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Review article

Convergent technologies to tackle challenges of modern food authentication

Jessica Frigerio [a,*], Luca Campone [a], Marco Davide Giustra [a], Marco Buzzelli [b], Flavio Piccoli [b], Andrea Galimberti [a], Ciro Cannavacciuolo [a], Malika Ouled Larbi [a], Miriam Colombo [a], Gianluigi Ciocca [b], Massimo Labra [a]

[a] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza, 2, 20216, Milano, Italy
[b] Department of Informatics Systems and Communication, University of Milano-Bicocca, viale Sarca, 336, 20216, Milano, Italy

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ABSTRACT

The authentication process involves all the supply chain stakeholders, and it is also adopted to verify food quality and safety. Food authentication tools are an essential part of traceability systems as they provide information on the credibility of origin, species/variety identity, geographical provenance, production entity. Moreover, these systems are useful to evaluate the effect of transformation processes, conservation strategies and the reliability of packaging and distribution flows on food quality and safety. In this manuscript, we identified the innovative characteristics of food authentication systems to respond to market challenges, such as the simplification, the high sensitivity, and the non-destructive ability during authentication procedures. We also discussed the potential of the current identification systems based on molecular markers (chemical, biochemical, genetic) and the effectiveness of new technologies with reference to the miniaturized systems offered by nanotechnologies, and computer vision systems linked to artificial intelligence processes. This overview emphasizes the importance of convergent technologies in food authentication, to support molecular markers with the technological innovation offered by emerging technologies derived from biotechnologies and informatics. The potential of these strategies was evaluated on real examples of high-value food products. Technological innovation can therefore strengthen the system of molecular markers to meet the current market needs; however, food production processes are in profound evolution. The food 3D-printing and the introduction of new raw materials open new challenges for food authentication and this will require both an update of the current regulatory framework, as well as the development and adoption of new analytical systems.

1. The authentication framework

Food authentication is the process by which a food item is verified in terms of compliance with its label description. This activity encompasses many aspects including the analytical assessment of product mislabeling, adulteration, and misleading claims about origin, composition, production strategies, processing method and distribution [1]. The authentication process involves all the supply-chain stakeholders (e.g. producers, transformers, distributors) and it is also adopted to verify food quality and safety. In recent

* Corresponding author.
E-mail address: jessica.frigerio@unimib.it (J. Frigerio).

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Abbreviations

HRM	High Resolution Melting
HTS	High throughout sequencing
LAMP	Loop-mediated isothermal amplification
RPA	Recombinase polymerase amplification
SSR	Simple Sequence Repeats
HDA	Helicase-dependent amplification
SNP	Single-nucleotide polymorphism
SALDI-MS	Surface-assisted laser desorption/ionization
SERS	Surface Enhanced Raman Scattering
CNN	Convolutional Neural Networks
PCA	Principal Comonent Analysis
PLS	Partial Least Square
OLPS-DA	Orthogonal Partial Least Square
LDA	Linear discriminant analysis
HCA	Hierarchical Clustering Analysis
MALDI-MS	Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
HS-GC/MS	Headspace Gas Chromatography Mss Spectroscopy
LC-HRMS	Liquid Chromaography- High Resolution Mass Spectrometry
LC-QqQ-MS/MS	Liquid Chromatography tandem with Triple Quadrupole Mass Spectrometry
IR FTIR	Fourier-Transform Infrared Spectroscopy
NIR	Near-Infrared Spectroscopy
MIR	Mid-infrared Spectroscopy
SERS	Surface-enhanced Raman Spectroscopy
NMR	Nuclear Magnetic Resonance spectroscopy
2D NMR	2 dimensional Nuclear Magnetic Resonance spectroscopy
QqQ-MS	Triple Quadrupole Mass Spectrometry
QTof_MS	Quadrupole Time-of-Flight Mass Spectrometry
MALDI-TOF-MS	Matrix-Assisted Laser Desorption/Ionization in tandem with Time-of-Flight Mass Spectrometry
PTR-MS	Proton-Transfer-Reaction Mass Spectrometry
DART-MS	Direct Analysis in Real Time Mass Spectrometry
ORBITRAP	Orbital Ion Trap Mass analyzer
CE	Capillary Electrophoresis
GC	Gas Chromatography
LC	Liquid Chromatography
HPLC	High Performance Liquid Chromatography

years, consumers are also increasingly aware and interested in this topic, especially concerning the ways in which authentication processes are useful in preventing health risks due to food fraud or misidentification.

Nowadays, the global current cost of food fraud for the industrial sector amounts to approximately US$ 40 billion [2]. This mainly consists in the deliberate adulteration of products, counterfeit, substitution, and misrepresentation with food products and ingredients increasing their apparent value and gaining an economic advantage to the producer. The food supply chains are long and extensively complex, and each step may cause quality and safety issues. Globalization has exacerbated the complexity of the food supply chain, paving the way for high occurrences of food fraud at the international level. Therefore, local and international regulatory mechanisms have been established to prevent or mitigate food fraud. Dedicated institutions such as the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) defined shared frameworks and effective control systems. For this reason, there is a growing demand for greater effectiveness of food authentication tools for consumer protection as they constitute a fundamental part of food safety and quality and an essential component of the food supply chain.

To achieve this goal, food authentication tools are an essential part of food traceability systems because they provide information on the credibility of origin, species/variety identity, geographical provenience, production entity (e.g., company, consortium), as well as systems capable of evaluating the effect of transformation processes, conservation strategies and the efficacy of packaging and distribution flows.

There are more complex elements to control, such as the compliance with production procedures, the recipes used, as well as the adopted short/long-term conservation strategies. For these reasons, fast, reliable, and user-friendly analytical techniques capable of controlling specific qualitative and/or quantitative parameters linked to the intrinsic characteristics of each product are necessary, starting from the chemical composition, up to the organoleptic and structural properties of the food.

In recent years, consumers, through specific associations, seem to be the major stakeholders in food traceability processes [3];

however, the authenticity of a product is relevant also for the producers as a mechanism to protect their brand and reputation and therefore the economic value of the sold items. Given that food authentication is a global issue and involves a plethora of stakeholders, effective analytical methods have been developed to certify the authenticity of numerous food products of interest. For these reasons, many production consortia have equipped themselves with standardized production protocols and analytical systems for food traceability along the supply chain. If production standards are adhered to, and individual producers undergo pre-visit inspections by the consortium, they are granted the authenticity mark, known as IGT (Indication of Controlled Origin), and PGI (Protected Geographical Indication).

1.1. Food authentication tools

Food authentication is directed to the confirmation of quality and safety traits of the products and is based on several diagnostic characteristics and procedures. Generally, such tools rely on molecular markers (i.e., chemical, biochemical, genetic), sometimes specific for the different products, and effective in describing food properties from food origin and identity, to the occurrence of contamination and adulteration. Therefore, the authentication tests provide relevant information on food quality, organoleptic characteristics, product consistency and food safety.

The most common authentication strategies are based on chemical analysis. Specifically, methods based on spectroscopy, spectrometry, and metabolomics, can provide accurate results to prevent food fraud and grantee the food authenticity. Spectroscopy technologies are funded on the interaction of electromagnetic radiation with matter and varies according to wavelength or frequency and energy. This wide range of radiation includes ultraviolet (UV), visible (VIS), near mid and far infrared (NIR, MIR, and FIR), and X-rays. An advantage of spectroscopic techniques is the recent development of portable spectrometers [4]. Although their measurement accuracy is lower than desktop instruments, their small size, low cost, and operative simplicity, make them widely useable in the market supervision. However, spectroscopy only measures the chemical information of samples and does not reflect spatial information. For this reason, the hyperspectral imaging technology has gained strong competitiveness in food identification [5].

Another approach to easily detect food fraud events, is the investigation of metabolic markers (e.g., metabolites, lipids, secondary metabolites). The study of metabolites concentration aims to detect the overall changes in a sample and can be classified in two complementary approaches: target and untarget analyses. The former ones focus on the analysis of a single or a small group of metabolites, whereas untarget approaches aim to characterize compare the highest number of metabolites of a sample. This approach is very useful in complex matrices such as honey, extracts, wine but also raw materials such as fish. The complexity of metabolites can be measured and detected using a broad variety of analytical methods. So far, metabolomics analyses have been carried out either with mass spectrometry (MS) or nuclear magnetic resonance (NMR). Although NMR is a very responsive and high-performance technology, it is time-consuming, and require expensive instrumentation. MS analysers provide a wider detection scope, including metabolites producing a unique chemical fingerprinting of either a polar or a non-polar nature.

The complexity of data, obtained by spectroscopic and spectrometric methods can be influenced by the physical state, and by the presence of interfering compounds. Therefore, sample preparation and separation techniques of matrix components are required prior to analysis. Several separation techniques have been used for the analysis of complex food items. Currently, the most used separation

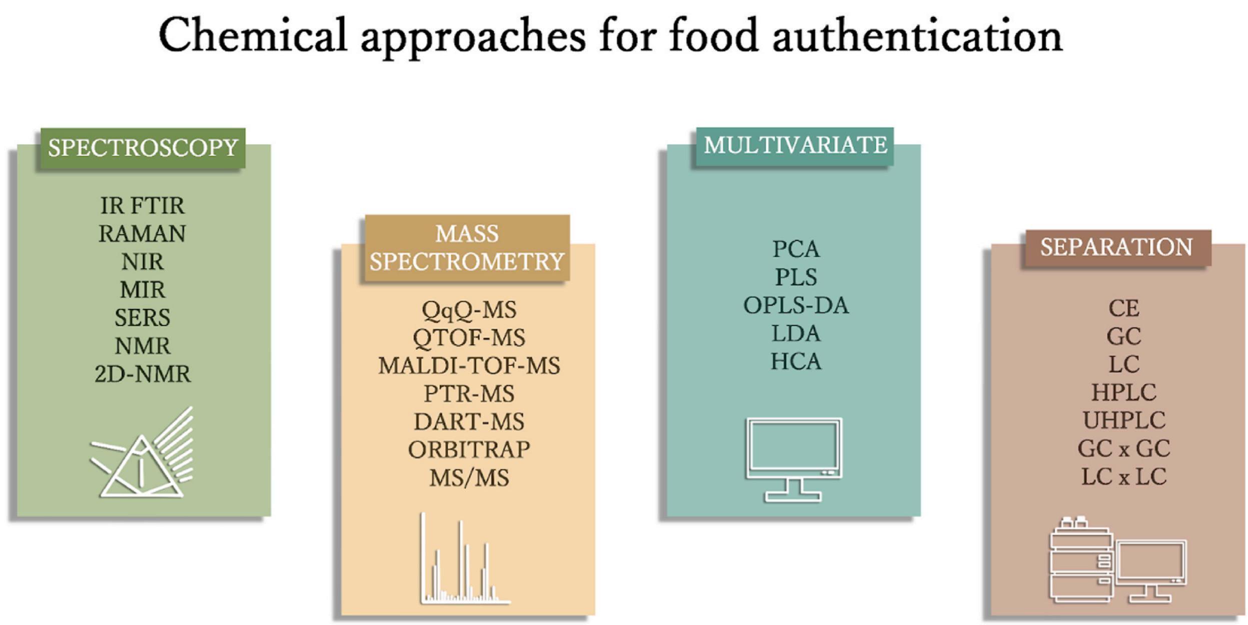

Fig. 1. Fig. 1 describes the main chemical approaches used for the most common foods susceptible to fraud.

technologies are capillary electrophoresis (CE), gas chromatography (GC) and liquid chromatography (LC). Improvements in chromatographic performance have been achieved by the introduction of comprehensive two-dimensional (2D) chromatography, a powerful analytical technique that uses two columns of different phase selectivity connected by a modulation device. The (2D) chromatographic improves peak capacity, resolution, and detectability than monodimensional chromatographic processes.

The analyses performed by different analytical techniques provided a huge amount of data, therefore, to highlight useful information, after data acquisition, pre-treatment of spectral measures must be carried out. Nowadays, with the development of computer science, multivariate statistical analyses of chemical information by chemometrics methods have been increasingly applied to foods authenticity. Chemometrics models uses mathematical and statistical methods to analyse spectral data from chemical systems such as foods, extracting relevant information to determine or quantify components. The main chemometric models used to analyse spectral data, extracting relevant information from foods are partial least squares (PLS) regression, principal component analysis (PCA), principal component regression (PCR), and multiple linear regression (MLR) Linear discriminant analysis (LDA), soft independent modeling of class analogy (SIMCA) (see Fig. 1).

Another important food authentication strategy is based on DNA analysis. DNA is generally resistant to food processing treatments and can thus be easily isolated and characterized. With the reduction in costs of sequencing technologies and the availability of a growing number of reference genomes, a large panel of DNA markers is available for many kinds of raw material and processed food items. Among the most universal approaches, DNA barcoding ranks first [6]. It is considered the gold standard for DNA-based identification, addressing one of the major issues in food fraud, mislabeling, through the analysis of standard markers especially in animals and plants (e.g., mtDNA *CO1* and *Cytb*; cpDNA *rbcL, matK* and *psbA-trnH*; and nrDNA ITS). Many reference sequences for addressing DNA barcoding identification are stored in international and publicly available databases (e.g., GenBank NCBI and BOLD Systems) and local reference datasets are also easy to assemble to achieve food validation purposes. This approach is particularly effective in the seafood market, but also for the traceability of meat, honey and vegetable products. In the food sector, it is also essential to analyse matrices of different types, both single-species and multi-species. Therefore, techniques based on High Throughput

Table 1
pro and cons of the most common chemical, biological, nanotechnologies and computer vision tools for food analysis. Moreover, the ranking in terms of the three criteria presented in this review (simplification, sensitivity and not destructivity) are indicated for each analysis.

Method	Pro	Cons	Simplification	Sensitivity	Not disruptive
MALDI-MS	Sensitive, fast, reduced sample-prep, chemical profiling of large and small molecules	<u>Disruptive, used for target or untarget analysis</u>	✓	✓	x
ICP-MS	Detection of heavy metals	<u>Disruptive, database or pure standards are needed</u>	✓	✓	x
HS-GC-MS	Sensitive, fast, chemical profiling of volatile molecules	<u>Disruptive, target or untarget analysis, database or pure standards are needed, chemometric evaluation is necessary</u>	✓	✓	x
LC-HRMS	Sensitive, fast, chemical profiling of small molecules	<u>Disruptive, untarget analysis, database or pure standards are needed, chemometric evaluation is necessary</u>	✓	✓	x
LC-QqQ-MS/MS	Sensitive, fast, chemical, marker-based methods	<u>Disruptive, taget analysis, pure standards are needed</u>	✓	✓	x
DNA barcoding	Reliable, universal, untarget	Databases are needed	x	✓	x
SSR	Reliable, universal, untarget	Databases are needed, specific primer pairs need to be designed	x	✓	x
LAMP/RPA	Fast, cheap, easy visualization, simple	Specific primer pairs need to be designed, nonspecific amplification	✓	✓	x
HDA	Fast, cheap, easy visualization, simple	Specific primer pairs need to be designed, nonspecific amplification	✓	✓	x
HRM	High sensitivity, fast, cheap	Specific primer pairs need to be designed, nonspecific amplification	x	✓	x
SNP	High sensitivity, genetically stable, highly reproducible marker	Expensive, long time for results	x	✓	x
HTS	High sensitivity, multispecies analysis, universal, untarget	Expensive, long time for results	x	✓	x
MinION	High sensitivity, fast, cheap, multispecies analysis, universal, target,	Potential base assignment errors	✓	✓	x
Fluorescence emission (i.e Quantum dots)	High sensitivity, selectivity, non-destructive, fast detection	Specificity Limitations, potential interferences	✓	✓	✓
SALDI-MS	High sensitivity and selectivity, Rapid analysis, non-destructive	Sample matrix effect, sample preparation and interferences	x	✓	✓
SERS	sensitive, selectivity, non-destructive, versatility and quantitative	Signal variability and interference, limited range, sample preparation	✓	✓	✓
CNN (Convolutional Neural Networks)	High sensitivity and selectivity, rapid analysis, non-destructive	Databases are needed	✓	✓	✓

Sequencing Technologies (HTS) like DNA metabarcoding can meet this requirement [7,8]. These approaches have for example been successfully used to identify the composition of honey [9], spices and herbal teas [10], complex matrices such as burgers [11], but also for insect-based novel food quality control [12].

1.2. The challenges in food authentication

The global dimension of the food market and the wide variety of both raw materials and processed products, requires increasingly flexible and universal authentication systems. Flexibility implies the transition from closed and fine-tuned analytical systems, dedicated to local products and based on species-specific markers, towards more universal approaches that can be adapted to different food matrices. The universality of the approaches concerns both the implementation of methodologies useful to different operational contexts (e.g., production companies, supply chain control laboratories, distributors), and the selection of shared and objective markers responsive to different aspects of food quality that could independently be adopted by different countries. To better meet the needs of the global food market in terms of food authentication, it is essential that innovative authentication methods respond to the following characteristics (Table 1).

1) Simplification of analytical and interpretation systems

Usually, chemical analyses require complex procedures of sample's preparation and expensive instrumentation. It should be considered to replace them with simplified sensor-based tools able to detect specific markers responsive of identity, quality and safety of food items. Among the most important factors, the shortening of analytical time and the availability of simpler protocols (e.g., colorimetric indicators) rank first but this transition requires methodological and technological innovation.

In this context, an effective simplification has recently occurred for DNA-based methods. Biomolecular methods have become increasingly simple and immediate [13] especially given to the use of target PCR analyses to detect specific contaminants and/or to certify the species origin. Simplified techniques such as the Loop-mediated isothermal amplification (LAMP), Recombinase Polymerase Amplification (RPA), and HDA Helicase-dependent Amplification) comply with these requirements, allowing for rapid food analysis (less than 2 h) even when equipped laboratory and instruments (e.g., thermocyclers) are not available. Therefore, these DNA-based procedures have increasingly being used in industrial and border customs settings for food control [14 and Fig. 2].

2) Increase in analytical sensitivity and ability to evaluate food quality

The fourth industrial revolution in the agri-food sector introduced the concept of "Food Quality". This refers to both the control in food preparation and composition, as well as the capacity to ensure accuracy and compliance with quality and safety standards of the final products [26]. The adopted analytical methods must therefore be able to control many factors linked to the organoleptic, nutritional, allergens and bioactive properties of a food item. Regarding known and common food allergens, numerous commercial systems based on antigen-antibody reactions are available. DNA-based analyses also allow the detection of potentially allergenic contaminants in raw material or into the final products by using, for example, Real Time PCR analyses [27].

The evaluation of the general quality of a food is, however, a thorny issue because it is made up of various characteristics that concern the entire chemical and organoleptic composition. Therefore, multiple markers should be measured at once. A partial response

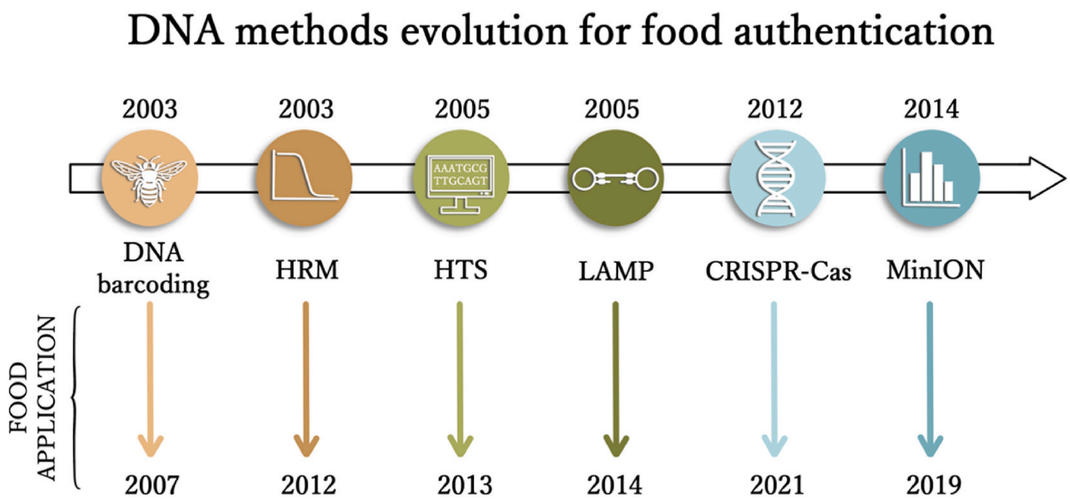

Fig. 2. In the figure, the most used techniques for food authentication are illustrated. The top part shows the date of the technique's introduction, while the bottom part indicates the date of its application to food authentication. This date has been verified based on one of the earliest uses presented in the literature [14–25].

to this need comes from multi arrays and multivariate analyses [28] and from the combination of chemical and biomolecular approaches [29]. Alternative analytical strategies, such as DNA metabarcoding, work well to ascertain the composition of a food, highlighting any contamination, substitutions and fraud. However, nowadays, these approaches still require high costs and the need for qualified operators both for analysis and for bioinformatic interpretation of the results.

3) Development of non-destructive food analysis systems

These methods allow the analysis of the same samples without pre-treatment, several times along the supply chain by different bodies (for example in the various customs offices where a product passes through) without altering its structure or quality, and thus reducing analytical times and the overall laboriousness of authentication processes. The non-destructive methods based on macro and microscopic characteristics of foods, are acquiring growing importance and are increasingly being used in the food industry [30]. Generally, these approaches consist in the analysis of raw material and/or final food products by using energy sources to yield relevant optical, magnetic or electrical parameters which are detected and interpreted to obtain quantitative and qualitative status of food and to certify food authenticity. Among the non-destructive tools, there are vibrational, hyperspectral, fluorescence, nuclear magnetic resonance (NMR) spectroscopy, and sensor techniques such as electronic tongues and electronic noses. These techniques are rapid, cost-effective, involve little-to-no sample preparation, environmentally friendly, easy to operate and can be adopted to identify target species and/or adulterants that have been added to the product.

Unfortunately, many of these approaches are not yet exhaustive and above all are not linked to chemical, biochemical and molecular markers. This often limits their use in pre-screening contexts or as early-warning indicators rather than definitive diagnostic tools [31].

1.3. How to strengthen authentication methods

Although chemical, biochemical and biomolecular methodologies have evolved over time, they are not always adequate to meet the challenges of the modern food industry and the complex network of international connections along each food supply chain. At the same time, digital transformation has dramatically increased the amount of data available to the food chain. Approaches such as the Internet of Things (IoT), cyber-physical systems, and artificial intelligence (AI) are very trendy and promising for food authentication. However, most of these technologies should be further developed to become more affordable, portable, and efficient to be easily adopted also by small/medium enterprises and reliable to be adopted in authentication activities from the field to the fork. Sometimes, the lack of technical skills in many food chain segments and the inadequacy of facilities, especially in developing countries, are also a critical limitation to concretely exploit such digital technologies.

The main trend in food authentication is the development of technological convergent approaches able to integrate conventional chemical, biochemical and molecular markers with advanced nanotechnologies and computer vision methods. In our vision, this convergence would result in the rapid development of simplified, exhaustive, and, in some case, non-destructive methodologies for food authentication. This vision discourages the research and development of the perfect analytical procedure and pushes towards the adoption of multi-marker systems detectable by a wide and integrated panel of technologies. Among these, nanotechnology has recently impacted the food industry. Nanoparticles have been used in various fields of food science, including food processing, food packaging, and functional food development [32]. However, the most interesting application for food authentication refers to detection of foodborne pathogens such as *Salmonella* spp. *Listeria monocytogenes*, and *Escherichia coli*. The food nano-diagnostics field can provide opportunities beyond the mere pathogens detection because it offers a few favorable properties, including.

i) *Versatility*. The physicochemical characteristics of nanoparticles make them promising candidates for generating biomarker-harvesting systems based on several chemical, biochemical and DNA-related markers. It is also possible to modify the nature of the nanoparticles, the ligands and therefore the molecular targets, thus personalizing the diagnostic systems.
ii) *Simplicity*. The miniaturization allows for the development of Lab-on-a-chip settings able to analyse several food matrices with reduced sample volume, low cost, portability [33]. Furthermore, simplified detection systems and signal amplification technologies allow the diffusion of this approach even for segments of the supply chain without expensive laboratory equipment.
iii) *Sensitivity*. The high surface-to-volume ratios of nanoparticles allows the generation of multiple nanosensors capable of detecting simultaneously more contaminants and/or recognizing species-specific markers of a food product. The large contact surface of these particles, the high ability to penetrate matrices with different densities and the potential for signal amplification make nanotechnologies very sensitive even for detecting trace compounds.

Therefore, nanoparticles can combine with traditional food markers, obtained through analytical chemistry approaches or DNA marker regions, to generate new effective tools for food traceability. However, to make the nanosensors even more effective it is necessary to bring this technology into an operational environment, evaluate the suitability within different segments of the supply chain. Furthermore, these sensors should also allow the operator to analyse highly processed matrices. For example, DNA nanobarcodes are very promising for food authentication and for protecting the brands and preventing food adulteration. However, DNA barcoding does not work in some processed foods and is subjected to chemical-physical treatments that can degrade DNA (high temperatures, pressure, treatments with denaturing chemical solvents). For this reason, mini barcoding-based systems have been developed and successfully adopted, for example in highly processed foods such as plant extracts [34]. Combining mini-barcodes and nanotechnology is an example of technological convergence that responds to the real needs of the food supply chain.

At the same time, the adoption of nanoparticles in electronic nose devices enhanced the efficacy of artificial sensing of smell and taste [35]. Therefore, analytical chemistry instruments could benefit significantly from technological convergence with nanotech.

To sum up, combining the versatility of nanomaterials with the wide range with target-specific bio-receptors such as enzymes, antibodies, antigens, hormone levels, bacteria and nucleic acid it will be possible to detect several 'indicators' of food authenticity which are normally analysed using complex chemical techniques rather than gene sequencing systems.

Another interesting technology consists in Computer Vision (CV) which allows an analysis of morphological features of food products. CV, combined with the automatic evaluation of chemical-physical characteristics (i.e., moisture, pH, temperature, pressure, humidity) based on Internet of Things (IoT) represent the two most promising trands of AI for the food diagnostics. Specifically, CV outperforms in its ability to detect discrepancies in color, texture, size, shape and defects useful to indicate food alterations which can be linked to natural deterioration processes and/or substitutions and counterfeits [36]. In the last years, image technologies have proven to respond to the modern challenges of food traceability and fraud prevention because they are.

i) *Non-destructive.* As previously suggested, image analysis allows the user to assess the consistency and integrity of food products, without carrying out invasive sampling of the products. This reduces the risks of contamination of the product itself during the analysis phases from an operator, allows the repeatability of the analyses without requiring additional samples, and makes the approach more easily automated [37].

ii) *Combine and integrate multiple analytical methods.* CV includes the capturing, processing and analysis of images. Most computer vision-based systems for food authentication use the electromagnetic radiation in the form of visible and infrared light (near-infrared - NIR, mid-infrared - MIR, Raman, terahertz - THz and hyperspectral imaging - HSI) [38].These systems return different images and information which can also be combined using modern algorithms and deep learning systems to quickly and thoroughly describe the food item characteristics [39].

iii) *Simplification and automatization.* Today, food image acquisition is an easy and cost-friendly approach for inferring information about food characteristics. The images can also be acquired via smartphone and by non-expert users. Using Convolutional Neural Networks (CNNs), the data can be combined and compared to classify the food and generate a reference database useful for future recognition tests. This also supports the development of machine learning systems which enables the development of food control approaches capable of comparing multiple data and information resources exportable to different areas and updateable over time.

CV systems could benefit from a link to chemical, biochemical and DNA-based markers. This is because most molecules absorb and emit electromagnetic radiation, and this allows a spectral image to be associated with the presence of a marker metabolite which can describe the quality of the food and/or any risks of alteration. CV systems can also increase the sensitivity of more conventional analytical methods. For example, dedicated technologies of CV and augmented visualization improve the level of information obtained through the process of two-dimensional chromatography [40].Tests performed on samples of raw sweet cream to ripened butter showed the abilities of this combined approach to assess the evolution of volatile components along the production chain and the impact of different microbial cultures on the finished product volatilome [41].

In general, CV approaches can be very useful for carrying out an initial screening of food products. For instance, this approach was used to evaluate the acceptability of mayonnaises [42], meat [43], chocolate [44], cheese [45] and other food product categories. Overall, the adoption of this novel strategy could significantly reduce the amount of traditional chemical and metabolomic analysis by directing them only towards less discernible or truly suspicious cases.

2. From tradition to innovation in the food supply-chain

The food sector is undoubtedly one of the most technologically advanced ones, inclined to exploit innovative processing, preservation and packaging systems [26]. There are sectors such as cereals where mechanization starts from the field and ends when the product reaches table. A the same time, many products require compliance with ancient recipes and procedures that do not always allow for rigorous controls or guarantee reproducibility and accuracy. For example, the production of high-quality wine often follows ancient procedures to highlight the local terroir. The same principle is valid for the dairy sector. Many dairy products have certain specifications that often do not match the automation of industrial technologies. Finally, there are sectors where the market is continuously expanding, such as the seafood one, where new species of fish are often not adequately labeled when they arrive on the market. These scenarios suggest that there is no single appropriate technology for addressing food authentication, traceability and fraud prevention. Sometimes the most exploited technology is the best known and most consolidated, but it could not necessarily be the best one. Therefore, a process of technological convergence, which combines biological or chemical marker-based systems with innovative technologies such as nanosensors and computer vision, is strongly necessary to meet the regulatory and technological needs of the food sector but also to quickly address new critical issues. In the next sections, we discuss the value of technological convergence in four highly interesting food supply chains that are widely subject to alterations and fraud.

2.1. The global seafood market

The seafood sector is considered one of the most at-risk to fraud [46] with a substitution and mislabeling rate of 36 % [47]. Fish fraud is relatively easy to carry out since products are often sold under a highly processed form (e.g., fillets), making it impossible to identify them by analysing morphological traits [48].

The chemical-metabolomic approach can effectively classify the quality of seafood by assessing the geographical origin and authenticity investigating complex lipids and/or proteic patterns of fish. For example, Wang & Bi (2021) developed a rapid method to distinguish rainbow trout and atlantic salmon by using multivariate analysis MALDI MS analysis comparing mass spectra of protein in muscle tissues [49]. Similarly, Hong et al., 2023 studied the geographical origin of salmon by creating a chemometric model by evaluating lipidomic and elementomic profiles both with Rapid Evaporative Ionization Mass Spectrometry (REIMS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

DNA barcoding is also considered one of the most effective analytical procedures for fish authentication [50] due to a high interspecific species distance value of mitochondrial COI regions, also among closely related species, and to the availability of a well-populated and validated DNA barcodes reference database. Simplified molecular systems have also been developed for identity fish species. These are based on loop-mediated isothermal amplification -LAMP [20] and Recombinase Polymerase Amplification -RPA- [13].

In the case of complex seafood products such as soups, the most performant approach is DNA metabarcoding also due to the adoption of simplified solutions such as MinION sequencer from Oxford Nanopore Technologies (ONT), which has the advantage of portability, real-time analysis, and lower cost compared to other sequencing technologies.

By combining DNA barcoding with nanotechnology, the NanoTracer approach was developed. This analytical strategy is based on the use of DNA primer regions, complementary to the DNA barcode region, linked to nanoparticles. When the primers recognize the target species, the nanoparticles pair up and have a color change. This system is versatile, low cost, rapid and reliable readout also to detect cases of food substitutions [51]. However, among the different technologies are those of CV that are widely exploited in the fisheries and aquaculture sector because they respond to the needs regarding the non-destructiveness of the approaches and the analytical speed. Multimode hyperspectral imaging methods were used to distinguish fish species as a whole or as fillets and to test the freshness of frozen filets. Qin and co-workers (2020) tested several filets including red snapper, vermilion snapper, malabar snapper by using multimode hyperspectral imaging techniques and, thanks to Machine Learning analysis, achieved an identification accuracy close to 100 % [52].Today, many images of fish are also available, even amateur ones, and this has favored the use convolutional neural networks (CNNs) which work to rationalize information and develop ever more precise identification systems [53]. More sophisticated computer vision systems also allow the user to connect the image to fish chemical characteristics such as specific type of fats and proteins [54].

These techniques have some limitations such as the need to have adequate image documentation, adopt efficient deep learning and effective machine learning approaches. The seafood sector is also experiencing great growth in terms of new species and processed foods, therefore once again, a process of converging technologies is recommended. In this context, the chemical characterization of the food matrices is expected to perform an initial assessment, providing the conditions for generating image reference databases on which developing effective and smart traceability systems.

2.2. The high processed food: spices

The global spice market is estimated to be worth $21.3 billion in 2021 [55].The EU imports more than 300,000 tons of spices yearly, often through long and complicated supply chains, leaving many opportunities for the occurrence of fraudulent products' adulterations [29].Generally, spices are sold in shredded or powdered forms, which alter their original appearance and increases the risk of fraudulent mixing with cheaper materials of plant origin [56].

Usually, spices are appreciated for the presence of various secondary metabolites that provide flavors and peculiar colors. Gas cromatography and UHPLC-ESI-MS/MS can used both to identify and quantify these metabolites [57] in raw material and processed samples (e.g., extracts). Isotopic analyses are instead used to determine the origin of spices [58]. To meet the needs of the modern market, rapid analysis based on electronic nose (E-nose) and the electronic tongue (E-tongue) have been developed [59]. These technologies are rapid and effective and the integration of nanomaterials has enhanced their sensitivity and selectivity toward specific molecules occurring in essential oils [60,61] (Recently, specific nanosensors have also been developed to detect contaminants in different spice products. For instance, a nanosensor taking advantage of the use of on carbon dots as fluorophores detectors was developed to find traces of tartrazine, a food additive used as fake saffron [61].

The image analysis sector has shown less success for spice identification than other fields; however, there are some interesting applications such as the adoption of smartphone images to estimate saffron characteristic based on different percentages of carotenoids [62] and to evaluate the presence of contaminants such as chickpeas powder [63]. CV authentication approach is therefore promising in this field, although the great variety of spices and their variability in colors and shapes, demands both a data collection effort and the development of efficient deep learning strategies to process it and generate automatic response systems [26].

Finally, concerning molecular-based approaches, DNA barcoding for spices traceability is very efficient on raw materials [14,65]. However, on alcoholic extracts and species subjected to industrial processing it could fail [34]. Integrating nanotechnological systems with mini-barcode tags could be a possible solution to increase the effectiveness of this system and to make it more suitable for industrial traceability applications.

2.3. The complex food matrices: the extra virgin olive oil

According to the 2019 Annual Report of the Food Fraud Network (FFN), the category of 'Fats and oils' was the most affected by fraudulent adulteration cases [64]. It has been reported that approximately 80 % of the Italian extra virgin olive oil (EVOO) available in the market is likely fraudulent [65]. Most of the fraud is attributed to the addition of lower quality oils from the same species (such as refined olive oil or olive pomace oil) or the addition of cheaper vegetable oils (such as palm oil, and palm stearin olein) [66].

The chemical composition of EVOO generally encompasses three major parts [67]. The main part is the saponifiable fraction (more than 98 % of the total oil weight), which includes primarily triglyceride (TGs) and secondarily diglycerides (DGs) and free fatty acids (FFAs). The second part is the unsaponifiable fraction (1–2% of the total oil weight). This fraction is chemically complex and could be subdivided into lipophilic and hydrophilic molecules. The lipophilic part contains hydrocarbons (e.g., squalene), tocopherols, sterols, and pigments such as carotenoids. On the other hand, the hydrophilic component is mainly composed of phenols. The third part is a volatile fraction which is a critical determinant of the particular aroma of EVOO. At the chemical level, volatile organic compounds and polar metabolites are considered the main markers to assess the quality and integrity of EVOO [68] and in most cases, the chemical information is sufficient to determine oil identification and authentication [69]. Nanotechnology could improve the authentication methods for olive oils starting from these chemical markers. Hybrid nanostructured surfaces, consisting of dense arrays of silicon nanowires (SiNWs) functionalized by Ag nanoparticles (AgNP/SiNWs), were used for the laser desorption/ionization time-of-flight mass spectrometry analysis of some typical unsaturated fatty such as squalene, oleic acid to distinguish different oil samples [70]. Yang et al. (2021) sprayed Ag NPs in oil samples to enhance TAGs (triacylglycerols) fingerprints in different vegetable oils, consequently developing a rapid and robust method to prepare samples [71].

Another technique that is well-matched with nanomaterials is RAMAN spectroscopy. Camerlingo et al. (2019) exploited SERS spectroscopy to estimate the abundance of saturated fatty acids in different Italian olive oils. They demonstrated the versatility of this rapid and non-destructive technique by quantifying olive oil components [72].

Differently from the technological convergence between chemical markers and nanotechnology, the analysis of DNA-based markers is much less used in oils analysis. The main reasons for this reside in the difficulty of extracting DNA from oily matrices and the poor information provided by these markers. Usually, DNA sequences can provide information on the original species and/or cultivar but it is not sufficient to identify organoleptic alterations and chemical-physical characteristics which are very relevant for this type of food product [73,74].

Concerning potential upgrades offered by CV technologies, Song et al. (2020) introduced a smartphone-based sensor system that generate a sequence of light, which is used to illuminate oil samples. The proposed system employed computer vision techniques to process input videos, converting them into sensor data in the form of a data vector. Machine learning approaches have made it possible to recognize oil samples with an accuracy of over 95 % [75]. Similarly, Pradana-Lopez et al. (2022) harnessed convolutional neural networks to train an algorithm for classifying different types of Extra Virgin Olive Oil (EVOO) with a success of about 96 % [76].

Finally, Mirhoseini-Moghaddam et al. (2023) introduced an innovative system combining the Enose with computer vision for detecting adulteration in extra virgin olive oil. Utilizing PCA-Quadratic Discriminant Analysis (PCA-QDA) an identification rate close to 100 % was achieved. A convergence action between quality chemical markers and image analysis systems would be desirable to make computer vision systems methods increasingly responsive to the market demand [77].

2.4. The case of traditional food: wine

The European Union is the world-leading producer of wine with about 180 million hectolitres. Reports from the European Commission detected instances of fraud involving more than 1 million liters of wine and over 1.2 million euros in the last six months of 2020 [78].

The most common forms of fraud are dilution with water, alcohol, coloring or flavoring substances and substitution with a different grape cultivar or the indication of a different geographic origin [79].

Geographic origin is an indicator of wine quality, and for this reason the European Union safeguards this product by granting different designations of origin, including Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Geographical indication (GI). For each of these labels there are specific regulations to follow, such as the grape varieties used, cultivation and vinification methods, aging, and both physical/chemical parameters and sensory characteristics [80]. Therefore, the traceability and authenticity analysis of the wine must start from the grape and continue to the bottle of wine. Since ancient times, wine quality evaluation has been based on sensory testing that is strictly related to chemical composition. The color, mouthfeel, and taste are largely influenced by the phenolic profile. Thus, at the chemical level, the phenolic compounds such as flavonoids (e.g., anthocyanins, flavan-3-ols, flavonols) and non-flavonoids (e.g., phenolic acids, tannins and stilbenes) can be used to assess wine quality and authenticity of grape varieties, the geographical origins, and the aging [81]. The combination of chemometric tools and LC–MS data allows the successful discrimination of grapevine [28].

Volatile organic compounds, or VOCs, are certainly elements of interest for distinguishing wines with different volatile aromatic molecules. Among these, monoterpenes are the main aroma-related terpenes, and they can occur in grape berries in the form of hydrocarbons, aldehydes, alcohols, acids, and esters [82]. In terms of wine color, anthocyanins are the most often considered wine markers; molecules such as 3-monoglucosides of malvidin, cianidin, petunidin, peonidin, and delphinidin are largely present in different percentages in many red wines.

Concerning DNA based approaches to wine authentication, the DNA microsatellite analysis represents the most adopted approach. Given their hypervariability and analytical simplicity they are largely used to distinguish between closely related cultivars and to register new accessions in the varietal catalog.

An emerging trend involves the study of single-nucleotide polymorphisms (SNPs) that offer the advantage to link the polymorphism to specific DNA region and to overcoming DNA degradation limitations, allowing for the use of more sensitive techniques like quantitative real-time polymerase chain reaction (qPCR). Boccacci and colleagues (2020) developed a methodology using SNPs TaqMan®-based detection for the authentication of "Nebbiolo" in musts and wine [83].

The need to find faster approaches to identify and analyse the quality of wines has supported exploratory research programs in the nanotechnology world. For example, the use of nanomaterials is encouraged by using Surface-enhanced Raman spectroscopy (SERS) to identify the components of a particular wine, determine its origin and assess its quality by excluding the presence of adulterations. Zanuttin and collegues (2019) exploited Ag nanoparticles as SERS enhancers to analyse three different white wines (Sauvignon Blanc, Ribolla Gialla and Friulano) and found that it is possible to distinguish wines and producers using SERS technique with high efficiency [84].

Qu and collegues (2020) used Ag nanoparticles in a different way by instead creating a silver colloidal mirror in contact with extracted phytochemicals from red wines (Gallo Family Vineyards Hearty Burgundy, Château de Chantegrive Graves, 2014, and Corley Family Cabernet Sauvignon State Lane Yountville, 2014) [85]. The authors identified important chemicals such as gallic acid, resveratrol and catechins, thereby establishing the basis for future experiments on more wine samples with the goal of producing a large database useful for quality assessment.

Non-Invasive Digital Technologies such as near-infrared spectroscopy is a promising approach to assess wine quality traits. Specifically, this approach has been used to assess ripening parameters of berry such as total soluble solids, pH, and total anthocyanins [86, 87] and to estimate wine quality traits such as alcohol content, sugar content, pH, volatile phenolic compounds [88], and sensory descriptors [89]. In addition, chemometric analysis combined with machine learning algorithms can be used to classify wine based on varietals and geographic origins [90]. Unfortunately, CV approaches on wine are more complex because it is most often contained in mirror bottles made with dark glass.

3. Every law has a loophole

The technology innovations described in the previous sections represent a real frontier in food authentication, even if they are not infallible. On one hand, the control systems of food supply chains are becoming increasingly widespread and capable of responding to global needs (e.g., movement of products around the world, speed of transport, processing in multiple countries and in multiple phases). On the other hand, counterfeiters are developing increasingly refined techniques. For instance, in the case of olive oil, innovative sophistication strategies have been developed based on the use of olive pomace oil and/or on fraudulent addition to other vegetable oils having a similar fatty acids and sterols (e.g. high oleic sunflower oil, high oleic safflower oil and hazelnut) [64]. This makes chemical controls less effective. Furthermore, if the consistency and colors of the counterfeit oils are very similar to the original olive oil, computer vision analysis is also not applicable.

In some cases, to find new fraudulent food adulteration strategies, continuous technological innovation and rapid updating of international directors on the new strategies adopted to evade controls is necessary. For example in the case of tuna, the image analysis has proven effective for detecting new frauds consisting in the histamine treatment to improve the appearance of spoiled flesh [91] and to modify the color of cheaper tuna species [92]. A non-invasive method based on digital imaging using a smartphone has been successfully adopted to clearly distinguish each of the most commercialized species of tuna (*Thunnus thynnus, Thunnus albacares* and *Thunnus obesus*) and their alteration with beetroot extracts [93].

Fraud cases could also appear to be linked to raw materials characteristics, especially if they do not involve species substitutions or the introduction of exogenous chemical compounds. In this context the most common fraud is adding water to meat or fish to increase weight. Dilution is another form of substitution applied to liquid foodstuffs such as the watering down of milk, coffee and wine. The analytical ability to detect these frauds is closely linked to the dilution percentages and the sensitivity of the methods adopted. Based on these considerations, it is possible to conclude that there is no universal technology for the identification and traceability of food products, but researchers should be able to get ahead of counterfeiters.

The same considerations also apply to regulatory processes linked to international food traceability which in many cases requires a long time before ascribing a contaminant or adulteration in a recognized catalog. Support in this context can come from blockchain [94] technologies as they require the definition of clear and universal rules for all countries and can also integrate analytical technologies for control of each segment. A growing amount of literature [95,96] suggest that blockchain technology can increase the efficiency and transparency of supply chains. This requires a lot of effort to gather information along the entire global food supply chains and the involvement of many actors that are spread around the globe to share common analytical procedures.

4. Conclusion

Concluding in a provocative way, further considerations should be devoted to the production of synthetic foods. Recently, 3D food printing technology is gaining global attention. Data Bridge Market Research analyses that the global 3D food printing market is growing at a CAGR of 52.30 % in the forecast period of 2021–2028. The 3D printing technology has a wide range of applications, from the production of food that meet the needs of elderly and children to the production of functional food for human wellbeing. This technology allows to produce meat without starting from animals, to integrate materials and foods at different levels of processing with functional compounds, to modify the texture of foods by integrating structural components. While this provides substantial progress in the food industry sector, at the same time it adds a further element of difficulty in the traceability context. Therefore, in the immediate future the food supply chain will no longer be composed of an original raw material and interested by specific transformation phases, but there will be a mix of various synthesis and manipulation segments useful to obtain the final food product. These technologies are often associated with the sustainability of processes and the personalization of foods, but it cannot be ruled out that this represents a new counterfeiting practice.

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No data was used for the research described in the article.

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Jessica Frigerio: Writing – review & editing, Writing – original draft, Conceptualization, Investigation, Validation. **Luca Campone:** Writing – review & editing, Writing – original draft, Conceptualization, Validation. **Marco Davide Giustra:** Writing – review & editing, Writing – original draft, Conceptualization. **Marco Buzzelli:** Writing – review & editing, Writing – original draft, Conceptualization. **Flavio Piccoli:** Writing – review & editing, Writing – original draft, Conceptualization. **Andrea Galimberti:** Writing – review & editing, Writing – original draft, Conceptualization. **Ciro Cannavacciuolo:** Writing – review & editing, Writing – original draft, Conceptualization. **Malika Ouled Larbi:** Formal analysis. **Miriam Colombo:** Writing – review & editing, Writing – original draft, Conceptualization. **Gianluigi Ciocca:** Writing – review & editing, Writing – original draft, Conceptualization. **Massimo Labra:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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