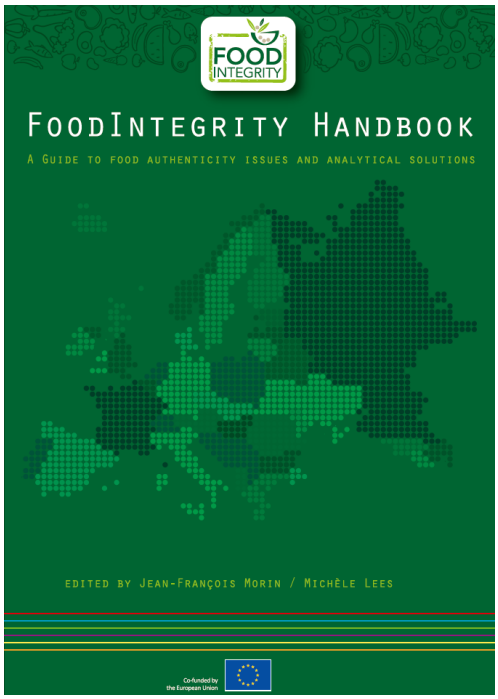
FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

Editors: Jean-François Morin & Michèle Lees, Eurofins Analytics France



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Meat and meat products

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General overview of the products

Demand for animal-derived food is increasing because of population growth, rising income and urbanisation, with poultry meat showing the fastest trend over the last decades and becoming, in 2016, the meat with the highest consumption worldwide (Figure 1). Estimates from the FAO show that the global production of meat in 2016 is around 330 million tons. At the global level, the three main types of meat produced are: poultry meat (36.5 %), pork (35.8 %) and bovine (21.1 %). The average annual growth rate for poultry meat over the last 45 years was 2.3 %, while it was only 0.7 % for beef and 1.8 % for pork [1].

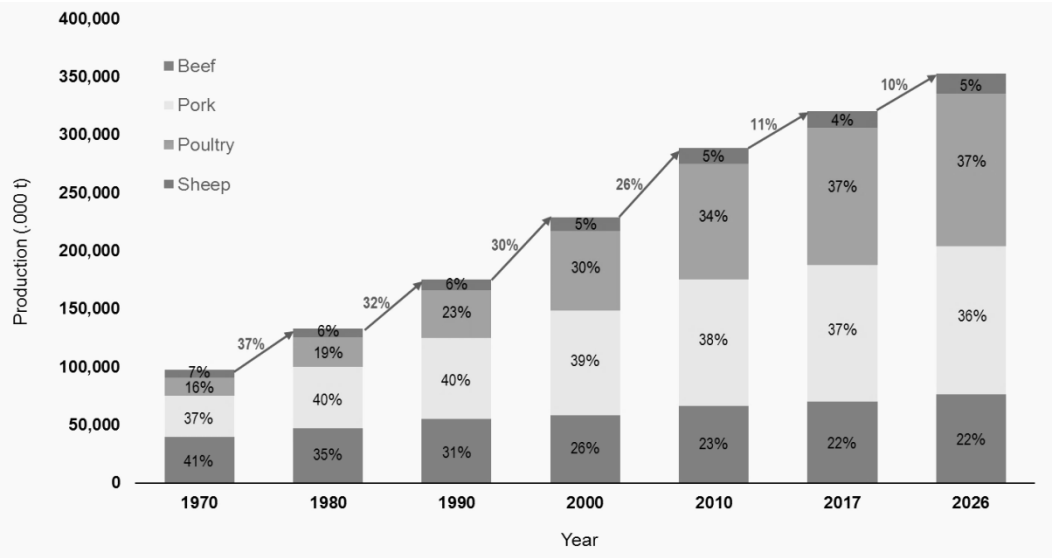

Figure 1: Evolution of global meat production from 1970 to 2016 (Own design, data source: Faostat)

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In the last few years, world bovine meat production has been increasing at a modest pace. The United States are the major bovine meat producing country in the world, with 11 million tons (Figure 2). The second producer is Brazil, with 9 million tons, with a herd expansion encouraged by international trade, despite a reduction in domestic demand. The European Union (EU) is the third beef producer (almost 8 million tons), followed by China, India and Argentina. In 2016, China produced about 55 million metric tons of pork which accounted for 47 % of total world production. The EU is the second world producer with almost 24 million tons followed by Vietnam, Brazil and the Russian Federation. The biggest poultry meat producers are the United States, with almost 21 million tons a year, followed by China, with 19 million tons, the EU and Brazil with about 14 million tons (Figure 2).

Global meat production is projected to be 13 % higher in 2026 relative to the base period (2014-16). This compares with an increase of almost 20 % in the previous decade (Figure 1). Developing countries are projected to account for the vast majority of the total increase, with a more intensive use of feed in the production process. Poultry meat is the primary driver of the growth in total meat production in response to expanding global demand for this more affordable animal protein compared to red meats. Low production costs and lower product prices have contributed to making poultry the meat of choice both for producers and consumers in developing countries. In the bovine meat sector, cow herds are being rebuilt in several major producing regions, but the decline in cattle slaughter in these regions is projected to be offset by higher carcass weights. Pork production will also increase after 2017, driven by slow herd expansion in China.

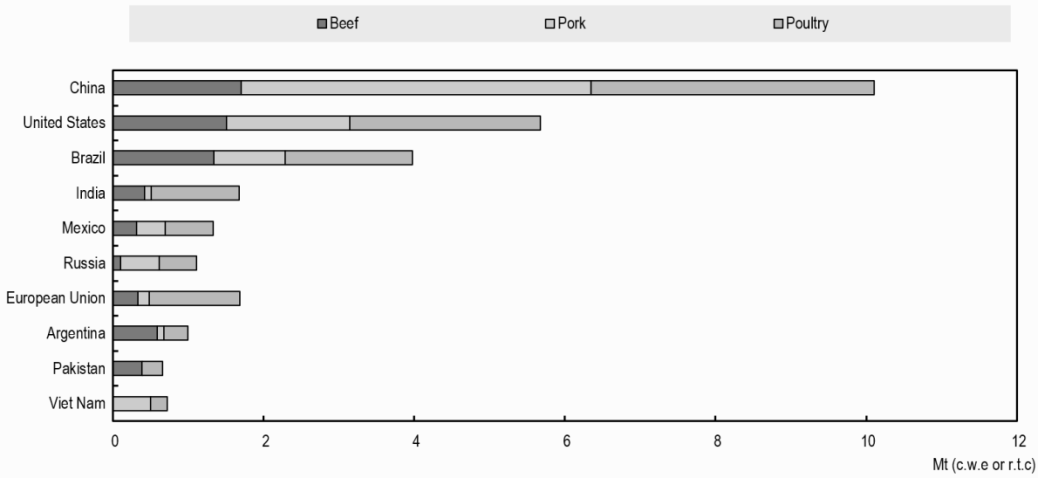

Figure 2: Countries with the greatest share of additional meat production by meat type [1]

In the EU-28, pork is by far the main meat produced, followed by chicken meat and beef (Figure 3). In the EU, beef is mainly produced from cattle breeds grown specifically for their meat, but it can also come from dairy cattle. France (19.0 %), Germany (14.7 %) and the United Kingdom (11.7 %) accounted for almost half (46 %) of the total EU-28 beef production (Figure 4).

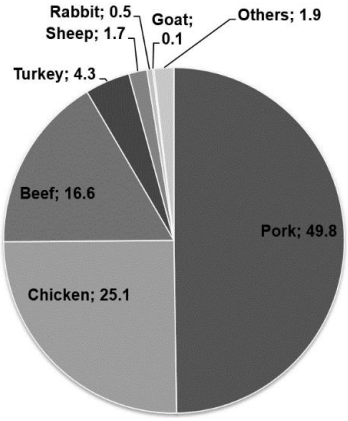

Figure 3: Production share of main meat produced in EU on 2016 (Own design, data source: Eurostat)

As for pork meat production, Germany produced around one quarter (23.9 %) of the EU-28's pig meat in 2016, while Spain produced one sixth (17.9 %) of the EU-28 total, equal to 23 million tons. Finally, Poland, France, the United Kingdom, Spain and Germany each contributed between 10 and 15 % to the EU-28 production of poultry meat in 2016 (Figure 4).

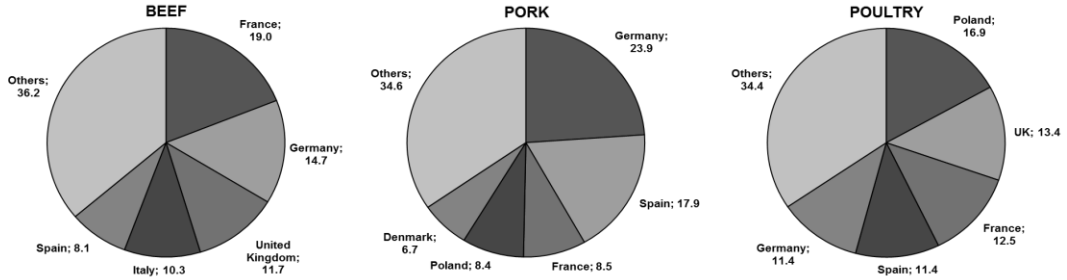

Figure 4: EU Countries with the greatest share of production by meat type on 2016 (Own design, data source: Eurostat)

Global meat apparent consumption per capita is expected to stagnate at 34.6 kg by 2026, an increase of less than half a kilogram compared to the base period (Figure 5). Beef consumption will gradually increase over the next ten years. By 2026, and relative to the base period, it is expected to increase by almost 6 % in developed countries, whereas in developing regions it is expected to increase by approximately 17 %. In per capita terms, beef consumption in the developing world remains low relative to developed countries, at about one-third in volume terms. High population numbers in Asia remain a major driver of growth, combined with the positive perception of Chinese buyers that bovine and ovine meat are healthier and disease-free; the result is an expected 44 % increase in beef consumed in Asia over the next decade [1].

Pork consumption on a per capita basis declines marginally over the outlook period with consumption in most developed countries reaching saturation levels (Figure 5). Among the developing countries, significant regional differences are evident in per capita pork consumption. Growth is sustained in Argentina, Brazil, Mexico, and Uruguay, albeit at a generally slower rate

than the past decade. Pork consumption has grown rapidly over the past few years in Latin America, fuelled by increased domestic production, improved quality, and favourable relative prices that have positioned pork as one of the favoured meats, along with poultry. Conversely, many countries with favourable economic conditions and expanding meat consumption do not traditionally consume high levels of pork relative to other meats, resulting in stagnant and even declining consumption on a per capita basis at the regional level. Population expansion still supports growth in total pork consumption in these regions [1].

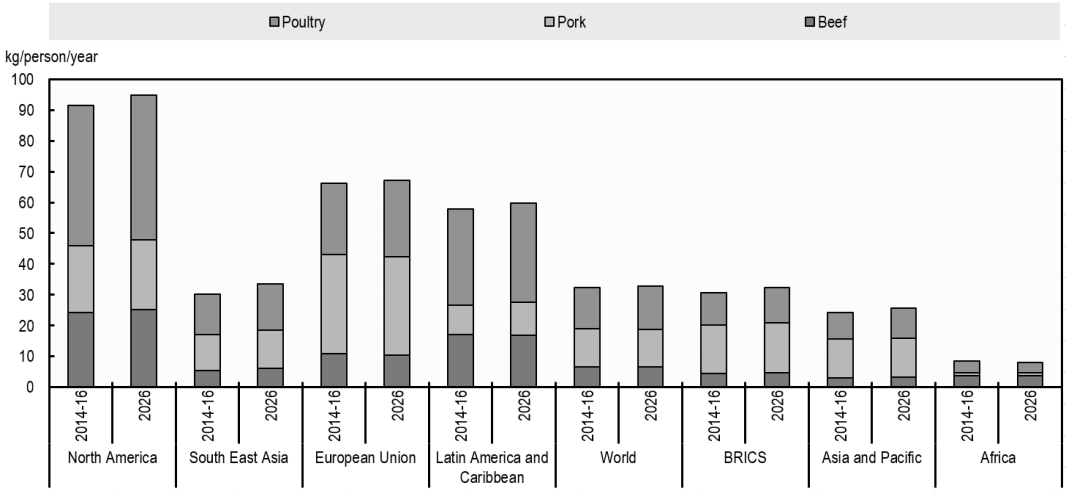

Figure 5: Annual growth in consumption of meat 2007-2016 and 2017-2026 [1].

Consumption of poultry meat increases regardless of region or income level. Per capita consumption will grow, even in the developed world, but growth rates will remain slightly higher in developing regions. Worldwide, poultry grew rapidly and surpassed pork as the preferred animal protein in 2016. This will remain the case during the outlook period and, of all the additional meat consumed over the next decade, poultry is expected to account for almost 45 % (Figure 5) [1].

Per capita consumption of meat is expected to slightly increase in the EU overall from 69.1 to 70.7 kilograms by 2026, whereas the individual big five countries (Italy, UK, Spain, France and Germany) are predicted to experience a decrease in consumption (Figure 5).

1. Product Identity

1.1. Definition of the product and manufacturing process

The first distinction is between fresh and processed meat. Fresh meat is defined as meat having undergone no treatment other than chilling and freezing, while processed meat is a very broad category of many different types of products, all defined by having undergone at least one further processing or preparation step such as, i.e. grinding, adding an ingredient or cooking, which changes the appearance, texture or taste. The main classes of processed meat are described below:

- Minced meat: boneless meat reduced in fragments which contains less than 1 % salt;
- Mechanically separated meat: obtained by removing meat from bones using mechanical devices (high-pressure application machinery) that contribute to the loss or modification of muscle-fibrous meat texture;
- Desinewed meat: obtained by removing sinews, tendons, cartilages and thicker collagen using mechanical devices (low-pressure application machinery) without modification of muscle-fibrous meat texture.
- Meat preparations: fresh meat (including fragments), containing flavourings, additives or subjected to treatments that do not modify the muscle-fibrous texture;
- Meat products: processed products derived from processed meat or further processing of other meat products subjected to treatments that modify the muscle-fibrous texture.

There are many meat products that are produced in different countries, but it is possible to categorise them in six groups, considering the processing technology used:

- Fresh processed meat products: products that are composed of muscle mixed fragments with different amounts of animal fat. They are salted, and small quantities of non-meat ingredients are added to improve taste and binding. All ingredients are added fresh and some of these products are filled in casings. They are cooked or fried immediately prior to consumption (e.g. hamburgers).
- Formed meat: products which may give the impression that they are made of a whole piece of meat, but actually consist of different pieces combined together by other ingredients, including food additives and food enzymes or by other means.
- Cured meat products: products that are submitted to a curing process and treated with small amounts of nitrite. These products are divided in two groups:
    - *Cured raw meat*: products that undergo a process of curing, fermentation and ripening in controlled conditions without any heat treatment (e.g. raw cured beef);
    - *Cured cooked meat:* products that undergo a curing process and then are submitted to heat treatment (e.g. cooked pork ham).
- Raw-cooked meat products: products composed of muscle meat, fat and non-meat ingredients which are reduced in fragments, mixed and portioned before being submitted to heat treatment (e.g. meat loaf);
- Precooked: cooked meat products; products composed of muscle trimmings, fatty tissues, meat from the head of the animal, animal skin, blood, liver and other edible parts, which undergo two different heating processes - precooking of raw materials and cooking of the finished product mix (e.g. corned beef);
- Raw fermented sausages: uncooked meat products obtained by a mixture of lean and fatty tissues combined with salts, nitrite, sugars, spices and other non-meat ingredients filled into casings. They are submitted to a fermentation process (drying and ripening) to obtain the typical flavour and are consumed raw (e.g. salami).
- Dried meat products: lean meat that undergoes a process of drying in natural or artificial conditions to prolong its shelf-life (e.g. dried meat strips or flat pieces).

1.2. Current standards of identity or related legislation

The definitions of "meat", "fresh meat", “carcase”, “offal”, “viscera”, “minced meat”, “meat preparations”, “meat products” and “mechanically separated meat” are laid down in Annex I of Regulation (EC) No 853/2004 (Table 1) [2].

With the Food Information Regulation (EU) No 1169/2011 (FIC) [3], uniform labelling requirements have been applied across the EU and include fresh meat and processed meat products (Table 1).

Specific rules for the origin of beef and beef products were introduced after the BSE crisis in 2000. More recently new rules on country of origin information for meat from sheep, pigs, goats and poultry have been issued in the EU (Table 2).

Definitions for meat are also given in the CODEX Standard for Luncheon Meat (Codex Stan 89-1891) and Cooked Cured Chopped Meat (Codex Stan 98-1891), which defines meat as “the edible part, including edible offal, of any mammal slaughtered in an abattoir”; poultry meat as “the edible part of any domesticated birds, including chickens, turkeys, geese, guinea-fowl or pigeons, slaughtered in an abattoir" ; and edible offal as “such offal as have been passed as fit for human consumption but not including lungs, ears, scalp, snout (including lips and muzzle) mucous membrane, sinews, genital system, udders, intestines and urinary bladder. Edible offal does not include poultry skin.” These definitions are used for the raw materials contained in these products.

However, many differences exist in the interpretation of ‘meat’ for use in meat products among different countries such as the EU, the USA, Brazil, and China. Therefore, methods to determine authenticity need to consider the legal requirements specific to each country.

Table 1: Meat definitions according to EU Legislation

EC Regulation	Basic definition of meat	Animal carcass components specifically excluded from the definition	Basis of meat content declaration
Regulation (EC) n. 853/2004	**Meat** Edible parts of the animals (including blood): - Domestic ungulates (domestic bovine including Bubalus and Bison species, porcine, ovine and caprine animals, and domestic solipeds); - Poultry (farmed birds, including birds that are not considered as domestic, but which are farmed as domestic animals, with the exception of ratites); - Lagomorphs (rabbits, hares and rodents); - Wild game (wild ungulates, lagomorphs and birds, as well as other land mammals that are hunted for human consumption); - Farmed game: farmed ratites and other farmed land mammals. Meat can be defined "Fresh meat" if it has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.	Genital organs of either female or male animals, except testicles; urinary organs, except the kidneys and the bladder; the cartilage of the larynx, the trachea and the extra-lobular bronchi; eyes and eyelids; the external auditory meatus; horn tissue; and in poultry, the head – except the comb and the ears, the wattles and caruncles – the oesophagus, the crop, the intestines and the genital organs.	Not appropriate
	Carcass Body of an animal after slaughter and dressing. The definition of 'carcass' for bovine, pigs, sheep, goat and poultry is reported in Regulation (EC) no. 1165/2008.		
	Offal Fresh meat other than that of the carcass, including viscera and blood		
	Viscera Organs of the thoracic, abdominal and pelvic cavities, as well as the trachea and oesophagus and, in birds, the crop		
	Meat preparations Fresh meat, including meat that has been reduced to fragments (minced meat), which has had other foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.	The same for meat and minced meat.	
	Meat products Processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat	The same for meat.	

<table>
<tr><th>EC Regulation</th><th>Basic definition of meat</th><th>Animal carcass components specifically excluded from the definition</th><th>Basis of meat content declaration</th></tr>
<tr><td></td><td>Minced meat
Boned meat that has been minced into fragments and contains less than 1 % salt. Raw material used to prepare minced meat must derive from skeletal muscle, including adherent fatty tissues.</td><td>Raw material used to prepare minced meat must not derive from: scrap cuttings and scrap trimmings (other than whole muscle cuttings); mechanically separated meat; meat containing bone fragments or skin; or meat of the head with the exception of the masseters, the non-muscular part of the linea alba, the region of the carpus and the tarsus, bone scrapings and the muscles of the diaphragm (unless the serosa has been removed).</td><td></td></tr>
<tr><td></td><td>Mechanically separated meat (MSM)
Product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure</td><td>For poultry, the feet, neck skin and head; and for other animals, the bones of the head, feet, tails, femur, tibia, fibula, humerus, radius and ulna
The use of bones or bone-in cuts of bovine, ovine and caprine animals is prohibited for the production (Reg. EC/999/2001 [4]).</td><td></td></tr>
<tr><td>Regulation (EC) no. 1169/2011</td><td>Meat
For labelling purpose, the term “meat” is referred to: skeletal muscles of mammalian and bird species (*) recognised as fit for human consumption with naturally included or adherent tissue, where the total fat and connective tissue content does not exceed the values indicated below and where the meat constitutes an ingredient of another food:
<table>
<tr><th>Species</th><th>Fat content</th><th>Collagen/meat protein ratio (1)</th></tr>
<tr><td>— Mammals (other than rabbits and porcines) and mixtures of species with mammals predominating,</td><td>25 %</td><td>25 %</td></tr>
<tr><td>— Porcines,</td><td>30 %</td><td>25 %</td></tr>
<tr><td>— Birds and rabbits,</td><td>15 %</td><td>10 %</td></tr>
</table>
(1) The collagen/meat protein ratio is expressed as the percentage of collagen in meat protein. The collagen content means the hydroxyproline content multiplied by a factor of 8.</td><td>Mechanically separated meat</td><td>If maximum limits are exceeded, but all their criteria for the definition of ‘meat’ are satisfied, the ‘… meat’ content must be adjusted downwards accordingly, and the list of ingredients must mention, in addition to the term ‘… meat’, the presence of fat and/or connective tissue.
Meat species is required on the label unless indicated by the product name.</td></tr>
</table>

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<tr><th>EC Regulation</th><th>Basic definition of meat</th><th>Animal carcass components specifically excluded from the definition</th><th>Basis of meat content declaration</th></tr>
<tr><td></td><td>Minced meat
Specific requirements concerning the designation of minced meat:<table><tr><th></th><th>Fat content</th><th>Collagen/meat protein ratio (1)</th></tr><tr><td>lean minced meat,</td><td>≤ 7 %</td><td>≤ 12 %</td></tr><tr><td>minced pure beef,</td><td>≤ 20 %</td><td>≤ 15 %</td></tr><tr><td>minced meat containing pigmeat,</td><td>≤ 30 %</td><td>≤ 18 %</td></tr><tr><td>minced meat of other species,</td><td>≤ 25 %</td><td>≤ 15 %</td></tr></table></td><td>Mechanically separated meat</td><td>In the case of minced meat and meat preparations made from pre-prepared minced meat, except for sausages and sausage meat, the label must indicate the appropriate % of fat and collagen in meat protein (i.e. “% of fat under.....”, “ % of collagen in meat under....”)
Meat species is required on the label unless indicated by the product name.</td></tr>
<tr><td></td><td>Formed meat
Meat products, meat preparations which may give the impression that they are made of a whole piece of meat, but actually consist of different pieces combined together by other ingredients, including food additives and food enzymes or by other means</td><td></td><td></td></tr>
</table>

Table 2: Rules about the labelling of meat products within European Union

Meat	Compulsory information to be provided on the label
Beef (except mince or trimmings)	As required by Regulation (EC) No 1760/2000 and Commission Regulation (EC) 1825/2000, as amended: - Reference number or reference code ensuring the link between the meat and the animal or animals - Member State or third country of birth - All Member States or third countries where fattening took place - Member State or third country where slaughter took place and where the carcass was cut
Pork, lamb, mutton, goat meat and poultry (except mince or trimmings)	As required by Regulation (EU) No. 1337/2013): - Reference number or batch code identifying the specific animal or group of animals from which the meat was derived - name of member state or non-EU country - Member State or third country where an animal has been reared in more than one country, the table below explains what should appear on the labelling. - Member State or third country where slaughter took place

2. Authenticity issues

2.1. Identification of current authenticity issues

In general beef is the main added-value meat product that is the most widely traded, and therefore where major authenticity problems can occur. This is reflected in the list of authenticity topics below. However, some authenticity issues do concern other meat types such as poultry or lamb. These are mentioned where relevant.

Food fraud is a global issue which damages the reputation of companies, disrupts markets and erodes consumer confidence. Food fraud surfaces more frequently in certain supply chains and that of meat is always present. The importance of studies covering these topics is mainly related to economic issues associated with fraud in high-value foods like beef, with cheaper ingredients added. However other fraudulent practices in the meat industry could occur such as: 1) the origin of meat and the animal feeding regime (as in the case of certified regional products of poultry and lamb, for example); 2) substitution of meat ingredients by other animal species, tissues, fat or proteins; 3) modification of the processing methods of meat products; and 4) addition of non-meat components such as water or additives.

2.1.1. Substitution

2.1.1.1. Species substitution

The correct description of the origin of meat and meat products is a common problem reported worldwide. This type of food fraud is, typically, an intentional act for economic gain, using sources of low-priced meats in high-value meat products. Consequences include economic, religious, but also health concerns: counterfeit components may be toxic and the undeclared addition of some ingredients (e.g. soy, wheat, dairy) can pose health risks for consumers with food allergies or intolerances. The most important authenticity issues are the species of meat, followed by specific cut, breed of the animal and geographical origin of the meat or meat product [5].

The flesh of many meat species differs only subtly in appearance and texture, making it difficult to identify the species just by visual inspection. Once meat is comminuted and incorporated into value-added products, however, identification based on appearance and other sensory parameters becomes virtually impossible.

Many countries have legislation for regulating such practice. The EC Minced Meat Hygiene Directive requires each species of the animal used in the minced meat to be listed. Similarly, the EC Meat Products Hygiene Directive requires naming of species in meat products. The EC Labelling Directive requires each species of meat used in products to be clearly identified in the ingredient list.

It is not possible to be specific about the extent of species substitution that is occurring. However, in the last few years, several meat adulteration scandals have had great repercussions worldwide, such as the recent horsemeat scandal in the EU.

2.1.1.2. Protein substitution

Proteins can be added to meat products in most countries within a prescribe limit, but the types and the amounts prescribed differ greatly. There are often regulations or requirements for a meat content declaration prescribing the minimum content of muscle meat in meat products.

Cheap animal protein might be fraudulently used to substitute more expensive animal protein. Casein is by far the most commonly used milk protein, sometimes in combination with excessive amounts of water and polyphosphates. Whey proteins are also used for this purpose.

Vegetable protein such as cheap and readily available soy is probably one of the most commonly used proteins: in recent years, the addition of soybean protein as a raw material replacing red meat in burgers for example has increased significantly due to its functional characteristics (which include increased water and fat binding capacity, emulsification ability), and improved organoleptic properties, such as appearance, (smooth texture, and cutability), nutritional value, as well as its low price. For these same reasons the addition of vegetable protein can be carried out fraudulently, leading to a potential safety concern due to its allergenic properties.

Another special sanitary issue has been the use of gluten which causes intolerance reactions in some individuals. Microbial proteins have been developed for use in foods but are not widely used in meat products.

Finally, the addition of melamine and urea to meat products is an unlawful method of increasing the apparent protein content [6].

2.1.1.3. Fat substitution

The replacement of animal fat with a cheaper vegetable may occur; however, the incorporation of these vegetable fats, especially in meat products, may be associated with a reduction in quality mainly due to a significant oxidative instability.

Substitution could also be used in order to make a claim about the nutritional quality of meat products in response to consumer demands for natural and healthy foods; in fact, the substitution reduces the level of saturated fatty acids (SFA) and increases the level of polyunsaturated, both essential for the prevention of heart disease. Nonetheless the oxidation of unsaturated lipid fractions along with oxygen presence during meat grinding and the need to add salt during processing could have a negative impact on the quality of these products, misleading the unaware consumer [7].

2.1.1.4. Tissue substitution

Offal represents any of various non-muscular parts of the carcasses of beef and veal, mutton and lamb, and pork, which are either consumed directly as food or used in the production of other foods. In countries where these parts of the animal can be designated as meat, there is a financial incentive to include them in products due to the difficulties in their detection [8].

Mechanically recovered meat (MRM or MRPM for mechanically recovered poultry meat) means removal of any remnant flesh from bone after manual deboning. MRM is used as an inexpensive product, with good nutritional and technological properties which has encouraged most meat processors to substitute meat partially or totally by MRM in most types of meat and poultry. The nutritional value and chemical components of MRM vary with raw materials (necks, backs, frames and skin) used in its production. Crushing of the bones and subsequent mechanical separation leads to changes in the chemical, physical, sensory and functional properties of the meat including the development of undesirable aromas (rancidity), loss of its characteristic red colour because of lipid and pigments oxidation, loss or modification of the muscle fibre structure, reduced stability during storage as well as its functionality and processing ability [9]. Moreover, consumer defence groups are concerned about the inclusion of bone fragments in mechanically recovered meat , where mechanical separation results in some bone particles (Ø<0.5 mm) remaining in the meat mass. Mechanically recovered meat is cheaper than raw meat, thus it has been incorporated into

many meat-derived products, where it is less easily detected, without declaring it on the label. EU regulations exclude mechanically recovered meat from the definition of meat and it should be separately identified in the ingredients list when it is used in meat products.

2.1.1.5. Breed substitution

The increasing demand for higher quality meat and the pursuit of desirable attributes such as tenderness or a low-fat content, make the fraudulent declaration of breed a common practice. Among the most popular breeds Charolais, Jersey and Aberdeen Angus and Piemontese are those most subjected to mislabelling. Similarly, meat from young bulls and steers may be regarded as superior to that from older cows [10].

2.1.1.6. Sex substitution

A current practice of producers, in order to meet the needs of the consumer, sometimes report the sex of the animals on the product label even if it is not required by legislation. Today there is considerable awareness of the part of consumers of differences in meat quality and people prefer to consume less meat but with higher sensory characteristics such as tenderness, flavour and appearance [11]. Many factors are involved in beef sensory quality, and variations can be induced by production factors such as breed, age, and diet as well as technological factors such as slaughter conditions, ageing time and cooking procedures. Sex is also related to meat quality, as it can affect pH, cooking loss, water holding capacity (WHC) and shear force (SF). In addition, meat and fat colour parameters, as well as sensory texture and overall acceptance attributes are related to sex [12].

2.1.2. Addition of substances

2.1.2.1. Additives

The purpose of Regulation 1333/2008 [13] and further amendments is to harmonise the use of specific preservatives in food products and it gives a list of both authorised and prohibited additives for certain foods, including some traditional meat products. The use of colours (Council Directive 94/35/EC [13]), antioxidants, preservatives and flavourings is generally not allowed in fresh unprocessed meat because they mask spoilage. Similarly, many meat products and preparations have restricted the use of these additives for the same reason.

2.1.2.2. Water

Water is the cheapest extender of meat and meat products and the water-holding capacity of meat proteins facilitates the binding of water. While the practice of 'enhancing', 'injecting' or 'plumping' has been around since the 1970s, particularly in the chicken industry, it is becoming a subject of concern in recent years. While many believe injecting meat with salt water helps give the product some added juiciness, there are some unpleasant truths about this practice. Besides the increase in product weight, both salt water or contaminated water represent a safety risk, due in the first instance to an unknown uptake of high quantities of sodium and in the second to the presence of pathogens in case of polluted water. When the amount of water is greater than 5 % of the finished product, the EU Regulation requires water to be declared in the ingredient list. Although the amount of water added to cured meats can be very different, very few countries have a requirement for a quantitative declaration of added water. However the debate continues to make consumers aware of the possible fraudulent addition [14].

2.1.3. Process/production/welfare deception

2.1.3.1. Fresh versus thawed meat

Fresh meat is a sensitive material, which is not able to maintain its desired sensory and microbial qualities for a long time-span. Long-term storage and transport between slaughterhouses, meat processors and consumers may take days or even several weeks, for instance in case of overseas imports. Freezing is an excellent way of extending the storage life of meat and makes transport easier. However, generally the retail price of frozen or thawed meat is lower than the price of its fresh counterparts. During thawing the meat loses moisture which contains components contributing to the characteristic flavour and nutritional value of meat. The texture of meat is also affected by the formation of ice crystals, which damage the muscle structure and increase the water activity on the meat surface [15]. Due to the perceived higher quality, consumers are willing to pay a higher retail price for fresh meat. Additionally, in the case of poultry meat, Council Regulation (EC) No 1047/2009 [13] defines "fresh poultry meat" as poultry meat which has not been stiffened at any time by the cooling process prior to being kept at a temperature not below –2°C and not higher than +4°C and prohibits the sale of previously frozen poultry meat as fresh poultry meat. There is also the question of added water where immersion chilling is used before freezing compared with air chilling for chilled birds. In many cases, there is a significant price differential between the frozen and chilled product giving rise to an incentive to deceive the consumer. The EC Labelling Directive requires a process or treatment of a food to be declared where it is misleading not to do so. Therefore, in most cases, it is a requirement to indicate if the meat has previously been frozen [16].

2.1.3.2. Slaughtering methods

Council Regulation (EC) N° 1099/2009 on the "Protection of animals at the time of killing" [17] requires, as a general rule, that "animals shall be spared any avoidable pain, distress or suffering during their killing and related operations". However, it allows slaughter without stunning for particular methods prescribed by the Jewish (kosher meat) and Muslim (halal meat) rites if it takes place in a slaughterhouse. Therefore, there must be a correct labelling system to avoid that meat obtained through Jewish or Islamic ritual slaughter may be purchased by unwilling consumers who prefer not to eat this meat, while vice versa, to avoid meat derived from stunned animals being sold to Muslim or Jewish consumers [18].

2.1.3.3. Geographic origin

For consumers, foods of animal origin, such as meat products, may have a particular value associated with the geographical origin or production system from which they derive, e.g. "Protected Designation of Origin (PDO)", "Protected Geographical Indication (PGI)". The authentication of regional and traditional foods made from meat poses a significant challenge. It continues to be a very difficult task which requires employment of quite advanced analytical techniques [19]. These products, despite a similar process of manufacturing, differ in taste and aroma. This happens due to the use of special breeds of animals, the application of appropriate feeding regimes as well as the effect of the place and climate. Obvious examples are Parma or Serrano ham, but provenance can also be important for raw meat, for example New Zealand lamb or Scotch beef, although this distinction is often associated with a specific breed such as Aberdeen Angus for Scotch beef, as well as the husbandry of the animal. Confirmation of geographical origin authenticity, as with other origin issues, is achieved by checks and audit trails carried out by the product buyer.

2.1.3.4. Organic versus conventional meat

In the case of animal-derived foods generally, and meat specifically, not only is geographical origin important but so also is the authentication of "biological" or "organic" meat and meat products, as well as those which involve less intensive rearing and husbandry methods. In this case the issue focuses on the dietary background of animals, since diet can be a distinct feature of certain production systems, e.g. "organic" or "grass-fed" and can have profound effects on the composition and quality (nutritional and sensory) of the meat and the sustainability of the production of animal-derived food products. In such cases, animal or birds should not be treated with growth hormones, which are illegal in many countries, nor should there be the prophylactic use of antibiotics and other veterinary compounds to improve growth rates. Furthermore, some consumers restrict their purchases to certain production chain. This has led to producers making claims such as "antibiotics free" to declare that animals have been farmed without the use of antibiotics or advertising specific animal welfare practices, such as free-range or other less intensive housing, as well as insisting on more humane handling of animals during transportation and slaughter even if not required by legislation. In some cases, veterinary drug residues may indicate mislabelling but generally authenticity can only be checked by audit trails [20].

2.1.3.5. Feed intake

It is possible to determine the feed intake by different chemical methods, which can detect in animal blood and fat the metabolised forms of typical feed constituents [21]. The most common procedures are:

- Carotenoids content (higher in pasture than in concentrate and hay) in heifer fat, detected by HPLC;
- Fatty acid composition in meat, detected by GC (higher ratio of polyunsaturated fatty acids than saturated ones and of n-3 polyunsaturated fatty acids than n-6 ones, in grass-fed animals than in concentrate fed animals);
- Vitamin and terpene contents in meat.

2.2. Potential threat to public health

In 2013 mislabelled meat products containing horse meat were discovered in many European countries (Ireland, UK, France, Norway, Austria, Switzerland, Sweden and Germany), inducing Member States to increase their surveillance. At that time, the main threat for consumers was linked to the presence in some samples of the horsemeat of an anti-inflammatory molecule, phenylbutazone, and this raised concerns that any commercial fraud could in effect hide a sanitary one. This issue led to the setting up of a special Anti-fraud Unit in charge of managing emerging risks by the European Commission.

To date other examples of common frauds in which a risk for human health can be present are:

- The presence of undeclared additives such as sulphites in fresh meat preparation, causing allergic reactions in sensitive peoples;
- Addition of prohibited substances, such as melamine, causing neurological deficits, renal failure and death in young children;
- The false declaration of geographical origin of the meat or meat product, in order to cover up a source in which a sanitary risk may be present (i.e. contaminants, hormonal treatment, infectious diseases).

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

The standards described in this section include official analytical methods dealing with the authenticity of meat and meat products; these are approved by the Association of Official Analytical Chemists (AOAC International) or by the International Organization for Standardization (ISO). Some analytical methods are also reported in the Codex Alimentarius: most of them refer to AOAC or ISO.

The official methods described here can be very helpful to find suitable analytical solutions for most of the authenticity issues described in session 2.1. However it is important to point out that recently developed methods have also been shown to be efficient in meat authentication.

3.1.1. Substitution

3.1.1.1. Species substitution

These two official methods are related to species identification:

- Multiplex PCR – ISO/NP 20148, still under development.
- Identification of beef and poultry adulteration of meat products by ORBIT (overnight rapid bovine identification test) and PROFIT (poultry rapid overnight field identification test) kits [22].

3.1.1.2. Protein substitution

These official methods deal with the determination of proteins. Most of these methods consists in digesting a test portion with concentrated acid, to convert organic nitrogen to ammonia ions. This is followed by an alkalisation, distillation of the liberated ammonia, titration using boric acid, and finally a calculation of nitrogen content of the sample from the amount of ammonia produced. Other approaches involve combustion, use of dye binding and enzyme linked immunosorbent assay (ELISA) as follows:

- Determination of nitrogen content (reference method) [23] (this standard was last reviewed and confirmed in 2001);
- Determination of nitrogen in meat by the Kjeldahl method [24];
- Determination of crude protein in meat and meat protein by a combustion method [25];
- Determination of crude protein by a digestion method [26];
- Determination of protein in raw and processed meat by an automated dye binding method [27];
- Determination of protein content in processed meat and poultry products, cooked cured ham and in cooked cured pork shoulder and in luncheon meat by titrimetry and Kjeldahl digestion – Codex Alimentarius [28], different AOAC and ISO methods;
- Determination of soy proteins in raw and heat processed meat by Enzyme Linked Immunosorbent Assay [29].

3.1.1.3. Fat substitution

The official methods dealing with the determination of fat and consist in extracting it under specific operating conditions; the total fat content is expressed as a percentage by mass:

- Determination of total fat content in meat and meat products by a gravimetric method [30];
- Determination of fat (crude) or ether extract in meat by a gravimetric method [31];
- Determination of fat (crude) in meat and meat products by a gravimetric method [32];
- Determination of fat content in processed meat and poultry products, cooked cured chopped meat, cooked cured pork shoulder, cooked cured ham and luncheon meat by a gravimetric method [28,30].

3.1.2. Addition of substances

3.1.2.1. Additives

Nitrites and nitrates

The methods consist in colorimetric and spectrophotometric determinations:

- Determination of nitrites content in cured meats by a colorimetric method [33];
- Determination of nitrites content meat and meat products (reference method) [34];
- Determination of nitrates content in meat and meat products by a colorimetric method [35];
- Determination of nitrates and nitrites content in meat by a spectroscopic method [36];
- Determination of nitrites and nitrate content in meat and meat products by spectrophotometric determination after enzymatic reduction of nitrate to nitrite [37];
- Determination of nitrites content in meat and meat products, processed meat and poultry products, canned corned beef, cooked cured chopped meat, cooked cured pork shoulder, cooked cured ham and luncheon meat by a colorimetric methods [28], different AOAC and ISO methods.

Ascorbic acid

Determination of total vitamin C in food – semiautomated fluorimetric method [38].

Phosphorus and polyphosphates

Different principles are on the basis of these methods, ranging from spectrophotometry to gravimetry:

- Determination of total phosphorus content in meat and meat products (reference method) [39];
- Determination of total phosphorous content in meat and meat products by spectrometric method [40] (this standard was last reviewed and confirmed in 2001);
- Determination of linear condensed phosphates in meat and meat products by thin layer chromatographic separation [41];
- Determination of total phosphorus content by gravimetric method [42];
- Determination of phosphorus in meat and meat products by spectroscopic method [43].

Colouring agents

Detection of synthetic, water-soluble colouring agents in meat and meat products by a thin layer chromatographic method [44].

Sulphur dioxide

Detection of sulphurous acid (free form) in meat by a titrimetric method [45].

Preservatives

Detection of preservatives (sorbates, ascorbates, benzoates, sulphites) in ground meat by a spectroscopic method [46].

3.1.2.2. Water

The official methods for the determination of water content are basically based on the measure of the loss in mass obtained for a sample under specific conditions, such as different kind of heat treatments, divided by the mass of the test portion; moisture content is expressed as a percentage by mass. NMR analysis can be applied as well:

- Determination of moisture content in meat and meat products (reference method) [47];
- Determination of moisture in meat and meat products by air drying [48];
- Determination of moisture and fat by microwave and NMR analysis [49].

3.2. Other commonly used methods

In this section, an overview of commonly used analytical methods for each of the current authenticity issues described in the section 2.1 is provided. A special focus is on the analytical methods used routinely in laboratories, and therefore widely available to industry. In addition to these, R&D methods can also be adopted with satisfactory results for some issues, although not described in this section.

3.2.1. Substitution

3.2.1.1. Species substitution

Species identification is mainly achieved by different kind of analytical methods:

- Chemical determinations, since content in certain components varies among species (e.g. glycogen, fat);
- Genetic methods based on nuclear or mitochondrial DNA, such as end-point PCR, multiplex PCR and nested PCR;
- Immunological methods, such as precipitation test – Overnight Rapid Beef Identification Test (ORBIT), Multispecies Identification Field Test (MULTI-SIFT), ELISA and immunoblotting.

3.2.1.2. Protein substitution

Animal proteins could be replaced with vegetable cheaper ones, such as soy, that can be identified using techniques such as ELISA and PCR. Histochemical analysis and immunohistochemical techniques are also routinely adopted in the laboratories. Analytical methods normally used to

measure total nitrogen content (e.g. Kjeldahl and Dumas) are not able to discriminate between nitrogen atoms derived from proteins or chemical compounds, thus chromatographic techniques are employed (HPLC or GC usually coupled to mass spectrometry).

3.2.1.3. Fat substitution

Vegetable fat contains phytosterols that are absent in animal fat; these compounds needs to be isolated from the fat through preparative steps and are routinely detected by different chromatographic methods, such as HPLC or GC coupled with several kind of detectors, as well as NMR.

3.2.1.4. Tissue substitution

h-caldesmon ELISA can be used as a histological method able to differentiate tissues (it is present in smooth muscles and absent in cardiac and skeletal muscles) to detect this type of fraud.

3.2.1.5. Breed substitution

Several analytical methods can be used to differentiate breeds, even if they are not so widespread and routinely used either in quality control laboratories or in industry:

- Genetic analysis;
- Analysis of the microsatellite DNA markers is used to identify, for example, Italian cattle breeds Chianina, Marchigiana, Romagnola and Piemontese [50];
- SNP array, adopted to detect the cattle breeds Holstein and Japanese Black [51].

3.2.1.6. Sex substitution

It is possible to determine the sex origin of meat by detecting sex-specific hormones using different analytical tools. For example, for pork meat, a routinely used method for detecting uncastrated pigs (boars) is based on indole/skatole quantification by HPLC and enzyme immunoassay (EIA).

Molecular techniques can also be used for sex specific identification of raw meat:

- End-point PCR to distinguish the DNA regions that differ between males and females (zinc fingers genes, sex determining region of the Y-chromosomal gene, tooth enamel amelogenin gene);
- Real time PCR to distinguish the DNA regions that differ between males and females (sex-determining region of Y-chromosomal gene, X-chromosomal proteolipid protein gene, tooth enamel amelogenin gene).

These methods can be applied to beef, chicken, pork, and other types of meat, such as goat and sheep. PCR-capillary electrophoresis (DNA analysis using PCR according to IRMM Guidelines and EC Regulation 765/2002 [47]) can be used as a test with four specific primer systems that amplify two loci on both chromosomes, the X and the Y, respectively. Other tests exist on the market that are able to prove the presence of a Y chromosome by amplification of Y-chromosomal regions only, but this technique may lead to false Y-negatives if the amplification itself fails.

3.2.2. Addition of unauthorised substances

3.2.2.1. Additives

Many additives could be fraudulently added to meat. Among these, colouring agents, flavours and preservatives can be detected using HPLC and GC, while fibrinopeptides A and B from thrombin addition are identified and quantified by HPLC.

3.2.2.2. Water

Water could be added to meat in order to increase its weight; thus, extraneous water in meat can be determined by measuring water and protein content, using several methods that are more or less sophisticated (simple determination in oven, NMR, etc.) and also through the determination of the water/protein ratio.

3.2.3. Process/product/welfare deception

3.2.3.1. Geographic origin

Different methods can be routinely used to determine the geographic origin of meat, such as inductively coupled plasma mass spectrometry (trace elements) and isotope-ratio mass spectrometry (stable isotopes ratios). They are based on the principle that the content of these substances in animal tissues depends on feed intake, drinking water, pollution and soil composition, which are strongly linked with the geographic areas in which the animal lives.

3.2.3.2. Fresh versus thawed meat, organic versus conventional meat and feed intake

Microscopy analysis can be used to differentiate fresh versus thawed meat. The method which is validated for poultry meat, is based on the principle that thawed meat present microscopic alteration of muscles fibres which can be related to freezing temperatures.

There are several analytical strategies in the literature showing the possibility to differentiate between animals bred using organic or conventional farming systems, as well as to determine feed intake, however these are not routinely used in the industry.

4. Overview of methods for authenticity testing

The following tables provide a summary of the official and commonly used methods respectively and the authenticity issues they address.

Table 3: Official methods for authenticity testing of meat and meat products

Analytical technique	Indicative data or analyte	Authenticity issue / information
Multiplex PCR	Molecular biomarker	Species substitution
ORBIT (overnight rapid bovine identification test)	Antibodies and antigens	Species substitution
PROFIT (poultry rapid overnight field identification test)	Antibodies and antigens	Species substitution
Kjeldahl	Nitrogen content	Protein substitution
Automated dye binding	Protein content	Protein substitution
Combustion method	Crude protein	Protein substitution
ELISA	Soy proteins	Protein substitution
Gravimetric method	Total fat content	Fat substitution
Colorimetric method	Nitrites and nitrates	Addition of nitrites and nitrates
Spectroscopic method	Nitrites and nitrates	Addition of nitrites and nitrates
Fluorimetric method	Total vitamin C	Addition of ascorbic acid
Spectrometric method	Total phosphorus content	Addition of phosphorus and polyphosphates
Thin layer chromatographic separation	Linear condensed phosphates	Addition of phosphorus and polyphosphates
Gravimetric method	Total phosphorus content	Addition of phosphorus and polyphosphates
Spectroscopic method	Total phosphorus content	Addition of phosphorus and polyphosphates
Thin layer chromatographic method	Synthetic, water-soluble colouring agents	Addition of colouring agents
Titrimetric method	Sulphurous acid (free form)	Addition of sulphur dioxide
Spectroscopic method	Sorbates, ascorbates, benzoates, sulphites	Addition of preservatives
Gravimetric method	Water	Addition of water
Nuclear magnetic resonance	Water	Addition of colouring agents, aromas and preservatives

Table 4: Non-official commonly used methods for authenticity testing of meat and meat products

Analytical technique	Indicative data or analyte	Authenticity issue / information
Genetic methods	DNA	Species substitution
Immunological methods	Protein	Species substitution
ELISA and PCR	Soy protein	Protein substitution
Chromatographic methods	Melamine and urea	Protein substitution
Chromatographic methods	Phytosterols	Fat substitution
Nuclear magnetic resonance	NMR spectrum	Fat substitution
ELISA	h-caldesmon	Tissue substitution
Genetic methods	DNA	Breed substitution
Genetic methods	Microsatellite DNA markers	Breed substitution
Genetic methods	SNP	Breed substitution
HPLC and enzyme immunoassay (EIA)	Indole/skatole	Sex substitution
End point and real time PCR	DNA	Sex substitution
PCR-capillary electrophoresis	DNA	Sex substitution
Chromatographic methods	Colouring agents, flavours and preservatives, fibrinopeptides A and B	Addition of additives
Gravimetric method	Water	Addition of water
Nuclear magnetic resonance	Water	Addition of water
Inductively coupled plasma mass spectrometry	Trace elements	Geographic origin
Isotope-ratio mass spectrometry	Stable isotopes ratios	Geographic origin
Microscopy	Morphological structure	Fresh/thawed conservation

5. Conclusion

Considering the growing demand for meat, related fraud is expected to represent an ongoing challenge in future years. The analytical tools to detect meat fraud will need to be improved based on a number of different strategies. First, those analytical procedures that are not included in existing standards (determination of additives, use of molecular techniques to determine species substitutions) need to be standardised and validated. Standardised methods need to be revised, such as the EU reference method to determine hydroxyproline content in meat. This is a simple spectrophotometric technique, while other more advanced ones such as LC-MS/MS are available but not recognised as reference techniques. Multi-screening and untargeted methods further development to detect simultaneously different and unknown adulterants. And finally, innovative analytical approaches have to be developed and validated to propose solutions for different old and emerging issues directly linked to fraud such as:

- Characterizing different animal breeds, using a larger data set to build effective models (NIR techniques);
- Determining animal feed intake, since the current analysis based on carotenoid content in fat and blood are influenced by other factors such as breed, gender, lactation and rumen environment;
- Determining the slaughter age of animals;
- Assessing animal welfare condition related to intensive vs traditional farming practices;
- Distinguishing different meat cuts (a possible solution could be the evaluation of collagen content that varies among different meat cuts, considering that visual inspection is useful only to differentiate primary beef cuts);
- Quantifying vegetable fat as adulterant in meat, not only revealing its presence by phytosterols detection;
- Establishing the geographic origin of meat, since the simple identification of breed may not be effective since individual breeds can be raised in different countries despite their origin;
- Detecting animal fat from different undeclared species;
- Developing methods to identify fresh-thawed products that are applicable to ground meat and temperatures higher than -12°C (the HADH method is not applicable to ground meat because the grinding process causes similar alterations to those induced by freezing and it is able to detect frozen-thawed meat only if the freezing temperature has been -12°C or below).
- Setting up reliable methods to detect mechanically deboned meat (MDM) and to distinguish among low pressure *vs*. high pressure MDM in meat products.

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