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How to Determine the Geographical Origin of Food by Molecular Techniques

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1.1 Linkage Between Food and Its Geographical Origin: Historical View

Historically, food products have always been linked with a specific geographical origin. Regional product identities have a long history. In ancient Egypt, places of origin were used to identify products and to signal their quality. In the Middle Ages, European guilds gave their products certain names to ensure consumers got consistent quality, assure market exclusivity, and protect producers legally (Institut National des Appellations d’Origine [INAO] 2005). The history of some well-known cheeses can be traced back to this period, for example Parmigiano Reggiano in Italy, Stilton in the UK, and Comté in France. The process of establishing a regional reputation went parallel with the emergence of the concept of individual brands. In both cases, producers tried to enhance their products’ value by associating consumers with a name: a single producer in the case of a brand, on a collective scale in the case of regional products (Boto et al. 2013).

Several regional products identified in the marketplace by geographical names date from the 19th century, including Opperdoezer Ronde potatoes (Netherlands) and Washington apples (USA). While such regional indications remained important, their significance gradually shrank with time. National and international trade evolved, and technical grades and standards developed and became more important in trade. During the 20th century, internationalization expanded rapidly. The urge for economies of scale meant that certain regions began to specialize in producing a few products. Firms marketed their products over an ever wider area. Product specialization also occurred; instead of producing a broad product assortment, companies specialized in a few standard products. This mass production resulted in the loss of many unique, specific regional products. In time, the globalization of business and markets increased further (Boto et al. 2013).

By the late 1990s, a new geographical diversity of foods had emerged. While the globalization of trade in food produce continues apace, Europe has experienced an increasing interest in foods with local and regional identities. Local food production
systems have indeed been characterized by various strategies to promote local/regional food products (Goodman 2004; Ilbery & Maye 2005; Marsden et al. 2002; Murdoch 2000). An image of the region and regional names are often used to market products that may have a strong reputation associated with their place of production (INAO 2005). As Bérard and Marchenay (2005) point out, products do not just “come from” a region; they “are” from a region. This means that they convey values and culture, that is, an identity. In general, these products have, to a greater or lesser extent, specific qualities based on human expertise and the natural environment where they are produced. The mix of these specific qualities and the regional image creates a unique identity for the product, therefore raising its value (van de Kop & Sautier 2006).

Food quality and authentication are becoming of primary importance for both consumers and industries, at all levels of the production process, from raw materials (farm) to finished products (fork). Moreover, consumers around the world have shown an increasing interest for typical food products with reliable indicators of geographical origin. Typical food products have an important economic role at both national and international levels, as confirmed by certifications and trademarks of quality (e.g., Protected Denomination of Origin, PDO; Protected Geographical Identification, PGI; and Traditional Specialities Guaranteed, TSG), assigned to guarantee typicity and quality standards (Longobardi et al. 2015).

Figure 1.1 highlights the significant stages in determining the geographic origins of products through human history.

1.2 Scope and Approach

This chapter focuses on how to determine the geographical origin of food. Figure 1.2 illustrates the scope of the chapter and the major issues related to determining the geographical origin of foodstuffs. The demand to know the geographical origin of food has been a driving force for implementation of determining the geographic origins of food. Technological innovations, the benefits of using molecular techniques, and the drawbacks of existing approaches are reviewed below.

1.3 Definitions Related to Tracking of Food Origins

1.3.1 Geographical Area

This is the area in which the production and/or processing take place. Generally, the limits of the area are defined by natural and/or human factors which give the final product its particular characteristics. Supporting documents, such as maps, must be provided (Patent Office of the Republic of Poland [PPO] 2010).

1.3.2 Regional Products

In a general sense, van de Kop and Sautier (2006) defined a regional product as a “local product based on a territorial identity and reputation, and/or a typical product based on specific mode of production and whose quality, reputation or any other characteristics
Figure 1.1 Developments in the history of geographical origin determination.

Figure 1.2 Analytic structure illustrating the scope of this chapter on determining the geo-origin of food.
are attributable essentially to its geographical origin.” The geographical origin can be a province, state, department or country, but also cross-border areas that are culturally, naturally or climatically similar.

1.3.3 Appellation of Origin (AO)

This term is defined through the World Intellectual Property Organization (WIPO 2013) as “The geographical name of a country, region, or locality, which serves to designate a product originating therein, the quality and characteristics of which are due exclusively or essentially to the geographical environment, including natural and human factors.”

Appellation of Origin was one of the earliest forms of Geographical Indication (GI) recognition and protection (WIPO 1979). Although mentioned in earlier treaties, the 26 contracting parties to the Lisbon System in 1958 first formally recognized the term “Appellation of Origin” as a form of GI, by using a single registration procedure, effective for all the signatories (Boto et al. 2013).

1.3.4 Geographical Indication (GI)

Geographical Indication is defined by the TRIPS Agreement 1994 as “Indication which identifies a good as originating in the territory of a member (country), or a region or locality in that territory, where a given quality, reputation or other characteristic of the good is essentially attributable to its geographical origin” (World Trade Organization [WTO] 2005).

1.3.5 Protected Designation of Origin (PDO)

The Protected Designation of Origin is for products closely associated with the area whose name they bear (European Commission 1992, Regulation No. 2081/92). Such a product must meet two conditions.

- Quality or characteristics of the product must be connected with the particular geographical environment of the place of origin; this environment includes inherent natural and human factors, such as climate, soil quality, and local know-how.
- Production and processing of the raw materials, up to the stage of the finished product, must take place in the defined geographical area whose name the product bears.

The PDO covers agricultural products that are produced, processed, and prepared in a given geographical area using recognized know-how. Well-known PDO products are prosciutto di Parma (ham) from Italy, Kalamata (olive oil) from Greece, and Camembert de Normandie (cheese) from France (Drivelos & Georgiou 2012).

1.3.6 Protected Geographical Indication (PGI)

Protected Geographical Indications also designate products attached to the region whose name they bear but the link is different from that between a product with a PDO
and its geographical area of origin (European Commission 1992). To be eligible to use a PGI, a product must meet two conditions.

- It must have been produced in the geographical area whose name it bears. Unlike the Protected Designation of Origin, it is sufficient that one of the stages of production has taken place in the defined area. For example, the raw materials used in production may have come from another region.
- There must also be a link between the product and the area which gives it its name. However, this feature need not be essential, as in the case of a designation of origin. It is sufficient that a specific quality, reputation or other characteristic be attributable to the geographical origin of a given product.

The PGI covers agricultural products and foodstuffs closely linked to the geographical area. At least one of the stages of production, processing or preparation takes place in the area. Typical products with recognized PGIs are Scotch beef from the UK, Calcot de Valls (onion) from Spain, and Budějovické pivo (beer) from the Czech Republic (Drivelos & Georgiou 2012).

1.3.7 Generic Name

A term or sign is considered “generic” when it is so widely used that consumers see it as designating a class or category name for all goods or services of the same type, rather than as referring to a specific geographical origin (Boto et al. 2013).

1.3.8 Food Safety

Food safety is defined as the style of preparing, handling, and storing food to prevent infection and to help ensure that food retains enough nutrients to support a healthy diet. Unsafe food means that it has been exposed to pathogens or is rotten, which can cause diseases or infections (e.g., diarrhea, meningitis, etc.) (El Sheikha 2015a; Food and Agriculture Organization of the United Nations [FAO] 2004).

1.3.9 Food Quality

Quality is a measure of the degree of excellence or degree of acceptability by the consumer. It can be defined as “a summary of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs” (FAO 2004). In simple words, the product should have attributes to “satisfy the wants/needs of the consumer or conformance with the user’s requirements.” Quality also covers safety and value for money.

Food quality can be considered as a complex characteristic of food that determines its value or acceptability to consumers. Thus it may be defined as “the composite of those characteristics which have significance in determining the degree of acceptability by the buyer. These characteristics should also have the ability to differentiate individual units of the product” (Leitzmann 1993). The important components of food quality are food safety, sensory characteristics, and nutritional value. Safety of food is a basic requirement of food quality.
1.3.10 Geo‐traceability

Geo‐traceability can be defined as the result of combination of geographic information and traditional data used in traceability procedures (El Sheikha 2010). In the agriculture‐food sector and more particularly in the field of plant production, geo‐traceability is concerned with the relationships between a production plot, its geographical location, its environment, and cultural practices. Geo‐traceability requires the implementation of spatial analysis and information acquisition and processing tools that will be combined in geographic information systems (GIS) (GeoTraceAgri [GTA] 2005).

1.4 Driving Forces for Determining the Geo-origin of Food

There are many driving forces behind the development and implementation of technologies for determining the geographic origin of foodstuffs. These forces can be put into five different categories: why do people buy “origin” food products, safety and quality importance, regulatory focus, economic and social concerns.

1.4.1 Why do People Buy “Origin” Food Products?

Food consumption habits were created by the local natural resources and the social or cultural factors of the community (Delamont 1995). Such links between food and origin have disappeared over time, the main reason for this being globalization of the food industry, following the extensive growth in technology over the past century (Montanari 1994). This has led to a similarity of lifestyles across regions such that food consumption patterns within a region no longer necessarily reflect food production of that area (Ritzer 1996). However, in recent years, consumers have renewed their interest in food strongly identified with a place of origin (Drivelos & Georgiou 2012; El Sheikha 2015b). There are a number of reasons for this increasing interest, including organoleptic qualities, health, psychology (patriotism and confidence in the product), media information, and concern about animal welfare and environmentally friendly methods of production (Gilg & Battershill 1998; Mitchell 2001).

Figure 1.3 shows the interaction between consumers, food product, and origin.

1.4.2 Food Safety, Food Quality, and Consumer Protection Linked to Geographic Origin

Food scares have occurred throughout history. Atkins (2008) has discussed that, in Europe, food scares (especially zoonotic hazards) have been present in the UK for at least 150 years. Saltini and Akkerman (2012) mentioned that in Europe, foodborne illness affects about 1% of the population (approximately 7 million people) each year. In 2011, approximately 16.7% of the US population (47.8 million people) experienced food‐related illness (Resende‐Filho & Hurley 2012). Other types of food scare such as contamination with radioactive materials disturb the food supply chain. After the release of radioactive materials from damaged nuclear plants due to earthquake in Japan in 2011 (World Health Organization [WHO] 2011), many countries implemented intensive food control measures concerning their food trade relationship with Japan while some countries suspended food imports from Japan.
In addition to the public health risk, food scares lead to economic crises due to direct and indirect (damage to reputation and brand name) costs of product recall. Recent studies in Europe and North America reported that commercial frauds range from 15% to 43% of total commercial seafood products, with 75% of fraud cases related to the red snapper (*Lutjanus campechanus*) (Hellberg & Morrisey 2011; Rasmussen & Morrissey 2008). Therefore, traceability is an important component of contemporary supply chains in the production industry in general and the food sector in particular as the food sector is sensitive from a human and animal health point of view (Olsen & Aschan 2010). Figure 1.4 represents the benefits of applying an effective traceability system.

![Figure 1.3 Interaction between consumers, food product, and origin. Source: Adapted from Vandecandelaere et al. Linking people, places and products: a guide for promoting quality linked to geographical origin and sustainable geographical indications, Food and Agriculture Organization of the United Nations (2010). Available at: www.fao.org/docrep/013/i1760e/i1760e.pdf. Reproduced with permission.](image-url)

Proof of provenance has become an important topic in the context of food safety, food quality, and consumer protection in accordance with national legislation and international standards and guidelines. Provenance means being able to identify and ensure the origin of a commodity and thereby the region where it was produced. Incidents such as the outbreak of *Salmonella* food poisoning via contaminated peppers from Mexico, which occurred in the USA in 2008, have demonstrated the need for effective traceability systems and the deficiencies in current paper-based systems. The failure to trace the contaminated batch of peppers to their origin resulted in a wide-scale, costly, and lengthy recall procedure involving many producers in Mexico and retail outlets in the USA (International Atomic Energy Agency [IAEA] 2011).

Therefore, origin of food is one of the most important criteria for ensuring food safety and quality. At the same time, origin is an indispensable basis in the concept of quality from farm to fork. In this context, origin detection tools are regarded as important...
IMPROVED QUALITY CONTROL
Traceability systems increase the standard of product management and quality control work by a system of records that can be accessed on demand. Having access to this information means that a product can be easily checked to confirm that it adheres to various quality scheme criteria.

BUSINESS EFFICIENCY
Traceability systems help increase the efficiency of product management and quality control work by managing products by ID numbers and by storing and offering information about the origins and characters of products. Ultimately it strives to achieve the harmonisation of all operators in the system.

INFORMATION STORE
New legislation stresses that all traceability systems must give easy access to vital points of recorded data. The primary function of a traceability system is to have the ability to locate products not reaching their specifications.

IMPROVED FOOD QUALITY
If an accident related to food safety occurs, traceability systems help trace the cause quickly and easily. The systems help collect and remove a problem food product correctly and promptly.

MINIMIZE PRODUCT LOSS
Being able to reduce the possibility of mass product recalls in the event of products not reaching their specifications could save a producer from being forced to withdraw perhaps a whole year’s stock. Effective traceability systems enable the zeroing in of the product batch and tracing it along both directions of the food chain.

TRANSPARENCY
Traceability systems have to work across the full supply chain and be easy to use by companies operating the system and those requiring access to the data.

Figure 1.4 What are the benefits of applying an effective traceability system on food chain components? Source: El Sheikha & Montet (2016). Reproduced with permission of Taylor and Francis.

aspects of food traceability. Providing early warning systems for avoiding safety and quality problems and recalling effectively are some of the benefits of determining the geographic origin of food products.

1.4.3 Regulatory Focus

The 1883 Paris Convention was the first international multilateral treaty to include provisions relating to indications of geographical origin. Article 1(2) of the Convention recognizes “indications of source” and “appellations of origin” as subject matter for industrial property (WIPO 1979). Indications of source offer a measure of protection
for origin-based product names and icons without the burdens associated with demonstrating specificity and/or reputation, developing a binding product specification and instituting a system of certification control. Indications of source are particularly applicable for marketing through nation branding (Boto et al. 2013).

European consumers are paying increased attention to the quality and authenticity of foodstuffs. European Union regulations allow food products to be distinguished by their specific characteristics and their geographical origin. In 1992, EU regulations 2081/92 and 2082/92 introduced an integrated framework for the protection of geographical indications and designations of origin for agricultural products and foodstuffs. Furthermore, laws enforce labeling of the geographical origin of agricultural products in many countries due to consumer demands for more information on foods. EU regulations 2081/92 and 2082/92 have been replaced by regulations 510/2006 and 509/2006, respectively, and EU regulation 1898/2006 was added (Koç et al. 2012). The EU regulations allow the application of the following geographical indications to a food product: PDO, PGI, and (TSG) (Mout 2004).

The EU has established regulations for every product available. Regulation CE 1760 17/07/2000 made the indication of origin on meat carcasses mandatory, Commission Directive 2001/110/CE posed the same condition for honey, Commission Regulation No 2065/2001 established rules for the application of Council Regulation 104/2000 to inform consumers about fishery and aquaculture products (Ghidini et al. 2006). EC Regulation 182/2009 has detailed rules regarding the geographical origin labeling of virgin and extra virgin olive oil in the European Community. As of December 2014, EU Council Regulation 1169/2011 EU made it obligatory that all fresh and frozen meat, as well as fish produce, be clearly labeled with the point of origin (Wilkes et al. 2016). This is also demonstrated by the existence of a European Wine Databank on authentic European wines (as foreseen in EC Regulation 2729/2000) (Luykx & van Ruth 2008). Figure 1.5 shows products with protected signature names in Europe.

In addition to regulations enabling producers to legally protect regional food specialties from counterfeit copycat products and name abuse, regulations also seek to achieve wider social and environmental objectives with respect to the rural economy.

1.4.4 Economic Concern

The market for GI food products is considerable, especially in the United States, Europe, and the more affluent countries. The estimated value for sales of GI products worldwide is well over USD 50 billion. A number of countries, ranging from Scotland to Australia and China to Chile, have GI exports in excess of USD 1 billion. In France, the market value of GI products is almost USD 24 billion, or close to 10% of the national food market’s total value. Products registered under Italy’s 430 GIs generate a value of some USD 13 billion and employ about 300,000 persons, while Spain’s 133 GI-designated products generate approximately USD 4 billion (Rondot et al. 2004).

Chever et al. (2012) published a study analyzing the value of the EU name protection scheme for all GIs (agri-food products, wines, and spirits). The products with GIs in EU countries are worth about USD 54 billion worldwide. The study also analyzes the value premium of products bearing a GI, that is, the premium that a GI can expect on the market, compared to similar non-GI products. On average, GI products were estimated to achieve a price 2.23 times higher than their non-GI counterparts.
1.4.5 Social Concern

Increasing confidence of consumers in their food, changing lifestyles, increasing consumer income, and increasing societal awareness about health are some of the social issues that motivate food companies to implement GI systems (El Sheikha 2017). The improvement in food crisis management enables concerned agencies to build capacity to safeguard food safety and security which in turn strengthens the social and political security of a nation. In contemporary food traceability systems, companies should not only attempt to comply with government rules but should also adequately provide information that consumers need to know such as a variety of food attributes, country of origin, animal welfare, and genetic engineering-related issues (Golan et al. 2004).

Some origin-linked products have been produced for a long period in the same social and cultural environment. They incorporate producer know-how regarding how to manage a sound production process and attain high specific quality within a particular local environment. The link between product, people, and place often makes the origin-linked product an element of identity for local populations, transcending even its economic impact. As a consequence, the social dimension of certain products has many aspects (Boto et al. 2013).

- The origin-linked product is related to the preservation of the natural and cultural heritage, traditions, know-how, and lifestyle in marginal areas.
- The collective dimension of the origin-linked product strengthens social linkages between local actors, not only through local organizations and greater equity in the production sector, but also externally, as all local stakeholders are involved (for example, public actors, stakeholders of the tourism industry, schools, etc.).
- The sustainable management of various local resources used for food and agriculture contributes to food and livelihood security, while the preservation of typical products offers consumers broader food diversity.

Figure 1.5 European food products with protected signature names. Source: Drivelos & Georgiou (2012). Reproduced with permission of Elsevier.
1.5 Geo-origin Determination ... Evolution of Molecular Techniques

1.5.1 New and Sophisticated Techniques are Increasingly Needed ... Why?

Reports on analytical methods for determining the geographical origin of agricultural products have been increasing since the 1980s. The initial focus was on processed agricultural products such as wine (Etiévant et al. 1988; Frías et al. 2003; Latorre et al. 1994; Martin et al. 1999), honey (Sanz et al. 1995), teas (Fernández-Cáceres et al. 2001; Marcos et al. 1998), olive oil (Angerosa et al. 1999), and orange juice (Mouly et al. 1999), while later studies examined fresh products such as potatoes (Anderson et al. 1999; Chung et al. 2016), Welsh onions (Ariyama et al. 2004a,b; Ariyama & Yasui 2006), pistachios (Anderson & Smith 2005), and garlic (Smith 2005), chiefly because worldwide trade in fresh agricultural products has increased year by year and the law now enforces labeling of their geographical origin.

The use of GIs allows producers to obtain market recognition and often a premium price. False use of GIs by unauthorized parties is detrimental to consumers and legitimate producers. From this point of view, the development of new and increasingly sophisticated techniques for determining the geographical origin of agricultural products is highly desirable for consumers, agricultural farmers, retailers, and administrative authorities. It is an analytically challenging problem that is currently the focus of much attention within Europe and the USA (Luykx & van Ruth 2008).

1.5.2 Overview of Molecular Techniques Used for Geo-origin Determination of Foods

Various techniques have been studied based on organic constituents, mineral contents or composition, light- or heavy-element isotope ratios, or combinations thereof. If the components have sufficient discriminatory power, the set of their concentrations will form a characteristic pattern or "fingerprint" relating to the geographical origin of the sample.

Molecular approaches that have been developed so far for determining geographical origin are outlined and evaluated below. For this overview, the molecular approaches have been subdivided into five groups: mass spectrometry techniques, spectroscopic techniques, separation techniques, molecular biology techniques, and other techniques. All techniques and abbreviations are summarized in Figure 1.6.

1.5.2.1 Mass Spectrometry Techniques (MS)

Isotope ratio mass spectrometry (IRMS) is a method that can be used to differentiate chemically identical compounds based on their isotopes (Brenna et al. 1997). This technique is applicable for the determination of the geographical origin of numerous food products. Geographical origin of food is determined also by ICP-MS which analyzes inorganic elements. Furthermore, ICP-AES (atomic emission spectroscopy) and AAS (atomic absorption spectroscopy) have also been successfully combined with ICP-MS to classify onions (Ariyama et al. 2004a, 2007) and tea (Moreda-Piñeiro et al. 2003) according to their regions of origin. On the other hand, qualitative and quantitative analysis and geographical origin determination can be administered by GC-MS technique (Luykx & van Ruth 2008).
Generally, MS, ICP-AES, ICP-MS, and GC-MS serve as traceable tools in combination with each other. Antimicrobial, antibiotic, and pesticide residues in food are detected by MS-based methods (Herrero et al. 2012).

1.5.2.2 Spectroscopy Techniques

Spectroscopy-based traceability tools are used for the analysis of semi-solid and liquid food. By these techniques, finding the specific fingerprint of each food sample is considered to be an easy method to trace its origin (Aarnisalo et al. 2007; Luykx & van Ruth 2008).

Site-specific natural isotope fractionation (SNIF)-NMR is often used in food analysis and allows determination of the geographical origin of foods based on the isotopic ratio of a given nucleus found in a constituent of the analyzed food (Reid et al. 2006). SNIF-NMR has particularly been used for the geographical authentication of various wines (Martin et al. 1999; Ogrinc et al. 2001). Furthermore, this technique was successfully applied to identify the geographical origin of natural mustard oils (Remaud et al. 1997). The main drawback of SNIF-NMR is that it requires laborious sample preparation involving many purification and concentration steps (Ibañez & Cifuentes 2001).

Infrared spectroscopy is the measurement of the wavelength and intensity of the absorption of infrared light by a sample (Putzig et al. 1994). With respect to mid-infrared (MIR), various wines (Picque et al. 2005), cheeses (Karoui et al. 2004a), olive oils (Tapp et al. 2003), and honey (Ruoff et al. 2006) have been differentiated on the basis of geographical origin. With near-infrared (NIR) spectroscopy, the geographical classifications of grapes (Arana et al. 2005), wines (Liu et al. 2006), rice (Kim et al. 2003), soy sauce (Iizuka & Aishima 1997), and olive oils (Downey et al. 2003) have been accomplished.

Fluorescence spectroscopy provides information on the presence of aromatic amino acids and their environment in biological samples (Luykx et al. 2004). In this way, fluorescence spectroscopy allows determination of the geographical origin of various cheeses (Karoui et al. 2004a, b, 2005a), milks (Karoui et al. 2005b), and olive oils (Dupuy et al. 2005).

Via AAS, it was possible to relate the selenium content of beef to a geographical region (Hintze et al. 2001) and to geographically discriminate honeys (González Paramás et al. 2000) and wines (Frias et al. 2001) by measuring various mineral elements. A combination of AAS and AES allowed determination of the geographical origin of orange juice, nuts (Schwartz & Heicking 1991), and potatoes (Galdón et al. 2012; Rivero et al. 2003).
1.5.2.3 Separation Techniques

By HPLC, GC and CE methods, sample molecules can be partitioned to mobile and stationary phases (Aarnisalo et al. 2007).

High-performance liquid chromatography is a chromatographic method used for determining the amount of soluble and insoluble contents in the solution such as carbohydrate, fat, protein, vitamins, mycotoxins, and proteins (Luykx & van Ruth 2008). HPLC is not only an accurate and quick analysis but is also considered as an ideal method for determining phenolic compounds and organic acids (Aarnisalo et al. 2007). European wines from different geographical origins have been correctly classified on the basis of their chromatography profiles obtained with HPLC in combination with a UV-vis and/or fluorescence detector (Luykx & van Ruth 2008). HPLC has also been used to geographically discriminate honey (Tomás-Barberán et al. 1993), nuts (Gómez-Ariza et al. 2006), olive oil (Stefanoudaki et al. 1997), and cheese (di Cagno et al. 2003) based on the HPLC profiles of flavonoids, metal-binding proteins, triglycerides, and peptides, respectively.

Volatile and semi-volatile structures, flavors, and pesticides have been analyzed by GC (Luykx & van Ruth 2008). The contamination of sample or column is one possible limitation. However, a rapid and reproducible operation and a high sensitivity on a small amount of sample are considered as GC advantages (Aarnisalo et al. 2007). By analyzing the GC profiles of various compounds (e.g., alkanes, aldehydes, alcohols, acids) present in wine, it is possible to classify wines according to their geographical origin (Étiévant et al. 1989; Shimoda et al. 1993). Determination of the fatty acid composition and corresponding concentrations by GC allowed the geographical discrimination of milk samples (Collomb et al. 2002) and olive oils (Oliver et al. 2003). Furthermore, determination of the geographical origin of cocoa masses (Hernández & Rutledge 1994) and orange juices (Ruiz del Castillo et al. 2003) was accomplished via GC analysis.

Capillary electrophoresis is an electrokinetic separation technique that separates components based on their different electrokinetic mobility. This method can be used in various analyses ranging from simple inorganic ions, small organic molecules, and peptides to viruses and microorganisms (Kvasnička 2005). Delgado et al. (1994) were the first to propose that CE be used for studying the geographical origin of a food product. Their study concerned the determination of flavonoids which accumulate in different proportions in honey depending on its geographical origin. In a similar way, CE has been applied to geographically discriminate Chinese fruit extracts (Peng et al. 2006). Furthermore, CE profiles were able to differentiate herb samples based on their geographical origins (Wang et al. 2005).

1.5.2.4 Molecular Biology Techniques

Enzyme linked immunosorbent assay is the most commonly used enzyme-based method with high sensitivity. It is economical and efficient (Ahmed 2002). Enzyme-based traceability tools are used in various implementations such as verifying suitability of meat and dairy products (Aarnisalo et al. 2007; El Sheikha et al. 2017), determining authenticity in fish, fish products and fruit juice and detection of GM products or allergens (Ahmed 2002; Asensio et al. 2008; Sass-Kiss & Sass 2000, 2002; Valdes et al. 2003; Williams et al 2004).

The characteristics of DNA make it a useful geo-origin marker for food. DNA-based techniques are more effective, and can also be applied to different food matrices...
(Lockley & Bardsley 2000; Mafra et al. 2008). Furthermore, DNA is more informative than proteins, and can be easily extracted in the presence of small traces of organic material (Hellberg & Morrisey 2011). PCR-based methods are extremely sensitive, often faster than other technologies, and are widely used in agriculture and zootechnology (Doulatly Baneh et al. 2007; Grassi et al. 2006; Labra et al. 2004; Mane et al. 2006; Teletchea et al. 2005). In recent years, PCR-denaturing gradient gel electrophoresis (PCR-DGGE) has been largely used in the field of food traceability and safety in order to characterize bacteria, yeasts, and molds in food products (Dalmacio et al. 2011; El Sheikha & Xu 2017; El Sheikha et al. 2009; Rychlik et al. 2017; Zheng et al. 2012).

More details regarding DNA-based approaches and other recent techniques which use innovative fingerprinting of food will be discussed in Chapter 19.

1.5.2.5 Other Techniques
Sensor technology, sometimes referred to as “electronic nose technology,” is based on detection by an array of semi-selective gas sensors of the volatile compounds present in the headspace of a food sample (Strike et al. 1999). The electronic nose has been successfully applied to differentiate geographical origins of olive oils (Guadarrama et al. 2001), wines (Penza & Cassano 2004), orange juices (Steine et al. 2001), and cheeses (Pillonel et al. 2003).

Sensory evaluation is considered as an important technique to determine product quality. It comprises a set of techniques for accurate measurement of human responses to foods (Pérez Elortondo et al. 2007). Appearance, odor, flavor, and texture properties are important characteristics determining the quality of food products. Sensory analysis requires panels of human assessors on whom the products are tested, and recording of their responses. By applying statistical techniques to the results, it is possible to make inferences about the products under test (Luykx & van Ruth 2008). Sensory analyses have also been applied to geographically discriminate a spirit drink (Lachenmeier 2007), cheeses (Pillonel et al. 2002), and olive oils (Stefanoudaki et al. 2000).

1.6 Pros and Cons of Molecular Techniques Used as Geo-Discriminative Tools of Food

Although conventional analytical approaches such as IRMS and ICP-MS can provide a good indication as to the likely geographical origin of a sample, instrumentation and running costs, plus the requirement for highly trained analysts, and a protracted workflow, make their use for routine sample analysis difficult. With lower cost implications and an increasing number of nucleic acid-based assays becoming available for food authenticity testing, DNA-based molecular methods have experienced a rapid adoption by many enforcement agencies. Recent technical advances with nucleic acid-based marker systems have made possible the exploitation of genetic variation, where present, which can be used to provide an indication as to where a product may have originated (Chauhan & Rajiv 2010; El Sheikha & Montet 2016; Leal et al. 2015; Lockley & Bardsley 2000; Wilkes et al. 2016; Woolfe & Primrose 2004). A number of techniques have been, or are currently in the process of being evaluated for this purpose. Table 1.1 presents an overview of these molecular methods with their pros and cons.
Table 1.1 Pros and cons of the molecular techniques used for determination of the geographical origin of food products.

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<th>Techniques</th>
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a For abbreviations of the techniques, see Figure 1.6.

b (+) Favorable;
c (+/-) moderate;
d (-) unfavorable.

* Possibility of identifying;
** possibility of profiling.

Source: Adapted from Luykx and van Ruth (2008). Reproduced with permission of Elsevier.

### 1.7 Conclusions

Consumer awareness is increasing day by day and the interest of consumers in high-quality foods with a clear geographical identity has grown rapidly. The concept of food traceability must be evaluated with total quality from farm to fork. In this context, food origin is the base point for ensuring the quality of the whole process. Therefore, participation in protected food name systems (PDO, PGI, TSG) is encouraged in the EU. This means that suitable techniques for determining the geographical origin of food products are highly desirable.
Unfortunately, it is difficult to develop a 100% accurate method for determining geographic origin, and the techniques which have been developed usually cannot avoid a certain number of mistakes. In the last 25 years, molecular tools for studying food have become more sensitive, reliable, and faster. These methods are capable of analyzing specific characteristics of a product which are influenced by geographically specific factors. Current molecular methods are quick, precise, and reliable, and as a result analysis of genetic variation has rapidly become the method of choice for a number of applications, including that of food authenticity. Consequently, the development of food authenticity is beneficial both for raising the awareness of consumers and for ensuring food safety.

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