Is Authentication of Regional and Traditional Food Made of Meat Possible?

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Authentication of regional and traditional food made of meat poses a significant challenge. It continues to be a very difficult task which requires employment of quite advanced analytical techniques. 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. In order to perform correct identification of geographical origin, a good solution is to determine both stable isotopes as well as trace elements. It is essential to collect detailed meteorological and geochemical data and information about farming practices and to compare them with the obtained results. In a majority of cases, the performed identification is confined to species and the determination of the animal breed is very limited. In the case of individual breeds a comparative analysis of SNPs appears to present the highest potential, especially genes affecting the coat color of animals may serve as markers. Experiments confirm that genes responsible for pigmentation underwent mutations in individual breeds. Authentication on the basis of the manufacturing process appears to be easier to realize than tracing geographical origins.

Keywords tracing of origin, species/breed identification, trace elements, stable isotopes, DNA analysis, manufacturing process

INTRODUCTION

More and more attention is being paid by consumers not so much to the quantity but rather to the quality of food articles. Regional and traditional products, perceived both as healthier and tastier, enjoy increasing demand among consumers. This trend finds support in the European Union which passed appropriate legislation protecting these foodstuffs by a system of their registration. These actions aim at supporting the diversity of agricultural produce, activization of local populations and at the prevention of migrations from rural areas, as well as an increase of income of farmers. Four different EU Regulations implement the principles of registration and protection of these products within the borders of the European Union as well as their labelling by means of placing on their packaging appropriate labels and confirming their authenticity on the one hand, and on the other hand, guaranteeing their quality. The purpose of these measures is to ensure fair competition as well as to increase the reliability of these products in the eyes of consumers.

The Council Regulation No. 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs refers to products whose characteristic features are associated with their geographic origin. Therefore, these products can, in short, be referred to as “regional products.” The above regulation takes care of two types of labels: Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). The Council Regulation No. 509/2006 on agricultural products and foodstuffs as traditional specialities guaranteed implementing principles of registration of Traditional Specialities Guaranteed (TSG) and defines the term “traditional” as the one that has been in use on the EU market for a period which indicates its “passage from generation to generation”; this period should correspond to a period commonly attributed to one generation and should amount to at least 25 years. Therefore, such products can, in short, be referred to as “traditional products.” Two successive Regulations of the European Union, Nos. 1216/2007 and 1898/2006, established detailed rules regarding the application...
of Regulations: 509/2006 and 510/2006, respectively. However, it should be noted that the European Food Information Resource Network project (2005–2010) (http://www.eurofir.net) founded under the EU 6th Framework aimed to define the term “traditional” (Trichopoulou et al., 2007). The EuroFIR working group specified the term “traditional” as “conforming to established practice or specifications prior to the Second World War” and this definition differs from that enacted by the Council Regulation No. 509/2006. According to the European scientists “traditional food” should be defined as “a food of specific feature or features, which distinguish it clearly from other similar products of the same category in terms of the use of “traditional ingredients,” or “traditional composition,” or “traditional type of production and/or processing method” and it should be applied to food produced prior to World War II, that is, before the era of mass production had been started (Trichopoulou et al., 2007).

In the case of PDO, PGI, and TSG, it is their quality that is protected and understood as the compatibility of these products with their description found in the specification accompanying the application for registration. Official control examines the compatibility of a given product with its specification. Whereas regulations concerning penalties connected with violations of PDO, PGI, and TSG are under the jurisdiction of individual member states and may comprise of administrative or civil-law procedures, the awarded label confirms the authenticity of a given product and is to guarantee its quality.

The observed growing interest of consumers in traditional and regional products and desire for quick and easy profits can encourage dishonest producers to adulterate them or to utilize their name to gain unfair advantage over competitors. Food articles are regarded as adulterated if they are inconsistent with the declaration of the salesman. In the case of meat and meat products, incompatibility may concern, among others, substitution of constituents by less valuable ones, addition of undeclared declaration of the salesman. In the case of PDO, PGI, and TSG are under the jurisdiction of individual member states and may comprise of administrative or civil-law procedures, the awarded label confirms the authenticity of a given product and is to guarantee its quality.

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or place or historically in association with culture or tradition, and (4) generating attraction through impacting subliminal desires of consumers, among others, design, texture, flavor, taste appearance, and premium price. Therefore, the quality concept should be considered not only from the point of view of food trust and safety but, equally importantly, it should also refer to social interactions.

In the European Union, over 873 agricultural products and foodstuffs have already been registered in accordance with the regulations mentioned earlier, of which 211 products and foodstuffs have been registered and are protected in such groups as “Fresh meat and offal” and “Meat products (cooked, salted, smoked, etc.)” (Table 1). The greatest number of such products have been registered in Portugal (63) followed by France (57) and Italy (34) (Fig. 2). So far, only 3 TSG have been registered, although further 7 applications have already been submitted and 4 have been published.

The market for regional and traditional products is huge. In France alone, the sale value of products marked with the PGI symbol amounted to €1 billion in 2007, while the number of manufacturers reached 12000 (INAQ, 2010). The number of registered articles continues to increase and individual member states support manufacturers and encourage product promotion. In Poland, a List of Traditional Products has been established at the Ministry of Agriculture and Rural Development. An entry on the List is connected with a distinction of high quality of agro-food products but is not accompanied by protection of production and name. At the present time, the total of 720 food products can be found on the list, of which 134 products have been registered in the meat products category (http://www.minrol.gov.pl). The entry in this registry constitutes the first step in the process of registration of a given product at the EU level.

DIVERSIFICATION OF PRODUCTS AND THEIR CHARACTERISTICS

Production of regional and traditional products depends on natural climatic factors, hence conservation by drying with the assistance of molds in the south of Europe and application of smoking in the north in regions with high air humidity. The

<table>
<thead>
<tr>
<th>Logos</th>
<th>Short</th>
<th>Fresh meat and offal</th>
<th>Meat products (cooked, salted, smoked, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Registered</td>
<td>Applied</td>
</tr>
<tr>
<td>PDOs</td>
<td>26</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>PGIs</td>
<td>85</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>TSGs</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1 The number of protected fresh meat and meat products in EU

Data from: Database of Origin and Registration (DOOR)
climate, soil quality, animal breed, appropriate feeding as well as unique production technologies all exert an impact on product specific composition and determine the characteristic complex of traits affecting its final quality.

Protected Designation of Origin (PDO) is awarded to agricultural products and foodstuffs whose quality and characteristic features are associated, to a considerable extent or exclusively, with a given geographic environment. This category comprises carcasses as well as meat from their partition derived from special breeds born, reared, and subjected to partition on a specified area. Example products include meat produced from poultry (Volailles de Bresse from the white variety of the French or Bresse Breed), cattle (Carne Maronesa of the Maronês Breed), goats (Cabrito Transmontano of the Serrana Breed), or swine (Carne de Bisaro Transmontano). In the last case, it refers to meat derived from the slaughter of piglets or partition of carcasses of the Bisaro breed using the traditional manufacturing method. The production area is restricted to specific communes in regions of Bragança and Vila Real in Portugal. The feed derives from this geographical region and constitutes a mixture of cereals supplemented with pumpkin, turnip, potatoes, beets, various fruits, sweet corn, cabbage, dried forage, and fodder plants. In autumn, the diet of these animals is chestnut. With the exception of the winter period, the animals are kept outdoors. The carcass structure, the amount and distribution of fat, as well as the taste and flavor of meat are all directly connected with the way of animal rearing and feeding. The meat, although not very fatty, has fairly frequent streaks of fat, its muscles are juicy and smooth of dense structure, pink fat. The above-mentioned sensory traits are associated with the application of a local breed adapted to severe climatic conditions, traditional, semi-extensive production system, existing soil-climatic conditions, and feeding with products derived from local cultivations. In order for products to be registered as PGI products, their specific quality, reputation, or other properties should be attributable to geographical origin. Carcasses and meat from their partition registered in this category possess a specific quality due to a definite climate, applied production method, and the reputation of this product frequently goes far beyond local boundaries and goes back to many centuries. Examples of such products include poultry (Volailles de Cholet), pork (Porc Fermier du Limousin), bovine (Ternera Asturiana), or Scotch lamb.

The most common meat products labelled with PDO and PGI logo comprise such raw aging products as raw sausages and hams (Table 2). Italian Prosciutto, French Jambon de Bayonne, or various kinds of salamis are best known and available outside regions of their production. These products, despite a similar process of manufacturing, differ in taste and aroma. This happens due to the use of special breeds of pigs, the application of appropriate feeding regimes as well as the effect of the place and climate. The applied meat is of high quality, is characterized by considerable marbling as well as a higher content of intramuscular fat (over 3%) thanks to which the desirable tenderness, juiciness, and taste are achieved.

Traditional Specialities Guaranteed (TSG) are traditional products whose specific character distinguishes them from other products of the same category with respect to their physical, chemical, and microbiological or organoleptic properties as well as the production method or conditions. At the moment, the only registered TSG product in the category of fresh meats is “Traditional Farmfresh Turkey.” The traditional slow growth of the turkeys ensures a good carcass with firm meat. Prior to evisceration, the carcasses are hung by both legs for 7 to 14 days which results in tender meat with a gamey flavor.

The production of traditional and regional products is frequently based on the breeding of old, primitive breeds well adapted to the environment, resistant to diseases, and long-lived. The development of genetics and modern breeding methods aiming at high productivity and based on a small number of highly productive animal breeds resulted in the disappearance of many valuable local breeds in the twentieth century. At the present time, attempts are being made to save old, primitive breeds by creating genetic reserves and gene banks, by establishing conservation breeding programs or by freezing sperm and embryos (Przybylski, 2008). An example of such a breed is the German Schwäbisch-Hällisches whose population was reconstructed from a couple of survived individuals. In Poland, in the 1950s, two lines of pigs were successfully bred thanks to rational selection work from several primitive pigs of fatty-type, pigs of Zlotnicka White of meat-type, and Zlotnicka Spotted of meat-fatty-type. Traditional products manufactured from these two breeds enjoy increasing popularity.

POSSIBILITIES OF AUTHENTICATION

Bearing in mind specific features of regional and traditional products manufactured from meat, adulteration of these products may refer to the application of raw materials derived from outside the declared geographic region and/or raw materials originating from breeds inconsistent with the specified ones. Secondly, it is also necessary to take into consideration the replacement of constituents by less valuable ones, addition of undeclared constituents, change in their proportions, or the employment of the manufacturing process inconsistent with the specified one. In consecutive sections, examples and possibilities of analytical techniques to investigate such adulterations will be discussed. The most frequent techniques employed to investigate geographic origin, to identify species, and to control the manufacturing process are presented in Fig. 3.

Tracing of Origin

Analyses of trace elements and stable isotopes present significant potentials in the identification of origin of regional products. Throughout their lives, animals absorb considerable quantities of chemical elements and compounds from the environment which are subsequently excreted but some of them accumulate...
Table 2  Characteristics of the chosen meat products

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Geographical area</th>
<th>Raw materials</th>
<th>Specific character</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDO</td>
<td>Prosciutto di San Daniele</td>
<td>production – ten regions of Italy, processing – the municipality of San Daniele del Friuli; Italy</td>
<td>legs of pigs born, reared and slaughtered in the production area; sea salt</td>
<td>pigs fed on with cereals and the processing milk; relatively dry climate with steady breezes which helps to form the local microflora species that determine the product’s aroma</td>
</tr>
<tr>
<td>PDO</td>
<td>Jamón de Huelva</td>
<td>production – some parts of Andalusia and Extremadura, processing – north of Huelva province; Spain</td>
<td>legs and blades from pigs of the Iberian breed or 75% crosses of this breed with 25% of the Duroc-Jersey Breed; salt</td>
<td>the natural habitat of the Iberian pigs fed on acorns, grass, and stubble; the curing process assisted with dry prevailing winds, low temperatures, and an altitude of 800 m above sea level</td>
</tr>
<tr>
<td>PDO</td>
<td>Szegedi Szalámi</td>
<td>production – five southern Hungarian counties; processing – the municipality area of Szeged; Hungary</td>
<td>carcasses of Mangalitza breed and from crossbreds of Cornwall, Berkshire, Hungarian Large White, Hungarian Landrace, Duroc, Hampshire, and Pietrain; salt</td>
<td>pigs fed on with grain and juicy feed grown in farmlands; salami is smoked with beech logs; the river Tisza creates a micro-climate with higher humidity which ensuring the growth of specific “house mycoflora” of different mold species and form the typical greyish-white cover</td>
</tr>
<tr>
<td>PGI</td>
<td>Canard à foie gras du Sud-Ouest</td>
<td>rearing and processing in South-West regions of France</td>
<td>livers from a male Muscovy duck or a male mallard duck; salt; duckfat or goosefat; duck meat</td>
<td>the traditional farming economy of the South-West France; force-feeding using only maize harvested in the geographical area; the confit method using duckfat or goosefat</td>
</tr>
<tr>
<td>PGI</td>
<td>Chourico de Portalegre</td>
<td>some municipalities in the District of Portalegre; Portugal</td>
<td>meat and hard fat from pigs of Alentejo Breed; salt; garlic; sweet pepper; red pepper paste; white wine</td>
<td>pigs are reared outdoors in the oak forests of the Alentejo region; traditional manufacturing process with smoking using wood from the region; specific micro-climate with cold, dry winters</td>
</tr>
<tr>
<td>PGI</td>
<td>Thüringer Leberwurst</td>
<td>the Federal Land of Thuringia; Germany</td>
<td>at least 51% of the ingredients originate in Thuringia; pigmeat; pig’s liver; nitrate pickling salt; braised onion; spice mix; smoke</td>
<td>the sausage has a centuries-old tradition; a home-made natural method of production with cold smoking in beechwood smoke; good skills and experience of Thuringian butchers; the highest quality raw materials</td>
</tr>
<tr>
<td>TSG</td>
<td>Jamón Serrano</td>
<td>Spain</td>
<td>pigs’ legs; salt</td>
<td>the traditional method of production gives the ham its characteristic taste and aroma from processes of microbiological and enzymatic nature</td>
</tr>
<tr>
<td>TSG</td>
<td>Kielbasa jałowcowa –</td>
<td>Poland</td>
<td>pork meat; cutting fat; pepper; juniper; sugar; curing mix; smoke</td>
<td>the use in the production process of juniper berries and juniper branches during the smoking ensure the specific taste and aroma</td>
</tr>
</tbody>
</table>

Data from published applications for registration

in their tissues. The accumulated materials derive both from feeds as well as from the surrounding environment. This is particularly important in the case of regional articles based on extensive production systems (housing outdoor or in a barn) in which animals spend the major parts of their lives in the natural environment and consume local flora or feeds derived from a given region.

“Light” bioelements (H, C, N, O, S) and “heavy” geoelements (Sr, Nd), despite the fact that they are geographically changeable, they are also place-specific. Their presence in plant and animal tissues depends on the biosphere (unpolluted environment), geosphere (rocks), and anthroposphere (polluted environment, e.g., Pb, V, Fe, Zn, Cu) (Hölzl et al., 2004). The way of animal feeding can be controlled by analyzing the \(^{13}\text{C}/^{12}\text{C}\) ratio because it depends on feeding them with \(\text{C}_3\) or \(\text{C}_4\) type of plants which vary with regard to the mechanisms of CO\(_2\) assimilation, leaf structure, and photosynthesis efficiency. Plants of \(\text{C}_3\) type (majority of vascular plants and crop plants) are commonly found in the temperate climatic zone, whereas the \(\text{C}_4\) types of plants (e.g. maize, sugar cane, millet, and sorghum) are dominant in the tropical climate. Therefore, the falling \(^{13}\text{C}/^{12}\text{C}\) ratio gradient from the equator towards poles in plant material can be used for the identification of geographic origin. On the other hand, local farming practices correlate with a higher or lower \(^{15}\text{N}/^{14}\text{N}\) and \(^{34}\text{S}/^{32}\text{S}\) ratios. Geographical latitude, atmospheric conditions (e.g., evaporation, condensation, precipitation) affect the \(^2\text{H}/^{1}\text{H}\) and \(^{18}\text{O}/^{16}\text{O}\) ratios in ground waters. This parameter changes as we move in the direction from sea shores deeper into the land and finds its reflection in animal tissues (Kelly et al., 2005). Trace elements and stable isotopes occur in very small concentrations; hence the obtained results depend much on measurement accuracy. Moreover, in the case of examinations of meat or bone tissues, a longer, several months long exposure to these factors is required in contrast to milk in which factors or conditions to which animals are exposed are reflected almost instantaneously (Franke et al., 2005).
Taking into consideration all of the above-mentioned relationships, researchers make attempts to assess on their basis, ways of animal feeding, and authenticity of their origin. Analyzing oxygen and hydrogen stable isotopes in beef samples (tissue water) derived from Germany and Argentina, Boner and Förstel (2004) reported the possibilities of tracing back the region of origin by comparing the obtained values with the D/H and 18O/16O ratio in ground waters of the examined regions. Identification of closer locations, namely beef samples (raw protein) from three different regions in Germany, proved possible thanks to another pair of isotopes: 15N/14N and 34S/32S, albeit with ambiguous results. In the case of Aachen, 100% of the samples were identified, while in the case of the two remaining regions, Dueren and Rhenbach, 75% and 79%, respectively. In addition, differentiation between beef from conventional and organic rearing systems was successful on the basis of 13C/12C, bearing in mind the fact that, in the case of organic rearing, no feeds containing maize are applied. The above reports are further corroborated by investigations carried out on beef (Renou et al., 2004; Schmidt et al., 2005; Heaton et al., 2008; Nakashita et al., 2008) and lamb (Piasentier et al., 2003). Piasentier et al. (2003), employing isotope ratio mass spectrometry (13C/12C, 15N/14N IRMS), analyzed lamb meat derived from 6 European countries divided into groups in relation to the way of feeding (suckled milk only, pasture without solid supplementation, and supplementation containing maize grain). $\delta^{13}$C was significantly different, being higher in protein than in fat (average difference 5.0‰) and $\delta^{15}$N values of the protein fraction were not indicative of a region sufficiently. The discrimination of the lamb meat according to the country of origin gave 79.2% results and, according to their feeding regimes, 91.7% used canonical discriminant analysis. Similarly, Camin et al. (2007) reported significant differences when analyzing stable isotopes of hydrogen, carbon, nitrogen, and sulphur using the IRMS method in the meat of lambs derived from 13 regions of 7 European countries (Austria, France, Germany, Greece, Ireland, Italy, and Great Britain). Differentiation of the majority of regions was possible and the proportion of correctly classified samples reached 78%. In investigations conducted by Renou et al. (2004) $\delta^{18}$O values of the meat from Charolais steers determined with the IRMS method differentiated the diet of animals but not the place of breeding, while individual $^1$H, $^2$H, and $^{13}$C parameters in the fat tissue triglycerides determined by the Nuclear Magnetic Resonance (NMR) spectroscopy failed to identify the place of production and diets of the examined cattle. However, a combination of four selected NMR parameters allowed the determination of geographic origins depending on the diet. All the pastured animals were identified correctly and 94% of them were fed on maize silage. The isotope ratio of oxygen in the beef from Japan (Nakashita et al., 2008) correlated positively with the isotopic composition of drinking water and, hence, this parameter made it possible to determine the origins of beef not only from different countries (Australia, Japan, and USA) but also from individual regions in Japan.
When analyzing origins of poultry meat and dried beef samples from various countries situated in different continents on the basis of assays of 66 chemical elements and 46 isotopes, it was demonstrated that the values of some of them showed significant variations among countries (Franke et al., 2007). A combined analysis of many elements with the isotope analysis of oxygen failed to improve the results in comparison with data analyzed individually (Franke et al., 2008). Recent reports from China (Guo et al., 2010) show that a combined analysis of δ^{13}C and δ^{15}N can provide sufficient information to trace the origins of cattle. In this case, the isotope content in defatted muscle tissue and crude fat in samples derived from four Chinese provinces were analyzed.

Therefore, it can be said that it is possible to identify the origins and to assess the way of feeding of animals on the basis of analyses of trace elements and stable isotopes in the case of PDO and PGI products. The employed methods and analytical techniques are similar and the examined material can include muscle tissue proteins, fat, or muscle juice. However, it is also clear from the above-given examples that the effectiveness of the applied methods varies considerably and the obtained average level of traceability of approximately 60–80% is far from satisfactory. Good results are obtained examining samples derived from different continents, whereas in the case of neighboring regions, the origin identification will not always be effective and the obtained results will depend largely on the sensitivity of the applied equipment. Very little research has been conducted on processed meat products. In order to obtain reliable information concerning the identity of origin, it is essential to base the identification on analyses of several parameters (multi-element and multi-isotopic measurements); hence the employment of this methodology is both expensive and time-consuming. In addition, it is also necessary to secure detailed meteorological and geochemical data as well as information about farming practices, ways of animal feeding connected with the analyzed region. In the case of small ruminants, plant biomarkers such as carotenoids, terpenes, and phenolic compounds (Prache et al., 2005) can also be employed.

Among advantages, it is worth emphasizing the fact that the examined factors do not undergo extensive changes in time. Examining changes in δ^{18}O in the drip during 14 days of beef and pork cold storage after slaughter,Horacek et al. (2010) failed to observe significant differences. When investigating authenticity on the basis of stable isotopes, it is important to pay additional attention to their seasonal variations in animal tissue as a result of changes in their diets in relation to the season of the year. Seasonal changes in δ^{13}C, δ^{15}N, and δ^{34}S were found to occur in Irish beef produced by the conventional and organic methods (Bahar et al., 2008). A comparison of heavy metal concentrations (Pb, Zn, Fe, Cu, Hg, As) can be used as a measure of ecological origin of constituents, which manufacturers of traditional and regional articles use frequently to emphasize the advantage of these products.

Attempts are also made to estimate the authenticity of origin with the assistance of genetic markers. Such an attempt was undertaken by Sasazaki et al. (2007) who tried to differentiate between beef derived from Japan and imported from Australia. Their investigations were conducted on genomic DNA using the PCR-RFLP method. On the basis of the performed analyses of the following 6 selected markers: two specific for Bos indicus (SRY and ND5 gene markers), one coat color gene marker (MC1R), and three AFLP-derived markers, it was possible to assess the origin of cattle muscle tissue with 0.933 probability.

### Control of Species/Breed

The application of molecular authentication based on DNA technology is possible in the case of regional and traditional products in situations when producers declare that the meat used to make them derives from specific breeds or species. DNA technology makes it possible to identify the admixture of undeclared species and can also be helpful in controlling proportions of meat components in processed products. Both proteins and DNA undergo considerable degradation in the course of processing. That is why, identification of highly degraded products treated with high temperature, pressure, or subjected to drying or pH changes should be based on the analysis of short DNA fragments (up to about a few hundred base pairs). In the case of methods based on DNA amplification, particular attention needs to be paid to the proper extraction in order to eliminate compounds which could act as amplification inhibitors (e.g., organic and phenolic compounds, polysaccharides, products of the Maillard reaction, glycogen, collagen, fats, milk proteins, iron, cobalt, fulvic acids, bacterial cells, non-target DNA) (Wilson, 1997; Teletchea et al., 2005). The key problem here is not to accept falsely negative or falsely positive results (cross-hybridizations).

Recently, a number of interesting review papers have been published regarding PCR methods and possibilities of examining authenticity of food articles (Mafra et al., 2008) and meat (Ballin et al., 2009). However, no regional or traditional products were analyzed in those studies. What is more, few papers on this subject were published during the last few years. Below, papers undertaking problems associated with PDO and PGI products authenticity are discussed.

PCR-RFLP was employed to identify fish roe of Messolongi, a famous product from Greece with PDO (Klossa-Kilia et al., 2002). PCR amplification of mitochondrial DNA 16s rRNA segment enabled discrimination of the fish roe of Messolongi from other four Mugilidae species coexisting in the same area. Colombo et al. (2002) sequenced a cytb of mtDNA of Italian goose breeds and the PCR method was able to identify the goose species (Anser anser) in Italian “Mortara” salami which is home-made in accordance with old traditions in Italy (the Lomellina zone). Spanish hams manufactured from Iberian pigs are appreciated higher than those made from crosses. That is why a test based on coat color genes (MC1R and OCA2) was elaborated which provides an appropriate tool that distinguishes unambiguously purebred pigs and crosses between closely related populations such as Iberian and Duroc (Fernández et al.,...
The analysis allowed detecting $\frac{1}{1}$ Duroc crossbred pigs with a probability of exclusion of the pure Iberian origin of 0.968. One of the most valuable and best recognized French regional products is foie gras. Rodríguez et al. (2004) elaborated a fluorogenic assay (TaqMan real-time PCR) to be used for quantitative determination of mule duck (Anas platyrhynchos x Cairina moschata) in two-component duck/goose foie gras. The method allowed quantitative determination of duck in foie gras mixtures in the range from 1 to 25%. RADP-PCR using OPL-04 and OPL-05 primers was employed successfully to differentiate five animal species (pork, beef, lamb, chicken, and turkey) in raw and processed meat products, including such regional animals as chorizo and salchichón obtained from Spanish supermarkets (Saez et al., 2004).

It is evident from the above examples that it is possible to identify meat species in highly processed articles or even to determine them quantitatively, especially using the real-time PCR method (Ballin et al., 2009). It is possible to determine closely related species, for example, different bovine species (Verkaar et al., 2002), horse and donkey (Chisholm et al., 2005), wild boar, and domestic swine qualitatively by the PCR-RFLP method (Fajardo et al., 2008a) or red deer, fallow deer, and roe deer by the real-time PCR method with a detection limit of 0.1–0.8% in mixtures (Fajardo et al., 2008b). In the last case, the method sensitivity when raw meat was used amounted to 1pg DNA in water. On the other hand, identification of very closely related animals, breeds, or strains, not mentioning single genotypes, still poses many problems. The issue is very serious because the number of farm animal breeds that are most frequently consumed is as follows: cattle–897, pigs–541, chickens–1077, sheep–995, goats–512, turkeys–78 (Commission on Genetic Resources for Food and Agriculture, FAQ, 2007). One of few attempts in this field, namely elaboration of a test to determine beef derived from four Italian cattle breeds, was undertaken by Dalvit et al. (2008). The four breeds concerned: Chianina, Marchigiana, Romagnola, and Piemontese are reared in central Italy and their meat is protected by a PGI label, “Vitellone bianco dell’Appenino Centrale”; however, the applied combination of a statistical method with the 21 microsatellite markers (STR) analysis improved the identification of only 52.5% of genotypes.

A given product may contain meat tissue derived from several individuals and, additionally, DNA from bacteria, fungi, plants, and other animals. Therefore, an ideal solution would be a technology allowing a simultaneous identification of DNA derived from all organisms present in a given product, not only from the point of view of species but also breed or genotype. Attempts are being made to employ microarray technology (DNA chips) for this purpose. The sequence-specific chip hybridization analysis of mitochondria cyt b PCR products developed by Peter et al. (2004) offers possibilities of simultaneous identifications in meat product of four out of six species (cattle, pig, chicken, turkey, sheep, goat) during one analysis. In experimental mixtures, 0.1% addition of beef or poultry meat was detectable. Much higher possibilities, namely simultaneous identification of 71 species (mammals, birds, fish), are offered by a cyt b-based microarray elaborated by Teletchea et al. (2008), although in this case, we can only talk about semi-quantitative detection. The SNaPshot minisequencing analysis, on the basis of mtDNA cyt b gene target sequences, simultaneously identifies the following nine species: roe deer, red deer, steinbock, chamois, goat, sheep, buffalo, cattle, and swine (La Neve et al., 2008). The detection limit for roe deer amounted to 0.05 pmol.

The scientific breakthrough took place in 2004 when the chicken genome (Gallus gallus) of red jungle fowl 256 line was successfully sequenced (Schmutz and Grimwood, 2004). The Swine Genome Sequencing Consortium (SGSC) established in 2003 sequenced approximately 60.5% of the pig genome (Sus scrofa) of the Duroc breed (http://www.piggenome.org). In 2009, scientists succeeded in sequencing the taurine cattle (Bos taurus) genome of the Hereford breed (Elsik et al., 2009). This sequence and comparative sequences of six other breeds served to elaborate 37470 single-nucleotide polymorphisms (SNPs) in 497 cattle from 19 geographically and biologically diverse breeds (The Bovine HapMap Consortium, 2009). Databases are continually complemented with consecutive sequences and SNPs. At the present time, identification and comparative analysis of SNPs between closely related animals appears to offer the greatest potential in the field of authentication of regional and traditional products. The first attempts have already been made. Negrini et al. (2008) combined 90 SNP markers with Bayesian statistics with the aim to trace cattle origin and verification of PGI. The examined animals belonged to 24 breeds from six European countries. This type of analytical approach made it possible to identify correctly animals which belonged to breeds from which “Vitellone bianco dell’Appenino Centrale” is manufactured with 97%, Ternera de Navarra–with 84%, Boeuf de Chalosse–with 80%, and Guaranteed Pure Highland Beef–with 100% accuracy.

It is interesting that researchers who tested the developed technologies on commercial products reported cases of adulteration of meat articles purchased in supermarkets. Reports referred, among others, to substitutions of donkey/horse (Teletchea et. al., 2008) and duck/pork (Calvo et al., 2002). Pascoal et al. (2004), using the PCR–RFLP method, assessed the labelling correctness of meat products subjected to various processing procedures, for example, smoking, cooking, and sterilization. Out of 50 tested products with different meats declared on their labels, 15 products were labelled incorrectly. The most frequent fault was an undeclared addition of turkey meat (7 products) followed by the absence of declared beef or roe deer (4 products), that is, species with the highest prices.

Another promising development is species identification with the assistance of short DNA sequences (barcodes) of a single gene. In this case, the applied genetic marker is the COI gene (mitochondrial gene cytochrome c oxidase subunit 1) since it is present in all eukaryotic cells. DNA barcoding is currently a very helpful tool in the taxonomy and systematics of species living on the earth. An international consortium was established (Consortium for the Barcoding of Life (CBOL)) in 2004 which, at the moment, embraces 150 organizations from 45 countries and
whose aim is to implement DNA barcoding all over the world as a technology which can easily become a simple standard tool for rapid and accurate species identification (Costa and Carvalho, 2007). Access to the following databases collecting DNA barcoding data is free: the Barcode of Life Database (BOLD), the GenBank of the National Centre for Biotechnology Information (NCBI), the European Molecular Biology Laboratory (EMBL), and the DNA Data Bank of Japan (DDBJ). This technology is currently being introduced for fish identification which is a very difficult task due to the number of species (over 35,000; this is the most numerous class among Vertebrata).

Wong and Hanner (2008) tested possibilities of DNA barcoding for the identification of seafood obtained from shops and restaurants in the United States and Canada. On the basis of the analyzed 652 bp sequence from the 5' region of the COI gene, they managed to identify 90 of 96 samples; the analyzed sequences were analyzed with those collected in the BOLD and GenBank databases. However, it was suspected that 25% of the samples could have been identified incorrectly and therefore it was concluded that DNA barcoding is effective for the identification of seafood to the level of species. Dawney et al. (2007) corroborated the fact that this technology is suitable for the identification of mammal species (cow, chicken), provided that there is an appropriate reference sequence in the database.

In the case of studies mentioned earlier, Sasazaki et al. (2007) and Fernández et al. (2004), the identification of cattle (Japanese Black and Holstein) and pigs (Iberian and Duroc), respectively, was based on the analysis of genes affecting the coat color of animals confirming that breed-specific coat color variations were the result of genetic variability. In the above papers, the authors analyzed genetic variants of genes involved in the regulation of melanogenesis (gene melanocortion 1 receptor–MC1R) and in the biosynthesis and transport of melanosomes (oculocutaneous albinism II gene–OCA2). These investigations confirmed that genes responsible for pigmentation underwent mutation in individual breeds. This hypothesis was also confirmed by recent publications. Mohanty et al. (2008) demonstrated variability in MC1R and TYRP1 (tyrosinase-related protein 1) genes in native Korean Hanwoo and Angus black cattle. Moreover, sequence variants in MC1R were associated with total melanin and eumelanin in Hanwoo phenotypes. Royo et al. (2008) studied differences in the agouti signalling peptide (ASIP) gene expression and black color in a rare Xalda breed sheep. Drögemüller et al. (2009) demonstrated a relationship between the white belt pattern of Brown Swiss cattle and the telomeric region of BTA3. In the case of pigs, thanks to the analysis of SNPs and insertions/deletions (InDels) within 44 genes, it was possible to identify unique mutations in EDNRB, ADAMTS20, and KIT genes in the Jinhua breed, in the MLPH gene of Clawn breed, in the SLC7A11 in the Duroc breed, and in the KITLG gene in the Berkshire breed (Okumura et al., 2009). The above experiments confirm that it is possible to identify individual breeds analyzing genes responsible for pigmentation, although at the moment our knowledge in this field remains insufficient. Nevertheless, these papers may be treated as a starting point towards the possibility of authentication of regional and traditional products.

Control of Manufacturing Process

Authenticity investigations should also be considered in the context of the control of the manufacturing process. Meat product adulteration may refer to change of constituents into less valuable, addition of undeclared components, changes in their proportions, or employment of the manufacturing process incompatible with specifications. This is particularly important in the case of products labelled as TSG in which the method of production, the quality of the ingredients, as well as the chemical composition are given in the specification and play a more important role than the origin of raw materials which frequently are not specified. In this case, adulteration control may be based on the assessment of protein, dry matter, fatty acid, individual amino acid, or connective tissue.

Chemical composition can be analyzed using conventional methods but, currently, new as well as more sensitive and more efficient techniques are being introduced. Using near infrared spectroscopy (NIRS), Fontaine et al. (2001) analyzed the content of essential amino acids, protein, and dry matter in such high-protein content products as fish meal and meat meal and obtained satisfactory results with mean standard deviation less than 5% in comparison with reference samples of protein and individual amino acids. A significant advantage of this method is the possibility of analysis of many samples in a short time. On-line NIR was employed to determine the content of protein, water, and fat in ground beef and pork (Tøgersen et al., 1999). Depending on the type of sample and meat species, the mean error fluctuated from 0.35–0.70% for protein, 0.94–1.33% for water, and 0.82–1.49% for fat. At the same time, it was also possible to determine the content of connective tissue and intramuscular fat in ground meat on-line with the assistance of autofluorescence spectroscopy at 332 nm wavelength (Wold et al., 1999). The determined hydroxyproline content was converted into total collagen.

Authenticity investigations can be based on fat analysis because the profile of fatty acids in muscles and stored tissue reflects the composition of fatty acids fed in the diet. Szabó et al. (2007) conducted fatty acid analysis in the subcutaneous fat tissue of different animal species (among others, red deer, wild boar, pig, rabbit, goose) using gas chromatography for this purpose. Their experiments revealed species differences in fatty acid positional distribution of triacylglycerols (TAG) and 2-monoacylglycerols (2MAG), for instance pigs had lower species-specific unsaturation in MAGs. The contents of intramuscular fat and composition of fatty acids with the aid of gas chromatography were analyzed in Spanish hams (Fernández et al., 2007). The following five products were examined: two hams manufactured from white pigs, that is, Jamón Serrano (TSG) and Jamón de Teruel (PDO) as well as three hams manufactured from Iberian pigs, that is, Dehesa de Extremadura...
(PDO), Jamón de Huelva (PDO), and Guijuelo (PDO). Iberian pigs are slaughtered, on average, at the age of 18 months, whereas white pigs, after reaching the age of minimum 9 months. Fat content in muscle tissue ranged from 17.2% in Jamón Serrano to 29.2% in Dehesa de Extremadura. In Iberian hams, a lower content of saturated fatty acids (SFA) and significantly higher percentage proportions of monounsaturated fatty acids (MUFA) were observed. Jamón Serrano exhibited the highest percent of C18:2n-6 and the lowest MUFA content. Iberian hams contained the highest numbers of long-chain PUFAs from the recommended daily intake, whereas the highest proportion of C18:3n-3 was recorded in Dehesa de Extremadura. This technique may be useful in investigations of meat product adulteration. Fatty acid composition will also differentiate wild animals from domestic animals as well as animals from ecological and intensive rearing.

Sensory traits of regional and traditional meat products frequently are the results of interactions of specific microbiological populations settling a given product and whose presence depends on the applied raw materials, manufacturing processes, and local environment. Authentication on the basis of microbiological profiles is particularly effective in the case of cheeses which are inoculated with local strains of microorganisms. Whereas in the case of meat products such as, for example, choríco, the microbiological profile is rather affected by the applied raw materials, type of spices, wine, and the degree of pork grinding (Lopes et al., 1999). The performed studies indicate that tracing geographic origin on the basis of microorganisms settling animal organisms is not sufficiently effective. Hartel et al. (2002) isolated from some animal species (cattle, horse, swine, chicken) derived from Idaho and Georgia states (USA) 213 rybotypes of Escherichia coli, but they were not specific for animal locations. The same conclusions were reached by Millán-Suazo et al. (2002) who analyzed 62 strains of Mycobacterium bovis isolated from dairy cattle carcasses derived from different regions of Mexico. In addition, genetic diversity of the E. coli population in cattle changed over time within individual animals (Aslam et al., 2003). In the case of meat, sensory quality depended more on animal live weight than on breed (Gorraiz et al., 2000).

Authenticity of meat products can be controlled on the basis of sensory traits. Taste, smell, color, tenderness, or juiciness depend on proteolytic processes taking place in muscle tissues and these are the result of the activity of endogenous muscle enzymes and enzymes of microbial origin. Examining physico-chemical and microbiological traits and texture of Italian low-acid sausages, Spaziani et al. (2009) came to the conclusion that their sensory properties result from their traditional process of manufacturing. The sausages, with their final pH of over 5.3, are manufactured in a rural region of Italy (Friuli Venezia Giulia) in winter time from pork, fat, spices, and red wine and are usually consumed after a 100-day period of drying. Sensory analysis revealed that they were easily distinguished from products manufactured industrially or by other butchers. Their characteristic texture features included low hardness and cohesiveness. From among 66 volatile compounds determined by chromatography and spectroscopy, 95–98% derived from spices (black pepper and garlic) and wine and only 0.7–4.26% resulted from the activity of microflora. Analyzing volatile compounds in different types of Italian salami using the GC-MS method, Procida et al. (1999) observed significant quantitative differences. Volatile compounds can be used as indicators of authenticity in processed products but in raw meat their concentrations are too low. Identification of the region of origin would be possible, if their presence resulted from the diet fed to animals. Investigations show that both diet and breed affect the formation of volatile compounds in lamb muscle tissue during cooking (Elmore et al., 2000).

A novel technique for the evaluation of volatile compounds in food is an “electronic nose” (e-nose). The electronic nose systems consist of various types of gas, organic polymer, or metal sensors and software for suitable statistical analysis (Schaller et al., 1998). This technique was successfully applied to quality control, freshness evaluation, shelf-life studies, or authenticity of meat and meat products (Schaller et al., 1998; Haugen and Kvaal, 1998). Recent publications show that the electronic nose may have a great potential in authenticity of regional and traditional meat products, especially processed products like sausages, thanks to possibilities of distinction of flavors specific to products or geographic origin and produced by bacteria, added from smoke, air, or incorporated from feed. The electronic nose has been successfully used to discriminate between cooked alpaca and llama meats (Neely et al., 2001), between different Italian salami, the same salami with different ripening and even between salami produced from male and female pigs (Taurino et al., 2003), as well as between odor profiles of beef samples obtained from cattle fed on pasture or grain diets (Descalzo et al., 2007).

Authenticity of meat and its products can be determined on the basis of proteins. With the assistance of electrophoretic, chromatographic, or enzymatic techniques, it is possible to detect additions of specific animal tissues or non-meat proteins and to identify meat by species (Kvasnička, 2005; Montowska and Pospiech, 2007; Asensio et al., 2008). Among their advantages is the possibility of confirmation of species, applied additives, as well as the type of raw material (milk/milk proteins or blood/its constituents). As indicated by some research, it is possible to identify animals between individual breeds on the basis of proteomic analyses. In their experiments, using the 2-DE technique, Hollung et al. (2009) analyzed 1125 sarcoplasmic proteins derived from two Norwegian breeds, Landrace and Duroc, and reported significant differences in the volume of 94 of them depending on breed and in 41 depending on age. Proteome analysis of indigenous purebred Meishan pigs and Large White carried out by Xu et al. (2009) revealed the differences in protein expression of 25 protein spots between these two breeds. It would be interesting to confirm these observations in studies on pork derived from pigs of other breeds, reared in other countries or environments. At present it is certain that proteomic analyses are very effective in studies of
metabolic changes taking place in muscle tissue, in analyses of meat quality and its sensory traits but very few proteomic studies were carried out involving authentication of processed meat products. Perhaps, advances that have been taking place in this field in recent years, in particular, new equipment possibilities, high resolution of 2-DE gels, progress in mass spectrometry techniques will allow investigations in the area of adulteration of regional and traditional meat products, especially in those cases when it will not be able to employ techniques based on DNA.

**CONCLUSION**

What then should be the answer to the question asked at the beginning: “Is authentication of regional and traditional food made of meat possible?” The truth is that it is very difficult to give an unequivocal answer to this question. It is our belief that confirmation of authenticity depends very much on the scope of the asked question, on the examined product itself, and on the available information on the subject we have at our disposal.

In order to perform correct identification of geographical origin, it is necessary to conduct analysis of a number of factors. A good solution is to determine both stable isotopes as well as trace elements and, therefore, the application of this methodology is expensive and time consuming. In addition, it is essential to collect detailed meteorological and geochemical data as well as information about farming practices, ways of animal feeding, and the type of applied feeds used in the analyzed region and to compare them with the obtained results. If the above information is unavailable, then the determination of origin will be very difficult, if not impossible.

At the current state of our knowledge and technical possibilities, species identification of meat tissue, both in meat as well as in processed products, is undoubtedly possible. In a majority of cases, the performed identification is confined to species and determination of animal breed is very limited. In the case of the identification of individual breeds, identification and comparative analysis of SNPs appears to present the highest potential, especially genes affecting the coat color of the animals may serve as markers because breed-specific variations in coat color are the outcome of genetic variability. Experiments confirm that genes responsible for pigmentation underwent mutations in individual breeds. Our knowledge in this field is still quite fragmentary. Nevertheless, studies in this area can provide a starting point in authenticity investigations of regional and traditional products.

Authentication of regional and traditional products on the basis of the manufacturing process appears to be easier to realize than tracing geographical origins. The procedure would be facilitated if world-wide database gathering information concerning PGO, PGI, and TSG were established. This type of database could collect necessary information about the natural environment in which a given product develops animal nutrition, production conditions, applied raw materials, DNA sequences, etc. Kelly et al. (2005) submitted a similar postulate of development database on a European scale. Regional and traditional products are characterized by high quality, they being quite expensive, and they frequently enjoy considerable reputation and hence the establishment of such a system would also be economically justified. Incurred costs would undoubtedly be smaller than losses of both manufacturers and consumers resulting from unfair practices of some enterprises.

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**REFERENCES**


AUTHENTICATION OF REGIONAL AND TRADITIONAL FOOD


