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Bettina M. Franke · Gérard Gremaud · Ruedi Hadorn · Michael Kreuzer

Geographic origin of meat—elements of an analytical approach to its authentication

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Abstract This review article discusses recent analytical developments with respect to the determination of the geographic origin of raw meat. The main emphasis is laid on lamb, beef and poultry. So far, some methods have shown quite promising potential (e.g. stable isotope ratios, trace elements), others have remained unsatisfactory in their discriminating power to authenticate the geographic origin of meat (e.g. microbiological profile, sensory traits, volatile compounds). Other methods (e.g. animal genotype, gross chemical composition) could be auxiliary criteria as they help to determine related indicators such as feeding or housing conditions but not directly the origin. The complexity of this question is large. An integrated approach simultaneously addressing various species and production characteristics such as environment, animal husbandry conditions, breed, feeding and drinking water has to be developed. Strategies have to be different for global and micro-regional scales.

Keywords Authenticity · Traceability · Lamb · Beef · Poultry · Meat · Region

Introduction

Meat is a high-priced but controversially perceived commodity. A recent evaluation, carried out by the Swiss Federal Office of Public Health [1], showed that the origin of food is important for the purchase decision of 82% of customers, with the origin of meat being a very relevant criterion for 71% of consumers. In Switzerland more than half of the poultry consumed is imported (approximately 41,000 tonnes per year). Poultry importation ranks first in Switzerland. The main countries of origin are France, Hungary, Germany and Brazil [2, 3]. China as a major previous contributor was excluded after the antibiotic scandal from 2002 on. Owing to the current prevalence of avian influenza, a ban on imports has been imposed since January 2004 for poultry from Thailand, an upcoming exporting nation, and other Asian countries. This enlightens part of the current image problem of meat, additionally to BSE and hormone scandals, which may be at least partially overcome by confirmed geographic origin. Acknowledging this, the recent EU Regulation 1760/2000 and the corresponding Swiss regulation (LMV, Art. 22a, VAPR Art. 2) demand that the geographic origin of meat and meat products, containing meat in a proportion of at least 20%, must be declared. Additionally, in 2000 the EU mentioned traceability as one of the basic principles of consumer protection [4], this, however, without yet having an appropriate set of analytical tools for the verification of the declaration of meat origin. Therefore, the detection of mislabelling mostly relies on controlling the available documents and on-site inspections. False or incomplete documents and remote production sites, inaccessible to inspection, are serious limitations of these methods. Reliable analytical tools would not only protect consumers but would allow producers of traditional regional specialties (often protected labels such as Emmental cheese, Bordeaux wines, Bündnerfleisch and Parma ham) to get their products clearly differentiated from imitations.

Approaches to determine authenticity of meat, including its geographic origin, have already been compiled in the past [5, 6]. In contrast to those overviews, the aim of the present article was to review the state of the art of meat authentication by analytical tools with the exclusive focus on the issue of geographic origin. Special emphasis

B. M. Franke · M. Kreuzer (✉)
Institute of Animal Science,
Swiss Federal Institute of Technology (ETH) Zurich,
ETH Zentrum, LFW, 8092 Zurich, Switzerland
e-mail: michael.kreuzer@inw.agrl.ethz.ch
Tel.: +41-1-6325972
Fax: +41-1-6321128

G. Gremaud
Swiss Federal Office of Public Health,
3003 Berne-Liebefeld, Switzerland

R. Hadorn
Agroscope Liebefeld-Posieux,
3003 Berne-Liebefeld, Switzerland

was laid on muscle meat, while offal and meat products were not considered. Advantages and constraints of various analytical approaches are discussed in the context of species-specific problems. For that purpose, one species mostly fattened on home-grown feeds (lamb), one receiving often complete feeds produced off-farm (poultry) and one representing both production systems (beef) were taken into consideration.

Animal-species-related aspects

Traceability of the geographic origin of meat is getting increasingly difficult the more the fattening of livestock is becoming independent from site-specific production factors, especially with respect to feed and its production but also to housing (outside or in a barn) and the declining use of traditional local genotypes. Animals continuously consume a multitude of elements and compounds from their environment. These are absorbed to different extents through the digestive tract and thus are partially retained in edible body tissues and offal and partially excreted through faeces and urine. Traceability is comparatively easy in milk and milk products [7–9] since milk immediately reflects the actual conditions the animal is exposed to, as there is a high transfer rate of nutrients and/or contaminants. By contrast, body stores respond less clearly and fast, and there may be a high or even complete depletion of the signatures from the tissues before slaughter once the exposure is terminated. Within meat, extensive systems of production (e.g. lamb, sometimes beef), especially organic production, are very much related to the local environment as animals are kept outside as much as possible (occasionally even in winter). Few supplements are used and even less feed is imported from outside the farm (prescribed in EU Regulation 2092/91 for organic farming). As a result, the animals incorporate elements into their body exclusively from one local area. In conventionally fattened beef, the situation is more complicated. Fattening systems range from free grazing in summer and feeding locally produced feeds in winter (quite similar to organic beef production) to systems based on full-time indoor housing where all concentrate originates from outside the farm. The concentrate ingredients may still come from the same region but may also be purchased from another region or even another part of the world. In particular, protein supplements such as soybean meal are traded worldwide. In such beef absorbed elements and compounds reflect the signature of two or more regions. The same holds true for mineral supplements. Poultry represents the other extreme compared with lamb. Very rarely chickens are fattened extensively. Owing to the low price and the high demand, imported poultry is highly likely to originate from intensive, sometimes industrialised, production. Globally there are only a few fattening genotypes (broiler strains) offered from just a small number of breeding companies with this stock being spread all over the world [10]. Moreover broilers are typically raised in closed units with little or no contact with their local environment, except through local drinking water, air and often litter. In broiler fattening, typically complete feed mixtures are used, where the feed manufacturers obtain the ingredients on the basis of economic competitiveness. The origin is highly variable from batch to batch and the proportion from regional origin is unclear. Poultry meat therefore requires the most sophisticated techniques to ensure verification of stated geographic origins.

The search for suitable species-specific chemical, physical and biological properties, related to geographic origin, includes the generation and utilisation of knowledge on how environment, breed, type of animal farming, feed quality, composition and processing influence these traits.

Trace elements

Trace elements are discussed as one promising group of meat constituents for the determination of the geographic origin taking into consideration their retention from the local environment. It is characterised by the trace element profile of the soil as well as the site-specific profile of drinking water, feed, litter and air. Generally, a migration through the food chain is known and quantified for many trace elements.

Trace elements in meat caused by natural deposits

The amount or the composition of elements in the soil is typical for certain regions [11–13]. For example, the Se concentration in American soil is known to be much higher than that in Europe. In a Swiss study [12], the Se contents of meat (milligrams per kilogram of dry matter) were the following: organic beef, 0.15; conventional beef, 0.26; poultry, 0.58. Beef from North America, by contrast, contained approximately twice as much Se (0.43 mg/kg) and Brazilian poultry contained as much as 0.73 mg Se/kg [12]. Hintze et al. [14] found close correlations between Se concentrations in soil, grass and beef skeletal muscle, and beef from areas of low and high soil Se concentration could be clearly distinguished. The meat Se concentration from animals not fed Se supplements and grown on soils either poor or rich in Se were 0.27 and 0.67 mg/kg, respectively, while Se supplementation of the feed increased meat Se only from 0.41 to 0.46 mg Se/kg. This was considered by the authors [14] as an indication that the Se concentration in skeletal muscle is more related to geographic origin than to mineral supplementation, i.e. feeding practices. Chilean researchers [15] were able to discriminate eggs by significant differences in the Se content of both egg white and egg yolk from five main areas, differing in their geographic and climatic characteristics. Another trace element determined by natural differences in soil contents is Rb. Anke et al. [16] showed that the Rb concentration in plants and drinking water was highest on granite and gneiss weathering soil. Herbivores

were found to store 37 and 33% more Rb in the liver than carnivores and omnivores, respectively. For unknown reasons [16], poultry meat was found to be rich in Rb, although the main feeds, rye and other cereals, contained little Rb. The Cd concentrations in horse meat originating from Poland, Lithuania and Hungary but slaughtered in Italy, depended on sex (only in muscle tissue) and age, and were also shown to be country-specific [17]. The reasons for the high Cd content of Polish horse meat (0.084 mg Cd/kg) and the lower contents of Hungarian meat (0.040 mg Cd/kg) remained unclear [17].

Trace elements through supplements in feed

A study carried out in 2002 [18] showed that supplemental Se influenced meat Se more than the Se content of soil mediated through locally produced pasture grass. In detail, steers originally from seleniferous and nonseleniferous areas were fed diets containing either moderate (0.6 μg Se/g) or high (11.9 μg Se/g) amounts of Se from a certain time-point on. These results did not agree with those published by Hintze et al. [14], which were described earlier. Accordingly, entire muscles of steers from seleniferous areas and fed the high Se diet showed the highest amount of Se in tissue after slaughtering (increasing from 580 to 621 mg during the supplementation period), while steers from nonseleniferous areas and fed with the high Se diet showed a much larger final level increasing from 110 to 406 mg Se in the muscle. The amount of muscle Se in steers from seleniferous regions and supplied with moderate Se amounts in the diet even expressed a decrease from 397 to 365 mg [18]. Another study [19] demonstrated that Se has to be supplied to laying hens in amounts clearly exceeding requirements in order to be temporarily stored. After withdrawal of the additional Se, the deposited amounts were continuously depleted and returned to normal levels within 9 weeks. Ni is an essential trace element in animal nutrition and therefore can be used as a supplement, but it also might be caused by pollution. Muscle tissue of calves was found to respond to a supplementary 5 mg Ni/kg diet, but increases were weak [20]. Only excessive dietary Ni doses (more than 200 mg Ni/kg diet) provoked clear responses of muscle Ni concentrations in rats [21]. Lanthanides were found to have performance-enhancing effects in pig fattening, knowledge which has been applied in China for several decades [22, 23]. Both studies showed that the amount of La found in muscle depends on the level of intake, while the amount of Ce is independent of the intake [22, 23]. Feeding practices like this might also be useful for geographic allocation of the meat but analysis is demanding and concentrations in muscle tissue are low as shown for Ce in mice [24].

Trace elements in meat from pollution

Unnatural effects, such as pollution from industry, mining or disasters like that at Chernobyl, have effects on soil which might also be used for determining the geographic origin of meat. As and Zn, from Kidston Gold Mine (North Queensland), turned out to increase accumulations of these elements in liver, muscle and blood of grazing cattle [25]. Similarly, Hg pollution occurring in the province Guizhou in China had effects on the Hg concentration in water, soil and fish, with a declining trend with increasing distance from the pollution source [26]. Although meat of land animals was not included in that study, a similar effect of that geographic origin can be expected. This might also be valid for other Chinese provinces as the atmospheric Hg concentration in Guiyang was found to be nearly 4 times the global average [27]. A study about Pb, Zn and Cd in biological tissue of sheep from a polluted area in southwest Sardinia showed that there were no significant differences in muscle concentrations of these elements from that of unpolluted areas [28]. The reason given for that observation was that muscle is not a specific accumulating tissue for these heavy metals.

In regions strongly contaminated by the Chernobyl fallout in 1986, such as northern Sweden, there is still a considerable transfer of ^{137}Cs from soil via grass to grazing animals as was shown for lambs [29]. It is also obvious from the still high contamination of reindeer meat [30]. A Polish long-term study showed that in 1999 the ^{137}Cs contamination of fruit, vegetables, poultry and eggs had returned to the same level as found prior to the Chernobyl fallout, while milk, meat, fish and forestry products still present higher radioactivity [31]. In the Alps, Gastberger et al. [32] found higher concentrations of ^{137}Cs in soil and grass after the Chernobyl fallout at high altitude than in the lowlands. Accordingly, at present there are still higher ^{137}Cs concentrations in milk of cows grazing high-altitude pastures compared with milk of cows grazing lowland pastures [33]. This is promoted by the typically lower K contents of soil and grass at high altitude: K is a competitor of Cs during absorption in the digestive tract [33]. Therefore, the ^{137}Cs concentration might be a suitable indicator to prove or disprove a stated high-alpine pasture origin or other origins of meat from particularly contaminated soils.

Potential and limitations of trace elements as analytical tools to determine geographic origin of meat

The examples given already demonstrated a certain potential for determining the geographic origin of meat. Some elements are of particular interest in order to discriminate among products on a small-scale level (e.g. ^{137}Cs), others for differentiation of the origin from different continents (e.g. Se). However, there are also serious limitations to this approach. The elemental composition of meat is influenced by various factors. Regional dif-

ferences in key trace elements are frequently, but not always, typical for one single area. Inconclusive results may arise when animals switch between areas during fattening, which is often the case for cattle where an extensive phase on pasture is followed by an intensive feedlot phase at another site. Adaptation to the altered supply, when sufficiently long, widely eliminates the geographic signature which is more likely to develop in the extensive phase.

A serious restriction of the use of certain trace elements for the determination of the geographic origin of meat is that animals often get feed supplements enriched with essential elements. These include particularly Zn, Cu and Mn, but sometimes also Se. Typically, these elements are often supplemented through a mineral mixture either given separately at ad libitum access or as a fixed component of the concentrate or complete feed [34–36]. There are species differences in the frequency and the amount of supplementing diet, declining in the order poultry, cattle and sheep. Accordingly, supplements of Zn, Cu, Mn and Se are most frequently added to poultry feed [37], either in the inorganic form or linked to organic substances such as amino acids. The results of the experiments already described [14, 18] concerning the factors affecting muscle Se mostly (geographic origin or supplementation) remained inconclusive. But it can be assumed that there is a response starting from a low level of intake from either source. Furthermore supplementary Se should be similarly or even better absorbed from the digestive tract compared with Se incorporated in the matrix of fodder plants. It is a general phenomenon that excessive intakes of most trace elements are not likely to result in elevated muscle stores. This is prevented by homeostatic mechanisms, while the excessive amounts are either excreted or stored in inner organs. Overall, this means that typically supplemented trace elements do not allow exclusive conclusions on geographic origin, also because a change in feed supplements of animals could change the content of these elements in the meat. This leaves those trace elements that are specific for local drinking water, air and litter as the most promising for the verification of the geographic origin at least in intensively fattened poultry and maybe beef.

The applicability of trace elements resulting from pollution is limited as well. These elements are not always specific for only one area and not constant across complete regions, let alone countries, and pollution can be temporary [31] or at least changing over time (radionuclides). As shown by Chessa et al. [28], the heavy metals Cd, Pb and Zn additionally have the limitation that they accumulate in inner organs (e.g. kidney, liver) and mostly not preferentially in muscle tissue, maybe with the exception of horse meat [17]. Techniques determining elements caused by pollution could at least help to cross-validate assumptions as there is a global difference (for instance with and without Chernobyl fallout) and a local difference. So maybe some origins can be excluded and in conjunction with other factors a clear authentication of a stated geographic origin can be made.

In conclusion, these considerations suggest that the analysis of one single trace element would be conclusive for geographic origin only in exceptional cases, while otherwise multielemental approaches are required [38, 39]. Trace element analysis could also be very helpful in cross-validation of other results. A disadvantage of trace elements as an analytical tool is the expensive and time-consuming sample preparation and analysis. Highly skilled staff are required, especially in cases of analysing volatile elements (e.g. Hg), where particularly high standards are necessary for sample collection.

Stable isotopes

Principle and evaluation of isotopic ratios as analytical tools to determine geographic origin

The ratios of stable isotopes (either given as proportions or as an excess, δ, of the respective rare isotope compared with its natural occurrence) provide an interesting analytical tool to confirm meat origin as there are sometimes region-specific patterns in environmental isotopic ratios (soil, water). Similar to the trace elements, isotopes are incorporated in local feeds and in the body of the animals. Therefore, these ratios may be specific for those areas. The ratios of hydrogen (H/D) and oxygen ($^{16}O/^{18}O$) isotopes in body tissues are primarily influenced by drinking water. Isotopic ratios of HC, N, S and Sy ($^{12}C/^{13}C$, $^{14}N/^{15}N$, $^{32}S/^{34}S$, $^{86}Sr/^{87}Sr$) are more indicative of soil and feed origin [40–42]. As early as 1978, DeNiro and Epstein [43, 44] demonstrated by comprehensive studies of $\delta^{13}C$ and, later, of $\delta^{15}N$ fractionation that animal products are usually enriched in ^{13}C and ^{15}N depending on their diet. This enrichment proceeds along the food chain in a stepwise manner from one trophic level to the next. This may help to link meat to its diet and, if the diet is unique to a certain area, to its geographic origin [44].

The principles described have been successfully applied in studies of migratory birds [45–47] where different isotopic ratios as determined in feathers, local breeds and winter quarter environments allowed their migrating routes to be traced back. Also the applicability for the determination of the origin of milk and cheese by this technique was repeatedly investigated [8, 48, 49]. The C isotope ratios of milk closely reflected the proportions of C_3 and C_4 plant material in the cows' diet [40, 41, 50–52]. The underlying principle is that C_3 and C_4 plants discriminate differently against ^{13}C as they have other metabolic pathways in photosynthesis [53]. The majority of grasses of tropical origin used in livestock feeding, including maize, are C_4 plants, while most other herbaceous forages are C_3 plants. A study of bone collagen $\delta^{15}N$ and $\delta^{13}C$ values of deer [54] showed that 25% of the animals showed a correlation between increasing ambient temperature and increasing $\delta^{13}C$ (r=0.592). For these animals, consuming at least 10% of C_4 plants, $\delta^{15}N$ increased with decreasing local precipitation (r=–0.858) as well, while in deer consuming low amounts of C_4 plants

no such relationship of precipitation and isotopic ratios was found. This demonstrates that climate influences the feed composition of deer and, in this way, the isotopic composition of their meat.

Attempts based on the isotopic ratios of H, O, N and S have been also applied to determine the origin of meat [55–58]. On the basis of O and H isotopic ratios it was possible to differentiate between Argentinean and German beef [55], while N and S isotopic ratios allowed differentiation between certain local geographic regions [58]. Another result of the first mentioned study [55] was that $\delta^{18}O$ values measured in beef did not completely coincide with the corresponding regional ground water values known from earlier research [59, 60]. It is therefore questionable whether a differentiation of beef from nearby countries is possible. In a very recent French report [61], meat from steers reared at three different sites but fed two different diets (either based on maize silage or on grass) at each site was analysed. The results obtained from analysing $\delta^{18}O$ values showed a link to diet but not to geographic origin. Another study [62] determined a relationship between the abundance of ^{13}C in lamb meat and the feeding regime in a way that grazing resulted in lower $\delta^{13}C$ values than a diet based on milk and concentrate. Suckling lambs had the least negative $\delta^{13}C$ value. In the same publication, $\delta^{15}N$ was reported to be different between lambs of different breeds fed on similar diets depending on country of origin and climatic conditions (humidity). In Japanese poultry, $\delta^{13}C$ did not differ among muscle tissue samples from different body parts [63], but the values clearly varied among samples from China, Japan and the USA with −18.5, −17.2 and −16.6‰ $\delta^{13}C$ on average [63]. No information on the diets fed to these animals was given.

Some studies had the determination of the production system rather than the geographic origin of the meat as their primary goal. Boner and Förstel [58] investigated the use of isotopic ratios of H, O, N, S and C for confirming the authenticity of organic beef. The $\delta^{15}N$ and $\delta^{34}S$ values in raw meat protein allowed the differentiation between two German areas (Rheinbach and Aachen), while $\delta^{13}C$ was indicative of the production system (organic versus conventional). Values below −20‰ $\delta^{13}C$ were found in organic beef, while conventionally reared cattle with maize as one of the main diet components showed values in the range of −14‰. This means that probably the diet type and not the organic production system as such was responsible for these differences. These differences proved to be quite persistent in muscle meat as, even after 230 days of fattening on maize (a C_4 plant), part of the characteristic C isotopic ratio from feeding of moderate climate grasses (mainly C_3 plants) could be found in meat but no longer in body fat tissue [64].

An especially promising isotopic ratio in order to determine the geographic origin of meat could be that of Sr as it is typical for the soil of certain regions. The local ratio of ^{86}Sr to ^{87}Sr depends on geological features (type, formation and age of geological underground, e.g. rocks) [13, 65].

Potential and limitations of stable isotopes as analytical tools to determine geographic origin of meat

Analysing stable isotope ratios appears to be a promising method to obtain conclusions on the geographic origin of meat. The isotopic ratios of H and O, depending on the amount of drinking water consumed, cannot be easily falsified or masked by feeding diet ingredients from an origin outside of the region. Additionally, a method based on the properties of drinking water is not influenced by grazing versus feeding in a barn. In turn, the isotopic ratios of C and N give some indication of the type of diet fed, particularly when the diet differs in the proportions of C_3 and C_4 plants. The isotopic ratios of C and N are often also characteristic for production systems and feeding intensity (increasing maize proportions with intensive fattening of cattle) and the isotopic signature of previous feeding seems to persist [64]. Conclusions on, for example, the proportion of maize in the diet could be helpful to confirm or disprove claims of a certain regional origin, but only when a certain type of feeding is very common in a certain area.

The stable isotope approach also has some important constraints. Conclusions made from such results must consider possibly similar features from the environment (e.g. climate, altitude, distance from oceans) allowing few or no differences in isotopic ratios of the meat to develop [42]. Therefore, meat from animals originating from different, but climatically or geologically similar areas might have an identical isotopic signature. Auxiliary data on feeding suffer from the possibility that the animals' diet could easily be changed once this type of analytical tool is known. There are also lots of intensive feeding systems based almost exclusively on C_3 plants (concentrate made of cereals) as also used in intensive lamb fattening systems. Additionally, beef or lamb imported from tropical regions may have the same C isotope signature as that of those animals intensively fed on maize-based diets in temperate climates. Another disadvantage of analysing stable isotopes is the time-consuming and expensive preparation of samples for some elements and the high costs of the analytical equipment.

Gross chemical composition

Less specific, but much easier to determine, is the gross chemical composition of the meat, particularly since a variety of methods based on different concepts, such as IR spectroscopy or NMR, allow a fast determination in contrast to traditional methods (Table 1). The components determined include fatty acids, amino acids and meat compounds such as connective tissue (collagen), but currently application takes place mostly in feed analysis. Recently, near-IR spectroscopy (NIRS) was suggested as a promising method for the determination of the amino acid composition in meat and poultry meals, being even more accurate than the crude protein estimate. This method allows the analysis of many samples in a short

Table 1 Methods tested for the determination of the gross chemical composition of meat

Determined variable	Test method	Reference
Fatty acid profile	NMR	[61]
Fat and water content	NIR spectroscopy	[70]
Fat and connective tissue content	NIR spectroscopy	[69]
Fat, water and protein content	NIR spectroscopy	[67]
Amino acid profile	NIR reflectance spectroscopy	[66]
Connective tissue content	Autofluorescence spectra	[68]
Fat and connective tissue content	Autofluorescence image	[69]

NIR near IR

time [66], which makes it potentially promising for meat authentication because of the possibility to describe many variables, such as the complex amino acid profile. Limitations of this approach are given by the invariable genetic determination of the amino acid sequences of muscle proteins and even the relatively strict coding of the ratios of individual muscle proteins. Beef and pork samples were reliably analysed for water, fat and protein contents by NIRS [67]. Alternatively, intramuscular fat content and connective tissue could be analysed by a combination of auto-fluorescence spectra and image features, with a wavelength of 332 nm being regarded as the most useful wavelength to determine both components [68, 69].

Feeding, genotype and housing may affect gross chemical composition, allowing a differentiation between geographic origins when conventions of these production systems differ clearly enough. Feeding is most likely to influence the fat content and the fatty acid profile of the meat, while the muscle structure and proteins might differ between genotypes (breeds, strains). Further effects are to be expected from the fattening intensity, which is often related to animal husbandry and the production system. The first attempts using this set of analytical tools are also available. IR spectroscopy was successfully applied to differentiate "slowly growing" chicken from chicken from industrial-like production systems, with the latter showing a more intensive absorption in wavelengths where lipids absorb [70]. A new study [61] analysing polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) by NMR was able to confirm the feeding of maize silage instead of grass in beef by elevated MUFA contents at the expense of PUFA and SFA contents, while high PUFA values were indicative of cattle kept at higher altitudes. In another study [71], beef originating from organic grazing systems and from grazed weaners showed higher concentrations of n-3 fatty acids and lower ratios of n-6 to n-3 fatty acids than beef from conventional intensive fattening, and there were no differences in the n-6 to n-3 ratio of beef purchased in spring and in autumn.

Although clear effects of production factors on gross chemical composition of the meat are known, their exclusive attribution to geographic origin seems to be difficult. This does not exclude that these traits are employed as valuable auxiliary criteria which can be obtained rapidly with little extra effort. Using for instance IR techniques, even samples are not destroyed by the analysis and can be used for other methods afterwards.

Animal genetics

Effective genetics-based approaches to determine the geographic origin of meat require a differentiation between breeds and genotypes that should be exclusively or preferentially used in different regions, countries or continents. Even more sophisticated would be the use of global animal identification systems based on gene chips which would allow the authentication of each single animal (or piece of meat) and which would yield information on the geographic origin as well. But traceability based on DNA profiling would require a well-organised worldwide animal identification and registration system [72]. For cattle there are approaches to do this, but attempts are still far from being practically realised. For chicken, with their overall low individual monetary value, this approach is likely to be much too expensive even from a medium-term perspective. However, as early as 1997 a gene map was created for chicken, which contains 617 genetic markers [73]. This might allow the application of less detailed approaches. In 2004 the bovine genome (Bos taurus) was sequenced [74]. At present there are three general genetic databases which continuously collect and save DNA sequences: GenBank, EMBL [75] and DDBJ. Data are permanently exchanged among these.

For cattle there have been several investigations on genetic differentiation. According to Loftus et al. [76] there are just two independent domestications of cattle, one from Europe and Africa, the other one from India. Surprisingly, both Zebu and Taurine breeds from Africa showed a high sequence divergence from Indian breeds (average of 5.01%) but a low divergence from European Taurine breeds (average of 0.73%). Differentiation between Bos taurus and Bos indicus was achieved by dividing these into two genetic clusters [77]. The mean pairwise genetic distance within Holstein Friesian, Italian Brown and historic Maremmana cattle was found to be 85% of the average distance across breeds and, within breeds, the genetic distance was higher between cows than between bulls. This is probably due to the more intensive selection for high genetic merit in bulls [78]. Concrete approaches to be used for the determination of geographic origin are still rare. In South American Creole cattle a gradient from east to west and from north to south was found, gradients where the introgression of Zebu decreases [79]. This explains why in Brazilian cattle there was a high prevalence of the Zebu Y chromosome, which was low in Uruguayan and Argentinean cattle [79]. Frequencies of the Msp I (-) allele in the third intron of the

bovine growth hormone gene were found to be lowest in northern European breeds, followed by breeds originating from eastern Europe and the Mediterranean basin, while the highest frequency was found for breeds from the Indian subcontinent [80].

These preliminary investigations illustrate a certain potential of genetic methods. The improvement and simplification of genetics-based methods currently taking place could make such analyses very interesting in the near future. However, the applicability has to rely on the assumption that meat exported from a distinct region is produced from the site-specific genotypes. Occasionally, this may be the case with lamb (provided similar databases are created in sheep as in cattle) and with beef, particularly in eastern and northern South America. The fast developments in genetic analysis may help to create reliable genetic markers soon. In poultry, the extremely intense long-term selection for meat yield by breeding companies has eventually resulted in very few remaining genetic lines which are globally distributed. This indicates that the search for geographic origin on a large scale in chicken meat with animal genetics-based methods might be inadequate. This could be different on a micro-regional level where occasionally characteristic strains of poultry are used, this often in association with a label strategy.

Microbiological profile

Genetic methods might also be applicable in a completely different approach. It is reasonable to assume that the microbiological profile of meat samples differs with geographic origin. The analysis of the target DNA sequences coding for certain species and strains of bacteria could allow this to be specified in order to determine the relationship between the actual microflora detected and the stated geographic origin of the meat. The first attempts have already been described. Genetic subtypes of *Escherichia coli* were shared among various types of meat, but some of them were found to be unique in certain animals; however, there were changes in the bacterial profile over time [81]. Genotyping of 62 isolates of *Mycobacterium bovis* originating from four different regions of Mexico and female Holstein cattle in fair to good bodily condition was performed by Milián-Suazo et al. [82]. However, the genetic diversity of this microbial species turned out to be too wide to be able to group isolates by geographic location. Hartel et al. [83] described a good ribotype separation in *Escherichia coli* among host animal species (cattle, horse, swine and chicken) at geographically different sites, but an allocation of *Escherichia coli* to certain regions was not possible. Berndtson et al. [84], studying the appearance of *Campylobacter* on Swedish chicken farms, found that a contaminated flock can be followed by an uncontaminated flock, illustrating that the occurrence of key bacteria is not consistent. There are more studies on the bacteriological profile in poultry and beef [81, 85–90] but none of them addressed geographic features. Although basically promising as an approach with the fast improvements in methodology to be expected, the demands are very high in order to cope with the constantly changing microbial profile, which makes it extremely difficult to remain up to date at individual geographic sites.

Sensory characteristics

Sensory characteristics of meat such as flavour, tenderness, juiciness and colour might also depend on the production region, but so far no links with the geographic origin of meat have been described. The utilisation of a distinctive geographic breed distribution, as already discussed, would for instance require that sensory traits can be differentiated between breeds. A comparison of sensory traits of meat from milk-fed lambs (12 kg of live weight at slaughter) and lambs fattened further after weaning (Ternasco, 24 kg of live weight at slaughter) from Spanish Lacha and Aragonesa breeds showed that live weight had more influence than breed, but still each category of meat tested had its own characteristics with regard to texture and flavour [91]. Another Spanish study showed that the Warner–Bratzler shear force (a trait indicative of tenderness) of lamb meat is clearly influenced by breed [92]. Within a breed, tenderness decreased with age and a certain effect of nutrition on tenderness was also demonstrated. In that study [92] very good agreement between tenderness and different countries was found as well (meat of Icelandic 4.3-month-old, pasture-fed lambs was the tenderest, that of two Italian lamb origins fed on concentrates and grass transhumance was the toughest). Tenderness differences among widespread beef breeds are also known, although these are not very pronounced when the animals are fattened with the same intensity [93]. Also in some other studies, relationships between breed and sensory traits were found [94–96]. It is well known that beef of Bos indicus cattle is tougher than that of Bos taurus cattle [97, 98]. Apart from breed, sex and feeding are important factors influencing sensory perception of beef [99, 100].

Qiao et al. [101] found relationships in broiler breast meat between nutritional contents and meat colour (light, normal, dark). Light meat had lower contents of protein and ash, lower levels of the fatty acids C18:0 and C20:4 and higher levels of C16:1. However, it remains unclear how a relationship to geographic origin could be established with these relationships. By contrast, in lamb and beef there is a relationship between darkness as well as redness and the production system since carotene and carotenoids from grass largely contribute to meat colour [102–105]. Accordingly, beef from an organic grazing system was shown to have the most intensive red colour compared with beef from conventional intensive fattening, which showed the lightest colour [71]. However, this effect is confounded with age as can be seen from the fact that the beef of grazed weaners in that study had the least intensive red colour. Older animals store more Fe in the muscle, which facilitates a higher content of myoglobin,

and the meat becomes darker. Within the same category or age class (veal, young cattle, mature cattle, etc.), colour data could quite reliably reflect the production system, keeping in mind that a high dietary proportion of maize, rich in carotenoids, might also contribute to colour intensity.

Overall, the combination of the effects of breed and production conditions on tenderness, which could be reliable and easily estimated by shear force [91], makes it difficult even to attribute the effects to the breed, where genetic determinations would be far more accurate. Furthermore, as already discussed, breeds are not strictly linked to a geographic origin and are easy to exchange. Other sensory traits are even less conclusive, maybe with the exception of meat colour on a small-scale regional basis. None of the sensory approaches seem promising in the case of chicken meat for reasons discussed before. However, sensory studies could be especially interesting for processed meat, as the distinct processing technique often affects certain taste, odour and further organoleptic features, which are important criteria for consumers and are easy to check.

Volatile compounds

Besides sensory tests of flavour, the instrumental determination of volatile compounds and their profile is an alternative. This is a well-known method in order to analyse compounds contributing to the flavour [106–110] and freshness [107, 111, 112] of different kinds of food. In order to determine the geographic origin of meat, especially of processed meat, studying the volatile compounds occurring in the headspace and in meat itself could be interesting. Haugen and Kvaal [113] observed a high potential of using the electronic nose for the development of products with certain flavour characteristics and of using gas sensor array technology to assess sensory quality, off-flavours and taints, shelf life, spoilage and even authenticity by analysing the compositions of headspace gas mixture. Another approach would be to determine the volatile compounds produced by bacteria during storage and processing of meat, as for instance is done for cold-smoked salmon [114], which showed a relationship between the microbial profile, the composition of volatile fractions and sensory quality. Blank et al. [115] determined and identified volatile compounds in Italian-type dry-cured meat products using gas chromatography (GC) combined with mass spectroscopy (MS) and olfactometry, but could not describe single-flavour compounds eliciting the typical salami or Parma ham flavour. Procida et al. [116] were able to draw conclusions on the stage of ripening from volatile components in salami determined by headspace capillary GC-MS. There have also been several studies using different methods investigating volatile compounds in raw and cooked chicken meat [117, 118] and during spoilage [119].

For authentication of meat, headspace analysis would be interesting when distinct volatile compounds either produced by bacteria or, probably only in exceptional cases, incorporated from feed could be related to certain regions. Distinct proportions of such compounds could also be indicative. However, as mentioned earlier, the bacterial population is continuously changing over time, and this might affect these key volatile compounds and their proportion. Therefore, a permanent adaptation of the target values might be necessary. Particularly promising is the analysis of volatile compounds for the determination of the geographic origin in the case of processed meat. It has to be emphasised that, in this case, these compounds code for the site where the processing is done and not for the origin of the raw meat, as these sites are not necessarily identical. Processing would add flavours, e.g., from bacteria, smoke or air, which do not only characterise the specific product but also may be specific for the geographic origin.

The approach relying on volatile compounds achieved much progress with the availability of the so-called electronic noses. Little sample preparation is necessary; the procedure is simple, fast and cheap compared with other methods. A disadvantage of this technique is that the identification of the chemical compounds detected is not possible and that the detection limit is high compared with that of other methods (e.g. GC-MS). This approach, therefore, seems unsuitable for unprocessed meat where the concentration of volatile compounds is very low [120].

Conclusions

A determination of geographic origin of meat based on analytical tools seems to be possible as various traits are influenced by geographically specific factors. An overview of these factors and the studies describing these interrelationships is given in Table 2. There are two types of indicators to determine geographic origin. Primary indicators are directly related to the area the animal comes from. Examples are isotopic ratios of H and O from drinking water, trace elements from the soil and volatile compounds originating from the environment. Secondary indicators are linked to production systems, including animal genotypes, feeding conditions, the environment or the microbial profile, which are supposed to be associated with certain regions but which may change for various reasons. In some cases, the determination of the origin through primary indicators might be sufficient, while in other cases secondary indicators must also be considered.

So far, there are few examples focusing on individual analytical tools where the question of geographic origin of meat could be conclusively answered. Analyses of stable isotopes and trace elements seem to be promising methods in that respect as they give a fraud-resistant, unique signature to the area the analysed meat comes from. An increasing degree of complexity arises when it is attempted to determine the geographic origin from information gathered on animal breed, feeding system or resident microflora. All the methods described had limitations in

Table 2 Factors characteristic for geographic origin of meat

Factor	Characteristic indicator	Lamb	Beef	Poultry	Others
Water	H/D	–	[57, 58, 61]	–	[8, 11, 45, 59, 60]
	$^{16}O/^{18}O$	–	[56–58, 61]	–	[8, 9, 11, 59, 60]
Feeding	$^{12}C/^{13}C$	[62]	[57, 58, 61, 64]	[63]	[8, 9, 11, 43–45, 47, 49–54]
	$^{14}N/^{15}N$	[62]	[58]	–	[8, 9, 43, 44, 46, 47, 49, 54]
Feed supplements	e.g. Se, Zn, Ni, lanthanides	[12]	[12, 18, 20, 25]	[12, 19, 34–36]	[21–24, 26, 27]
Breed, strain	Genotype	–	[72, 73, 76–80]	–	–
Geology	e.g. Se, Rb, Cd, S, Sr, N, Kr	[12	[12, 14, 58]	[12, 15, 16, 19]	[9, 13, 16, 17, 46–49, 65]
Pollution	e.g. As, Zn, Hg, Pb, Cd, Cs	[28, 29]	[25, 33]	–	[26, 27, 30–32, 45]
Housing system	Organic/conventional	[90, 91]	[59, 69, 71]	[13]	–

preventing the doubtless linkage of meat to its geographic origin. There is no feasible ideal method for all purposes, in particular not for simultaneous differentiation between micro-scaled regions. This, for example, is the case when protected denomination of origin (PDO) or protected geographic indication (PGI) statements of meat producers are to be verified, and between products made out of globally imported material. An accurate determination of the geographic origin of meat seems feasible only when a combination of parameters is applied. Thereby the number of traits required is likely to be lower in animals which are connected closely to their origin than in animals where production systems are rather independent of site-specific factors. By way of such a multifactorial approach, all data must be carefully interpreted and cross-validated with tools of multivariate statistics in order to establish links to the origin. With other commodities such as cheese [7, 8, 49], olive oil [121] and wine [122, 123] this strategy has been successfully established.

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