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Food authentication: Techniques, trends & emerging approaches

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ABSTRACT

Food authentication is a rapidly growing field due to increasing public awareness concerning food quality and safety. This review presents critically the analytical techniques which are used for authenticity assessment, explaining how and why they give plausible solutions. Classification of different methodologies is based on authenticity indicators providing insight into future developments. Analytical breakthroughs and novel techniques that emerged recently are discussed, along with their applications on food authentication. We have discussed current limits and gaps, related to informatics needs for data analysis of large quantities. Reporting standards and reference database are elaborated indicating urgent needs for the progress of this field. A scientometric evaluation highlighted the research trends and emerging approaches of this evolving field. Popular analytical techniques are commented, while the potential of the field is depicted in the temporal evolution of the research output focusing on geographical distribution of research activity and preferred journals used for dissemination.

Contents

1. Introduction

Food authentication is the process that verifies that a food is in compliance with its label description. This may include, among others, the origin (species, geographical or genetic), production method (conventional, organic, traditional procedures, free range),

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or processing technologies (irradiation, freezing, microwave heating). The declaration of specific quality attributes in high-value products is of particular interest since these products are often target of fraudulent labeling. Proof of provenance is an important topic for food safety, food quality and consumer protection, as well as the compliance with national legislation, international standards, and guidelines [1]. Due to the globalization of food markets and the resulting increase in variability and availability of food products from other countries, consumers are increasingly interested in knowing the geographical origin along with the assumed quality of the products they eat and drink. The quality assurance and the methods used to authenticate foodstuffs are of great interest both from commercial and legal points of view [2].

Authenticity has been a major concern of consumers, producers, and regulators since ancient times [3]. Modern instrumentation, advances in basic sciences and in information and communication technologies provide means for precise measurement and elucidation of origin of foods [4]. Since the beginning of 20th century, organizations that set standards for and control the origin of ingredients and the production process, have appeared all over the world e.g., the French 'Institut National des Appellations d'Origine (INAO), Italy's 'Denominazione di Origine Controllata', Spain's 'Denominación de Origen', South Africa's 'Wine of Origin' or the United States' 'American Viticultural Areas'. The production of consumer goods according to these standardized procedures normally results in better products and is rewarded with higher prices at the point of sale. Unfortunately, these financial benefits attract the production of counterfeit food and illegal food trades.

In Europe, origin is one of the main authenticity issues concerning food. European Union legislation reserving specific names for foods and beverages of a particular quality or reputation has been abundant since the dawn of the European integration process (Council Regulation, EEC No 2081/92). These legislations introduced regulatory framework for wines and spirits and quality schemes for food products including PDO (Protected Designation of Origin) that links products to the defined geographical area where they are produced, PGI (Protected Geographical Indication) that links products to a geographical area where at minimum one production step occurred, and TSG (Traditional Specialities Guaranteed) that protects traditional methods of production. Furthermore, recently defined optional quality terms such as OQT, "mountain product" and "product of island farming" were defined (1151/2012 EU Regulation). The purpose of these EU schemes is to protect the reputation of the regional foods and to promote good practices in rural and agricultural activity. Such practices help producers obtain premium prices for authentic products and minimize the unfair and misleading competition from non-genuine products, usually of inferior quality or of different flavor (1151/2012 EU Regulation). The information includes a characterization of the geographic region and reinforces the consumer perception of special quality attributed to mountain and island products. In case of cultivated species, EU indicates that a reference should be made to the country in which the food undergoes the final production stage. Vigorous research activities in EU are supported by the coordination actions of the European Union, including "Food Integrity", "MoniQa" and "TRACE" within HORIZON 2020.

Determination of food authenticity is an important issue in quality control and food safety. Authenticity testing is a quality criterion for food and food ingredients, increasingly a result of legislative protection of regional foods. Thus, there is a pressing need for accurate standardized food authentication techniques [3–6]. Food authenticity testing does not serve only consumers; the stakeholders include food industries who are seeking the opportunity to assure their food products labeling compliance and branding. Regulatory authorities are asking for an extended and updated list of the analytical methods for confirmation of authentic food products and to support law enforcement.

This review presents latest techniques used in food authentication and related research trends with the emphasis on recent analytical breakthroughs in this area.

2. Analytical techniques

2.1. Molecular techniques, genomics – proteomics

A variety of analytical techniques, for verification of foodstuff origin have been developed and tested. Molecular analysis for discrimination of original (authentic) food products from non-original is a major authentication methodology. Even though traditional methods have being extensively used for food authentication, genomic and proteomic techniques are rapidly complementing or outright replacing earlier methods. Nucleotide- and protein-based methods for food authentication are mostly used for species detection and identification. Since DNA is identical in all somatic cells of a given organism, it is invariant whether the DNA is extracted from blood, muscle, liver or any other tissue. High stability of DNA allows the analysis of highly processed food products, as well as trace contaminants. DNA-based methods for food authentication depend on the highly specific amplification of DNA fragments by the Polymerase Chain Reaction (PCR). This method belongs to "genomics", because the whole genome of the sample is used [7]. On the other hand, proteins can act as markers for many properties of the food products all along the food chain from farm to fork, and therefore proteomics can be applied for a systematic search of new marker proteins or peptides. The advantage of genomics is that it can amplify minute traces of nucleotide material, while proteomics identifies specific products encoded by DNA. The sensitivity of these methods is very high since the amount of required material can be as small as a few cells [8]. After the first discovery step using reference samples, reliable analytical methodologies are needed for targeted detection and quantification of characterized markers in real unknown samples [9]. These proteogenomic techniques are constantly being improved, examples including PCR Single Strand Conformation Polymorphisms, (PCR-SSCP), random amplified polymorphic DNA (RAPD), or the emerging field of Peptide Nucleic Acid (PNA), and DNA fingerprinting that are used for food authentication [10]. Genomics and proteomics are usually applied to identify false description and mislabeling of foods. Interesting examples are: detection of GMOs, seafood authentication, authentication of kosher and halal meat, detection of horse meat and pork in food labeled as beef, game meat authentication, botanical origin of foods (olive oil, wine, tomato products, tea, and cocoa), species origin authentication (meat, milk, fish).

Another emerging sub-field of proteogenomic is microbial fingerprinting for food authentication. PCR Denaturing Gradient Gel Electrophoresis technique (PCR-DGGE) is used for these type of studies. Microbial flora fingerprints of specific food products such as cheese [11]. This possibility arises from the production technology of these foodstuffs, in which the use of starter cultures is indispensable. Other examples where microbial fingerprinting can be used for identifying the authenticity of foods concern fruits, milk and dairy products, wine, cocoa and organic foods.

2.2. Chromatographic techniques

Chromatographic analysis provides rapid and reliable separation of chemically similar compounds in complex food matrices [12]. In food authentication, chromatographic techniques must overcome several challenges inherent to food matrices. Food substrates consist of a great number of compounds, including peptides, lipids, carbohydrates, amino acids, fatty acids, organic acids, nucleic acids, phytochemicals and other small molecule (additives, such as colorants, aromas, preservatives and other exogenous compounds) [13].

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These compounds are chemically diverse, ranging from the small organic molecules (usually up to 1000 Da) to macromolecules (biopolymers), that can possess a wide range of polarities – some are apolar (like oils) while some others are strongly polar (like amino acids). Chromatographic methods produce unique chemical fingerprints that differentiate and authenticate foods. The authentication is based, on identification of minimal analytical differences between patterns or identification of unique marker compounds.

Due to the chemical complexity of foodstuffs and high consumer demand for food quality and genuineness, high-resolution chromatographic techniques, such as gas (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS), have emerged as useful food authentication tools. Double MS (triple quadrupole) is replacing older instruments and most instruments principally used are Gas Chromatography Mass Spectrometry (GC-MS/MS), Liquid Chromatography Mass Spectrometry (LC-MS/MS) and Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOF-MS).

LC separation is typically performed by targeting three primary characteristics of the chemical compounds: polarity, electrical charge, and molecular size. It is mostly used to detect proteins, amino acids, carbohydrates, vitamins, phenolic compounds, tryglycerides, chiral compounds, and pigments, while Gas Chromatography is more suited to the analysis of naturally volatile or semi-volatile molecules [14]. Authentication by chromatography is based on the profile of specific compound profiles for each food product, such as fatty acids, triglycerides, waxes, sterols, hydrocarbons, alcohols, tocopherols, and volatiles, which form profile characteristic for food identity origin.

Examples where chromatographic techniques are used for identifying the authenticity of foods include adulteration of high-quality products with inexpensive or sub-standard ingredients [14] such as honey, wines, vegetable and olive oils, spirits, coffee, milk, cheeses, saffron, nuts and mushrooms. Such authentication is usually done by matching measured compound profiles with the predetermined target values.

2.3. Isotopic techniques

Isotopes are atoms of the same element that differ by the mass from each other. Different isotopes of the same element have equal number of electrons (and protons) but different number of neutrons resulting in different mass. Stable isotopes are separated into two groups by atomic mass, light (bio-elements) and heavy isotopes. In the light isotope group, the ratios mostly investigated are $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$, whereas $^{34}S/^{32}S$ is less commonly used. In heavy isotopes group, most commonly used ratio in food authentication is $^{87}Sr/^{86}Sr$ and more rarely $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, $^{143}Nd/^{144}Nd$ [15].

The analysis of isotopic ratios uses various methods such as Isotope Ratio Mass Spectrometry (IRMS), Multi Collector – Inductively Coupled Plasma – Mass Spectrometry (MC-ICP-MS), and Thermal Ionization Mass Spectrometry (TIMS). IRMS interfaced with Elemental Analyser, Pyroliser, Equilibration devices, GC or HPLC is used for the determination of light isotopes ratios, while heavy isotopes are measured by MC-ICP-MS and TIMS. The ratio $^{2}H/^{1}H$ is analysed also site-specifically in small molecules such as ethanol, using an NMR (Nuclear Magnetic Resonance) equipped with a deuterium probe.

The isotopic ratios are applicable to food authentication because stable isotope ratios change with the climatic conditions, geographical origin, soil pedology, and geology of the locations of food ingredients origin. As a primary indication, H and O isotopic data for organic matter in food are linked to the H and O isotope data of water from the source region which have geographical variability, N and C isotopes are related to the climate and the agricultural practices, and S isotopes are affected by geology, volcanism, distance from the sea, and certain anthropogenic effects [2].

The analysis of stable isotopes of bio-elements have been recognized by EU, OIV, CEN and AOAC as official methods since the 1990s to detect adulteration of wine, honey, fruit juice, or maple syrup with cheaper extenders, such as water or sugar syrup made from maize or sugar cane. Other examples of isotopic ratio applications include the discrimination of natural *vs.* synthetic vanillin and discrimination of champagne CO_2 produced naturally by adding sugar to bottles from direct injection of industrial CO_2. More recent applications of multi-isotope ratio analysis ($^{2}H/^{1}H$ or D/H, $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, $^{87}Sr/^{86}Sr$) include geographical origin verification studies of wine, olive oil, orange fruit, honey, tomato, Chinese cabbage, meat, dairy products, eggs, seafood, and coffee. Remarkably, isotopic fingerprints are used as indicators for organically grown products [16].

Furthermore, the isotopic fingerprinting can be combined with other indicators (e.g., elemental analysis, NMR and GC) to improve the determination of the origin of a variety of food products.

2.4. Vibrational & fluorescence spectroscopy

Spectroscopy, in particular vibrational spectroscopy, is a fast and inexpensive method for both the assessment of food quality and food authenticity. Novel instrumental techniques combined with chemometric methods have enabled the development of rapid methods that apply multivariate (MVA) analysis, to near infrared (NIR) and mid infrared (MIR) data to analyze food matrices. In Infrared radiation (IR) region, solid, liquid or gaseous samples can absorb some of the incoming infrared radiation at specific frequencies producing a spectral 'fingerprint' of the sample. The MIR fingerprints result from fundamental stretching, bending and rotating vibrations of the molecules, whilst NIR spectra result from complex overtone and high frequency combinations at the shorter wavelengths. Raman spectroscopy, another emerging methodology, is based on fundamental vibration modes that can be assigned to specific chemical functional groups within a sample molecule and therefore can provide useful information for sample fingerprinting. Qualitative identification is mostly done because of high detection limits featured by vibrational techniques, mostly Raman. Analytical techniques deploy Fourier Transform to – Infrared (FT-IR) and Raman (FT-Raman) fluorescence. A major advantage of IR and Raman techniques is the rapid, non-destructive analysis of samples [17]. Surface Enhanced Raman Spectroscopy (SERS), in contrast to Raman spectroscopy, provides low detection limits for certain specific molecules, allowing applications to food adulterants determination.

Fluorescence spectroscopy is a simple, non-destructive, non-invasive and relatively inexpensive analytical technique. It features low to very low detection limits as compared to other spectroscopic techniques. Molecules detected by fluorescence spectroscopy are polyaromatic hydrocarbons and heterocycles with rigid molecular skeletons. Recently, simple accurate and low cost fluorometers combined with advanced analytical software, gave the opportunity for fast, reliable, repeatable measurements and elaboration of the spectra. Hence, many fluorometric methods have been developed to check the authenticity, adulteration, quality and composition of foods [18]. A variant, Synchronous Fluorescence (SyF) utilizes excitation-emission plots increasing the discrimination power of fluorescence. This allows applications to food authentication, for example to olive oil adulteration [18].

Characteristic examples of spectroscopic methodologies deployed for food authentication include milk and soya bean meal adulteration by melamine, honey adulteration by syrups (high fructose corn, maltose, or jaggery syrup) and sugar solutions, adulteration of olive oil by vegetable oils or lampante/pomace olive oils, ground black pepper mixing with buckwheat and millet, culinary spices adulteration by Sudan I dye, and meat adulteration. Authenticity identification of milk, olive oils, honeys, wines, spirits, spices and other food ingredients, saffron and lentil seeds have been reported.

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2.5. Elemental techniques

Elemental profiling is increasingly applied to assessment of food authenticity. Elemental profile refers to macro-elements (such as sodium, calcium and potassium), trace elements (such as copper, zinc and selenium), rare earth elements (such as lanthanum, cerium and samarium), or other elements occurring only at very low abundance (such as iridium and gold). Plants derive their mineral content from the soil. Fertilization, harvesting, botanical origin, soil type, pollution, and production year all cause variations of elemental concentrations. However, these variations are smaller than variation observed between production areas and geographic regions. Rare-earth elements have great potential for geographical origin determination because their fingerprints are directly linked to the geology of the area and could be minimally influenced by different agricultural practices and harvest year. The elemental composition of foods of animal origin reflects, to some extent, the mineral content of the fodder and vegetation they eat. Beyond feed intake, elemental content depends on various factors such as drinking water, pollution and soil composition, all of which depend on geographic origin. Thus, vegetation is the compositional reflection of the bioavailable and mobilized nutrients present in the underlying soils from which they were cultivated [2,6,19].

The elemental fingerprint of foods is measured by a variety of analytical techniques. Even though Atomic Absorption techniques have been used in the past, nowadays Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) and Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) are almost exclusively used due to their ability for multi-element measurements [2,9,19]. Food authenticity applications of ICP-MS and ICP-AES include discrimination of geographical origin, organic vs conventional products, and free range vs. cage egg production. Other examples of elemental fingerprinting analysis of food authenticity are the discrimination of origin of wine, honey, olive oil, coffee, cheese, fruits and vegetables, and also spices and food additives.

2.6. NMR

Foodstuffs contain compounds such as amino acids, fatty acids and sugars. NMR is one of the most suitable methods to obtain "high-throughput" spectroscopic and structural information on a wide range of molecular compounds. It enables determination of complex compositional matrices of foodstuffs, with high analytical precision. The amount of any selected metabolite in a mixture can be assessed with minimal sample preparation. In past, sensitivity of NMR was considered as a main limitation, but continuous developments in hardware resulted in high sensitivity of NMR. Therefore, NMR enables a collection of comprehensive metabolic profiles that can be used for food authentication. Site-Specific Natural Isotopic Fractionation (SNIF-NMR), enables robust fingerprinting of natural molecules. A well-known application of SNIF-NMR is the determination of geographical origin of wine, developed by the EU in 1990 (EU regulations 2670/90, 2347/91 and 2348/91). Profiling methods such as non-targeted ^{1}H-NMR analysis have been applied for assessing geographical provenance of food [20]. NMR analysis has been used for assessing adulteration, such as red wine adulteration with anthocyanins, synthetic flavors sold as natural, addition of cane or corn sugar to maple syrup. Discrimination of origin/adulteration cases by their metabolic profile using NMR includes wines, coffee, olive oils, honey, fish, spirits, vinegar, and saffron.

2.7. Sensory analysis

Nowadays, consumers increasingly demand products that are not only safe and nourishing but also are desired to have high organoleptic quality. Sensory analysis has become important in many food sectors. Traditionally reliable results in sensory analysis require a well-trained panel of human assessors. Organoleptic test panels comprise a set of techniques for accurate measurements of human responses to foods [21]. Appearance, aroma, flavor and texture properties are important characteristics determining the quality-authenticity of food products. These panels require extensive training of judges, adequate replication and detailed statistical analysis of the observations. In all cases, the response obtained has to be properly evaluated because the sensory evaluation varies both among panellists – they are individuals with different sensitivities, preferences, and product knowledge. Assessment may change within a given panellist with time – depending on his fatigue, stress, health, and other factors. Therefore, panellists are required to have a reasonable level of sensory perception, commitment and motivation but they should also be trained in the use of standardized and systematic sensory methods to get reliable results.

However, even if are perfectly trained, there is still need for the panellists standardization of sensory analysis. That is possible with the development of instrumental techniques that could recognize objectively and quickly specific sensory perceptions in the same way as an expert tasting panel does. Instrumental test of food quality using perception sensors instead of human panel test is attracting massive attention recently. Novel cross-perception multi-sensors data fusion imitating multiple human perception has been proposed [22]. Amongst the techniques, there is a clear need to refer to Gas Chromatography Olfactometry (GCO), biomimetic sensors: electronic tongue (e-tongue), electronic nose, (e-nose), electronic eye, (e-eye). The "e-nose" uses detection of the volatile compounds present in the headspace of a food sample by an array of semi-selective gas sensors [23]. First, the headspace (volatile compounds) of a sample is generated and the headspace is injected into the detection system (sensors set). Each sensor is sensitive to all volatile molecules but each in their specific way. Most "e-noses" use sensor-arrays that react to volatile compounds on contact: the adsorption of volatile compounds on the sensor surface causes a physical change of the sensor. A specific response is recorded by the electronic interface transforming the signal into a digital value. Recorded results are then computed based on statistical models. Further, e-nose data can be correlated to those obtained with different methods for example sensory panel. The major benefits of sensory analysis are the simple procedure, the quite minor sample preparation, rapidness and low cost.

The scientific community has just started to accept that we can define analytical instrumental specifications that characterize the quality of the sample in the same way that the tasting panel does. Detailed analysis of instrumental variables, with the help of sensory information and, in some cases, of GCO will provide chemical information about the markers, compounds responsible for the behavior of a given attribute. Examples, where sensory analysis can be used for identifying the authenticity of foods are wines, olive oils, tea, beers and cheeses.

2.8. Non chromatographic mass spectrometry

Another noticeable class of methodologies that should be mentioned is non chromatographic mass spectrometry techniques. Recent MS applications include the use of stand-alone techniques for elemental or molecular profiling and imaging. Among more efficient methods for food authentication are Proton transfer reaction mass spectrometry (PTR-MS), Matrix-assisted laser desorption/ionization Time-of-Flight Mass Spectrometry/MALDI-TOF-MS and Ambient Mass Spectrometry techniques such as Direct Analysis in Real Time/DART-MS.

PTR-MS allows quantitative on-line monitoring of volatile organic compounds (VOC), by using soft chemical ionization method for ionization of organic molecules. VOC molecules react with charged ions,

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in most cases hydroxonium ions (H_3O^+). At that time, H_3O^+ ions transfer their proton exclusively to VOC molecules that have proton affinities higher than that of water, yielding protonated analyte VOCs. Then, an electric field accelerates the ions through the reaction chamber, leading to collision-induced dissociation of ions. After scanning a mass range, fingerprints of the volatile compounds are obtained. Therefore, PTR-MS gives instantaneously the absolute concentrations of VOCs. PTR-MS enables rapid detection of a variety of organic species, such as alkenes, alcohols, aldehydes, aromatics, ketones, nitriles and sulphides, in complex matrices with very low detection limits. Nowadays, there is also a new hyphenation, PTR-TOF-MS, which found considerable applications on food authentication [24].

MALDI has demonstrated a great potential in fast screening analyses for food quality, safety and authentication, since chromatographic separation is usually not needed. Concerning sample preparation, a matrix constituted by a weak organic acid, is mixed with the sample and the resultant solution is deposited on a microtiter plate and allowed to crystallize. Then the plate is loaded in the mass spectrometer and a laser beam hits the spot where co-crystals of the matrix and the analyte are present. Due to the laser energy, a part of the matrix, which has a strong absorption of the laser wavelength, is vaporized together with the analyte in a "plume" that expands at high velocity. The sample ions formed in the ion source are extracted and accelerated in an electric field with high voltages. After passing the charged grid, the ions fly into the TOF mass analyzer. Finally, the ions reach the detector where the conversion and the amplification of ion current in an electrical signal are accomplished. With MALDI-MS techniques require a low amount of sample. They are very sensitive with sample preparation without the need of analyte derivatization [25].

Ambient Mass Spectrometry is performed in an open atmosphere either directly on samples or matrices in their natural environments or by using auxiliary surfaces. Ambient-MS has greatly simplified and increased the speed of MS analysis. Nowadays there are a variety of different desorption and ionization mechanisms available. Most types of molecules with a large range of masses and polarities can be ionized with great ease and simplicity with the outstanding combination of the speed, selectivity, and sensitivity of MS detection. Direct analysis in real time mass spectrometry (DART-MS) is one of the variants of ambient mass spectrometry. It has become an established technique for rapid mass spectral analysis of a large variety of samples. The ionization process of DART-MS takes place at atmospheric pressure and takes only few seconds. In DART, an electrical potential is applied to a gas with a high ionization potential (typically nitrogen or helium) to form plasma of excited-state atoms and ions, and these desorb low–molecular weight molecules from the surface of a sample. It is suitable for fast analysis, with minimal sample preparation and high salt tolerance. DART-MS can be applied to compounds that have been deposited or adsorbed on to surfaces or that are being desorbed therefrom into the atmosphere. DART could also be hyphenated with TOF-MS, giving solutions in authenticity studies [26].

Non chromatographic MS techniques have been applied in food authentication to uncover incorrect description and mislabeling of foods with specific geographical label such as saffron, truffle, honey, beer, olive oils, juices, and botanical origin of spices and species. These techniques are also used to prevent food fraud such as dilution of olive oil by cheaper vegetable oils, and adulteration of donkey milk, of higher value types of milk (sheep's and goat's) with milk of lower value (cow's milk), of fresh cow's milk with powdered milk, of coffee, and of animal feed .

2.9. Immunological techniques

Immunoassays are analytical tools that rely on the specific interaction between antibodies and their cognate antigens. They were originally developed to facilitate the study of immunology but are now finding widespread applications in many other fields as they can be used to detect a host of molecules, ranging from proteins to small organic molecules in a complex sample matrix present in foodstuffs [27]. Immunoassays became popular tools for verifying identity standards of various types of food and food ingredients because they are fast, sensitive, highly specific, and cheap. In addition they are user-friendly, have a high throughput, and are amenable to field-testing. In food industry antibodies are developed against specific antigens (allergens, toxins, pathogens, etc.) [28] and then used as capture molecules to trap their target antigens. The production of specific antibodies is thus the first crucial step in the development of an immunoassay. A major step forward that opened the door for the more general use of immunoassays was the development of enzyme labels. Enzyme-linked immunosorbent assay, ELISA, is the most used of immunological techniques [29]. It has been used to verify the authenticity of several food commodities such as meat, fish, and dairy products. It can also detect presence of genetically modified organisms (GMOs) and undeclared processes like food irradiation. Food authenticity assessment by immunological techniques includes determination of osteocalcin in meat and bone meal, detection of glucomannan (use is banned in Europe) in konjac plant products, detection of melamine and bovine IgG in milk, and detection of pork in ground beef and soybean proteins in meat products.

2.9.1. Chemometrics – bioinformatics

All fingerprinting techniques produce a large volume of information. Chemometrics [30] and bioinformatics [31] tools are fundamental for food authentication studies since huge amounts of data need to be handled. Data mining, data fusion and feature selection are essential for the making sense out of the huge data set generated through various analytical methods.

Informatic infrastructure in the field of food authentication is in its infancy and its development is critical for systematic, comprehensive, and broadly applicable assessment of food authenticity. Food authentication must address multidimensional challenges. First, there are no standard guidelines for description of the workflow, so the design of experiments and reporting of results in the literature show large diversity. Thus, our ability to compare the results from similar studies is very limited. Most of the reports have limited description of study design limiting the value of the report. An example of a well-designed study with comprehensive description is [32], where conventional, organic, and courtyard eggs were compared. In this study, the data collection was matched between three production methods and the chicken species, their diet, and housing conditions were described. In contrast, many studies report that, for example, food sample was bought in supermarket with little, if any, description of the samples. Although, the situation is improving in recent years, most reported studies do not have sufficient number of samples for meaningful analysis. The databases of reference materials do not exist, so the reported results either demonstrate the separability between the classes in the data (such as organic vs. non-organic), or simply report the data, such as mean value of observed trace elements. Food authentication involves technical, structural and legal concerns [5]. The methods and results must be:

- Accurate to provide correct interpretation of data. This is mainly achieved using standard certified reference materials to ensure the accuracy of measurements.
- Robust to take care of natural variability of sample content, measurement error, and effects of processing, storage, and handling. Reference materials for this step do not exist and the number of studied samples is typically too low.

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- Mutually comparable across different studies to ensure that they can be compared in a meaningful way to database references.

Food authentication will critically depend on establishment of databases that contain comprehensive and standardized information about origin of foods including geographic origin, species/subspecies, production methods, and other critical information. Most studies done to date are of exploratory or sample classification nature – they analyze preliminary data and show that the data are separable across classes [2]. Predictive models that can map unknown sample to known class depend on existence of reference samples and appropriately defined databases. While effort has been made to record food ingredient fraud and adulteration [33] and the European DOOR database (ec.europa.eu/agriculture/quality/door) lists more than 1500 foods of protected origin, an appropriate database needed for classification of unknown samples is lacking.

Chemometrics is definitely needed for solving the problem of authentication or identity confirmation. It must be combined with database infrastructure and appropriate mathematical tools for food authentication [34].

3. Research trends

More than 80% of food authentication publications are original work published in research journals while review articles and conference proceedings account for around 7–8 % each and book chapters/books account for 5%. Determination of geographical origin, adulteration, mislabeling and food safety are amongst the main aspects in food control. Published research depicted in Fig. 1 shows that growth in food authentication after 2000 is exponential. We identified 409 articles that were published during 2006–2008 and 907 during the period 2012–2014.

3.1. Analytical techniques used for food authentication

Determining the food authenticity involves a range of verification approaches depending on the level of sophistication of the suspected fraud. This section provides insight on analytical techniques to verify the origin of our food, in terms of regulatory and more recently consumer and industry requirements. Concerning geographical origin determination, analytical methods rely largely on determination of chemical compositions, which may be quite similar even when the matching materials come from different geographical areas. Attempts have been made by determining some components as typical for certain areas or production methods. Other methods that are applied include molecular methods when different strains/breeds of organisms are used in production.

Various analytical techniques have been assessed on their suitability for food authentication studies throughout the years. Chromatographic and molecular methods are the major approaches to food authentication solutions (Fig. 2). These two groups account for almost half of published research. Isotopic, vibrational, UV & fluorescence spectroscopy, elemental techniques and NMR are also prominent. Some other technologies such as non-chromatographic MS, microbial fingerprinting and sensory analysis, have not been exploited to the maximum, yet our opinion is that they will find extensive use in the near future. This warrants attention of the analytical community that has to benefit from cross scientific collaborations. A trend depicted by the increase in popularity during the last four years is that chromatographic, molecular, vibrational and fluorescence spectroscopy techniques are emerging in food authentication (Fig. 3).

3.2. Research activity spreads to different countries

South European countries such as Italy, Spain, France, Portugal and Greece are involved in food authentication studies, as can be seen in Fig. 4. This is expected as these countries produce a majority of foodstuffs and wines registered as PDO, PGI, or TSG. Yet some countries with high scientific capabilities and large food production, for example USA, stay behind in food authentication. China is an emerging country in the field, showing a rapid growth during the last 5 years (Fig. 5). It is interesting to note that all countries in the top 10 list, except the USA and China, are European. This indicates the interest of Europeans in food authenticity that is supported by national and EU legislation in European countries. Interest in Europe concerning food authentication is also shown by continuous funding from FP 5 to the Horizon 2020 initiative.

Fig. 5 depicts the temporal evolution of food authentication research per country. Italian scientists have been active in this field

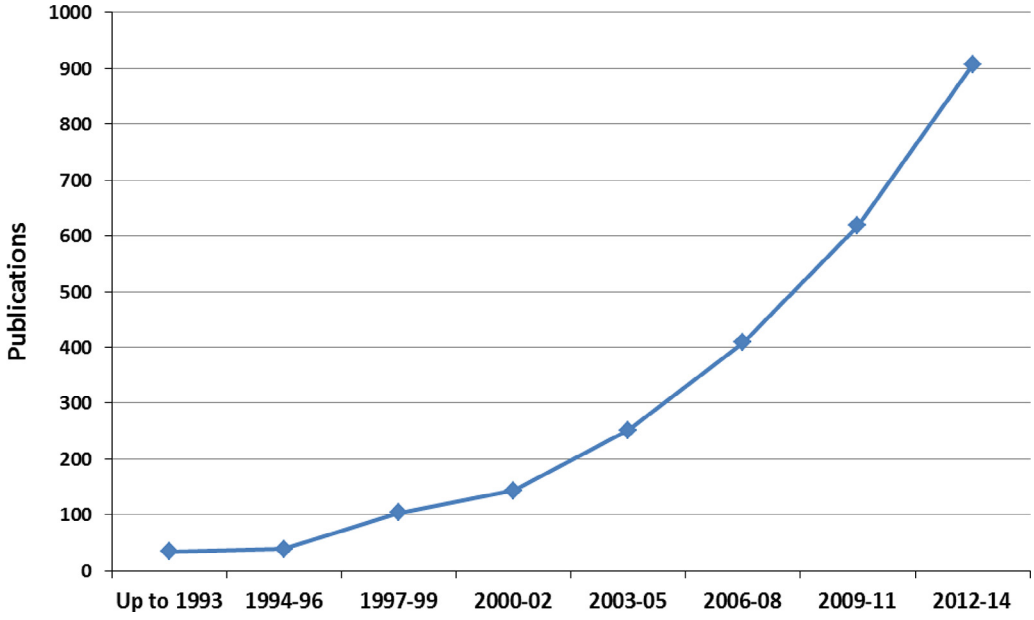

Fig. 1. Temporal evolution of published work.

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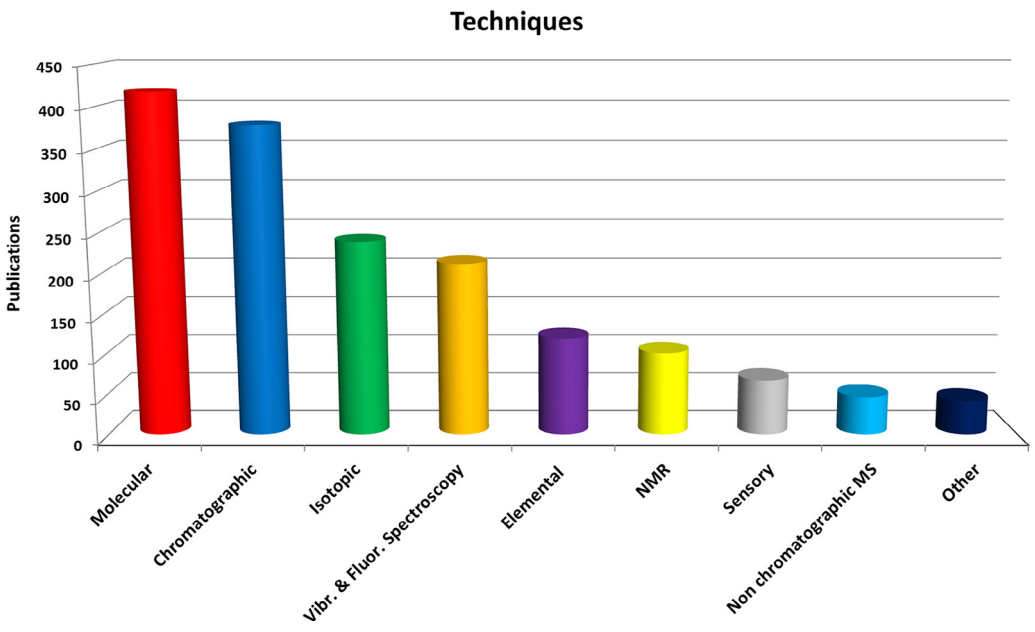

Fig. 2. Publications assessed in scopus 9–2015 distributed between different techniques.

for many years, while Chinese are strongly involved nowadays. This could be correlated to the high number of Italian authentic food products. Italy produces more PDO-PGI-PGI food products than any other country. A possible explanation for the intensively growing activities in China could be attributed to the growth of the gross domestic product per capita and the interest associated with quality food products such as olive oil. Spain also has many authentic food products.

Another measure that provides useful insight to research efforts of individual countries in food authentication is the number of relevant publications per million of population, shown in Fig. 6. Data presented this way are normalized concerning country size. This measure shows similar trends as other research activities on food – Europeans are most active, while USA and China come behind. South European countries, according this measure, are more active than Northern European countries – this is attributed to large number of high quality food products produced there. Northern European countries have big biodiversity and unique climatic features that contribute to the production of quality/authentic foods. Noticeable is the leading position of Switzerland.

3.3. Journals

Publications on food authentication are scattered across more than 150 journals! Food authentication papers are published by a variety of journals, although preferences for specific journals have

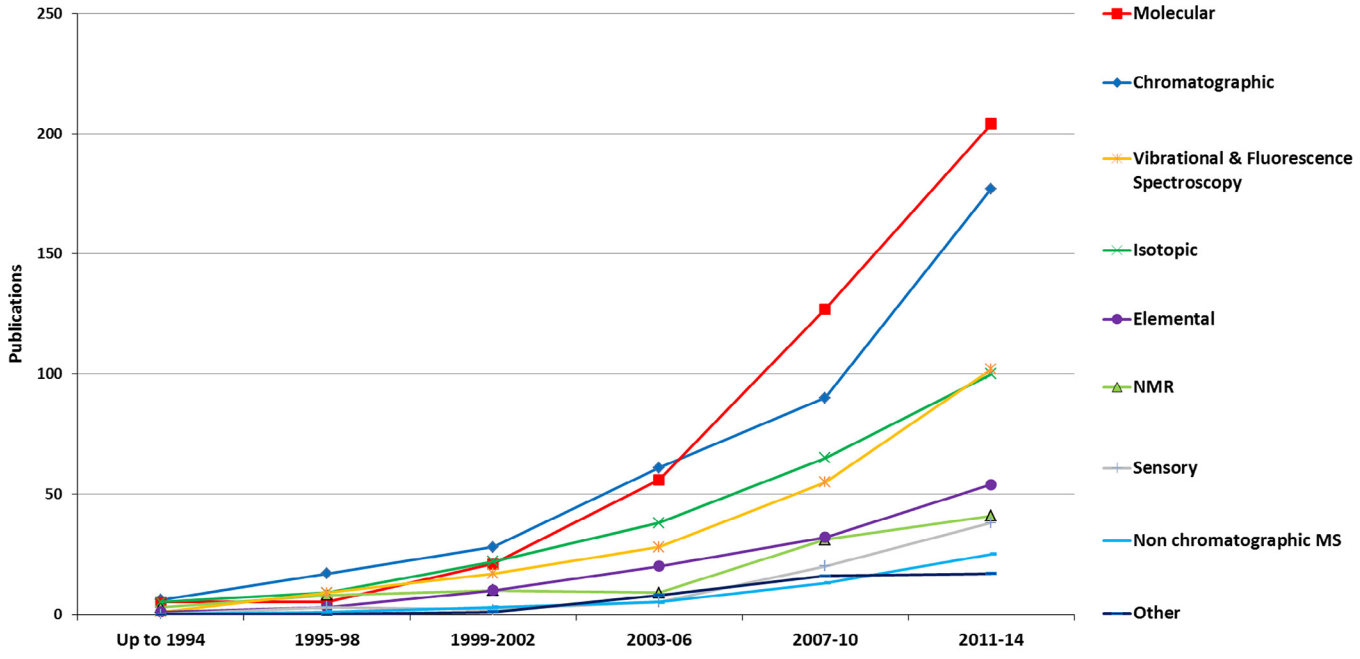

Fig. 3. Temporal evolution per technique.

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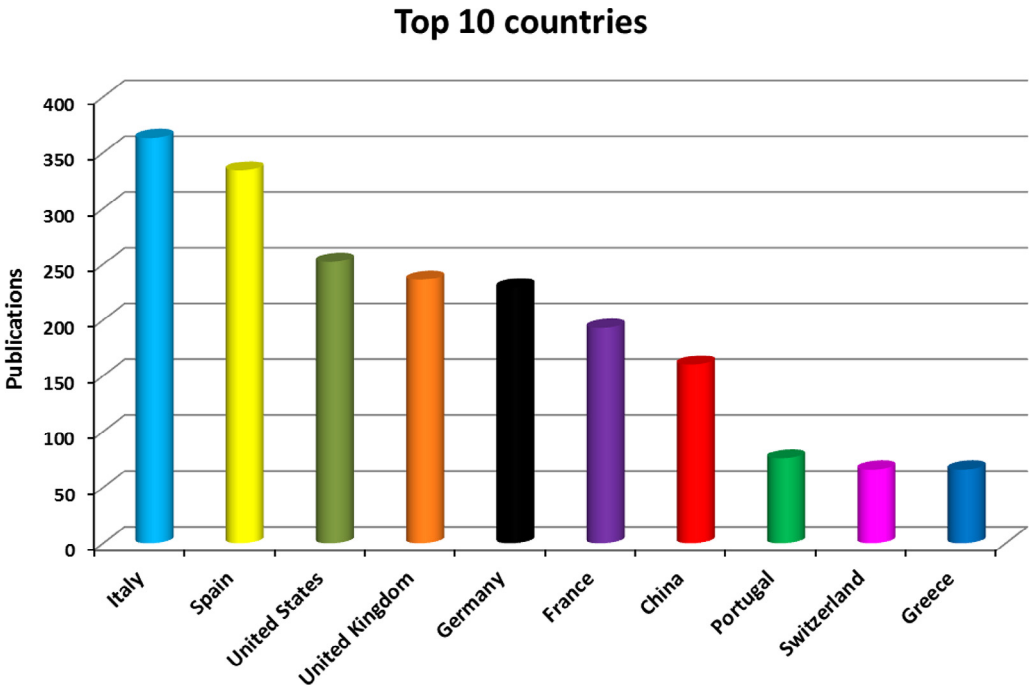

Fig. 4. Top 10 countries on food authentication.

been noted. A significant volume of manuscripts accounting for twenty percent of the total (Fig. 7), are published in the *"Journal of Agricultural and Food Chemistry"* and *"Food Chemistry"*. These two journals are the most highly cited food journals. This highlights the appreciation of food authentication as industrial and scientific topic. Food authentication is strongly based on chemical analytical technologies is shown by the presence of the highly cited "pure" Analytical Chemistry journals, such as "*Analytica Chimica Acta*", *"Analytical & Bioanalytical Chemistry"* and *"Journal of Chromatography A"*. Yet, the need for reliable and robust new analytical methods for the verification of food authenticity is larger than ever.

4. Conclusions

Analytical Chemists, based on their knowledge of methodologies, lead the research and technology development for food authentication. However, food authentication is a multi-disciplinary field that has input from instrumentation, biology, informatics, mathematics and statistics, agriculture, and food technology. This article provides a brief survey of analytical methods, information needs, and an up-to-date scientometric evaluation of the field. This article is also a valuable source of information for food scientists that would like to be exposed to different analytical technologies used for food

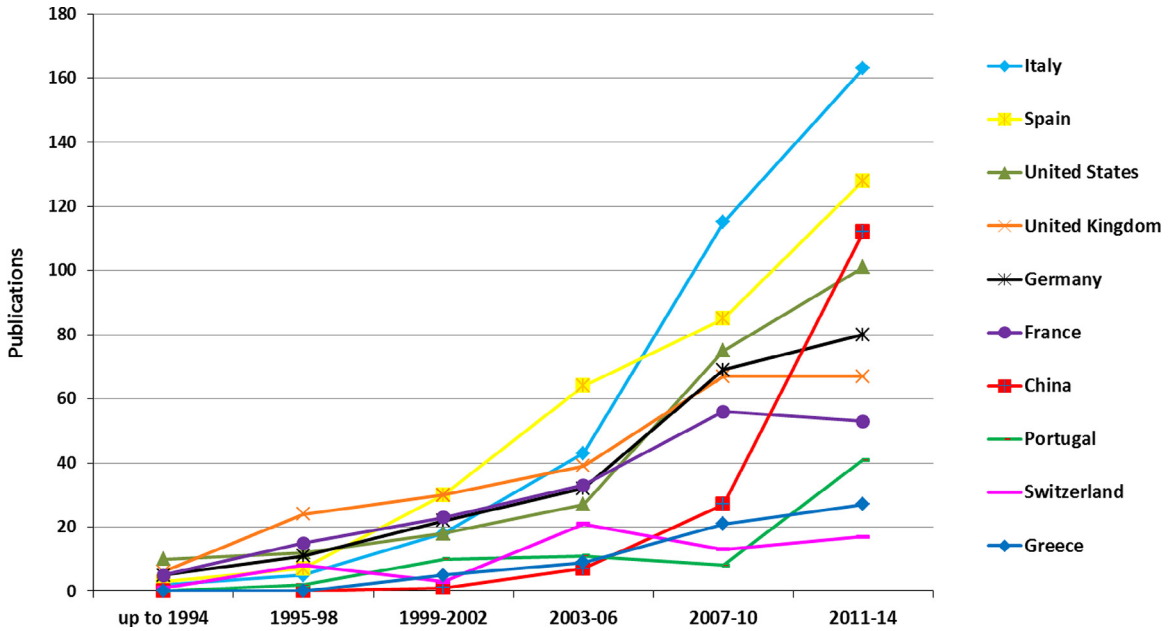

Fig. 5. Temporal evolution of food authentication research in different countries.

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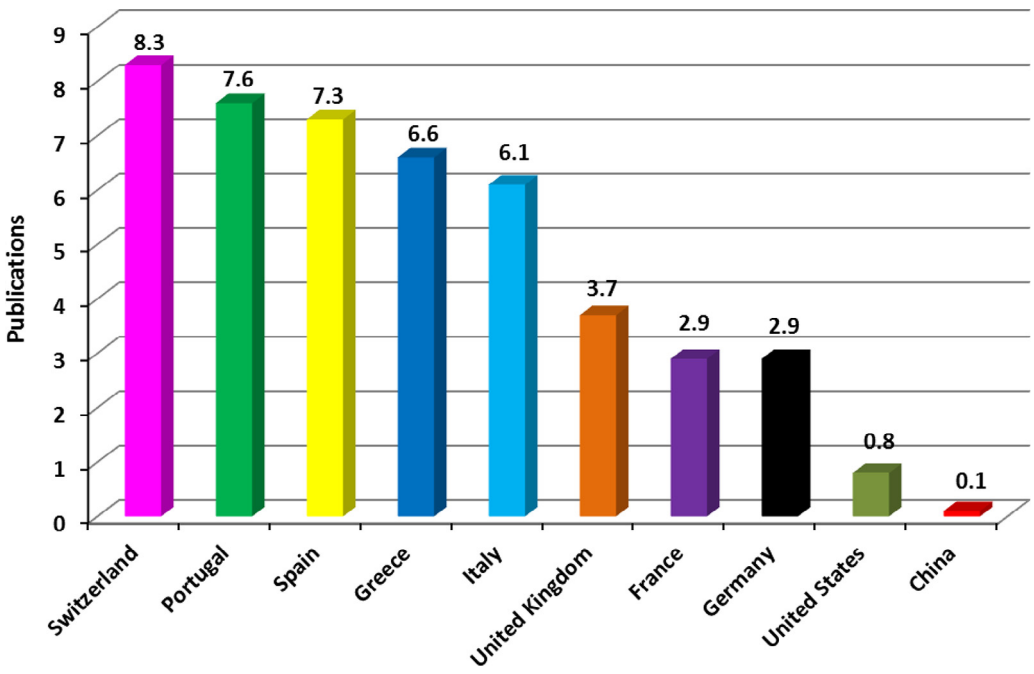

Fig. 6. Food authentication publications per million capita of the top 10 Countries.

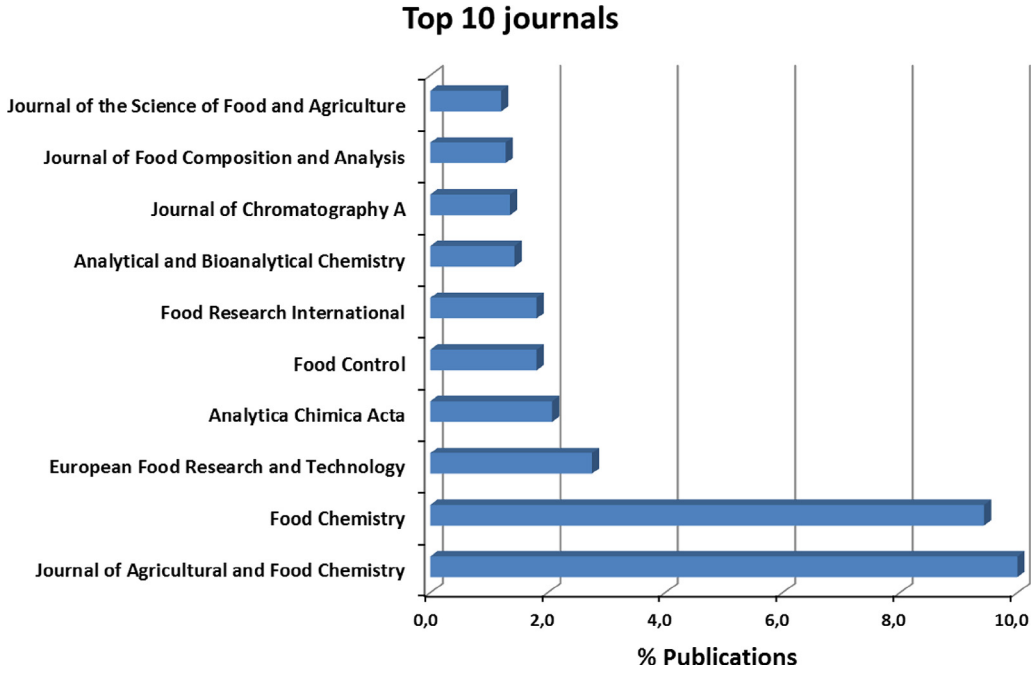

Fig. 7. Top 10 Journals for food authentication.

authentication. The most extensive use of molecular techniques is for determination of species and botanical origin, while all other techniques are mainly dealing with geographical origin and adulteration. Mass spectrometry is a frontline technology rapidly replacing other methods in many fields of food science. This trend extends to food authentication, due to unsurpassed advantages such as high sensitivity, selectivity, throughput and multi-analyte capabilities of MS techniques [4]. Multi-analyte capabilities are essential for food authentication studies since they provide more descriptors and thus facilitate better classification. We are at a point where vast volumes of data are generated, but our ability to manage and analyze these date are falling behind the ability to generate these data. To this end, various techniques described either under the term chemometrics or data analytics are crucial for future successful development of prediction models.

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