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Analytical Methods for the Authentication of Meat and Meat Products: Recent Developments

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Introduction

Authentication was defined by Dennis (1) as the process by which a food is verified as complying with its label description. Indeed, labels on meat and meat products give allegations on issues such as species, type of feed given or composition of meat products. These allegations may be verified in some cases, using analytical methods. However, as shown in Table 1, the analyst is faced with a very broad spectrum of possible frauds and mislabelling and the development of an applicable analytical tool is not always feasible. As shown in some recent reviews (see e.g. (1, 2)) the analytical tools involved in the assessment of authentication, which can be highly complex in some cases, still are under development for some authenticity issues.

The aim of this paper is therefore to assess which new developments occurred recently, trying to emphasise on what are the weaknesses and the strength of the currently available methods, and trying to identify which authenticity issues are still lacking an adequate analytical tool.

This paper focuses on authenticity issues such as determination of the geographic origin, animal species, type of feed, as well as treatment (such as irradiation, freezing) and composition of meat and meat products (issues are listed in table 1). Further aspects, such as contamination of meat with veterinary drugs or detection of processing aids and additives are beyond the scope of this review.

It was found that the various authenticity issues are very unevenly covered by the existing analytical methods and that quality labels and organic meat are among the issues that are currently most badly missing efficient analytical tools for their authentication.

Table 1
Some authenticity issues linked with meat/meat products

Production step before end consumer	*authenticity issues*
animal feed production farm	type of feed (GMO-free, hay, protein concentrate), animal husbandry issues: "biological" or "organic" meat, quality labels
slaughterhouse	flow separation between meat for human consumption/other types of meat, flow separation between organic/conventional, geographic origin, + issues mentioned above
transformation/process	addition of blood proteins, addition of mechanically recovered meat, addition of non-meat proteins, animal species/breed/sex, irradiation, meat content, + issues mentioned above
retailers, restaurants	specific cuts, freezing-then-thawing, + issues mentioned above

Analytical methods for the determination of the geographic origin

Despite of the crises that stroke the meat and meat product sector, the international trade of these items is permanently growing. Every day large amounts of beef, poultry and pork meat travel across Europe. The major European producers of meat are Netherland (pork, beef, poultry), Belgium (pork), France (beef, poultry), Germany (beef) and Danemark (pork). The European community also imports significant amounts of meat from Brazil (beef, poultry) and Argentina (beef) (3). On the other hand, the enlargement of the European community also could mean that the meat trade with eastern Europe, with Russia and with Ukraine will be strongly increased. This calls for adequate analytical tools allowing the control of the declared origin.

Trace Elements

Elemental composition and especially heavy metals may be relevant indicators for the region of origin as they may be transferred from soils to meat through forage. Indeed, the trace element *selenium* is found in higher concentrations in beef when forage and soil present higher levels in these elements. A study on 138 cull cows (4) showed that selenium concentrations in soil and grass were positively correlated to selenium content of skeletal muscle. Selenium concentration in whole blood, diaphragm and liver also were significantly correlated to its concentration in skeletal muscles. In a previous study performed on food items from different countries including meat from beef, pork, veal, horse, sheep, game and chicken (5), American beef showed markedly higher selenium concentrations (435±56 ng/g) than Swiss beef (256±89 ng/g). However, Swiss and French beef samples laid in

the same range. For sheep meat, Switzerland (219±117 ng/g) was similar to Newzealand (291±165 ng/g), but both were markedly lower in selenium than Australian sheep meat (484±10 ng/g). The largest differences were observed for horse meat, where Swiss origin (337±115 ng/g) was characterised by less than half the selenium concentration found in meat of American origin (876±601 ng/g).

In a study where *cadmium* was analysed in organs and tissues of horses slaughtered in Italy (6), the geographic origin was recognised as the main factor influencing the cadmium content of the equine species analysed. The differences between horse meat imported from Poland, Lithuania and Hungary were statistically significant. In opposite, a study on Canadian wildlife including deer and caribou did show that the concentration of cadmium in tissue samples was not correlated to cadmium in exposed bedrock close to the animals nor to the proximity to anthropogenic sources of cadmium but was correlated to the age of the animal (7). Finally, in a recent Swedish study on the transfer of *radiocesium* from fallout to reindeer meat it was suggested that both the extent of transfer of ^{137}Cs to reindeer meat and its subsequent decline with time, were affected by the different geographic origin of samples (8).

However, metals in meat are not fully governed by natural enrichment of local forage and soils but also on supplementation from feedstuffs and probably metabolic factors and age, making the interpretation of differences difficult. Moreover, animals are not only fed local grass or hay but also agricultural by-products such as peanut hulls, almond shells, waste form bakeries and poultry manure. These commodities are shipped all over the world (9).

Stable isotope composition/analysis

As mentioned in recent papers (10, 11) the relevance of *site-specific natural isotope fractionation* and of *stable isotope ratio analysis* to authentify the geographic origin of meat is obvious and these techniques will probably play a key role in this field. The abundance of lighter elements (H, C, N and O) is most frequently used. However, heavier stable isotopic tracers such as ^{208}Pb, ^{87}Sr or ^{143}Nd may be more closely correlated with the geological parameters and soil composition but are less often measured as the lighter ones. The type of samples amenable to isotopic analysis include raw or processed meat as well as meat water and also protein and lipid fractions.

In two recent communications it was reported (12, 13) that the abundance of ^{13}C in meat can be used to assess the amounts of C_3 or C_4 plants that were included in the local cattle's diet. Moreover, the abundance of ^{15}N was found to be related to the type of fertilizers applied to the acreage. The abundance of ^{18}O in body fluids was influenced by drinking water and the latter was related to local climate conditions. Using the isotopic ratios of $^{2}H/H$ and $^{18}O/^{16}O$, meat samples from Germany and Argentinia showed a perfect separation of the geographic origins, and even a clear differentiation between southern and northern Germany (14). In a further study

using the same principle, the abundance of ^{18}O exhibited significant differences in beef meat samples from Germany, United Kingdom and Argentina (15). In a study on lamb meat, the isotopic data from ^{15}N and ^{13}C of protein produced a good separation between UK, Iceland and France. Moreover, these origins could be discriminated from a cluster including southern countries Italy, Spain and Greece (16). It should be objected here that isotopic methods using $^{18}O/^{16}O$ and $^{2}H/H$ work apparently fine on fresh meat, but that their reliability is unknown in the case on processed meat products, such as dried meat and cooked sausages where technological treatments may significantly alter the isotopic composition. Moreover, these parameters are bound to local climatic conditions, which are likely to change from year to year.

DNA Analysis

The geographic origin may be in some cases connected with the *breeds,* which are domestic in a certain geographic zone. Analysis of genomic or mitochondrial DNA may discriminate species and breeds within species. Methods proposed include RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) and polymorphic microsatellites or STS (sequence tagged sites) (17). However, the proposed identification of "robust markers" for breeds still has to be done.

Traceability systems are one of the most direct way to relate a piece of meat with its geographical origin. Indeed, using these systems, an unbroken chain of information should link a cut of beef that landed in the consumer's plate with the place where the animal was born and grown up, with the type of animal husbandry, what kind of fodder it received and where it was butchered. In Switzerland, every head of cattle shipped elsewhere will travel with a "passport" and all animal movements will be integrated into a database. Species such as beef, pork, goats, sheep, and captive game should bear an ear tag in order to follow each animal from its birth to the place where it is butchered. However, full-traceability stops, except for organically produced meat, at the slaughterhouse's door. Meat and meat cuts being thereafter pooled in batches, irrespective of the production site. Finally, it is always possible to go around any traceability system. As observed in UK, some pieces of cattle were never declared at birth, were then illegally butchered without the documents allowing a cattle entering a slaughterhouse and were illegally purchased by retailers (18). For these reasons, traceability systems should be completed using laboratory measurements. In some countries, the sliver of displaced ear is collected and used as a DNA link between shelf meat and farm animal (19). The DNA sample will be stored and only accessed where disputes arise or for random checking. The electronically marked tube will be also cross-linked to farm records such as health and drug treatments. This DNA tagging may also be used to certify a PGI product (Protected Geographic Indicator). A cheaper version of DNA tagging named digital DNA-signatures was also recently proposed as an alternative (20). Using a robot-

ized version of DNA-analyser, the measurement of genotype on up to 60000 samples of bovine ear/day was feasible (21).

Analytical methods for the determination of species

As shown by reports of Swiss food control authorities, false declaration of species still occurs regularly, though often by ignorance of the regulations. In year 1999 food control authorities found out, on the base of random controls, a rate of false declaration of species between 7 % and 15 %. In some meat products (sausages) it was up to 40 % (22). Two years later, the percentages were from 13 to 27 % (23). It is nevertheless clear that these figures do represent only a very small sample size and may not be representative of all the meat and meat products sold on the market.

The differentiation of animal species is a theme which has given birth to many scientific papers and a broad variety of methods are currently available. They are mostly of two types. Methods of the first type are protein-based (immunoassays, electrophoretic techniques). They use markers such as soluble proteins, structural proteins and/or heat-stable proteins. The second type of methods are DNA-based (hybridisation, Polymerase Chain Reaction, fingerprinting, sequencing, PCR-RFLP). Finally some further methods are based on physical or chemical parameters. A review on PCR methods can be found for example in the abstracts of the conference on Food Authenticity (issues and methodologies) (24).

Protein-based methods

The well established electrophoretic methods are widely used to differentiate meat species. They offer the possibility to differentiate between a large number of species (25). Isoelectric focusing (IEF) is described as a quick and accurate method for identification of closely related species (26). Using specific monoclonal antibodies in an Enzyme Linked Immunosorbent Assay (ELISA), it was possible to detect 5 g/kg of pork, beef, lamb and horse meat in cooked poultry products (27). This was a marked increase in sensitivity compared to previous methods based on monoclonal antibodies and ELISA (100g/kg chicken meat in mixed meats (28). Moreover, chicken could not be distinguished from turkey, duck or pheasant. Specific assays for immunoglobulin G in pork, chicken and beef was also previously developed to detect the adulteration of ground meats by meats of other origins (29).

DNA-based methods

Working on meat and bone meal samples, a PCR assay based on the amplification of the *mitochondrial ATPase 6/8* gene in bovine, ovine, porcine and poultry species (30) showed a detection limit of 0.3 % for bovine and ovine specific primers and 1 % for porcine specific primers. More recently, the methods using PCR of the *mitochondrial cytochrome b sequence* with subsequent restriction fragment length polymorphism (RFLP) have been re-examined (31) and sequencing of the PCR fragment together with a BLAST (Basic Local Alignment Search Tool) search in a

public database for meat was proposed to solve problems such as unavailability of reference material. Using *Quantitative Competitive (QC) PCR* (32), it was possible to differentiate between contamination and intentional admixture of pork meat. This *QC-PCR* could also be used in detection of minimal amounts (1g/kg) of pig meat in products which must not contain any porcine residue. Using an actin gene-related PCR test for chicken meat mixtures, detection of admixtures with other meat species at a level of 1 % was feasible in fresh and cooked meat (33). Random amplification of polymorphic DNA *(RAPD)* was the technique chosen to differentiate meat from beef, buffalo, pork, lamb, horse, mule, donkey, elk, reindeer, goat, kangaroo and ostrich. Each meat species generated a species-specific RAPD fingerprint, allowing identification even in frozen, boiled and canned meat and in salami (34).

The performances of DNA-based and proteins-based methods have been recently reviewed and compared (35). The authors concluded that both ELISA and PCR were able to detect raw and poorly heat-treated (100° C, 15 min.) raw materials and finished products if there is a certain amount of ruminant material present. Detection limits were about 0.5 to 1 g/100 g for beef and 1 to 2 g/100 g for sheep. However, both types of methods cannot detect bovine, ovine or caprine material in very strongly heat-treated material. Detection limit went down to 0.1 g/100 g for beef (wet petfood) in some cases but all sensitivity was lost when a more intensive heat treatment was applied (125° C, 125 min).

Many PCR-based assays use DNA targets in the mitochondrial genome. These non-nuclear targets possess several advantages over nuclear genes. They are generally more abundant in any given sample tissues than single copy nuclear genes (by a factor up to 10000), thus lowering the threshold of detection. Then, mitochondrial DNA has a relatively high mutation rate compared with nuclear DNA and thus contains more sequence diversity, facilitating the identification of closely related species. Mitochondrial DNA tends to be inherited through the maternal germline and the resulting lack of heterozygosity in the alleles simplifies analysis (36). Thus, the use of mitochondrial DNA with target genes such as cytb in conjunction with PCR-RFLP or multiplex-PCR provides specific and sensitive analytical tools to distinguish between species, breed and sex. However, the sensitivity of the PCR approach depends on the type of food/meat matrix being examined. Indeed, *Rüggeberg et al.* (37) reported that the sensitivity of PCR analysis for various admixtures of meat heated for 100° C for 20 minutes varied between 1 % – 10 %. Longer incubation periods and higher temperatures also markedly affect the quality of data (38). In addition, successive rounds of baking procedures result in a marked decrease in PCR efficiency (39).

Although DNA is a relatively stable molecule and can survive quite severe food manufacturing processes, it can be degraded by chemical, physical or enzymatic factors. Prolonged heat-treatment such as autoclaving used in some canning processes may result in DNA hydrolysis, which fragments the DNA or modify its chemistry

in such a way that the PCR steps may not work. This may also occur at low pH (e.g. products in vinegar). Enzymatic degradation of DNA by nucleases may also occur on prolonged storage of fresh foodstuffs. A well recognised problem in using PCR methods with foods is the presence of PCR inhibitors that reduce the efficiency of the genetic amplification process. These include many common food components such as cations (e.g. Ca^{2+} or Fe^{3+}), trace heavy metals, carbohydrates, tannins, phenolic compounds or salts (e.g. sodium chloride or nitrites).

All the PCR-based methods described in this article require some form of secondary analysis that is generally based on gel electrophoresis. Despite the development of automatic systems, this type of analysis remains laborious and is usually only qualitative or at the best semi-quantitative. There is however a particular need for methods allowing to quantify the relative amounts of different genomes in a mixed food product. The rapid development of Real Time PCR provides an elegant alternative to classical PCR. The method makes use of fluorescent primers and probes, meaning that the amplification products can be monitored quantitatively during the course of the reaction. The size of the amplified products being also smaller, a higher sensitivity can be reached since specific targets can potentially be amplified in partly altered materials. Real Time PCR is gradually being adopted for use in food-related testing (40).

Further analytical methods

Other methods for species identification, based on physical or chemical properties, were also developed. Authenticity of species designation may be an important issue for some religious communities, therefore a specific method based on *HPLC-RI profile of triacylglycerols* was developed to detect the presence of pork fat (lard) at a level down to 5 % (on a fat basis) in process food (41).

As screening method *infrared reflectance spectroscopy* has been successfully used to distinguish lamb in beef mixtures (42). In a study on 230 homogenized meat samples (chicken, turkey, pork, beef and lamb) species identification was attempted using mid-infrared (2000–800 cm^{-1}), near-infrared (400–750 cm^{-1}) and visible (400–750 nm) reflectance combined with statistical techniques such as discriminant PLS, FDA and KNN. Turkey and chicken meat could not be separated using this technique. Otherwise, correct classification rates in excess of 90 % were achieved in all cases (43).

Analytical methods for the determination of breed

To distinguish between 3 different beef cattle *breeds*, RAPD was successfully used working with genome sample collected from meat (44). In a study on five indigenous chinese goat breeds used for meat production (45), microsatellites markers allowed to identify the breed.

Analytical methods for the determination of sex

Using an *electronic nose* of the type MOS (Metal Oxide Semiconductor gas sensor), it was possible to rapidly detect *sex-linked differences* in meat products composition (46).

Analytical methods for the determination of the type of feed

Here will methods deal with the detection in the meat of a prolonged use of some feed types and not on methods for analysing feed themselves. By the European council decision of 2000/766/EC of 4 dec. 2000, processed animal proteins (meat and bone meal, meat meal, bone meal, blood meal, dried plasma and other products) are totally banned in feed destined to farmed animals which are kept, bred or fattened for the production of food. The regulations are even stricter for organically fed animals.

Using ^{15}N *abundance in cattle hair* (47) a marked difference was observed between animals fed with protein concentrates and those fed only with hay. The method however integrates the isotopic composition over a long period of time and is unlikely to detect short term feeding with protein concentrates.

Plant pigments such as *carotinoids* offer an interesting alternative to this isotopic method. A study with lambs fed either with green grazed grass (G group) or concentrate and hay (S group) showed the two groups could distinguished using the reflectance of adipose tissue at 450 and 510 nm, measured using a portable spectrophotometer (48). Again, this method is unlikely to detect short term feeding with concentrate and may be easy to fool by adding plant pigments in concentrates.

Using *Curie point Pyrolysis-MS (Py-MS) techniques,* samples of subcutaneous fat, lamb fed milk or concentrate or pasture could be correctly classified in 92 % of cases (49). The free fatty acid and volatile compounds of Iberian swine subcutaneous fat was also used as an indicator of different feeding systems. Using such an approach (50), it was possible to differentiate swine fed on a traditional diet based on acorns from those fed on concentrates. Depending on the type of botanical flora present in hay, various *terpenes* may accumulate in meat. Based on this principle, it was proposed that terpenes in meat could be used as tracer for both, type of food and geographic origin of the animal (51).

Near Infrared Reflectance Spectroscopy (NIRS) (52, 53) has been tentatively used to identify high quality Iberian pig meat and meat sausage. These pigs are fed mostly with acorns from *Quercus Ilex* and *Quercus suber* and also some grass as protein supplement. On the other hand some lower quality pigs, fed with a mixture of corn, barley or compound feedingstuff are also produced to satisfy the market demand. NIRS and PCA statistical treatment was proposed as fast, cheap and non-destructive alternative to more complex techniques (GC-based) already allowing a correct classification. The method proved however to be unsuccessful in this instance and results showed that only a considerable intake of acorn during a long

period of time may produce enough difference in fat spectral properties. A method is currently being developed (12) to assess the use of meat flours by isotopic analysis of meat, hair and horns. The stable isotopes of carbon (^{13}C) and sulfur (^{34}S) have been used to characterise products of iberian swine in relation to feeding regimes (54). Isotopic composition of adipose tissue and of liver have been used in the mentioned study.

Analytical methods for the determination of quality label and organic meat

The numerous scandals that occurred in the last ten years, such as bovine spongiform encephalopathy disease crisis, dioxin and more recently antibiotics, led to a marked loss of consumer's confidence in meat and meat products. Since then, many quality labels have flourished in the hope of restoring consumer confidence. By definition, quality labels imply a defined type of feed, breed and keeping. However, these labels may actually lead to further deceptions and frauds. Therefore there is an obvious need to develop adequate analytical tools to control these products.

For the authentication of label chicken (such as Loué, label Rouge or others) a study was published by the Gembloux Agricultural Research Center (55). This work reports the use of *NIRS* for the authentication of fast or slow-growing chicken breeds. The NIRS could resolve about 80 % of the cases. Nevertheless, the drawback of the NIRS is that it requires to build up a database of authentic samples which the unknown ones has to be compared with. *AFLP* was also used successfully for the same purpose. In the same study, an attempt with PCR-RFLP to detect admixtures of turkey and chicken meat was assessed, but the method was found to be inappropriate for this scope. Using muscle development index and fat score together with intramuscular fat composition, a 100 % correct attribution could be performed for 2 populations of chicken ("label fermier" vs "standard"). For ducks, the classification was correct at a 98 % level (56).

Approaches using multiple parameters such as proposed by *Marquina* (57) may offer a greater separation power than those relying on one single parameter. In an attempt of characterization and differentiation of commercial type of Aragon sausages, several measurands have been considered such as moisture, ash, fat, total protein, nitrates, non-protein nitrogen, collagen, free fatty acids, hydroxyproline, *p*H-value, curing conversion index and water activity. However the statistical tools involved in data reduction of these multi-parameter studies are often trickier to handle.

These multi-parameter approaches for authentication may include physical, isotopic, chemical and microbiological data and are being used with much success on other matrices such as Emmental cheese from Switzerland (58). However, it also implies the creation of databases of authentic samples, which could not be done on a general basis for all meat products.

Isotopic data from (12, 13) may also be used to assess the types of feed that were used to feed the animal. Indeed, ^{13}C-data can be used to assess the amounts C_3

plants (such as grass) or C_4 plants (such as maize) included in the fodder and that ^{15}N is related to the type of fertilizers applied to the acreage.

The authentication of quality labels and organic meat is very much related to legal definition of these production modes and these definitions may be different from one land to the other. Moreover, it is obvious that some criteria required by organic labels (such as number of days spent outside and space allocated to each animal) could hardly be analytically measured, but can be better controlled by on-site inspections.

As stated in the regulation EC 1804/1999, organic fed herbivores must receive at least 60 % of dry matter in daily rations in roughage. Commercially available fodder usually contain synthetic α-tocopherol (8 isomers), whereas cattle fed with natural food receive mostly the natural form (RRR- α-tocopherol). Based on this fact, a method using chiral HPLC was designed to compare the tocopherol composition of organic and conventional beef meat (59).

Analytical methods for the determination of frozen and then thawed meat

In Switzerland, recent analytical results showed that the freezing-then-thawing practices have not disappeared. The canton laboratories of Appenzell, Glarus, Schaffhausen investigated the question of frozen-then-thawed meat. As a result, out of 43 samples of "fresh" meat, 15 % actually contained frozen-then-thawed meat but were not declared as such (23). Storage in frozen form is the best technique for preservation of meats, however even a short time storage implies a deterioration of quality. Thus, meat that was previously frozen must be labelled accordingly. Methods often cited for detection of previously frozen meat use HADH activity or near IR reflectance spectroscopy of drip juice. The latter uses a combination of near infrared (1100–2498 nm) spectroscopy and chemometric methods (60). The former HADH-activity method proved to be efficient to detect freezing in selected cuts of beef, pork, lamb, chicken, turkey and duck breast, but not in frozen liver or kidneys from red meat species (61).

Analytical methods for the determination of irradiation

Several European standard methods are currently available from CEN to assess the irradiation of food. The EN 1786:1996 "CEN standard for the detection of irradiated food containing bone" is based on the detection by ESR of the radicals that are formed by ionising radiations. These radicals are stable for a period up to several months in foodstuffs. The EN 1784:1996 "CEN standard for the detection of irradiated food containing fat, GC analysis of hydrocarbons" is based on the fact that irradiation lead to the formation of alcenes or alcadienes by some triglycerides, which are measured by GC-FID or GC-MS (62). The standard EN 1785:1996 "CEN standard for the detection of irradiated food containing fat, GC analysis of 2-alklylcyclobutanones" is more complex to use and should be regarded as a confirmation method. An alternative method has been recently published for irradiated

chicken (63). It permits simultaneous determination of 2-alkylcyclobutanones (RCBs) and hydrocarbons (HCs) using cleanup of fat extract by Soxhlet extraction and Florisil chromatography followed by GC/FID analysis. For chicken again, a fluorometric HPLC method was proposed, based on the fact that irradiation of protein-rich foods produces an increase of *o-tyrosine* production (64). This method was compared with ESR method (on bones). The ESR signal intensity correlated with the logarithm of *o-tyrosine* production.

As a screening method, the DNA comet assay (EN 13 784:2001) should be mentioned for the detection of irradiated meat.

However, as it appeared in the data published by food control authorities, unlawful irradiation of meat/meat products seems not to be an issue in Switzerland (22).

Analytical methods for the determination of addition of non-meat proteins or addition of blood proteins

Near infrared techniques may be used to detect adulteration of beef hamburgers with adulterants such as skim milk powder or wheat flour. In a recent study, 5–25 % admixture of these products in beef hamburger could be detected with an accuracy up to 92.7 % (65). Some other, older methods, mostly based on the search for target protein structures by binding of specific antibodies, are still valid (24).

The methods available for determination of addition of non-meat protein are numerous as they are related with specific adulterants. It requires a practical knowledge of the potential adulterants. Methods for blood protein may be easier to define as the type of protein structures to be expected are well defined. Semiquantitative detection of albumin with electroimmunodiffusion is mentioned as an example for the detection of blood plasma or serum in meat products (66).

Analytical methods for the determination of meat percentage in meat products

From 14 February 2000, the directive 97/4/EC (QUID directive) requires for food, which is sold with reference to a characterising ingredient, a quantitative declaration of this ingredient on the label. However, there are no direct analytical techniques allowing the determination of fruit, meat, milk, etc. as such. In the case of meat content, *Farnell* (67) measured nitrogen and fat, and converted these analytical data into information about the composition of the sample. This requires in turn the collection of considerable amounts of background information to ensure that the interpretation is valid, as chemical substances in the meat are subjected to natural variation due to age, sex, breed, cut, and species. A thematic network has been set up, managing the efforts of European food law enforcement laboratories. As a result, procedures and factors have been developed to calculate meat content from nitrogen and fat. Several pitfalls are remaining both on analytical (example: other sources of proteins) as well as on the interpretational side (example: contribution of N-factor variability to uncertainty).

As 3-methylhistidin is only present in actin and myosin, the HPLC determination of this trace amino acid was proposed as a potential indicator of the amount of meat protein (without protein from conjunctive tissues) in meat products. In a study of the German federal bureau for meat research, cooked ham samples were measured for amount of meat with a method based on 3-methylhistidin. The results were compared with the indirect method proposed in the German directive for meat and meat products. In most cases the results agreed within ±0.6 % (68). As clearly shown in *Farnell* (64), the question of analytical assessment of meat amount in meat products is not yet solved. A clear and universally accepted definition of what is meat should be found before an analytical solution is proposed.

Analytical methods for the determination of prohibited parts in meat products (central nervous system (CNS), spinal marrow, mechanically recovered meat (MRM))

The outbreak of bovine spongiform encephalopathy (BSE) and the occurrence of a new variant of Creutzfeldt-Jakob disease have focussed the attention on the use of tissues of the central nervous system (CNS) in food. Indeed, CNS tissue such as brain, spinal cord and some tissue of the peripheral nervous system contains more than 95 % of BSE-activity in animals approaching the end of incubation period. Therefore, to effectively control the banning of CNS tissues for human consumption, several methods for the detection of CNS tissue in meat products have been developed and are quoted in this chapter.

Commonly used are the methods based on the detection of specific marker proteins. Available is, on one hand, the method detecting the neuron specific enolase (NSE) using the Western Blot technique (see for example 69, 70, 71) and on the other hand, the ELISA-based test using antibodies against the glial-fibrillary-acidic protein (GFAP) (72). Both of them are very sensitive (detect 2.5 g/kg of brain tissue) and are commercially available as test kits.

The Western Blot technique mentioned above is highly specific and detects semiquantitatively the neuron-specific γ-enolase NSE (standard samples are included in the kit). Compared to other enolases, NSE is relatively heat-stable and mainly found in CNS tissue. Eventhough NSE is also present in peripheric nervous tissue, its low concentration does not affect the interpretation of the results. For the detection of NSE, monoclonal antibodies are used, but these not only bind to γ-enolase but also to α- and β-enolases. This together with the problem of matrix-dependent background may complicate the interpretation of the immunoblots and may lead to false positive results. On the other hand, negative results of immunoblotting must be carefully interpreted in the case of intensively heat-treated meat products (canned food). Despite the fact that NSE is relatively heat-stable, high heat exposure of the product may damage native proteins, and therefore higher concentrations of NSE are required to test products as positive. Thus, there is a need for further studies to increase the sensitivity of immunoblotting (71). Further-

more the application of the Western Blot technique is in general time-consuming and requires some practice.

The ELISA-based test detecting the glial-fibrillary-acidic protein GFAP is easy to perform, automated and rapid, and has a high sample throughput. It is an enzyme immunoassay for the semiquantitative analysis of CNS in raw and processed meat (83) as well as on contaminated surfaces (73). Standards are also included in the test kit. The limits of detection are 0.2 % for brain tissue and ≤0.01 % for spinal cord tissue. When results are interpreted, it has to be considered that the contents of marker proteins in different risk materials (brain, spinal cord) may considerably vary. Therefore, spinal cord will lead to positive results even in lower concentrations. Because of native marker proteins, samples containing raw meat will be detected positive at lower levels. The method worked as well for items cooked at 80° C for 60 min. However, heating the products to 115° C for 100 min. prevented the detectability of GFAP (73). In a study with 220 samples of commercial meat products (including salami, raw and cooked, sausage), 41 samples (18.6 %) were found positive (including 27 samples positive on MRM). Test based solely on neurone specific enolase appeared to give false positives as they sometimes could not be confirmed by the glial-fibrillary-acidic protein method (74). It is now established that raw poultry meat may cause false positive NSE- resp. GFAP-results (75).

Both tests differentiate neither between brain and spinal cord tissue nor between species like beef, pork, chicken, sheep and goat, which would require additional tests.

The facts, that the methods cannot be applied to all matrices and that intensively heat treatment like sterilisation of the product may affect the structure of the marker proteins and therefore reduces the sensitivity of the tests, are restrictions and need to be mentioned. New developments are on their way to cure the problems encountered with current methods. In a recent study, an immunohistological approach for the detection of tissues of the central and peripherous system (CNS/PNS) was proposed (76). In the latter work, NSE and GFAP were used, but also other marker proteins: myelin basic protein (MBP), neurofilament (NF) and peripherin. NF proved to be relatively less sensitive to heat treatment when compared to other markers. More efforts are still needed to achieve the requested standards of reliability.

Further analytical methods

Other methods based on totally different principles were developed for detecting CNS tissue in meat products. Results obtained by quantification of brain specific fatty acids *(nervonic acids)* using GC-MS with a detection limit down to 0.1 g/kg CNS indicate that the method may serve as a reference for immunochemical and immunohistochemical determination of CNS tissue in processed meat products (77). Compared to other methods, the differentiation of beef, pork and sheep species is possible down to a level of 0.05 % brain tissue based on the ratio of cis/trans nervonic acid (78).

Because concentration of cholesterol in CNS tissue is higher than in muscle meat, quantification of cholesterol provides another and low-cost screening method for the rapid analysis of meat products suspicious with regard to CNS tissue addition (67, 79). Prior to the application of the method, normal cholesterol concentrations in authentic samples of meat products need to be defined. Intensive heat treatment while processing meat products does not affect cholesterol contents compared to protein-based methods. However, further food components like egg or other tissues such as liver with increased levels of cholesterol could provide false results, if found in the product. Therefore increased concentration of cholesterol is more an indication than a proof of addition of CNS tissue to food products. With regard to preventive health protection and correct labelling, integrated procedures for the detection of CNS tissue propose to use cholesterol, NSE and GFAP as markers (67).

Mechanically recovered meat may contain parts of the spinal column, but the contamination with spinal cord might be lower than a detectable level. The usefulness of different methods like immunohistochemistry, histochemistry and polarization microscopy was investigated for the detection of spinal cord in products containing MRM (80). Generally if MRM is found using histochemistry, the product is considered to contain traces of CNS tissue.

Analytical methods for the determination of admixture with offals and/or cheaper cuts in pure muscle tissue

The admixture of offals (heart, tripe, kidney, liver) in cooked "pure beef" meat products at a niveau of 20 % w/w of adulterants was investigated using mid-infrared spectroscopy. The results showed a 97 % discrimination between all five sample types (pure beef and beef containing each one of the four adulterants) (81). Once again, the level of cooking had a negative influence on the precision of the method. In a similar study from the same author, but on minced beef adulterated with kidney or liver, samples containing 10 to 100 % of either liver or kidneys were detected. The same author reported previously on a ^{1}H NMR method to detect the adulteration of beef cuts with offal (82).

Aknowledegment

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Summary

This review includes 83 references encompassing most of the recent analytical developments in the field of meat and meat products authentication. It shows that most authenticity issues concerning meat or meat products can be at least partly addressed using the presently available analytical techniques. An overview of these methods is given in table 2. However, in many cases, the methods proposed are only

Table 2
Overview of methods for authenticity testing of meat and meat products

Analytical Method	*Analyte*	*Applicability*	*Literature Reference*
ICP-MS	Trace elements	Geographic origin	4, 5, 6, 7
Stable isotope ratio analysis	^{2}H, ^{13}C, ^{15}N, ^{18}O ^{208}Pb, ^{87}Sr, ^{143}Nd	Type of feed and Geographic origin	10, 11, 14, 15, 16 12, 13, 47, 54
DNA-based methods	Genomic or mitochondrial DNA	Species identification	17, 20, 21, 24, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 44, 45
		Irradiation	EN 13 784:2001
Protein-based methods	Protein patterns	Species identification	25, 26, 27, 28, 29
		Blood protein	66
	NSE	Central nervous syst.	69, 70, 71, 68
	GFAP	Central nervous syst.	73, 74, 75, 76
HPLC-RI	Triacylglycerols profile	Species identification	41
HPLC-UV	Tocopherols in meat	Organic meat	59
HPLC-fluorimetry	o-tyrosine	Irradiation	64
	3-methylhistidin	Meat percentage	68
Infrared spectroscopy	Reflectance signals at specific wavelength	Species identification	42, 43
		Type of feed	52, 53, 55
		Frozen-then-thawed	60
		Non-meat protein	65
		offals	81
UV-visible spectroscopy	Reflectance signals at specific wavelength	Type of feed	48
Electronic nose	Volatile compounds	Sex identification	46
1H-NMR	meat	offals	82
GC-MS	Volatile compounds	Type of feed	50
	Terpenes	Type of feed	51
	Alcenes or alcadienes	Irradiation	62
	Nervonic acids	Central nervous syst.	77, 78
	cholesterol	Central nervous syst.	79
GC	2-alkylcyclo-butanone	irradiation	63, EN 1785:1996
Pyrolysis mass spectrometry	Fingerprint spectrum of pyrolysed meat	Type of feed	49
Muscle development index	Muscle meat	Label chicken	56
Enzyme activity	HADH	Frozen-then-thawed	61
ESR	radicals	irradiation	EN 1786:1996
microscopy	Spinal cord	Mechan. Rec. meat	80

valid for specific applications or may lack robustness. The analytical methods for authentication issues are rather unevenly developed. Some issues such as detection of irradiation are covered with international analytical standards (e.g. irradiated meat) whereas some other issues mostly have been the subjects of preliminary work (e.g. authentication of organic meat, labels).

Zusammenfassung

Der vorliegende Review-Artikel umfasst 83 Referenzen und beschreibt sowohl die aktuelle Situation als auch die neuen Entwicklungen auf dem Gebiet der Analytik für die Prüfung der Authentizität von Fleisch und Fleischerzeugnissen. Dabei wird festgehalten, dass diesbezüglich mit den heute vorliegenden analytischen Methoden bereits annähernd alle Fragen zu diesem Thema behandelt werden. Eine Übersicht der Methoden ist in der Tabelle 2 gegeben. Die vorgestellten Methoden jedoch sind in vielen Fällen nur für ganz bestimmte Anwendungen verwendbar oder es fehlt ihnen eine gewisse Robustheit. Zudem ist die Analytik zur Überprüfung der Authentizität von Fleisch und Fleischerzeugnissen nicht für alle Bereiche gleichermassen entwickelt. Einige Aspekte sind heute bereits durch internationale analytische Standards abgedeckt (z.B. bestrahltes Fleisch), während andere Aspekte erst in präliminären Studien bearbeitet wurden (z.B. Authentizität von Bio-Fleisch, Labels).

Résumé

Une revue de littérature incluant 83 références est présentée sur les nouveaux développements et la situation actuelle des méthodes existantes pour l'authentification de la viande et des produits à base de viande. On constate que la plupart des questions liées à l'authenticité peuvent déjà être partiellement ou totalement traitées en recourant aux méthodes disponibles aujourd'hui. Ces méthodes sont résumées dans le tableau 2. Cependant, dans beaucoup de cas, les méthodes proposées ne sont valables que pour certains produits carnés donnés et peuvent manquer de robustesse. Le développement de l'analytique dans les différents aspects de l'authenticité est aussi inégal. Certains aspects de l'authenticité sont couverts par des standards analytiques internationaux (par ex. la détection de l'irradiation) alors que seulement des travaux préliminaires existent pour d'autres sujets (comme l'authenticité de la viande bio et des labels).

Key words

Meat authenticity, Analytical method, Review article

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Abbreviations used

AFLP	Amplified Fragment Length Polymorphism
CEN	European Committee for Standardization
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Factorial Discriminant Analysis
GC-FID	Gas Chromatography Flame Ionisation Detection
GC-MS	Gas Chromatography Mass Spectrometry
GFAP	Glial Fibrillary Acid Protein
HPLC	High Performance Liquid Chromatography
HPLC-RI	HPLC-Refractive Index
KNN	Kohonen Neural Network
MBP	Myelin Basic Protein
MOS	Metal Oxide Semiconductor Gas Sensor
MRM	Mechanically recovered Meat
NF	Neurofilament
NIRS	Near Infrared Reflectance Spectrometry
NMR	Nuclear Magnetic Resonance
NSE	Neuron Specific Enolase
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PLS	Partial Least Squares
Py-MS	Pyrolysis Mass Spectrometry
QC-PCR	Quantitative-Competitive PCR
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RT-PCR	Real Time PCR
STS	Sequence Tagged Sites