Chapter 18

Trends in Food Authentication

Wen-Hao Su*, Ioannis S. Arvanitoyannis† and Da-Wen Sun*

**Food Refrigeration and Computerized Food Technology (FRCFT), School of Biosystems and Food Engineering, University College Dublin, National University of Ireland, Agriculture and Food Science Centre, Dublin, Ireland, †University of Thessaly, School of Agricultural Sciences, Department of Agriculture Ichthyology & Aquatic Environment, Volos, Greece*

Chapter Outline

1 INTRODUCTION

Food authenticity has been an issue since the early 1800s and the study core of food authenticity comes from South Europe, while China has made a big step forward during past 10 years (Danezis et al., 2016). This issue is mainly related to improper labeling and economic adulteration (EA), that is, the substitution, in part or whole, of cheaper and inferior food products for high-cost foods in order to defraud the consumer. An authentic food, defined as a food that "conforms to the description provided by the producer or processor," includes the process history of a product or ingredient, its geographic region of origin, or the species or variety of the ingredient. Although rarely a health hazard, EA is driven by the demand for higher-value goods, global trading, and price fluctuations, factors that provide an opportunity for illegal profits. For these reasons, food processing industries and regulatory agencies have pushed for analytical methods to confirm food product authenticity (Su and Sun, 2016b).

The development of new and increasingly sophisticated techniques for the authentication of food products continues apace with increasing consumer awareness of food safety and authenticity issues. Food authentication is also of concern to food processors who do not wish to be subjected to unfair competition from unscrupulous processors who would gain an economic advantage from the misrepresentation of the food that they are selling (Arvanitoyannis, 2016).

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Taking the fishing industry as an example, the development of worldwide high-seas fishing vessels, the improvement in food processing and storage, and the establishment of fishing industries in developing countries have increased the variety of seafood species, both fresh and processed, currently available in markets. These factors have contributed to an increase in total catches from fisheries and, thus seafood consumption worldwide. The demand for a year-round seafood supply, however, has negatively impacted the number of some valued and appreciated species due to exploitation. Because most consumers are not very familiar with the taxonomical and morphological characteristics of seafood species, such as skin pattern, body appearance, and size, eyes, shape, and number of fins, they are subject to being defrauded by buying a seafood product that is not what it claims to be. In addition, the processing of seafood products, which often requires the removal of significant morphological characteristics, hinders species recognition. Because of these reasons, species substitution has become the main form of adulteration in the seafood-processing industry (Floren et al., 2015).

Food authenticity is a major issue for producers, processors, distributors, consumers, and regulators. Many consumers are prepared to pay a premium for products that they consider to be of superior quality, whether that is because of the manner of production, as with organic food, or for other reasons, such as the geographical origin of a product. Food manufacturers need to ensure that tested and valid methods are available to meet the needs of industry and to protect the public from misleading or fraudulent labeling (Spink et al., 2016).

Spectroscopy, protein-based methods and DNA-based methods are the main techniques for detection of food authenticity. Some polymerase chain reaction (PCR)-based techniques that address the problem of establishing a relationship between the concentration of target DNA and the amount of PCR product generated by the amplification have been recently developed. The two principal techniques of quantitative PCR in use at the moment are: quantitative competitive PCR (QC-PCR) and real-time PCR (Kyriakou et al., 2017; Garcia-Bardon and Thal, 2016). The most commonly employed methods for the evaluation of food authenticity issues are given in Tables 1 and 2 and Figs. 1 and 2, respectively.

2 EMERGING AUTHENTICATION METHODS

2.1 Physicochemical/Chemical Fingerprinting Methods

2.1.1 Nuclear Magnetic Resonance/SNIF-NMR

Nuclear magnetic resonance (NMR) spectroscopy is nowadays being used more and more to analyze foods. NMR spectra of food products can act as "fingerprints" that can be used to compare, discriminate, or classify samples. Selected variables that characterize the samples in some specific way are also used instead of the whole spectra (Kim et al., 2015; Su and Sun, 2016a).

TABLE 1 Authenticity Testing of Variety and Geographical Origin and Traceability Testing

Test Method	Item of Interest	Authenticity Factor	Reference
NMR spectroscopy	Orange juice	Grapefruit juice	Cuny et al. (2008)
MIR spectroscopy	Trappist beer	Identification of brands	Engel et al. (2012)
FT-MIR spectroscopy	Cod liver oil	Identification of vegetable oils	Rohman and Man (2011)
NIR spectroscopy	Honey	Commercial variety	Guelpa et al. (2017)
FT-NIR spectroscopy	Veal product	Pork adulteration	Schmutzler et al. (2015)
Hyperspectral imaging	Potato	Identification of the organic variety	Su and Sun (2016b)
Raman spectroscopy	Meat	Origin	Boyaci et al. (2014)
FT-Raman spectroscopy	Milk powder	Whey adulteration	Almeida et al. (2011)
Raman chemical imaging	Milk powder	Melamine and urea	Qin et al. (2017)
DNA technology	Potato	Commercial variety	Ashkenazi et al. (2001)
PCR	Fish	Detection of white fish species	Dooley et al. (2005)
SNIF-NMR	Basmati rice	Geographic location	Verma et al. (1999)
Real-time PCR	Mozzarella cheese	Milk variety	Dalmasso et al. (2011)
AFLP	Fish and seafood	Identification of the species of origin	Maldini et al. (2006)
HPLC	Cheese	Detection of rennet whey	De La Fuente and Juárez (2005)
ELISA	Cow, ewe, goat, buffalo	Identification of the species of origin	De La Fuente and Juárez (2005)
DNA analysis	Rice-based food products	Identification of GMOs	Ren et al. (2006)

TABLE 2 Examples of Food Authenticity Issues

Commodity	Issue
Herbs and spices	Adulteration with water to increase weights
	Incorrect botanical declaration
	Intentional addition of low value materials
Fruit and vegetable	Undeclared water, sugar, and acid addition to fruit juice
	Undeclared pulpwash or peel extract addition into fruit juice
	Incorrect declaration of fruit type
Grains	Basmati rice replaced with nonbasmati rice
	Undeclared replacement of durum wheat with common wheat and impurities in wheat flour
	Discrimination of viable-germinating corns and soybeans from dead seeds
	Incorrect declaration of geographical and cultivar origin of cereal rice and wheat
Oils and fats	Undeclared addition of other vegetable oils to single seed oils
	Undeclared addition of poorer quality oils to extra virgin olive oil
	Butter adulterated with hydrogenated oil and animal fat
Milk and dairy	Undeclared addition of water to milk
	Cow milk in sheep, goat or buffalo milk yoghurt, or cheese
	Distinction between cheese made from raw or heat-treated milk
	Discrimination of milk and cheese from different regions, varieties and manufacturing processes
	Addition of melamine or nonmilk fat/oil into dairy products
	Mislabeling of conventional milk as a product from organic farming
Meat and fish	Incorrect declaration of species
	Labeling previously frozen meat as fresh
	Undeclared water addition to meat and fish in excess of legally permitted amounts
	Distinction between fresh and thawed meat
Beverages	Single malt whisky replaced by blended one
	Inappropriate sugar addition to increase alcohol content in wine
	Incorrect declaration of vintage or geographical origin of wine

TABLE 2 Examples of Food Authenticity Issues—cont'd

Commodity	Issue
Miscellaneous	Incorrect declaration of floral or geographical origin of honey
	Undeclared sugar addition to honey
	Undeclared use of genetically modified food

Based on Kvasnička, F., 2005. Capillary electrophoresis in food authenticity. J. Sep. Sci. 28(9–10), 813–825, Kamal, M., Karoui, R., 2015. Analytical methods coupled with chemometric tools for determining the authenticity and detecting the adulteration of dairy products: a review. Trends Food Sci. Technol. 46(1), 27–48, and Su, W.-H., He, H.-J., Sun, D.-W., 2017. Non-destructive and rapid evaluation of staple foods quality by using spectroscopic techniques: a review. Crit. Rev. Food Sci. Nutr. 57(5), 1039–1051.

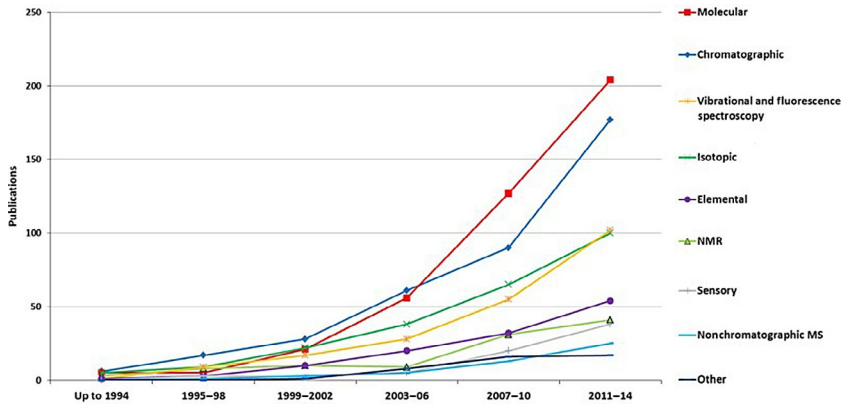

FIG. 1 Temporal evolution of different techniques for the authentication of food products (Danezis et al., 2016).

Chemometric techniques are often employed to analyze the data as the information contained in the spectra is of a high degree of complexity (Otto, 2016). One definition of chemometrics is as follows: the chemical discipline that uses mathematical and statistical tools for handling, analyzing, interpreting, and predicting chemical data (Varmuza and Filzmoser, 2016). Chemometrics represents a wide range of statistical methods aimed at tackling three different main objectives: simplifying complex and massive data sets, classifying objects, or predicting analytical parameters. Several reviews have already been published on the subject of NMR and chemometrics (Borràs et al., 2015; Remaud and Akoka, 2017; Marcone et al., 2013; Kelly et al., 2005). NMR spectroscopy involves the analysis of the energy absorption by atomic nuclei with nonzero spins in the presence of a magnetic field. NMR spectroscopy can therefore

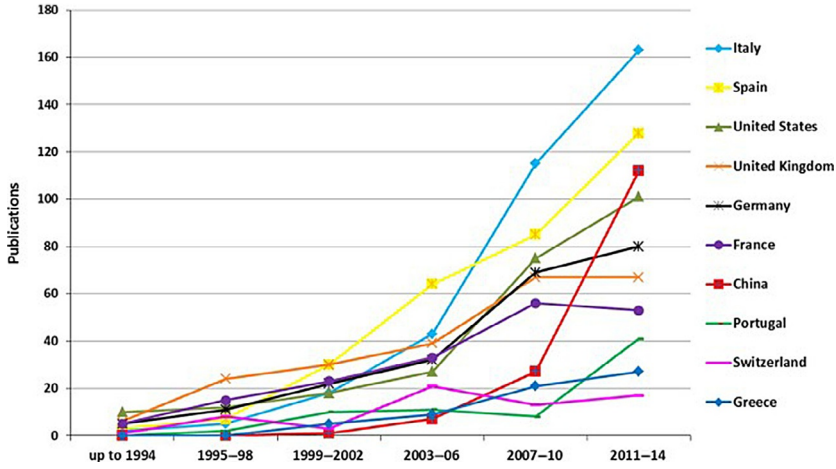

FIG. 2 Temporal evolution of food authentication research in different countries (Danezis et al., 2016).

provide detailed information about the molecular structure of a food sample, given that the observed interactions of an individual atomic nucleus are dependent on the atoms surrounding it (Bertelli et al., 2010).

There are several independent approaches for the conversion of the raw NMR spectra into data suitable for chemometric analysis. One procedure is to transfer the transformed spectra to a PC and use them as they are (Köbler et al., 2011, Duarte et al., 2002). Another option is to divide the spectra into segments and to sum the intensities of the data points in each segment (Gao et al., 2008; Wang et al., 2015). There is also the popular alternative of selecting a series of signals from the NMR spectra using the spectrometer's own "peak-picking" routine which gives peak positions and heights (Würz and Güntert, 2017; Mannina et al., 2001). In chemistry, it is possible to record many pieces of information such as spectroscopic and chromatographic intensities for each sample.

The authenticity of a food product is essentially defined by legally recognized descriptions that concern its characteristics (quality, origin, and process). An authentic food product must be properly labeled according to the appropriate national and international regulations when presented for sale in the marketplace. Analytical protocols based on high-resolution multinuclear (^{1}H, ^{13}C, ^{31}P) NMR spectroscopy for food authentication have been described in many articles (Spyros, 2016; Kaffarnik et al., 2013). Both ^{13}C NMR and ^{31}P NMR have been used for the authentication of virgin olive oil and oils from different plants (hazelnut, corn, soybean, rapeseed, and sunflower oils) (Dais and Hatzakis, 2013). One of the advantages of ^{31}P nucleus is the wide range of chemical shifts. Compared with ^{13}C nucleus, ^{1}H NMR

spectroscopy is more sensitive and shows shorter relaxation time of proton nuclei. Kim et al. (2015) highlighted the potential of ^{1}H NMR to verify sesame oil authenticity. The sample preparation and acquisition were straightforward and eight variables selected from values of NMR peaks could correctly verify authentic sesame oil samples, suggesting that NMR spectroscopy is an effective approach.

NMR spectroscopy has also been extensively applied to classify wines produced by grapes belonging to the same cultivar from three South Korea regions (Son et al., 2009), German wines harvested in different areas (Godelmann et al., 2013), and Cabernet Sauvignon wines produced in California, France, and Australia (Son et al., 2008). Beer has also been analyzed by ^{1}H NMR. Mannina et al. (2016) have identified various compounds, including propanol, acetic acid, adenosine, uridine, 2-phenylethanol, and alanine in beer to trace the origin of different beer samples. Regular and nonalcoholic lager beers were compared and the organoleptic characteristic of each type of beer was greatly affected by their chemical compositions (Sánchez-Estébanez et al., 2017). Based on NMR metabolic fingerprinting, the authenticity of Greek spirits could be effectively achieved by chemometrics (Fotakis and Zervou, 2016).

NMR spectroscopy cannot only be used to differentiate fish oils according to the species or wild/farmed and geographical origins, but also to confirm the labeling information of various fish products (Erikson et al., 2012; Standal et al., 2009; Fasolato et al., 2010). On the basis of the level of oleic and linoleic acid contents in fresh and processed (frozen, canned, and smoked) Atlantic salmon (*Salmo salar*), ^{1}H NMR with supervised multivariate analysis was correctly applied to differentiate wild and farmed fish (Capuano et al., 2012). Besides discriminating between farmed and wild Atlantic salmon, ^{13}C NMR also showed a great ability for verifying fish geographical origins (Aursand et al., 2009). Other conventionally and organically grown food products such as tomatoes have also been successfully separated by using NMR spectroscopy (Hohmann et al., 2014).

The specific proportions of the particular isotope of hydrogen and oxygen present in molecules are dependent mainly on climatic and geographical conditions and, to a lesser extent, the photosynthetic metabolism of plants. The effect of these conditions on the final isotopic composition of a molecule is known as isotopic fractionation. This natural phenomenon is exploited by two particular analytical techniques—SNIF-NMR and IRMS—which are perhaps the most sophisticated and specific techniques for determining food authenticity (Grégrová et al., 2014). Both techniques are capable of determining the exact proportion and location of specific isotopes within a food sample (Jiang et al., 2015b). However, the financial cost of purchasing and operating such high-specification NMR and mass spectrometry (MS) instruments is quite high and time-consuming sample preparation is required before analysis can take place (Vinci et al., 2013).

2.1.2 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) analyzer measures the absorbed radiation at different frequencies and obtains spectra which, similar to fingerprinting, reveal the identity of the specific molecule. FT-IR provides detailed information about the chemical structure and composition of a sample. FT-IR analysis can also be associated with GC; for example, GC-FT-IR was used to determine chemical compounds with biological activities in *Origanum vulgare* seeds (Al-Tameme et al., 2015). FT-IR together with near-infrared (NIR), mid-infrared (MIR), far-infrared (FIR), and Raman spectroscopic techniques fall among the fingerprinting techniques; the latter are the ones which provide a means for rapid and high-throughput monitoring and would be ideally suited for rapid characterizations if prominent changes can be captured in a reproducible manner (Gautam et al., 2015).

There is an evolving trend for the use of profiling methods combined with chemometrics for the determination of authenticity. The advantage of the profiling methods would be the evaluation of the overall components in a sample rather than looking for a single marker compound. Contemporary FT-IR spectroscopy has gained the capability of rapidly obtaining reproducible biochemical patterns that would allow for the composition-based statistical classification.

Infrared (IR) spectroscopy is ideal for rapid screening and characterization of chemical composition variation. Distinct and reproducible components exist in different commodities of fruits (Taruscio et al., 2004); thus, biochemical fingerprints may be produced by FT-IR to allow for the differentiation of subtle differences. Variation in the chemical composition attributed to variety, geographical origin, or alien ingredients might be elucidated through chemometric analysis of spectral-based grouping (Rodionova et al., 2016; Korifi et al., 2011; Su and Sun, 2017b). Advances on instrument design and auxiliary optics as well as the development of powerful supervised chemometric software have made FT-IR spectroscopy a suitable tool for the assessment of quality and authenticity in various foods (Lu et al., 2014; Su et al., 2018). This technique requires low sample volume and is environmental friendly. It does not require a large amount of hazardous organic solvents as liquid chromatography. Minimal or no sample preparation is required, which greatly speeds up sample analysis.

Such FT-IR fingerprints can be useful for assessing bacterial contamination of meat (Alexandrakis et al., 2012) and for confirming food authenticity in general (Rohman and Man, 2012). Metabolite information obtained from FT-IR fingerprints of mutant strains may also be useful in evaluating and assessing gene function (Kaderbhai et al., 2003). The major advantage of this technique is its rapidity and ease of spectral acquisition, enabling noninvasive measurements to be made with little or no sample preparation. However, sufficient signal resolution is to be ascertained for the desired effect to be monitored, in order to use spectral information as protein or metabolic fingerprints.

Debebe et al. (2017) applied FT-IR for the direct determination of ethanol levels in fermented alcoholic beverages. This method provided accurate results in the determination of ethanol in alcoholic beverages without requiring any previous chemical treatment of the sample. Implementation of FT-IR spectroscopy on the extracted polymeric materials of Portuguese white wines revealed that this technique can be effectively used to characterize white wine polysaccharide composition (Lees, 2003). Overall, it is possible to use FT-IR combined with multivariate techniques for an in-depth characterization of white wine colloidal stability (Versari et al., 2011).

2.1.3 Near-Infrared

NIR measurement technology offers an amazingly diverse capability for the analysis of many different constituents or properties of food products. Moisture, fat, protein, and sugar contents are perhaps the most well-known applications in products such as grain, flour, cereals, dairy products, snacks, and coffee, but NIR has also found application for the measurement of chocolate thickness on refiner rollers, the thickness of sausage casings, the alcohol content of beverages, the maturity of peas, and even the quality of fruit juices (Su et al., 2017; Baiano, 2017; Sun, 2016).

NIR measurement is a well-established analytical technique, and many examples of its applications can be found in the literature going back as far as the 1950s and before. Specifically, early laboratory applications of NIR concentrated upon quantitative and qualitative studies of liquids and solvent mixtures (Kaye, 1954). NIR measurement has broadly evolved on two fronts, those being laboratory and online application of the technique. Laboratory measurement has the benefit of offering very controlled measurement conditions. The measurement range of the NIR is well suited to the needs of food processing since a wide range of different constituents may need to be measured, such as moisture, fat, sugar, caffeine, oil, and protein. In the NIR, the most important and prominent absorptions are due to the —OH, —NH, and —CH groups. In addition, the technique has one further important strength, that is, a choice of absorption sensitivities for a given constituent. Each of the absorbing groups characteristically exhibits three main absorption bands in the NIR (Benson, 2003).

When considering the possibility of using the NIR technique to solve a particular measurement problem, it is necessary to appreciate both its scope and its limitations. The noncontact characteristic is considerably attractive, since the measurement will not normally interfere with product flow. Also, for food processing, noncontact measurements are favored by hygiene considerations. In the application of NIR to moisture determination, the response to free or associated moisture and bound water should be appreciated. In most materials, the difference in wavelength between the absorption bands for these two forms of moisture is very small and therefore they cannot usually be treated separately.

Microwave measurement can be a useful alternative technique when the limited penetration of an NIR measurement is a problem (Corredor et al., 2011). The future for the application of the on-line NIR technology looks very good with increasing opportunity for its application as the food industry becomes ever more concerned with accurate process control (Porep et al., 2015).

2.1.4 Hyperspectral Imaging

Hyperspectral imaging seems to have integrated the main features of spectroscopic and imaging techniques, which is considered as a very efficient inspection technique to cope with the increasing demand of rapidly acquiring both spatial and spectral information (Su and Sun, 2017a). Normally, a hyperspectral image can be generated in three approaches, namely, point (whiskbroom) scanning, line (pushbroom) scanning, and area scanning (tunable filter or staredown). Associated with multivariate analyses, the hyperspectral imaging technique has shown great potential for rapid and nondestructive quality assessments of food powders (Fu et al., 2014). Specifically, the melamine in milk powder was quantified by applying NIR hyperspectral imaging (938–1654 nm) and band ratio (BR) ($R^2 = 0.980 - 0.976$) (Huang et al., 2016). In their study, it was demonstrated that the ratio of two spectral images at 1447 and 1466 nm was sufficient to generate distribution maps to visualize the authentication of melamine particles in milk powders. Also, hyperspectral imaging and pattern recognition have demonstrated great capability for categorization and authentication of fruits and meats (Kamruzzaman et al., 2012; Su and Sun, 2016b).

Hyperspectral imaging is irrelevant for checking the composition of homogeneous samples, but is more valuable for detecting particulate impurities that tend to random probability distribution. However, the development of real-time inspection based on hyperspectral imaging has encountered bottlenecks, because there is a speed limitation in acquisition and processing of hundreds of continuous and narrow band images in a hyperspectral sensor (Serranti et al., 2013). As the successor of hyperspectral imaging, multispectral imaging deals with narrowband images over a discrete spectral range and can generate feature wavelengths of each pixel in a targeted object. Given that multispectral imaging could capture image data within discontinuous spectral ranges, this technique has now been acknowledged as a more advanced means to meet the speed demand in food industry (Su et al., 2017).

2.1.5 Raman Spectroscopy and Raman Chemical Imaging

Raman spectroscopy can yield complementary information in regions of MIR and FIR by relying on sample scattering light from a laser in the visible (VIS), NIR, or near-ultraviolet (NUV) range (Larkin, 2011). Spontaneous Raman scattering is typically quite weak, but advanced types of Raman spectroscopy, including surface-enhanced Raman, tip-enhanced Raman, and Fourier transform Raman, can be effectively used to observe vibrational, rotational, and

other low-frequency modes in a system, and thus provide a fingerprint which is less influenced by moisture (Yang and Ying, 2011). However, conventional measurements of Raman spectroscopy, with relatively small point-source assessments, cannot give the spatial information of chemical compositions of particles in a large area (He et al., 2014).

Raman spectroscopies can been used in spectral imaging configurations by combining corresponding vibrational spectroscopic method and digital imaging into one system (Ravikanth et al., 2016). Based on line scanning or point scanning, Raman chemical imaging systems were investigated to detect the presence and the distribution of various particulate adulterants within food materials (Qin et al., 2014, 2016). In a recent study, a powdered milk sample with a surface area of 150×100 mm (0.2 mm/pixel) was analyzed by a line-scan Raman chemical imaging system (Qin et al., 2017). Based on the thresholding method and linear regression analysis (LRA), chemical adulterants including melamine and urea in milk powder were not only detected with higher R^2 of 0.995 but was also *v*isualized at an adulteration level as low as 50 mg kg^{-1}. By utilizing Raman chemical imaging in the wavenumber region of 2538–102 cm^{-1}, the feature bands for urea (1009 cm^{-1}), ammonium sulfate (973 cm^{-1}), melamine (673 cm^{-1}), and dicyandiamide (212 cm^{-1}) were extracted to detect and visualize those adulterants with R^2 values of 0.988–0.992 (Qin et al., 2014). Also, L-cysteine is additive that is forbidden in wheat flour in many countries. Because of compositional differences of adulterated flour samples, Cebi et al. (2017) selected eight feature bands (1426, 1399, 1344, 1200, 823, 774, 693, and 640 cm^{-1}) related to L-cysteine. They concluded that the L-cysteine could be determined in wheat flour with 100% success by using Raman chemical imaging combined with chemometrics of principal component analysis (PCA) and hierarchical cluster analyses (HCA). Compared with VIS/NIR hyperspectral imaging system, Raman chemical imaging systems can obtain higher resolution images. It is possible to accurately inspect samples to ensure the authentication of food products using Raman chemical imaging (Su et al., 2017).

2.1.6 High-Performance Liquid Chromatography

The high-performance liquid chromatography (HPLC) technique is highly sensitive and very fast in response. The efficiencies of separation are also very high. A wide range of compounds can be separated by HPLC because the technique has a wide range of selectivity through the availability of many solvent combinations and packings. In most cases, sample preparation is small in time and size. Detectors can work continuously and can detect very small amounts. The combination of HPLC and mass detectors opens new and wide horizons. A further trend is the miniaturization of apparatus resulting in less solvent use. For the detection of phenolic compounds, anthocyanins, and organic acids, HPLC is the ideal technique.

Chromatography is a separation method of components between two phases. One phase is a stationary bed (the stationary phase) and the other phase is a fluid moving through the stationary phase (the mobile phase). In HPLC, the mobile phase is pumped through the column. The parts of a LC instrument are: (i) solvent reservoirs, (ii) a solvent delivery system, (iii) an injection device, (iv) the column, (v) a detector, and (vi) a data-acquisition system. The MS detector has many applications with LC. Different techniques to interface or hyphenate LC and MS are available. A widely used interface is atmospheric pressure ionization (API) (Beltrán et al., 2014).

Electrospray MS has three steps: nebulization of the solution into electrically charged droplets, liberation of ions from the droplets, and transportation of ions into the vacuum of the analyzer. Sample preparation and cleanup are very important steps of analyses. The components of interest have to be isolated from the sample matrix and interfering substances have to be removed. Accuracy, detectability, and selectivity are highly improved. Frequently employed procedures are: lyophilization, ultrafiltration, and liquid-liquid extraction. In fruit and fruit products, different compounds can be monitored for authenticity purposes: phenolic compounds, organic acids, carotenoids, amino acids, anthocyanins, and sugars. Phenolic compounds, a diverse class of compounds containing a hydroxyl group on a benzene ring, include, among others, flavones, flavanols, flavonoids, polyphenols, and chalcones. Resurreccion (2009) analyzed the phenolic compounds by reversed-phase HPLC. The use of soybean proteins in bovine milk is forbidden in many countries and other countries have regulations on maximum allowance levels. Based on HPLC method, the adulteration of milk with soymilk was detected by Sharma et al. (2009). Other adulterants such as whey protein, melamine, aflatoxin could also be effectively separated from eggs, bovine milk, meat, and meat products (Rodríguez et al., 2010; Wang et al., 2012; Herzallah, 2009; Cao et al., 2013). The presence of soy protein, caseinate, and whey protein in unheated beef, pork, chicken, and turkey was possible as was that of added nonmeat protein. Added soy protein could also be detected at levels of 6 g/kg in cheese samples.

Downscaling or miniaturization of columns and sample treatment is the challenges of the future. Miniaturization involves less sample volume, fewer solvents, and less time. Regulations and government commitments require more separation efficiency; the hyphenation of HPLC and MS, allowing the development of new methodologies, is a move in the right direction. Automation of the separation and detection and lower costs of the apparatus will be helpful (Głowacki and Bald, 2009).

2.2 Protein-Based Methods

2.2.1 ELISA

Microarray-based approaches involve miniaturization of standard assay procedures in multiple arrays to allow simultaneous analysis of multiple

determinants/analytes (Al-Adhami and Gajadhar, 2014). Such assays are very popular in transcriptomics, but have also been extended to proteome analyses. Microspots of "bait" molecules are immobilized in rows and columns onto a solid support and exposed to samples containing the corresponding binding molecules. The complex formation within each microspot can be detected using readout systems based on fluorescence, chemiluminescence, electrochemistry, MS, or radioactivity. Large-scale assessment of protein profiles can be carried out by the use of immunoassays on microarrays (Janzi et al., 2005).

The analysis involves a scale-up of enzyme-linked immunosorbent assay (ELISA) that have been in use for protein analysis. Antibodies immobilized in an array format onto specifically treated surfaces act as "baits" to probe the sample of interest to detect proteins that bind to the relevant antibodies, using, say, fluorescence detection (Blanco et al., 2017). The majority of reported studies on immunological techniques for food authentication concern the use of ELISA (Asensio et al., 2008). This technique involves the cultivation of antibodies or antisera that are capable of binding to a protein of interest, thereby enabling the detection of that protein, both qualitatively and quantitatively. The major advantage of this approach is that antibodies or antisera can be manufactured to respond specifically to the protein of interest, thereby enabling recognition and quantitation of that protein exclusively. The disadvantages of the ELISA approach include the initial difficulty in producing an antibody specific to a particular protein. However, this is a relatively minor difficulty to overcome when the selectivity of the technique is considered.

Recent research studies using ELISA-based techniques include detecting the presence of aflatoxin B_1, ornidazole, and pirlimycin residues in food products such as beef muscle, milk, and honey (Liu et al., 2013; Jiang et al., 2015a; Zhao et al., 2017). There have been promising results for the use of ELISA to detect staphylococcal enterotoxin A and G proteins in milk (Liang et al., 2015), as well as to detect the adulteration of sheep and goat milk with cow milk at levels as low as 0.1% (Hurley et al., 2004). This technique holds much potential for the authentication of food products but, to date, limited advances have been made in extending its authentication capabilities (Ali et al., 2012).

Protein microarrays can also be developed for assessing protein-protein, enzyme-substrate, and other protein-metabolite interactions (MacBeath and Schreiber, 2000; Moore et al., 2016). In one study, 119 yeast protein kinases were arrayed in microwells and examined for kinase activity with 17 substrates, demonstrating the value of microarrays in multiplexed protein functional assessments (Zhu et al., 2000). Carbohydrate-based microarrays have also been devised and applied to ensure food quality (Baum et al., 2017). As a powerful and reliable screening tool, microarray technology has shown great potential for the examination of proteins on a large scale, but there are still several challenges to be considered particularly with respect to retaining the dynamic nature of proteins (Atak et al., 2016).

2.2.2 Western Blot

Western blot uses three elements to identify specific proteins from a complex mixture of proteins extracted from cells: (1) separation by size, (2) transfer to a solid support, and (3) marking target protein using a proper primary and secondary antibody to visualize (Mahmood and Yang, 2012). However, there are no available validated methods to quantify dystrophin protein in muscle biopsies (Schnell et al., 2016). The Western blot based on the proteins demonstrated difficulty in the interpretation of results from heat-treated retail meat products (Lücker et al., 2000). Incidentally, a reverse transcription PCR coupled with restriction fragment length polymorphism was described to successfully detect GFAP mRNA in (stored) minced meat and pasteurized meat products fortified with 0.5% brain tissue and higher (Seyboldt et al., 2003). Like GFAP, NF is a marker for intermediate filament, but is present in equal amounts in spinal cord and in peripheral nerves (Kelley et al., 2000). Immunohistochemical detection of brain tissue was obtained with anti-NF in processed and heat-treated bovine and porcine brains, but diminished rapidly in raw as well as in heated luncheon meat (Tersteeg et al., 2002). Western blot assays were performed to obtain reliable quantitative data using standardization procedures coupled with the updated reagents and detection methods (Taylor et al., 2013).

2.2.3 Lateral Flow Strip

Lateral flow strip tests provide advantages in simplicity and rapidity of the detection of a variety of biological agents and chemical contaminants (Ngom et al., 2010). A typical lateral flow strip comprises a microporous material, such as nitrocellulose (NC), cellulose acetate, or a glass fiber membrane. NC is typically brittle and fragile as a pure sheet, so it is laminated onto a semirigid plastic substrate, such as polyester, styrene, or polyvinyl chloride (PVC), often using a pressure- or heat-sensitive adhesive (Green and Rathe, 2005).

For protein-based detection, specific monoclonal and polyclonal antibodies have been mainly developed for immunochemical detection, Western blot analysis, and ELISA. The immunochromatographic assays, also known as lateral flow strip tests, Reveal CP4 and Reveal Cry9C detect 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS), derived from *Agrobacterium* sp. strain CP4 that confers resistance to the herbicide glyphosate in soybeans and corn, and *Bacillus thuringiensis* Cry proteins that confers protection against insects in corn plants, seeds, and grains, respectively. Both kits, which are commercialized by Neogen (http://www.neogen.com), detect GMO presence in 5–20 min at a low price, with high sensitivity ($<0.125\%$ mass fraction of GMO); both are reliable field tests for controlling the distribution of biotechnology-derived products (Ahmed 2002). Rather than changing different physical parts, mathematical models are now being used to optimize and improve quantitation ability of lateral flow strip tests (Sajid et al. 2015).

2.3 DNA-Based Methods

2.3.1 Microarrays

The starting point for a microarray is a set of short oligonucleotide probes representing genomic DNA (Malone and Oliver, 2011). Microscopic array of oligonucleotides or cDNA containing up to several hundred thousand different sequences are starting to influence methodologies and paths to discovery in genomics. Microarrays of DNA and oligonucleotides are beginning to have the same impact on the biological sciences that integrated circuits have already produced on the physical sciences, and for the similar reasons, they can do many things in parallel, with very little material and with a modest investment of labor. They can be used, for example, in expression analysis, polymorphism detection, DNA resequencing, and genotyping on a genomic scale (Marmiroli et al., 2003).

Various biological problems have been solved by using microarrays (Matson, 2013). These arrays consist of many microscopic spots, each of which contains identical single-stranded polymeric molecules of deoxyribonucleotide attached to a solid support such as glass or a polymer. The utility of these spots arises from the tendency of their component bases to pair up or hybridize with a second strand containing a complementary sequence. It is easy to see how an array of different sequences can be used to identify one or more pieces of DNA or RNA in a solution. When the array is examined, one can tell which molecules are present in the solution by determining which spots fluoresce. Although microarrays have been successfully applied in a range of applicca-tions, the challenges (such as noise, normalization, and large number of genes) in using this technique are still noticed and should be eliminated in future studies (Drăghici, 2016).

2.3.2 Southern Blot

The need to monitor and verify the presence and the amount of GMOs in agricultural products has generated a demand for analytical methods (Çakır et al., 2016). The methods of detecting target DNAs with PCR were reliable and highly sensitive, although it was difficult to use the routing agarose gel analysis and Southern blot confirmation of the PCR products for massive samples. Here, we described an improved liquid-phase hybridization (LPH) PCR-ELISA technique for specific detection of PCR products. In this method, the biotinylated PCR products were in situ hybridized with the digoxigenin-labeled probe in the PCR reaction mixtures, and then captured with Streptavidin-coated tubes. The PCR products were analyzed with agarose electrophoresis and then verified with Southern blotting (Liu et al., 2004). In PCR-based assays, the results are usually analyzed by electrophoresis and Southern blotting (Orpana et al., 2013). Gel electrophoresis method is rapid but hazardous, and Southern blotting method makes the testing of multiple samples tedious and time consuming.

The emergence of plant transformation and molecular marker analyses in genome studies has greatly enhanced the speed and efficacy of crop improvement and breeding program (Sundar and Sakthivel, 2008). A prerequisite for taking advantage of these methods is the ability to isolate genomic DNA of superior quality and quantity for analyzing through PCR, restriction enzyme digestion, and subsequent Southern blot hybridization. To fulfill this criterion, a rapid, simple, and reliable DNA isolation method is highly solicited. Since size, content, and organization of genome and contents of metabolites of different plant systems vary from each other to a great extent, a single DNA isolation protocol is not likely to be applicable for all plant systems (Loomis, 1974).

2.3.3 Qualitative Polymerase Chain Reaction (PCR)

The PCR has been used in many different applications because it has a very great flexibility in the field of molecular biology (Erlich, 2015). Its principal use is to generate a large amount of a desired DNA product starting from a given template, but it can be used also to amplify very long fragments of DNA and in such a way to synthesize whole genes, to amplify and quantify specific RNA species, to produce RNA fingerprinting, or PCR-mediated cloning, to screen DNA libraries, and to produce DNA sequences (Marmiroli et al., 2003).

Long-distance PCR amplifies and detects routinely and specifically PCR products ranging in size from <1 to >50 kb (Dieffenbach and Dveksler, 2003), regardless of target template sequence or structure. Long-distance PCR facilitates the amplification of eukaryotic genomic DNA segments containing introns of varying number and lengths, thus permitting the definition of intron/exons boundaries. The PCR starting from RNA detects or measures a defined RNA species, ranging from mRNAs for gene products to the level of viral RNA in plasma. The extension of arbitrarily primed PCR (AP-PCR) fingerprinting to RNA has resulted in a tool with exciting potential for detecting differential gene expression (Olkhov-Mitsel and Bapat, 2012). When applied to the screening of highly complex DNA libraries contained within either bacteriophage or plasmid vectors, PCR offers the opportunity to identify rare DNA sequences in complex mixtures of molecular clones by increasing the abundance of a particular sequence (Israel, 1993).

2.3.4 Quantitative Competitive PCR

Compared with qualitative PCR reaction, quantitative PCR analysis is more complicated (Köhler et al., 2012). The QC-PCR is the co-amplification of a target analyte with an internal standard. In particular, it involves the co-amplification of unknown amounts of an internal control template in the same reaction tube by the identical primer pair. The reaction conditions can be maintained to generate amplification products that should differ by >40 bp. Multiple PCR reactions are needed as each sample is amplified with increasing amounts of competitor, while maintaining constant the sample

volume/concentration. Qualification is achieved by comparing the equivalence point at which the amplicon from the competitor gives the same signal intensity of the target DNA on stained agarose gels (Hardegger et al., 1999).

QC-PCR is applicable for the quantification of pathogenic microorganisms in the environment (Manome et al., 2008). To confirm that the developed QC-PCR assay could be applied quantitatively, DNA was extracted from an overnight culture and diluted 100- and 200-fold, resulting in DNA samples I and II, respectively. Constant amounts of DNA from each sample were co-amplified with corresponding sets of serially diluted competitor DNA in QC-PCR. The DNA concentration in each sample was determined and compared to confirm if the results predicted by QC-PCR were equivalent to the actual twofold difference in DNA concentration (Li and Drake, 2001).

2.3.5 Real-Time PCR

Real-time PCR was originally developed in 1992 by Higuchi et al. (1992) and has rapidly gained popularity due to the introduction of several real-time complete instruments and easy-to-use PCR assays. With this technique, the amplification of the target DNA sequence can be followed during the whole reaction by the indirect monitoring of the product formation. Real-time detection strategies rely on continuous measurements of the increment in fluorescence generated during the PCR reaction. The number of PCR cycles necessary to generate a signal that is significantly and statistically above noise level is taken as a quantitative measure and is called cycle threshold (Ct). Real-time PCR has replaced double competitive PCR as the preferred quantitative PCR-based technology for several reasons: it is faster; the quantitative results are produced without the need for error-prone pipetting and image analysis; the risk of carry-over contamination is minimized by the lack of post-PCR pipetting; and the production and calibration of competitors is not required (Holst-Jensen, 2003).

Real-time PCR approach allowed for early detection and quantification of the pathogen prior to the occurrence of first symptoms (Hinze et al., 2016). It was found that there was a clear correlation between bacterial abundance and subsequent disease development. Also, molecular beacons have been successfully employed in real-time PCR and for the generation of melting curves, including the multiplex PCR format, and they are widely used for discriminating single base pair (SNP) differences. The growing number of commercially available real-time thermocyclers is an indicator of the success of this technology. Presently, real-time PCR tests quantifications can be considered as the more powerful tool for the detection mealworm in a wide variety of food and feed products (Debode et al., 2017).

2.3.6 Random Amplified Polymorphic DNA

Random amplified polymorphic DNAs (RAPDs) are in fact just one example of a whole set of PCR-based molecular markers, which have been collectively

termed as multiple arbitrary amplicon profiling (MAAP) (Caetano-Anolles, 1993). RAPDs involve use of single arbitrary primer in a PCR reaction, resulting in amplification of many discrete DNA. RAPD technology provides a quick and efficient screen for DNA sequence-based polymorphism at a very large number of loci (Kumari and Thakur, 2014). The major advantage of RAPD includes that, it does not require presequencing of DNA. The vast range of potential primers that can be used, give the technique great diagnostic power.

The techniques differ in the length and sequence of the primers, number of amplification cycles, temperature of the annealing stage, and methods for evidencing polymorphisms. This technique has been applied to all types of organisms for different purposes. The disadvantages are the dominance of RAPD patterns in different laboratories.

A different example can be derived from the identification of marine mammals (seal and whale) in processed seafood products performed with species-diagnostic molecular markers, including RAPDs (Martinez and Daníelsdóttir, 2000). In another study, the RAPD method was developed for efficient detection of adulterant breeds in milk mixtures used for fraudulent production of this cheese (Cunha et al., 2016).

2.4 Enzyme Immunoassays

Enzyme immunoassays have been used in a broad range of applications in food analysis and bioanalytical science (Marquette and Blum, 2009; Yu et al., 2013). Immunochemical methods are based on the ability of antibodies to recognize three-dimensional (3D) structures and play a major role in biochemical research (Ferenčík, 2012). Antibodies represent a group of glycoproteins possessing two distinct types of polypeptide chains linked by both covalent and noncovalent bonds. Most applications in this field focused on identification of meat and milk of numerous animal species, either directly in the raw material or in processed food (Anfossi et al., 2008). Enzyme immunoassays utilizing blood or serum proteins, such as albumin, as the target antigen show limited suitability in testing heat-treated sample materials. As well as the identification of meat and milk of different mammalian species. Substitution of canned sardine with other species and adulteration of canned tuna with bonito were two other specific authentication issues, which have been addressed (Taylor et al., 1994). Also, a number of applications were focused on the detection of plant proteins (soy in meat products or soy, wheat and pea proteins in milk powder) (Haasnoot et al., 2001). In addition, rapid analyses of enrofloxacin, chloramphenicol, or other aflatoxins in milk and egg were achieved by using enzyme immunoassays (Xu et al., 2012; Ni et al., 2014; Anfossi et al., 2015). The enzyme immunoassay microtiter plate assay may be automated, thus allowing a large number of samples to be processed, whereas rapid tests like dipsticks may be used as field tests to screen suspicious samples. The main advantage of enzyme immunoassay

over immunodiffusion or immunoelectrophoresis procedures is reduced assay time, requirement of only small amounts of antisera, and the possibility of obtaining quantitative results.

3 CONCLUSIONS

Food authentication is the process with which a food is verified as complying with its label description. Labeling and compositional regulations, which may differ from country to country, have a fundamental place in determining which scientific tests are appropriate for a particular issue. Of course, claims concerning the species of origin concern mainly the genetic makeup of the organism and the definition of a species may make this a rather arbitrary classification. Some claims may go beyond the species barrier to the variety of the organism. Another authenticity issue which may commonly arise is the need to determine whether food products from one species have been mixed with similar material from a cheaper species. ELISA and agar-gel immunodiffusion were the techniques employed for the analysis. The development of DNA methods continues to have a major place in food authentication. Spectroscopic techniques including IR spectroscopy, Raman spectroscopy, NMR spectroscopy, and spectral imaging are described in their great potentials in authentication of various food products. Such spectroscopic techniques are being increasingly applied to food authentication. They can be used in a number of different ways. The entire spectrum can be used to generate a database which is subsequently interpreted by statistical techniques. However, IR, Raman, and NMR spectroscopy can be used to measure small amounts of specific compounds in the sample which are then used as markers of authenticity. Given that spectral imaging (such as VIS/NIR hyperspectral imaging, FT-IR imaging, and Raman imaging) could capture large image data within spectral ranges, the imaging spectroscopy has now been acknowledged as a more advanced means to meet the speed demand in food industry.

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