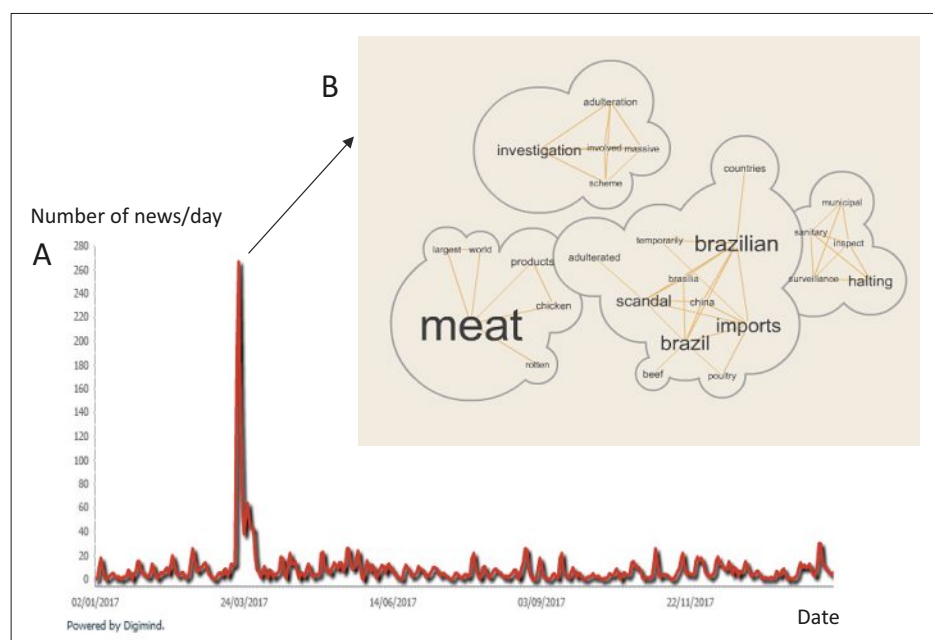


Fig. 2. A and B. Number of meat food fraud news reported using a commercial tool (Digimind) in 2017. Data showed constant daily news on meat fraud (between 0–20 news reports) with (A) a major event identified on March 24<sup>th</sup> 2017 corresponding to (B) the beef adulteration crisis in Brazil.

DNA amplification is of course applicable on raw meat where DNA is intact, knowing that some industrial processes like heating, acidic or alkaline treatments can more or less significantly degrade DNA. Meat PCR testing is applicable on cooked meat, meat powder and canned meat provided the amplification targets a short DNA fragment, *e.g.* below 150 base pairs (bp). Meat gelatin is a particular case where gelatin samples can contain a huge amount of DNA, or on the contrary can be absent of residual DNA depending on the manufacturing treatment. Also, it is usually unlikely to retrieve residual DNA from meat flavor, meat juice, meat sauce and meat bouillon.

PCR methods have been extensively described and are daily applied by service laboratories and by official control laboratories. However these approaches target the common meat species, like beef, pork, horse, chicken and turkey, and are limited to 5 or 6 meat species only.<sup>[23,24]</sup> Taking into account adulteration cases where a common meat species has been substituted by a more exotic one (*e.g.* fox or rat meat<sup>[16,17]</sup>), the range of targeted species has to be extended to cover adulteration and substitution with more exotic species.

Restriction Fragment Length Polymorphism (RFLP) and Random Amplification of Polymorphic DNA (RAPD) have been described as more flexible approaches than targeted PCR, and able to screen for more species.<sup>[25,26]</sup> However, these methods have the tendency to lead to ambiguous and non-reproducible results when DNA digestion is incomplete, for instance in the case of RFLP, or when

the obtained amplification profiles are too similar in the case of RAPD.<sup>[27]</sup> Due to these biases and lack of reliability, these approaches are thus less popular and less used in routine testing.

More recently, several macro- and micro-arrays have been described to identify meat species. These systems are simple and specific, and becoming more and more affordable. A generic meat PCR amplifies a meat DNA fragment of hundreds of base pairs, then the amplicons are hybridized on arrays spotted with species-specific probes. A biochemical reaction (*e.g.* biotin-streptavidin) reveals the positive hybridization(s) and the presence of the different meat species depending on their position on the array. Using a cheap macro-array, up to 32 meat species can be specifically identified, including exotic meat species such as kangaroo, ostrich, kudu, and dog.<sup>[28]</sup> This approach can reach similar sensitivity to classical PCR, and has the advantage of detecting many more species in a single test. Although these systems are easy to use, the hybridization step is critical and can affect the quality and reliability of the results when not rigorously respected.

These previous approaches are targeted methods, whereas untargeted methods are increasingly being developed and used for food fraud detection. For DNA-based testing, Sanger DNA sequencing is used by the International Barcode of Life initiative (iBOL) as an untargeted species identification tool. It is based on the DNA sequence variability of 650-bp fragments, named DNA barcodes.<sup>[29]</sup> DNA barcoding is now regularly applied to detect meat mislabeling



and allowed the detection of up to 20% of meat substitution in United States.<sup>[30]</sup> Highly reliable on single/pure meat species, DNA barcoding is however not applicable to meat powder or ground meat, which may contain several species. Sanger sequencing of a mixture leads to an overlap of their sequencing spectra and an unreadable final chromatogram. As another limitation of DNA barcoding, industrial processes tend to degrade DNA and to cut it in small fragments ( $\leq 200$  bp), which prevent its application on processed meat samples. Amplification of shorter DNA fragments, named mini-barcodes, has been described but is not yet considered as a reference approach.

Recently, Sanger DNA sequencing and DNA barcoding have evolved to Next Generation Sequencing (NGS) and DNA meta-barcoding, respectively, and have been shown to reliably identify species not only on pure/single meat species but also on mixtures<sup>[31]</sup> (Fig. 3). Following a PCR step to amplify a meat consensus DNA region, DNA meta-barcoding uses NGS to sequence all individual DNA amplicons from a sample and to record them. Since all PCR fragments are sequenced and numbered, species identification of a meat mixture is not only possible, but relative quantification can be considered. However, meat quantification by DNA-based methods has been reported as potentially unreliable due to the variability of DNA content per kind of meat tissues (muscle, tendon, fat, etc.).<sup>[9]</sup>

NGS technology and DNA meta-barcoding usually uses mini-barcodes, which is more appropriate to analyze processed samples. In addition, pooling samples is another advantage of NGS which finally becomes affordable for the analysis of several samples altogether.

DNA barcoding and meta-barcoding are considered as untargeted DNA approaches and evaluation of meat species identification by NGS have been shown to successfully identify common meat species and exotic ones (Table 1). Some species have been spiked at 1% (w/w) in beef or chicken matrix, and were successfully detected. This proof-of-concept showed promising results, not only in terms of precise species identification but also in terms of sensitivity in order to comply with regulatory requirements.

Since DNA meta-barcoding can identify thousands of species and is applicable on processed samples and mixtures, it is expected to become the reference approach for species identification. It can be applied for meat species identification, but also on fish, spices, herbs or botanical supplements<sup>[32]</sup> where risk of substitution and fraud are well known. In addition, application on insects has already been described, knowing that insects can be considered as a hygienic issue in the food supply chain, but can also be considered as a raw food material rich in protein and will certainly become prone to some adulteration when it becomes more popular.<sup>[33]</sup>

### Complementary Technologies for Meat Adulteration Detection

New technologies have been developed to be more appropriate to the detection of food adulteration issues. However, the analytical tools to detect food fraud are numerous and of varying degrees of sophistication. In this regard, it is important to combine the development and application of simple methods that can be applied in material reception with more sophisticated technologies requiring more sophisticated laboratory facilities and human expertise.

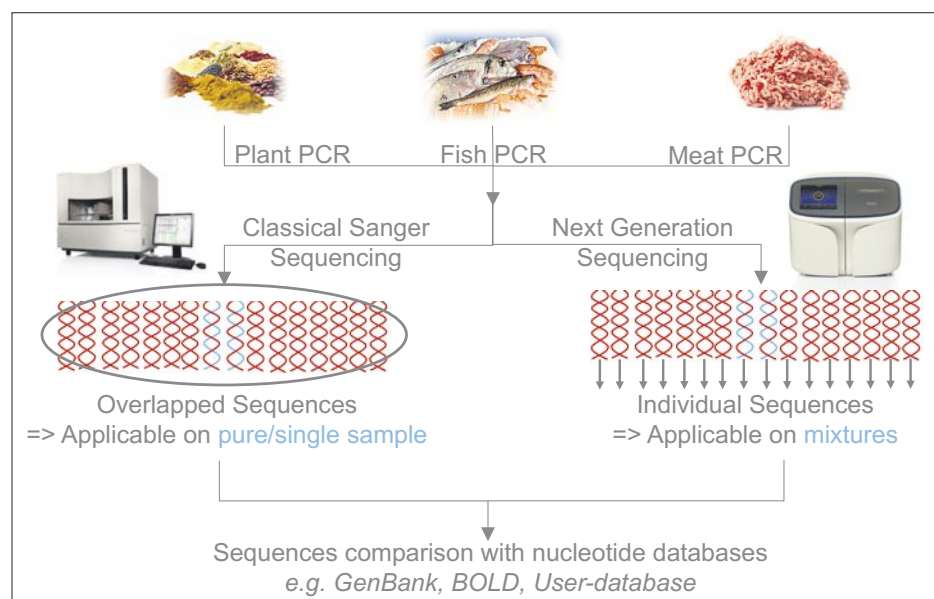


Fig. 3. Schematic difference between classical Sanger DNA sequencing (DNA Barcoding) and NGS (Meta-Barcoding) for species identification.

The former are usually based on immunoassays, sensors or simple spectroscopic techniques such as near- or mid-infrared (NIR, FTIR) instruments. In this context, ELISA tests have been developed for rapid detection of plant protein additions in meat powders and processed meat (e.g. soy, pea, wheat). An application has also been described for meat tissue discrimination using specific tissue proteins as markers.<sup>[8]</sup> Prieto *et al.*<sup>[34]</sup> recently performed an extensive review of NIR spectroscopy to characterize meat and meat products and to show the potential regarding food authenticity (e.g. species, breed, geographical origin, specific feeding). Several studies combined with chemometric analyses successfully classified (90–100%) homogenized meat, meat juices or specialties such as meat pâtés from different species origin.<sup>[34–36]</sup> Moreover, Schmutzler *et al.*,<sup>[37]</sup> applying three different IR experimental approaches (laboratory desktop device, industrial in- and on-line with a fiber optic probe and on-site using a handheld instrument), showed that NIR spectroscopy was able to detect the presence of pork meat or pork fat in veal sausages at adulteration levels up to the lowest level tested (10%). One exception was the detection of pork fat adulteration using the handheld spectrometer which was limited to 20%. Looking at geographical origin, NIR and multivariate approaches correctly classified (100%) lamb meat from pastoral and agricultural regions and identified meat from five individual regions at percentages varying between 75% (agricultural) to nearly 90% (pastoral samples). NIR spectroscopy using multivariate analyses has improved the understanding and characterization of meat properties by allowing classification without in-depth meat composition analysis.<sup>[33]</sup> Current data suggest that NIR spectroscopy is a valuable analytical approach for rapid and non-destructive screening of meat and meat products quality and authenticity. In food authentication, untargeted analytical methodologies are required for rapid detection of raw material abnormalities before confirmation of a potential issue with more sophisticated, cost and time-consuming technologies. For example, authentication of a meat geographical origin may require further analyses using stable isotope ratios which are predominantly used for such issues.<sup>[9,38]</sup>

As identified in the raw meat material vulnerability assessment, protein adulteration by addition of bulking agent and cheaper protein sources is of significant concern. Detection using approaches such as immunoassays, which have been described previously, remains limited due to their targeted nature.<sup>[9,39]</sup> Moreover, they have limitations in the analysis of processed meat because processing may

Table 1. Meat species identification by NGS: (species detected <1% (w/w) or analyses still in progress at Nestlé Research Centre). Up to now all meat species tested have been detected down to 1% (w/w).

Tested species	Identified species by NGS	Detected down to 1% (w/w)
Beef	<i>Bos taurus</i>	✓
Pork	<i>Sus scrofa</i>	✓
Horse	<i>Equus caballus</i>	✓
Sheep	<i>Ovis aries</i>	✓
Donkey	<i>Equus asinus</i>	✓
Goat	<i>Capra hircus</i>	✓
Bison	<i>Bison bison</i>	✓
Water buffalo	<i>Bubalus bubalis</i>	✓
Chicken	<i>Gallus gallus</i>	✓
Turkey	<i>Meleagris galopavo</i>	✓
Muscovy duck	<i>Cairina moscata</i>	✓
Mallard duck	<i>Anas sp.</i>	✓
King quail	<i>Coturnix japonica</i>	Not tested
Goose	<i>Anser sp.</i>	✓
Pigeon	<i>Columba livia</i>	✓
Guinea fowl	<i>Numida meleagris</i>	✓
Partridge	<i>Alectoris chukar</i>	Not tested
Pheasant	<i>Phasianus inornata</i>	Not tested
Ostrich	<i>Struthio camelus</i>	✓
Rabbit	<i>Oryctolagus cuniculus</i>	✓
Hare	<i>Lepus capensis</i>	✓
Red deer	<i>Cervus elaphus</i>	✓
Roe deer	<i>Capreolus capreolus</i>	✓
Fallow deer	<i>Cervus dama</i>	Not tested
Reindeer	<i>Rangifer tarandus</i>	✓
Elk	<i>Alces alces</i>	Not tested
Crocodile	<i>Crocodylus sp.</i>	Not tested
Emu	<i>Dromaius novaehollandiae</i>	✓
Lama	<i>Lama glama</i>	✓
Camel	<i>Camelus dromedarius</i>	Not tested
Kudu	<i>Tragelaphus strepsiceros</i>	Not tested
Kangaroo	<i>Macropus robustus/rufus</i>	✓
Rat	<i>Rattus norvegicus</i>	✓
Dog	<i>Canis familiaris</i>	✓
Cat	<i>Felis catus</i>	✓
Fox	<i>Vulpes vulpes</i>	✓
Oryx	<i>Oryx leucoryx</i>	Not tested
Gnu	<i>Alcelaphus buselaphus</i>	Not tested
Badger	<i>Meles meles</i>	Not tested
Springbok	<i>Antidorcas marsupialis</i>	✓
Impala	<i>Aepyceros melampus</i>	Not tested
Bushbuck	<i>Tragelaphus scriptus</i>	Not tested
Crow	<i>Corvus macrorhynchos</i>	Not tested
Weasel	<i>Mustela erminea</i>	Not tested
Muskrat	<i>Ondatra zibethicus</i>	Not tested

alter protein structure and affect the recognition of the target protein. Therefore, it is worth noting that meat samples (e.g. powder protein concentrate, isolate and other processed meat samples) normally contain sufficient DNA for detection by the NGS technology (see previous section on DNA-based approaches). The reported lower efficiency of the DNA-based approach in processed samples (significant degradation due to thermal, pressure processing conditions) is in principle overcome by this new technology using very short primers. In this regard, NGS could be an interesting alternative and used as an important screening tool to detect the presence of added cheap foreign protein. However, confirmation of the presence of a foreign protein source may require more sophisticated and time-consuming methodologies such as LC-MS/MS to identify the specific protein(s) added.<sup>[40]</sup>

New ambient mass spectrometry (AMS) technologies have been developed recently for rapid and direct analysis of samples. No or limited sample preparation is required due to their new ionization technique.<sup>[41]</sup> In this context, the Rapid Evaporative Ionization Mass Spectrometry (REIMS) with the hand-held *i-knife*, which was originally developed for rapid characterization of biological tissues in oncology, has now interesting potential for application in the detection of food fraud. In REIMS, a high frequency electric current is applied to the sample (tissue) which causes localized heating. Molecules are subsequently ionized at the heated surface and pass into the Q-ToF mass spectrometer. Balog and co-workers<sup>[42]</sup> showed the use of REIMS for rapid characterization of meat products with no requirement for sample preparation. The multivariate statistical algorithm was developed and successfully tested for the identification of breed and species with 100% of accuracy at species level and 97% accuracy at breed level. REIMS technology is currently being evaluated for its potential to detect tissue substitution, another major meat vulnerability. Data using minced beef muscle meat versus cheap beef offal such as liver, kidney, heart, stomach and large intestine showed a clear discrimination of the beef tissues by application of partial least squares discriminant analyses (PLS-DA) (Fig. 4). Furthermore, the presence of kidney in minced beef by substitution varying between 5% and 20% was identified in a few seconds by the recognition software based on PLS-DA model. Additional sample analyses will be required to further determine the limit of detection of kidney and other offal tissues in minced meat. Montowska *et al.*<sup>[13]</sup> have shown the applicability of another ambient technology (liquid extraction surface analysis mass spectrometry, LESA-

MS) to identify peptide markers resistant to thermal treatment in different types of processed meat products. The procedure is significantly simplified over other peptidomic methodologies. In addition to meat speciation detection, these specific markers can help to identify malpractices linked with the substitution of ingredients.

ed to meat adulteration. Spectroscopic techniques such as NIR combined with multivariate analyses have the potential to work as initial screening approaches providing a first line of defense. Different chemometric models have been validated to detect abnormalities associated with different meat vulnerabilities. Moreover,

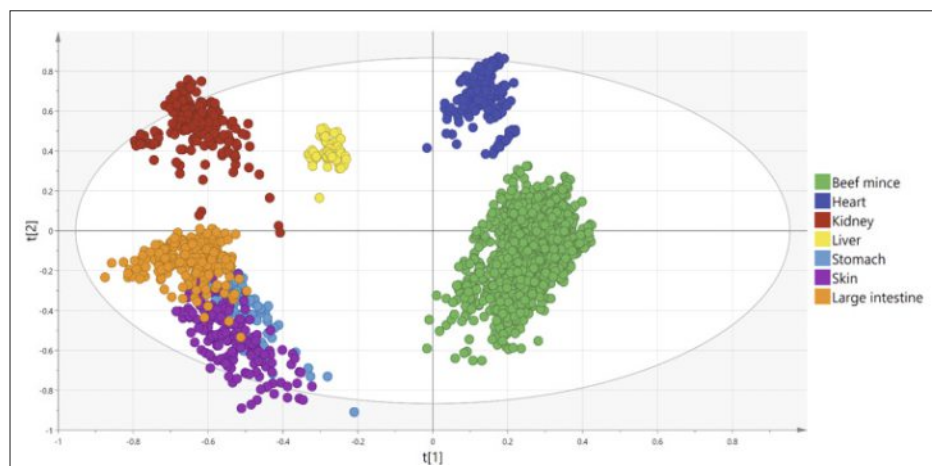


Fig. 4. Rapid Evaporative Ionization Mass Spectrometry (REIMS) showed a clear discrimination by PLS-DA analysis between minced beef muscle meat and beef tissue adulterants such as heart, kidney, liver, stomach, skin and large intestine.

Common increase of water content in meat was previously detected using the water/protein ratio, however, such adulteration can easily be masked by addition of exogenous proteins and polyphosphates. In addition to the methods of foreign protein detection mentioned above, nuclear magnetic resonance (NMR) has been shown to be a valuable approach to study the water distribution in meat, detect substances used to retain water in meat and in differentiating fresh from thawed meats.<sup>[39]</sup>

## Conclusions

The meat vulnerability assessment showed that this food category is at significant risk of adulteration. Meat species substitution is of major concern, and a fraudulent practice, which is still frequently detected in raw meat materials and finished products worldwide. Moreover, many other meat vulnerabilities and thus possibilities to adulterate meat and meat products have been identified. In recent years suppliers, food processors, retailers and regulators have taken significant initiatives to prevent food fraud all along the supply chain and to better manage these vulnerabilities. Significant efforts have been made to improve the supply chain transparency and increase raw material traceability. In addition, more targeted supplier audits are performed based on identified meat vulnerabilities. Analytical methods of detection are also better adapt-

such spectroscopic techniques can be combined with rapid targeted methods used at reception areas such as ELISA or lateral flow. More sophisticated technologies like DNA-based methods such as NGS have also evolved rapidly towards untargeted approach for a broad detection of animal species. Furthermore, such DNA-based evolution may provide broader applications for detection of potential foreign protein addition *via* detection of an abnormal presence of plant or insect DNA in meat samples. In parallel, the development of a peptidomic approach with identification of heat stable peptide markers may be very complementary to the DNA-based approach for discriminating meat species in highly processed raw materials. In addition, new ambient technologies are developed which are considered as emerging solutions for food fraud. They may have a significant impact on meat adulteration detection providing solutions such as REIMS, LESA-MS and others which provide data in a few seconds or are considerably simplifying current sample preparation. Moreover, initial data on meat adulteration detection showed broad applications and accurate results which are similar to traditional methods (LC-MS/MS).

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