In recent years, interest in meat authenticity has increased. Many consumers are concerned about the meat they eat and accurate labelling is important to inform consumer choice. Authentication methods can be categorised into the areas where fraud is most likely to occur: meat origin, meat substitution, meat processing treatment and non-meat ingredient addition. Within each area the possibilities for fraud can be subcategorised as follows: meat origin—sex, meat cuts, breed, feed intake, slaughter age, wild versus farmed meat, organic versus conventional meat, and geographic origin; meat substitution—meat species, fat, and protein; meat processing treatment—irradiation, fresh versus thawed meat and meat preparation; non-meat ingredient addition—additives and water. Analytical methods used in authentication are as diverse as the authentication problems, and include a diverse range of equipment and techniques. This review is intended to provide an overview of the possible analytical methods available for meat and meat products authentication. In areas where no authentication methods have been published, possible strategies are suggested.
1. Introduction

Today, many consumers are concerned about the meat they eat, and accurate labelling is important to inform consumer choice. The choice of one product over another can reflect aspects of lifestyle (e.g. vegetarianism and organic food), religion (e.g. absence of pork from some diets), diet and health concerns (e.g. absence of allergens). In addition, accurate labelling is important to support fair-trade. Additional descriptive label information can be added as a consequence of branding, product marketing purposes and regulations. While regulations enshrined in national and international law underpin mandatory label information, unfortunately, regulations are not sufficient to prevent food fraud. To ensure adherence to regulations, and to enforce punitive measures when needed, robust analytical tests are required. The prevalence of meat fraud today is difficult to measure. However, the examples of mislabelling and its abundance in meat and meat products illustrated in Table 1 make analytical authentication highly relevant.

This review provides an overview of the possible analytical methods used for authentication of meat and meat products and identify areas of future research needs. The analytical methods include polymerase chain reaction, chromatography, mass spectrometry, microscopy, spectroscopy, electronic spin resonance, and enzymatic assays. An overview of the analytical methods is provided in Table 2–4. In the few cases where no authentication methods have been published, possible analytical strategies are suggested. For simplicity, authentication problems with respect to meat and meat products are grouped into four major categories: meat origin, meat substitution, meat processing treatment and non-meat ingredient addition. Within each area the possibilities for fraud can be subcategorised as follows: meat origin—sex, meat cuts, breed, feed intake, slaughter age, wild versus farmed meat, organic versus conventional meat, and geographic origin; meat substitution—meat species, fat, and protein; meat processing treatment—irradiation, fresh versus thawed meat and meat preparation; non-meat ingredient addition—additives and water. Fig. 1 presents an overview of the different categories and subcategories.

Analytical authentication of food products often requires sample preparation such as extraction of proteins, DNA, and organic compounds. In this review, preparation prior to actual analysis is excluded for simplicity. However, it is often an important step that must be carefully considered.

2. Identification of meat origin

2.1. Sex

Analysis of sex-specific hormones is a conventional method used to determine the sex of meat. The level of sex specific hormones varies not only among individuals, but also within a given individual (Zeleny & Schimmel, 2002). It is therefore challenging to establish sex determination methods based on hormone analysis alone. However, gas chromatography-mass spectrometry (GC-MS) (Hartwig et al., 1997), high performance liquid chromatography-mass spectrometry/ mass spectrometry (HPLC-MS/MS) (Draisci et al., 2000), and enzyme-linked immuno sorbent assays (ELISA) (Simontacchi et al., 1999) have been found effective to measure bovine sex hormones and thereby determine the sex. Nevertheless, the most common methodology used to determine the sex of animals is unquestionably traditional polymerase chain reaction (PCR) that involves gel electrophoresis of DNA amplicons. An overview of the analytical methods is provided in Table 2.

DNA regions that differ between male and female individuals are essential features in PCR sex determination. Such DNA regions include the zinc finger genes (ZFX and ZFY), the sex determination region of the Y chromosomal gene (SRY), and the tooth enamel amelogenin gene (AMELX and AMELY). Traditional PCR amplification of ZFX and ZFY genes have been used to determine sex in cattle (Aasen & Medrano, 1990; Kirkpatrick & Monson, 1993; Zinovieva et al., 1995), sheep (Aasen & Medrano, 1990), pig (Lockley et al., 1997), and goat (Aasen & Medrano, 1990). PCR amplification of the Y chromosomal SRY gene has been reported to be successful in sex determination of cattle (Lu et al., 2007) and buffalo (Fu et al., 2007), while the AMELX and AMELY genes have been used to determine sex in cattle (Chen et al., 1999; Ennis & Gallagher, 1994) and pig (Fontanesi et al., 2008). Different bovine sex determination methodologies have been comprehensively described (Zeleny & Schimmel, 2002) and evaluated (Zeleny, Berneuether, Schimmel, & Pauwels, 2002).

Currently the focus seems to have shifted from traditional PCR towards real time PCR. Real time PCR based TaqMan technology directed toward the specific Y chromosomal SRY and the X chromosomal proteolipid protein gene has been used in sex determination of cattle (Parati et al., 2006). Furthermore, the real time PCR based SYBR Green technology combined with the melting curve analysis of amplified AMELX and AMELY genes has been used in bovine sexing (Ballin & Madsen, 2007), and a similar assay that amplifies genes encoding chromodomain-helicase-DNA binding protein (CHD) was described in avian sexing (Chang et al., 2008).

2.2. Meat cuts

Staff educated with this purpose in mind can easily differentiate between primary beef cuts such as chuck, brisket, sirloin, and shank through visual inspection. Steaks, as a result of secondary meat cuts, are more difficult to visually authenticate than primary cuts. Authentication is further complicated since the names of primary and secondary meat cuts vary across countries. In addition, meat cuts vary considerably over time because of various consumer demands and market trends. These factors make it difficult to establish visual objective criteria for authentication of specific meat cuts. To my knowledge, no analytical authentication methods have yet been published. However, the fact that some chemical constituents in meat differ between different meat cuts might aid in authentication. The

<table>
<thead>
<tr>
<th>Investigated product</th>
<th>Country of investigation</th>
<th>Authentication problem</th>
<th>Percentage of mislabelling cases (number of analysed samples)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamburgers</td>
<td>Brazil</td>
<td>Undeclared soy protein</td>
<td>30.8% (39)</td>
<td>Macedo-Silva, Shimkomaki, Vaz, Yamamoto, and Tenuta-Filho (2001)</td>
</tr>
<tr>
<td>Hamburgers</td>
<td>Mexico</td>
<td>Undeclared animal species</td>
<td>39% (23)</td>
<td>Flores-Munguia, Bermudez-Almada, and Vázquez-Moreno (2000)</td>
</tr>
<tr>
<td>Sausages</td>
<td>Mexico</td>
<td>Undeclared animal species</td>
<td>29% (17)</td>
<td>Flores-Munguia et al. (2000)</td>
</tr>
<tr>
<td>Meat products</td>
<td>United States of America</td>
<td>Undeclared animal species</td>
<td>15.9% raw samples 22.9% cooked samples</td>
<td>902</td>
</tr>
<tr>
<td>Meat products</td>
<td>Turkey</td>
<td>Undeclared animal species</td>
<td>22% (100)</td>
<td>Ayaz, Ayaz, and Erol (2006)</td>
</tr>
<tr>
<td>Meat products</td>
<td>Switzerland</td>
<td>Thawed meat declared as fresh</td>
<td>15% (43)</td>
<td>Anon (2001)</td>
</tr>
<tr>
<td>Meat products</td>
<td>United Kingdom</td>
<td>Thawed meat declared as fresh</td>
<td>8% (534)</td>
<td>Anon (1996)</td>
</tr>
</tbody>
</table>
difference in the amounts of collagen present in different meat cuts is a case-in-point.

2.3. Breed

Authentication of breed is primarily based on PCR and a subsequent analysis of amplicons. An overview of the analytical methods is provided in Table 2. Microsatellite DNA markers and a Bayesian statistical model identified the Italian cattle breeds Chianina, Marchigiana, Romagnola, and Piemontese (Dalvit et al., 2008). Another study that used single nucleotide polymorphism (SNP) markers directed toward the SRY gene and the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene identified the cattle breeds Holstein and Japanese Black (Sasazaki et al., 2004). Amplified fragment length polymorphism (AFLP) (Alves et al., 2002) and multilocus genotyping of repetitive sequences (Garcia et al., 2006; Vega-Pla et al., 2003) successfully distinguished Duroc and the crossbred Iberian–Duroc from the purebred Iberian pig. Interestingly, Iberian pigs are used for the dry-cured Iberian ham, which is a Spanish speciality. This ham must consist of 100% of the Iberian genome or a 50:50% mixture of the Iberian and Duroc genome. Random amplified polymorphic DNA (RAPD) has successfully differentiated between horse breeds (Martinez & Yman, 1998). With regard to RAPD, it is relevant to consider that electrophoretic profiles from mixtures of breeds might be difficult to interpret, specifically for species that can interbreed. For example, the RAPD pattern of a hybrid is similar to the 50–50% mixture of the RAPD patterns of the parental species (Martinez & Yman, 1998). In addition, a RAPD assay designed with primers toward mitochondrial DNA might fail in the identification of hybrids as mitochondrial DNA is primarily maternally inherited (Adam, 2000; Gyllensten, Wharton, Josefsson, & Wilson, 1991). PCR and a subsequent ampiclon analysis is not the only possibility in identification of breed and, for example, near infrared reflectance spectroscopy (NIRS) was used to study Friesian and Hereford beef; spectral differences were observed, especially in the region between 1449 and 1974 nm (Alomar et al., 2003). A larger data set should, however, be obtained to build a model capable of discriminating between Friesian and Hereford beef samples.

2.4. Feed intake

There are various means of tracing animal feed intake. Traceability is possible because different chemical constituents are present in feeds such as milk, pasture, hay, maize, and concentrate (mixed dried constituents), which upon consumption, shows up as different chemical constituents or metabolised forms in the animals’ blood and fat. An overview of the analytical methods is provided in Table 2. A group of chemical constituents that can be used as authentication markers is the carotenoids. The carotenoids, xanthophylls and carotenes, are much more abundant in pasture when compared to hay and concentrate (Prache et al., 2003). HPLC was used to measure carotenoid content in sheep’s blood (Prache et al., 2003) and in heifer’s fat, and the carotenoid content was dependent on whether the diet was based on pasture or concentrate (Dunne et al., 2006). Carotenoid differences also allowed reflectance spectroscopy between 400 and 700 nm to distinguish between lambs fed on grass and lambs fed on concentrate (Priolo et al., 2002). However, intrinsic factors such as breed, gender, lactation, and rumen environment also affect the carotenoid content (Dunne, Monahan, O’Mara, & Moloney, 2009) and must be considered in the development of these methods.

The composition of fatty acids in meat fat is also dependent on an animal’s diet. Gas chromatographic studies describe a higher ratio of polysaturated fatty acids to saturated fatty acids (Duckett et al., 1993; French et al., 2000), and a lower ratio of n−6 to n−3 polysaturated fatty acids (French et al., 2000) in steers fed on grass compared to steers fed on concentrate. A study on lambs found differences in saturated fatty acids and mono- and polysaturated fatty acid between lambs fed on milk versus a concentrate and hay diet (Okeudo, Moss, & Chestnutt, 2004). Differences in saturated fatty acids (C14:0, C16:0, C17:0, C20:2, and C23:0) and unsaturated fatty acids (18:1, 18:2, C18:3, and C23:3) were also found in another study that has successfully been used to discriminate between weaned and unweaned calves in a multivariate discriminant procedure (Moreno et al., 2006).
Furthermore, head space GC-MS was used to study volatile compounds in lambs’ fat, wherein 33 compounds were identified as being relevant in identification of meat from pasture and concentrate fed animals (Vasta et al., 2007). Another possibility is to investigate the content of vitamins and terpenes that are found in higher concentrations in meat from animals fed on grass compared to animals fed on concentrate (Descalzo et al., 2005; Larick et al., 1987).

2.5. Slaughter age

Slaughter age is important as meat sold from young animals is often more highly valued than meat from older animals. Examples are the higher priced veal compared to beef and the higher priced lamb compared to mutton. The definition of veal and lamb meat compared to beef and mutton meat differ among countries. This difference complicates analytical testing. In the European Union, veal must originate from an animal not older than 12 months. A limit of 12 months is, however, impossible to measure with analytical methods. If the veal label is also related to feed intake, such as the milk-fed veal calves and the grain-fed calves, analytical testing becomes feasible as specific constituents from the feed can be found in the meat (see previous section Feed intake).

2.6. Wild versus farmed meat

Wild pig (Sus scrofa scrofa), for example, differs genetically from domesticated pig (Sus scrofa domestica) hence differentiation between wild and domesticated animals can in this case be done by (sub) species identification, see section Meat (species and tissue). Game, such as crocodile and ostrich, is farmed in some countries and though fraud is a possibility, the scientific community has given this little attention as this is not done on a large scale. In fish, gas chromatographic measurement of differences in fatty acid composition (Almeida & Franco, 2007; Karapanagiotidis et al., 2006) in combination with multi element isotopic analysis (Thomas et al., 2008) was used to differentiate wild and farmed fish. This strategy might also be applicable to meat.

2.7. Organic versus conventional meat

One restriction in production of organic meat is the use of veterinary drugs and rules dictate how and when such drugs may be used (Anon, 2008a). Fluorescence microscopy (Kelly et al., 2006) of cross sectional bone cuts can estimate the number of tetracycline doses administered to pig and chicken, and illegal serial or prophylactic dosing of tetracyclines can help verify whether the animal complies with the specified organic rules. This strategy is not conclusive as absence of tetracyclines is not a guarantee of organically produced meat.

One strategy is to explore the differences in animal fat from organically and conventionally raised animals. A gas chromatographic study of fatty acid methyl esters showed an increase in polyunsaturated fatty acids in organically produced lamb (Angood et al., 2008), pig (Kim et al., 2009), and broiler (Castellini et al., 2002; Husak et al., 2008) as compared to conventionally produced meats. However, fatty acid composition is also dependent on specific dietary intake such as pasture and concentrate (Pérez-Palacios, Ruiz, Tejeda, & Antequera, 2009). Again, this strategy is not conclusive. A high level of polyunsaturated fatty acids could result from the specific dietary intake and not in from organically produced feed alone.

Another strategy could be analysis of isotopic composition. Stable ratio mass spectrometry of carbon, nitrogen, and sulphur isotopes succeeded in differentiating between organic and conventional Irish beef (Schmidt et al., 2005). The differences in isotopic composition in organic and conventional beef are partly due to the difference in the feed intake (grass or concentrate) (Schmidt et al., 2005). However, the higher content of 15N in conventional beef compared with organic beef (Bahar et al., 2008) might be a result of the mineral fertilizers applied to the soil where conventionally grown animals are fed (Watzka, Buchgraber, & Wanek, 2006). An overview of the analytical methods is provided in Table 2.

2.8. Geographic origin

Inductively coupled plasma mass spectroscopy (ICP-MS) of trace elements and stable isotope ratios are predominantly used in authentication of geographic origin, and an overview of the analytical methods is provided in Table 2. The content of trace elements and isotopes in animals depend on various factors such as feed intake, drinking water, pollution, and soil composition, all of which depend on geographic origin.

Franke, Haldimann, et al. (2008) tested fifty elements and 75As, 21Na, 85Rb, 77Se, 88Sr, and 205Tl for poultry meat and 75As, 109B, 137Ba, 42Ca, 111Cd, 63Cu, 161Dy, 167Er, 57Fe, 7Li, 55Mn, 104pd, 85Rb, 77Se, 88Sr, 128Te, 207TI, 238U, and 51V for dried beef, were shown to differ significantly between countries. Poultry discrimination was possible among samples from Brazil, France, Germany, Hungary, and Switzerland. In dried bovine meat, linear discriminant analysis was applied to a number of elements and used to establish a classification matrix. The classification matrix was used to predict geographic origin of bovine meat from Australia, Austria, Switzerland, Canada, Brazil, and the United States of America. Prediction of geographic origin was performed with varying degrees of success, and the accuracy might be improved by combining different approaches (Franke, Haldimann, et al., 2008). The oxygen isotope ratio (18O/16O) in meat’s water fraction was used to differentiate between beef and chicken when geographic conditions were clearly different (Australia vs. Europe vs. North America) (Franke, Koslitz, et al., 2008). A combination of multi trace elemental analysis (Franke, Haldimann, et al., 2008) and oxygen isotope analysis (Franke, Koslitz, et al., 2008) would seem appropriate; however, a recent attempt did not improve correct geographic classification compared to the individual methods (Franke, Hadorn, Bosset, Gremaud, & Kreuzer, 2008). Measurement of carbon and nitrogen isotopes has also shown potential in differentiating between beef originating from Japan, Australia, and the United States of America (Nakashita et al., 2008), as well as beef originating from Europe and the United States of America (Schmidt et al., 2005).

Some cattle breeds are country specific and DNA analysis has been used in indirect identification of geographic origin. Analysis of 24 breeds was used to build a Bayesian statistical model based on single nucleotide polymorphisms (SNP) markers, and indirect differentiation between Italy, France, Spain, and the United Kingdom was possible (Negrini et al., 2008). Another study, also using SNP markers, differentiated between breeds from Japan and Australia (Sasazaki et al., 2007). For species that have country specific breeds, this strategy could be useful in combination with elemental analysis. However, identification of breed alone is not evidence of geographic origin as individual breeds can be raised in different countries. An overview of European breeds and their origin is described elsewhere (Dalvit, De Marchi, & Cassandro, 2007).

3. Identification of meat substitution

3.1. Meat (species and tissue)

Fraudulent substitution of meat can involve both species and tissue. In species determination, analysis of DNA and protein are common practices. Detection of protein has traditionally been the most suitable method for animal species determination. Detection of animal protein is covered later in the section Protein. An overview of the analytical methods is provided in Table 3.
Rapidly evolving DNA-based techniques have resulted in a change in species determination from protein to DNA analysis. Degeneracy of DNA has the advantage that differentiating among different animal species can be done solely using DNA analysis. Furthermore, when compared to proteins, DNA has a higher thermal stability, is present in the majority of cells, and potentially enables identical information to be obtained from the same animal regardless of tissue of origin (Lockley & Bardsley, 2000). PCR is capable of amplifying very few.

Table 3
Examples of analytical techniques applicable in authentication of meat, fat, and protein substitution in meat and meat products.

<table>
<thead>
<tr>
<th>Authenticity problem</th>
<th>Analytical technique*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat substitution</td>
<td>ELISA</td>
<td>Gonzalez-Cordova, Calderon de la Barca, Cota, &amp; Vallejo-Cordoba, 1998; Koppelman, Lakemond, Vlooswijk, &amp; Heffe, 2004</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>Ashoor, Monte, &amp; Stiles, 1988; Ashoor &amp; Osman, 1988; Chou et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Isoelectric focusing</td>
<td>Skarpeid, Kvaal, and Hildrum (1998)</td>
</tr>
<tr>
<td></td>
<td>Capillary gel electrophoresis</td>
<td>Vallejo-Cordoba and Cota-Rivas (1998)</td>
</tr>
<tr>
<td></td>
<td>Traditional PCR</td>
<td>Calvo, Rodelar, Zaragoza, &amp; Osta, 2002; Colgan, O’Brien, Maher, Shilton, McDonnell, &amp; Ward, 2001; Hopwood, Fairbrother, Lockley, &amp; Bardisley, 1999; Pascoal, Prado, Calo, Cepeda, &amp; Barros-Velasquez, 2005; Rodriguez et al., 2003; Sun &amp; Lin, 2003</td>
</tr>
<tr>
<td></td>
<td>Real time PCR</td>
<td>Brodmann &amp; Moor, 2003; Christholm, Conyers, Booth, Lawley, &amp; Hird, 2005; Fumiere, Dubois, Baeten, von Holst, &amp; Berben, 2006; Hird, Chisholm, &amp; Brown, 2005; Laube, Zagon, &amp; Broll, 2007; Lopez-Andreu, Carrido-Pertierra, &amp; Puyer, 2006; Martin et al., 2009; Mendoza-Romero et al., 2004</td>
</tr>
<tr>
<td></td>
<td>RFLP</td>
<td>Verkaar, Nijman, Boutaga, &amp; Lenstra, 2002; Wolf, Rentsch, and Hubner (1999)</td>
</tr>
<tr>
<td></td>
<td>RAPD</td>
<td>Calvo, Zaragoza, &amp; Osta, 2001; Martinez &amp; Yman, 1998; Rastogi et al., 2007</td>
</tr>
<tr>
<td></td>
<td>SSCA</td>
<td>Tartaglia et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>CSGE</td>
<td>Rastogi et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Mid-infrared spectroscopy</td>
<td>Al Jowder, Defernez, Kemsley, and Wilson (1999)</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td>Chen, Hsieh, &amp; Bridgman, 2002; Kim et al., 2004, 2005; Muldoon, Onisk, Brown, &amp; Stave, 2004</td>
</tr>
<tr>
<td></td>
<td>LC-MS/MS</td>
<td>Colgrave, Allingham, and Jones (2008)</td>
</tr>
<tr>
<td></td>
<td>GC-MS</td>
<td>Nair, Kanfer, and Hoogmartens (2006)</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td>Szucs, Sarvary, Cain, and Adany (2006)</td>
</tr>
<tr>
<td></td>
<td>GC-FID*</td>
<td>Precht (1992a,b)</td>
</tr>
<tr>
<td>Fat (animal)</td>
<td>GC*</td>
<td>Precht (1992a,b)</td>
</tr>
<tr>
<td></td>
<td>Protein (vegetable)</td>
<td>Castro, Garcia, Rodriguez, and Marin (2007)</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
<td>Gonzalez-Cordova et al., 1998; Koppelman et al., 2004</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td>Haasnoot and du Pre (2007)</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>Asahoro et al., 1988; Ashoor &amp; Osman, 1988; Chou et al., 2007</td>
</tr>
<tr>
<td>Protein (animal)</td>
<td>Microsphere-based flow cytometric immunoassay</td>
<td>Chen et al., 2002; Kim et al., 2004, 2005; Muldoon et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Head space GC-MS</td>
<td>Frick, Dubois, Chabert, and Amperou (2009)</td>
</tr>
<tr>
<td></td>
<td>Head space GC-MS</td>
<td>Vareis and Jeskeis (2008)</td>
</tr>
<tr>
<td></td>
<td>Head space GC-MS</td>
<td>Frick et al. (2009)</td>
</tr>
</tbody>
</table>

*Abbreviations used: APPI, atmospheric pressure photoionization; CSGE, conformation sensitive gel electrophoresis; ELISA, enzyme-linked immuno sorbent assay; FID, flame ionization detector; GC, gas chromatography; HPLC, high performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism. These methods are described for dairy products but the same methodology might be applicable to meat.

Rapidly evolving DNA-based techniques have resulted in a change in species determination from protein to DNA analysis. Degeneracy of DNA has the advantage that differentiating among different animal species can be done solely using DNA analysis. Furthermore, when compared to proteins, DNA has a higher thermal stability, is present in the majority of cells, and potentially enables identical information to be obtained from the same animal regardless of tissue of origin (Lockley & Bardisley, 2000). PCR is capable of amplifying very few...
copies of DNA and the limit of detection is therefore often lower than what is observed with protein based assays. Amplified PCR products can either be visualised on a gel (end point PCR) or in real time PCR, sometimes referred to as qPCR (quantitative PCR). Real time PCR data can be collected at the early exponential phase of amplification, which allows quantitative results to be obtained. In contrast, species determination by end point PCR is only qualitative (Calvo et al., 2002; Colgan et al., 2001; Hopwood et al., 1999; Pascoal et al., 2005) or in some cases semi-quantitative (Rodriguez et al., 2003; Sun & Lin, 2003). Currently, real time PCR is generally the method of choice and a number of qualitative (Brodmann & Moor, 2003; Fumiere et al., 2006; Hird et al., 2005; Laube et al., 2007; Mendoza-Romero et al., 2004) and quantitative (Chrisholm et al., 2005; Lopez-Andreo et al., 2006; Martin et al., 2009) species determination methods have been published.

Other common PCR based techniques are restriction fragment length polymorphism (RFLP) (Verkaar et al., 2002; Wolf et al., 1999) and random amplified polymorphic DNA (RAPD). RAPD analysis takes advantage of short arbitrary primers that are able to produce a range of PCR products. RAPD is a powerful technique where little or no information on the DNA sequence is available as long as reference material for comparison is available. This technique is relevant to differentiate not only between domestic animals (Calvo et al., 2001; Martinez & Yman, 1998) but also among rare species (Rastogi et al., 2007) since no prior knowledge of the DNA sequences is required. For more information, the applications of RAPD analysis in livestock species are reviewed elsewhere (Cushwa & Medrano, 1996).

Characterisation of animal species by sequencing depends on availability of known sequences used for comparison. Much information is present in databases, such as the National Center for Biotechnology Information, (http://www.ncbi.nlm.nih.gov/), which contains a large number of sequences from common animal species, breeds, and other genetic variations. Sequencing can therefore be used to identify species in unknown samples, even if no reference material is available. It only requires that the species have a unique DNA sequence and that data are available for comparison. Sequenced PCR amplicons and identification through database comparison has in a number of cases established the identity of the species (Bartlett & Davidson, 1992; Forrest & Carnegie, 1994; Iijima et al., 2006; Imazumii et al., 2007). In addition, single-strand conformational analysis (SSCA) (Tartaglia et al., 1998) and conformation sensitive gel electrophoresis (CSGE) (Rastogi et al., 2004) helps provide post-PCR speciation evidence. Species determination with special focus on quantification is reviewed elsewhere (Ballin, Vogensen, & Karlsson, 2009). General DNA based authentication methods including species determination have also been reviewed (Lockley & Bardsley, 2000; Mafra, Ferreira, & Oliveira, 2008).

Besides substitution of one species with another, fraudulent substitution of tissue with collagen and offal might also be profitable to the food industry. In collagen, the natural amino acid, 4-hydroxyproline (hyp) is a major component and therefore useful in determination of collagen content. Collagen contains about 8% hyp, which is significantly more than other proteins e.g. elastin contains about 1% hyp (Etherington & Sims, 1981). Different amounts of hyp in different meat parts can be used to calculate the content of collagen in meat and meat products (Anon, 2008b). The EU reference method for quantification of hyp (Anon, 1978a) is based on a simple spectroscopic method. Other more advanced methods are also available and include chromatographic techniques such as LC-MS/MS (Colgrave et al., 2008). Offal has successfully been discriminated from muscle tissue with use of mid-infrared spectroscopy (Al Jowder et al., 1999). Another way to discriminate between tissues is the use of bovine h-caldesmon, which is present in smooth muscles (Sobue, Tanaka, Kanda, Ashino, & Kakiuchi, 1985) but absent in cardiac and skeletal muscles (Bretschger & Lynch, 1985). This makes ELISA useful for tissue differentiation (Chen et al., 2002; Kim et al., 2004, 2005; Muldoon et al., 2004).

3.2. Fat (vegetable and animal fat)

In meat products, vegetable fat might fraudulently substitute animal fat. However, vegetable fat often contains phytosterols such as stigmasterol and β-sitosterol, which are absent in animal fat. Thus, the occurrence of stigmasterol and β-sitosterol in meat products is indicative of the presence of vegetable fat. Techniques, such as HPLC (Nair et al., 2006), GC-MS (Szucs et al., 2006), and atmospheric pressure photoionization (APPI) LC-MS/MS (Lembcke et al., 2005) can be used to detect phytosterols in various matrices. It should be stressed that the ratio between total vegetable fat and stigmasterol or β-sitosterol varies and quantitative determination of vegetable fat is hence impractical.

In meat products, animal fat from one species might fraudulently be used to substitute animal fat from another species. However, both animal fat and vegetable fat contain species-specific relative amounts of fatty acids (Precht, 1992a). Gas chromatographic-flame ionization detection (GC-FID) methods based on these relative amounts of fatty acids have so far been used to quantify foreign animal and vegetable fat in milk (Precht, 1992b); the same principles might also be applicable to meat products.

3.3. Protein (vegetable protein, animal protein, and organic compounds)

Vegetable protein such as thecheap and readily availablesoy is probably one of the most commonly used proteins for fraudulent substitution of animal protein. Soy protein can be detected by ELISA methods (Gonzalez-Cordova et al., 1998; Koppelman et al., 2004). In addition, commercial immunoassays for soy protein detection are also available; these include—“BioKits Soya Protein Assay” (Tepnel, Stamford, Conn) and “Alert for Soy Flour Allergen” (Neogen Corporation). Other proteins might also be used as adulterants and a triplex immunoassay for simultaneous detection of pea, wheat, and soy protein has been described for milk, and this method might be relevant to meat and sausage as well (Haasnoot & du Pre, 2007). The triplex immunoassay is based on microsphere-based flow cytometric immunoassay with MultiAnalyte Profiling, which allows measurement of up to 100 different bio molecular reactions (Haasnoot & du Pre, 2007). Also HPLC has shown potential in determination of soy even in highly processed meat mixtures (Castro et al., 2007). Analysis of soy protein in meat products is reviewed elsewhere (Belloque, García, Torre, & Marina, 2002; Koppelman et al., 2004).

Cheap animal protein might be fraudulently used to substitute more expensive animal protein. Animal protein can be detected by ELISA methods. Commercial ELISA methods are available and include “Reveal for Ruminant in MBM” (Meat and Bone Meal; Neogen Corporation), “MELISA-Tek” (ELISA Technologies), “FeedChek” (Strategic Diagnostics Inc.), and the “Tepnel BioSystems BioKit” (Stamford, Conn.). All four kits were evaluated with focus on selectivity, sensitivity, ruggedness, and specificity but failed to meet acceptance criteria set up by the US Food and Drug Administration’s Centre for Veterinary Medicine Office of Research, and hence were regarded inadequate for regulatory use (Myers et al., 2007; Myers, Yancy, Farrell, Washington, & Frobish, 2005). Hopefully, other ELISA methods will perform better in future evaluations. Normally, ELISA tests do not differentiate between proteins from animal derived substances such as milk, whey, casein, cheese, eggs, and meat. It is therefore difficult to identify whether animal protein has been fraudulently substituted in meat products that contain labelled animal derived substances other than meat. In this regard, one option would be to develop tissue specific ELISA methods. Interestingly, bovine h-caldesmon present in smooth muscle (Sobue et al., 1985), is absent in body fluids, cardiac and skeletal muscles (Bretschger & Lynch, 1985). Practically, distribution of h-caldesmon enables ELISA test methods to differentiate between added bovine meat and other bovine derived substances (Chen et al., 2002; Kim et al., 2004, 2005; Muldoon et al., 2004). Detection of animal protein is not restricted to ELISA. Liquid
chromatographic (LC) methods that focus on protein profiles for qualitative detection of a variety of meats, which include beef, pork, and chicken from raw and cooked products have been published (Ashoor et al., 1988; Chou et al., 2007). Furthermore, protein profiles from LC analysis of unheated chicken and turkey mixtures can give quantitative results (Ashoor et al., 1988; Ashoor & Osman, 1988). Examination of the dipeptides, carnosine, anserine, and balenine can qualitatively detect pork, beef, lamb, and chicken as long as different species are not mixed (Aristoy & Toldra, 2004). Isoelectric focusing (Skarpeid et al., 1998) and sodium dodecyl sulfate (SDS) capillary gel electrophoresis (Vallejo-Cordoba & Cota-Rivas, 1998) of proteins also successfully differentiates between beef, pork, and turkey in meat mixtures.

Detection of vegetable and animal protein is dependent on the nature of proteins. However, detection of protein might be impossible if proteins are degraded or severely altered during the processing of meat. In that case, species-specific methods such as PCR can assist in identification of protein-adulterated meat. The advantage of DNA based methods as compared to protein based methods is that DNA is more stable as compared to protein under most conditions. However, since the amount of residual DNA in a formulation varies from detectable to non-detectable levels; PCR results should be considered indicative of qualitative character.

In addition to substitution with vegetable and animal protein, melamine and urea have been used in fraudulent substitution of protein (Frick et al., 2009; Sivaraman, 2007). Melamine and urea are effective adulterants as they contain a high level of nitrogen. Melamine and urea contain 67% and 43% nitrogen, by mass, respectively, and cyanuric acid synthesized with stabile isotopes and used as internal standards in liquid chromatographic tandem mass spectrometry, applicable to chicken, pork, catfish, and pet food (Varelis & Jeskeles, 2008). Head space GC-MS (Frick et al., 2009) has been used to study food ingredients adulterated with urea. An overview of the analytical methods is provided in Table 3.

4. Identification of meat processing treatment

4.1. Irradiation

Electron spin resonance (ESR) spectroscopy has been found to be useful in detection of irradiated poultry meat (Marchioni et al., 2005a; Marchioni, Horvatovich, Charon, & Kuntz, 2005b) while gas chromatography can be used to measure volatile hydrocarbons and 2-alkylcyclobutanones present in irradiated poultry meat. (Horvatovich et al., 2000). Another possibility is the Comet assay (single-cell gel electrophoresis) (Ostling & Johanson, 1984; Singh, McCoy, Tice, & Schneider, 1988), which is based on electrophoresis of lysed cells embedded in agarose on a microscopic slide. The intensity of the Comet tail relative to the Comet head reflects DNA damage (Collins, 2004). The Comet assay finds application in the study of irradiation-induced DNA degradation (Klaude, Eriksson, Nygren, & Ahnstrom, 1996); however, the Comet assay cannot be used as a confirmatory tool as different treatments such as freeze-thaw cycles also cause DNA damage (Park et al., 2000). Analytical methods for determination of irradiation have been reviewed elsewhere (Delincee, 2002).

Table 4

<table>
<thead>
<tr>
<th>Authenticity problem</th>
<th>Analytical technique*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation</td>
<td>Electronic spin resonance spectroscopy GC</td>
<td>Marchioni, Horvatovich, Charon, and Kuntz (2005a)</td>
</tr>
<tr>
<td>Fresh vs. Thawed meat</td>
<td>Comet assay Enzymatic</td>
<td>Park et al. (2000)</td>
</tr>
<tr>
<td>Fresh vs. Thawed meat</td>
<td>Comet assay Infrared spectroscopy NMR</td>
<td>Al Jowder, Kemsley, and Wilson (1997)</td>
</tr>
<tr>
<td>Preparation</td>
<td>LC-MS/MS</td>
<td>Erola, Hollebekkers, Hallikainen, &amp; Petlone, 2007; Paleologos &amp; Kontominas, 2007; Granby and Fagt (2004)</td>
</tr>
</tbody>
</table>

*Abbreviations used: GC, gas chromatography; HPLC, high performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance.

4.2. Fresh versus thawed meat

One method capable of distinguishing between fresh and thawed meat is the enzymatic β-hydroxyacyl-CoA-dehydrogenase (HADH) method (Gottesmann & Hamm, 1983). The HADH method has been widely used and discussed (Anon, 1996; Billington, Bowie, Scotter, Walker, & Wood, 1992; Chen, Yang, & Guo, 1988; Toldra et al., 1991). Originally, one spectrophotometric and one colour test method based on the reaction (1) were published (Gottesmann & Hamm, 1983).

\[
\text{Acetoacetyl—CoA + NADH + H}^+ \xrightarrow{\text{HADH}} \beta-\text{hydroxybutyryl—CoA + NAD}^+ \]

The spectrophotometric method measures the conversion rate of NADH to NAD⁺ by monitoring the decrease in absorption at 340 nm. Gottesmann and Hamm (1983) reported threshold HADH enzyme activity values for beef, veal, pork, mutton/lamb, game, and poultry; surpassing of threshold values indicates a freeze-thaw treatment. The HADH method is applicable to press juice from whole meat, and measures β-hydroxyacyl-CoA-dehydrogenase released from the freezing induced disruption of mitochondria. A grinding procedure of whole meat also induces disruption of mitochondria, whereby the HADH content is released to the press juice. In this case, a significant HADH difference between fresh ground meat and thawed meat is absent and discrimination therefore impossible. It is also important to note that differentiation between fresh and thawed meat is only possible with the HADH method if the freezing temperature has been −12 °C or below (Gottesmann & Hamm, 1983). Enzymatic methods have not been restricted to the HADH method. Other research groups have used the API-ZYM system, which is based on a semi-quantitative method for simultaneous measurement of 19 different enzymes (Ellerbroek et al., 1995; Toldra et al., 1991). Toldra et al. (1991) investigated press juice from pork and showed that enzymatic activity of esterase-lipase, β-glucuronidase, and α-glucosidase differed significantly between fresh and thawed pork. A freeze-thaw regimen also induces structural alteration in DNA and protein. Altered DNA was correlated with freezing and thawing regimens also induces structural alteration in DNA and protein. Altered DNA was correlated with freezing and thawing regimens also induces structural alteration in DNA and protein.
(Al Jowder et al., 1997) was used to study ground meat from pork, chicken, and turkey after storage at −30 °C. Successfully, a principal component analysis was performed subsequently on spectroscopic data in the region 1000–1800 cm⁻¹. Nuclear magnetic resonance (NMR) (Evans, Nott, Kshirsagar, & Hall, 1998; Guiheneuf et al., 1997) and electron microscopy (Sen & Sharma, 2004) have also shown potential in fresh vs. thawed discrimination. An overview of the analytical methods is provided in Table 4. A review on analytical methods to determine fresh vs. thawed meat is available (Ballin & Lametsch, 2008).

The use of sensory methods for detection should not be forgotten. Storage of meat either unwrapped or wrapped in vapour permeable material at subzero temperatures might result in freezer burn. Freezer burn is caused by sublimation of ice from the surface region. An opaque dehydrated surface is easily noticed in freezer burns (Kaess & Weideman, 1967) and clearly indicates meat stored poorly at subzero temperatures. Another obvious visual feature is decolouration induced by freezing (Deman, 1999). Studies on bone marrow and meat showed a red colour decrease and a brown colour increase after freezing, while little or no changes occurred for refrigerated samples (MacDougall, 1982; Nicolalde, Stietzer, Tucker, Mc Keith, & Brewer, 2006). A colour change might therefore indicate whether meat is fresh or thawed but also odour and tenderness of meat are changed (Jakobsson & Tsson, 1973; Khan & Berg, 1967; MacDougall, 1982).

4.3. Meat preparation (baking, cooking etc.)

With regard to preparation of meat products, a low economic benefit from fraudulent behaviour could perhaps be the reason why scientific discussions are limited. No analytical methods have to my knowledge been published in this area. However, production of Maillard compounds (Skog, Johansson, & Jagerstad, 1998) during heating could perhaps distinguish among different procedures used in boiled, fried, and grilled meat. Maillard products form above certain temperatures, which, for example, make it possible to distinguish between long-term cooking at a low temperature from short term cooking at a high temperature. For instance, acrylamide is formed above 120 °C (Mortram, Wedzicha, & Dodson, 2002) and could be used as an indicator of products exposed to temperature above 120 °C. HPLC (Eerola et al., 2007; Paleologos & Kontominas, 2007) and LC-MS/MS (Granby & Fagt, 2004) can be used to quantify acrylamide.

5. Identification of non-meat ingredient additions

5.1. Additives

The vast number of organic compounds used as additives makes it difficult to present a detailed description of each. These organic compounds might be colurants, aromas, or preservatives. Colorants and some reducing chemicals can be used to increase fresh meat appearance. Aromas, such as smoke aroma can fraudulently be used instead of natural smoking of meat. Preservatives can be used to preserve meat and make it appear fresh much longer than normal. The nature of the chemical compound determines the appropriate analytical technique. Since colour, aroma, and preservative relate to organic compounds added to the meat products both HPLC and GC methods may be appropriate.

Another group of additives are enzymes that take part in blood clotting. These can be used as blood-based binding agents and added to meat cuts or minced meat to form portions of desired mass and shape. In the binding process, thrombin cleaves fibrinogen to fibrinopeptides A and B. LC-MS/MS can be used to detect bovine (Grundy et al., 2007) and porcine (Grundy et al., 2008) fibrinopeptides A and B in concentrations down to 5%. As the fibrinopeptides A and B are species-specific the methods are capable of discriminating between blood-based binding agents of bovine and porcine origin.

5.2. Water

If water is added fraudulently to meat it effectively amounts to selling water for the price of meat. Addition of water is therefore a real problem (Elliot, 2007) and regulations dictate the permitted amount of extraneous water in meat. A standard method to determine extraneous water in meat is to study the water/protein ratio. For instance, added water can be determined from a plot of water/protein ratio against extraneous water in boneless, skinless chicken breast (Anon, 2005). Water content can be determined by ISO 1442 (Anon, 1997), which describes the measure of mass before and after drying of meat, and protein content can be determined by ISO 937 (Anon, 1978b), which describes an indirect protein determination method based on Kjeldahl analysis. If water is added, the water/protein ratio may be too high and serve as a clear indicator of addition of water. However, protein and phosphate can be added to meat products to increase water binding, leaving the water/protein ratio close to the natural ratio. In case of detection of foreign protein or phosphate might provide proof of fraudulent practice.

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References


