

Chapter 11

Food Safety and Quality Testing: Recent Areas of Focus and Research Perspectives



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1 Introduction

Globalization of trade and post-harvest processing enables continuous supply and access to almost any food material across the globe most of the time. It has supported overcoming food insecurity and hunger at many locations, especially where resources for food production are lacking. On the other hand, it has led to the introduction of food hazards, entirely new to a particular location due to various activities involved in food production (Nardi et al. 2020). Advances in food processing methods are responsible for reduced loss of agricultural produce and in enhancing the shelf-life of food products without compromising their safety and quality attributes. To an extent, it is accountable for achieving food security in some parts of the world (Augustin et al. 2016). However, certain processing conditions, additives, quality of raw materials, and their combinations lead to the formation of processing contaminants, which pose a risk of health hazard to humans (Ragavan et al. 2016). Estimation of the contaminants in food matrices is essential to ascertain the safety of processed food. A wide range of agrochemicals has helped increase food production to feed the human population. However, it has also led to a serious food safety issue due to agrochemical residues in the food matrix beyond permitted levels (Carvalho 2017; Medina-Pastor and Triacchini 2020; Thakur and Ragavan 2013). In the case of industrialized animal farming, extensive use of antibiotics and

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growth promoters results in their residues in animal products such as milk, meat, and meat products. It is held responsible for the rising concerns of antibiotic-resistant pathogens impacting human health (Boeckel et al. 2019; Moore 2019).

One of the most neglected food safety issues is related to the food-borne parasites, especially protozoans and helminths transmitted through contaminated majorly through pork, vegetables, dairy products, freshwater fish, and crustaceans (FAO 2021; Koutsoumanis et al. 2018). The global food supply chain increases the risk of introducing these food-borne parasites to new environments (Robertson et al. 2014). It causes around 90 million infections and is responsible for 52,000 deaths worldwide every year. However, regulations or testing protocols for these food-borne parasites are not in place (Chalmers et al. 2020; Torgerson et al. 2015). Due to the quantum of impact, regulatory agencies such as FAO/WHO and EFSA have come up with committees and recommendations to bridge the gap (Codex Alimentarius 2016; FAO 2021; Koutsoumanis et al. 2018).

In recent years, food fraud in terms of adulteration and food authentication is increasingly reported. The consumer is at a loss for receiving a less valuable product and, in some cases, a low-quality and unsafe product. Gaps in food regulations, economic status, and lack of traceability are reasoned for food fraud (Danezis et al. 2016; Manning 2016). Addressing food fraud requires the most advanced analytical techniques and continuous communication of raw material sourcing and processing (Danezis et al. 2016; van Ruth et al. 2017). Many groups are actively working to bring in practical solutions to prevent and identify food fraud.

The current generation of consumers is more aware of their food in terms of nutrition, source, quality, and safety attributes. Even though the awareness in middle and low-income countries is lower than in high-income countries, it might gradually increase due to the access to information through the internet and smartphones (Hoffmann et al. 2019; Huang et al. 2018a). It is worth noting that a section of consumers is willing to pay a premium price to ensure food safety (Alimi and Workneh 2016). Advancements in camera optics, wireless data transfer, and processing capacity in the smartphone are equipping it into mobile-based testing platforms. It renders the consumers test the food they consume for safety and quality parameters (Purohit et al. 2020).

Food safety issues discussed so far highlights the need for rapid and easy-to-use food safety and quality testing methods. Global food regulations are helping to an extent to overcome the food hazards in the supply chain and recommend a set of safe practices to produce safe food. However, it has mandated the necessity to have a robust food testing infrastructure across the globe to ensure the safety and quality of food obtained by agricultural practices, trade, and processing. It is a critical challenge to developing and poor economies to create and maintain the infrastructure. Advancement in electronics and material science has driven sensors and biosensors research to develop novel and straightforward food testing methods. Developed methods have the role of fulfilling the need of the consumer to test their food tested with accuracy. Overall, the development of food testing devices requires a highly multidisciplinary approach, with inputs from basic science and engineering topics. The link between advancement in food processing and food analysis is discussed concerning food quality and safety in the following sections.

2 Advances in Food Processing

Food processing is advancing with novel technologies to bring in required sensory attributes, food structures and ensure food safety. On the other hand, outbreaks of food-borne illness due to pathogens including *Salmonella*, *Staphylococcal* strains are reported every day, especially in ready-to-eat (RTE) foods, fresh and fresh-cut foods, and fish, meat, dairy products, and seafood. Flawed implementation of Hazard Analysis & Critical Control Points (HACCP) in processing plants coupled with Food fraud and Food adulteration for economic gain are commonly reported (Manning et al. 2019; Tibola et al. 2018). Intense collaborative efforts among the industry, regulatory, and research stakeholders to strengthen strategies and identify the best tools to ensure food safety is effectively implemented across the food supply chain (Castro-Ibáñez et al. 2017; WHO 2018; Zeaki et al. 2019). Food safety stakeholders are well aware that there is no “silver bullet” technology that can fully eliminate pathogens/contaminants from the food supply chain.

Nevertheless, substantial progress has been made in recent years, both in terms of enhancing existing prevention tools and developing novel technologies for microbial inactivation and detection of food contaminants. Hybrid techniques such as “Hurdle technologies” incorporate multiple processing operations to inactivate pathogens in foods (Chen et al. 2012). Along with the processing operations, researchers persistently report a range of food analytical techniques for food quality and safety. It includes chemical, biological, and nanomaterial-based sensors including bacterial adenosine triphosphate (ATP) based bioluminescence sensors and nucleic acid-based methods like polymerase chain reaction (PCR), etc., (Böhme et al. 2019; Cesewski and Johnson 2020; Parate et al. 2020; Zhang et al. 2017). Biosensors and chemical sensors are potential techniques to ensure food quality and safety assurance in the global food supply chains, especially in detecting pathogens or determining quality attributes such as shelf-life (Cesewski and Johnson 2020). Similarly, biosensors based on imaging and spectroscopic methods are onsite monitoring and screening of food products and raw materials for quality and safety attributes (Rady and Adedeji 2018). Free radicals and DNA are the most desirable targets for biosensor-based food analytical methods (Law et al. 2015; Poltronieri et al. 2014).

Mass spectroscopy techniques are powerful tools to detect adulterated components and detect inferior meat (presence of substantial pathogenic microorganisms and poor quality), called ‘zombie meat’ in China, which poses significant health risks. Huang et al. (2016) developed two-dimensional gel electrophoresis coupled with mass spectrometry (2DE-MS)-based proteomics system for detecting meat type and its quality. They identified 450 protein spots in the meat exudates, along with 22 proteins. Among them, myofibrillar protein and myoglobin, were chosen as markers to distinguish between freeze-thawed and fresh pork.

Global milk production is expected to grow at a rate of 1.6% per year, reaching 997 Mt. by 2029, outpacing major agricultural commodities (OCED/FAO 2020). Dairy products, second only to green leafy vegetables in terms of adulteration, account for 14% of all food-borne illnesses (Painter et al. 2013). Adulterants and

the rate of adulteration in milk and milk products are reported higher than earlier with the notorious Chinese milk scandal containing melamine to artificially inflate the protein content of dairy products. Chronic melamine exposure can lead to nephropathy and various other health problems, and the detection of this adulterant is crucial for food safety. Some of the recently reported methods for rapid detection of melamine include silver nanoparticles (Daniel et al. 2017), magnetite nanoparticle-based immunochromatographic strip (Huang et al. 2018), smartphone-based optical sensor containing fluorescent gold nanoparticles and carbon quantum dots nanocomposites (Hu et al. 2019). Raman chemical imaging (RCI), a novel technique that combines Raman spectroscopy (signals from vibrational modes of a molecule) and digital imaging capabilities, has the advantage of accurate detection of adulterants/contaminants and their distribution in a food matrix. RCI with NIR chemical imaging is reported to increase the accuracy of melamine detection in skim milk powder (Betz et al. 2012).

2.1 Novel Interventions

Cold plasma is an emerging non-thermal food processing technique applied to decontaminate vegetables, fruits, dairy, and animal products from pathogenic and spoilage microbes. Plasma is commonly generated through the application of high potential difference, high voltage alternating current (AC), direct current (DC), radio frequency (RF), or microwave (MW) across a non-conducting dielectric fluid/gas. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the two effective primary species responsible for antimicrobial action. (Tappi et al. 2014). UV light and reactive chemical species generated by the cold plasma ionization process are the primary mechanisms of action for decontamination. Cold plasma inactivates pathogens by three main pathways (Niemira 2012):

- (i) Interaction between free radicals, charged particles or reactive species with microbial cell membranes
- (ii) UV radiation damages cell membranes and internal cellular components
- (iii) UV radiation has the potential to break DNA strands.

Cherry tomatoes subjected to dielectric barrier discharge (DBD) plasma at 80 kV for 5 min effectively reduced the microbial load (*E. coli*, *S. typhimurium*, and *L. monocytogenes*) by 3.5, 3.8, and 4.2 log CFU, respectively (Ziuzina et al. 2014), while in fresh strawberries a 2 log reduction in aerobic mesophilic bacteria, mold, and yeast population was reported (Misra et al. 2015). Degradation of carotenoids was found to be responsible for color loss in food products after plasma treatment (Bagheri and Abbaszadeh 2020; Misra et al. 2015).

Pulsed electric field (PEF), a non-thermal technology that has been commercialized since 2005, has proven to be an effective microbial inactivation tool for liquid foods as well as wastewater treatment. PEF uses intense electric pulses to break down the cell membranes of vegetative bacteria, molds, and yeasts. Besides the

inactivation of microbes, PEF treatment has shown to be successful in certain in-package microbial decontamination, allergen reduction, and shelf-life extension of certain foods while maintaining their nutritional and quality attributes (Alirezalu et al. 2020). Pasteurization of foods such as milk, soups, juices, yoghurt, meat, and liquid eggs has been successfully tested using PEF technology (Bhat et al. 2019). However, it is restricted to foods that don't have any air bubbles and have a low electrical conductivity. A recent study on the application of PEF in pasteurization of liquid whole egg or liquid egg white to inactivate *L. monocytogenes*, *S. typhimurium*, and *S. enteritidis*. (Bricher and Keener 2007). From the above discussions, it is evident that PEF is a powerful processing technique to ensure the safety of high-risk food products prone to rapid pathogen/microbial contamination.

Other promising intervention technologies for food preservation and inactivation of spoilage and pathogens include, irradiation or ionization radiation treatment (Albert et al. 2021), UV for surface sterilization (Bintsis et al. 2000), and high-pressure processing (HPP) (Rajashri et al. 2020; Rastogi 2013; Zhang et al. 2019). At present, irradiation as a pasteurization method for fresh produce is being debated by industries and regulatory organizations such as the USDA. Irradiation of ready-to-eat (RTE) meat products and juices has recently received regulatory approvals. However, low-level ionizing radiation to inactivate microorganisms, yeasts, spores, molds, naturally occurring chemical toxins, and parasites in spices are followed for several years. Ionizing radiation was used in a study conducted by Food Safety Intervention Technologies Research Unit, USA, to reduce potential carcinogens (furan and acrylamide) in foods. Furan and acrylamide in water were entirely destroyed by low-dose ionizing radiation (2–3.5 kGy), and the levels of furan in RTE meats were substantially decreased from 25% to 40%, whereas a minor effect on the inactivation of acrylamide in potato chips and oil were observed (Fan and Mastovska 2006).

The effect of food processing methods on the physiology and behaviour of microorganisms in foods, such as homeostasis, stress reactions, and metabolic fatigue, has recently been studied, leading to the development of the novel concept of multi-target food preservation techniques (Leistner 2000; Peleg 2020). Studies involving combined intervention technologies demonstrate the significance of using a multiple hurdle strategy (Singh and Shalini 2016). Hurdle technology comprising antimicrobial agents, thermal processing, advanced non-thermal processing methods and antimicrobial packaging is expected to play a major role in retaining nutrients and ensuring food safety.

3 Advancement in Conventional Analytical Methods

Food authenticity testing is a primary criterion for food and food products becoming more common due to global food legislation. From quality and authenticity stand-points, product analysis in the food and beverage industry is critical to ensure that the products have the appropriate nutritional levels, contain all the required constituents,

are what they claim to be (to avoid food fraud), and adhere to international and domestic standards. Its goal is to classify foods based on their chemical composition, nutritional value, sensory perception, traceability, protection, and consistency. Among different food categories, milk and milk products, oils and fats, fish and seafood, meat and meat products, fruit juice, alcoholic drinks, coffee and tea, sweeteners (including honey), spices, cereals, and pulses, were reported to have the highest numbers of adulteration incidence (Hong et al. 2017). Conventional food quality analytical techniques have lower precision, efficiency, time-consuming at quantifying or predicting food fraud activities. In the last two decades, some of the most widely used techniques for detection of food fraud and adulteration are: (i) chromatographic techniques: gas chromatography (GC), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), (ii) mass spectrometry (MS) methods: gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), (iii) spectroscopic methods: Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), Raman, mid-infrared (MIR), near-infrared (NIR), and (iv) electrophoretic techniques: polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD) (Fig. 11.1). Among all techniques, mass spectrometry (MS) constituted the most

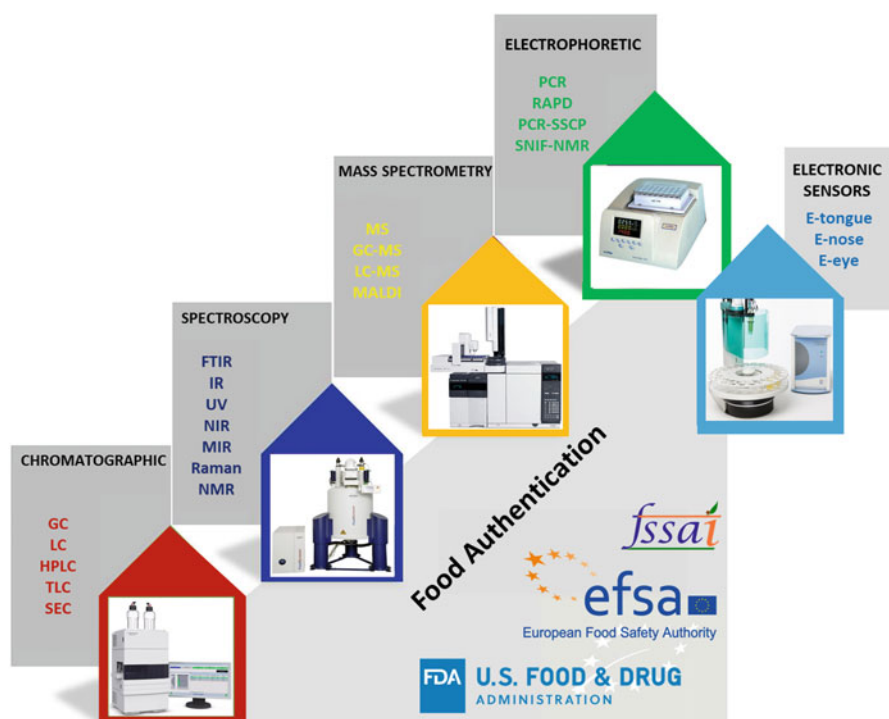


Fig. 11.1 Different analytical techniques followed by several regulatory organizations for the authentication of food samples

extensively and frequently used technique for cereals, spices, grains, and pulses. PCR techniques are most commonly employed in samples where DNA/RNA has to be tested, and it includes animal products such as meat and meat products and fish and seafood. Liquid chromatography (LC) and HPLC are regularly used for analyzing sweeteners, alcoholic beverages, fruits, and fruit juices. Owing to the chemical complexity of food products and high market demand for food quality and safety, high-resolution chromatographic techniques, including gas chromatography (GC) or liquid chromatography (LC) coupled with mass spectrometry (MS), have been identified as important food authentication methods.

Apple juice is among the most widely consumed juices in the world. For detecting the addition of low-cost commercial sugar syrups (beet and cane syrup) to pure apple juices and similar products, an advanced technique for determining ^2H and ^{13}C isotope ratios using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) has been developed (Kelly et al. 2003). This technique can precisely detect added sugars such as inverted cane sugar, glucose, and fructose in authentic apple juices by confirming the variation in sugar contents in the juices. On the other hand, DNA-based methods, such as real-time PCR, species-specific PCR, and multiplex PCR, are undoubtedly the most common techniques used to assess the authenticity of meats and meat products. The Food Safety Authority of Ireland released a press report on January 15, 2013, announcing the application of real-time PCR to detect horse and donkey DNA in ground beef items such as sausage, burgers, and meatballs (Chisholm et al. 2005; Meira et al. 2017; O'Mahony 2013; Walker et al. 2013). Another example of meat fraud is murine meat as a replacement for mutton meat, frequently reported in China (Fang and Zhang 2016). The TaqMan@ real-time PCR method was used to detect the adulteration in which the study suggested a limit of detection of fewer than 1 picograms (pg) of DNA per reaction and 0.1% murine contamination in the adulterated meat. DNA and mass spectrometry methods are reported to be frequently employed methods for detecting food fraud. Along with these methods, it is very crucial to share the validated information among the concerned stakeholders for better traceability and monitoring (Huck et al. 2016; Ulberth 2020).

Due to the production of olive oils with unique regional and varietal features (protected designation of origin-PDO) and customer demand for high quality, authentication and quality control of olive oil are of primary importance. NMR spectroscopy and stable isotope analysis can reveal a pool of information on chemical composition and the chemical structure of oil metabolites (Dais and Hatzakis 2013). Stable isotope ratios can determine isotopes whose relative abundance is influenced by isotope fractionation in nature. Different elements in olive oil, including $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$, enabled Italian oils to be differentiated as per their geographical origin and between PDOs from the same area in some cases (Camin et al. 2010).

Consumers are increasingly looking for foods that are safe and nutritious and have a high organoleptic quality. Generally, acceptable sensory analysis findings needed a well-trained panel of human sensory analyzers. Even if the panellists are well trained, there is still a requirement to standardize the sensory interpretation,

which is highly subjective. Instrumental food quality testing using perception sensors rather than human panel testing has recently gained popularity. An innovative cross-perception multi-sensors data fusion method has been proposed that mimics multiple human perceptions (Ouyang et al. 2014). Data were collected from rice wine samples using three sensors: an electronic tongue, eye, and nose. Principal components analysis (PCA) and multiple linear regression (MLR) were used to establish three cross-perception parameters: color, scent, and taste, used as inputs to models. Furthermore, a team of scientists from the UK has designed the first-ever 3D printed synthetic soft biomimetic surface that duplicates the wettability, elasticity, and topography of a real human tongue (Andablo-Reyes et al. 2020) (Fig. 11.2). The biomimetic tongues allow researchers to test newly developed products and speed up new development processes without expensive and time-consuming preliminary human testing. Oral tribological research with this advanced tongue-like surface can set the standard for understanding fundamental oral lubrication pathways, allowing basic mechanobiological questions to be addressed. At the same time, experimental and computational insights from this study can be extended to the biomimicry of other biological surfaces in the future to match the desired biophysical performance requirement.

Different food safety and control authentication techniques generate a humongous volume of data. Since a large volume of information needs to be analyzed, chemometrics and bioinformatics tools are essential for food authentication studies. Food science and technology have recently embraced novel and promising multivariate statistical methods such as chemometrics designed for analytical chemistry. Chemometric tools enable optimal applications of analytical techniques (chromatography, mass spectrometry, spectroscopy, PCR, calorimetry, wet chemistry, etc.) by extracting and interpreting valuable data from large and complex data sets. It also helps identify patterns in the data and develop calibration models (Capuano et al. 2014). However, the chemometric methods, such as principal component analysis (PCA), factorial discriminate analysis (FDA), quadratic discriminant analysis (QDA), and partial least squares discriminant analysis (PLS-DA), have certain shortcomings in delivering efficient and robust prediction models. Especially in cases involving large datasets presented in different formats (databases, images, texts, sounds, and video), which can be solved by incorporating statistical learning theories including artificial neural network (ANN), support vector machine (SVMs), probabilistic neural networks (PNN) (Kamal and Karoui 2015; Medina et al. 2019).

The development of databases containing standardized and comprehensive information regarding the origin of foods, such as geographical origin, species and subspecies, processing methods, and so on, will be critical to food authentication. Simultaneously, the availability of reference samples and well-identified databases is essential for predictive models that can relate an unknown sample to a known product. Although various regulatory organizations have compiled databases of food fraud and adulteration, there is a greater need for a pool of databases that can be used to classify these unknown samples. Some of the governmental/non-governmental platforms and databases that provide food authentication-related information are DOOR (records origin and registration of traditional specialties

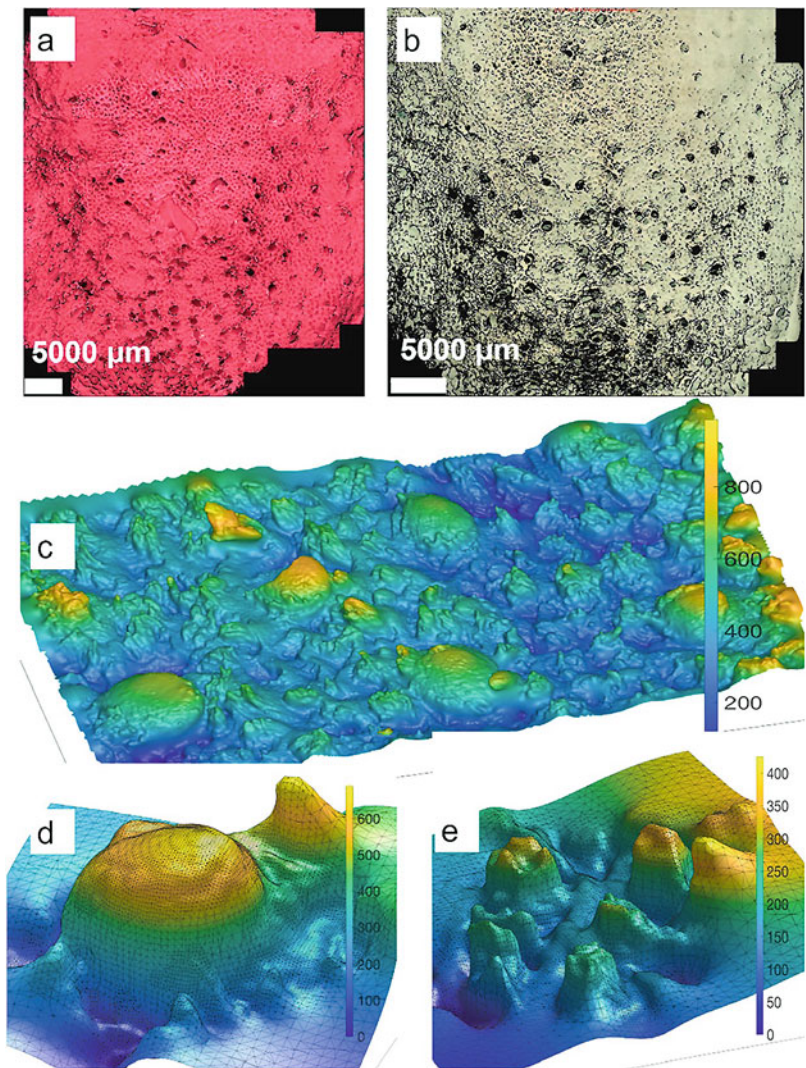


Fig. 11.2 3D printed biomimetic tongue surface to quantify oral friction and function as tribometer. Tongue impression on (a) polyvinyl siloxane (b) alginate masking materials imaged through negative 3D optical scans. (c) Tongue impression on polydimethylsiloxane obtained through positive 3D optical scan. (d) Fungiform papillae and (e) Filiform papillae on the surface of masking material reconstructed using screened Poisson surface. (Andablo-Reyes et al. 2020) (CC-BY)

guaranteed, protected designation of origin (PDOs), and protected geographical indication (PGIs), and Food Fraud Database (Medina et al. 2019). These databases are made available to the public through online websites such as Rapid Alerts System for Food and Feed (RASFF), which the public can access for recent incidents and any problems documented previously, thereby helping to identify food frauds.

The conventional analytical model involves sending samples to a laboratory and receiving results in several days or weeks. Today, food industries greatly benefit from several rapid testing techniques that can be performed at the point of use and provide a real-time result. The next expected paradigm shift will be towards testing kits/methods that customers can perform on supermarket shelves or at home. The use of small immunoassay test kits (similar to home pregnancy test kits) connected to smartphones to upload results into public databases is a specific area of interest for verifying “free-from” claims. One such example available in the market is AlerTox (<https://glutentox.com>) allergen test kits for detecting common allergens in soy, milk, and peanut. Although the R&D of advanced analytical techniques is going at a fast pace, most of the food authentication testing will continue to be performed in specialized laboratories due to the need for purchase and disposal of specific reagents, the intrinsic capital cost of equipment, stricter food regulations, the need for expert interpretation and so on.

It is evident from the literature that there has been considerable improvement in sample preparation, process automation, operating cost, and efficiency of conventional analytical techniques. Among them, supercritical fluid-based chromatography is gaining prominence because it requires less solvent (modifier and co-solvents, CO₂ being the major component) for sample preparation. When it is combined with mass spectroscopy techniques, it has increased its application in analytical chemistry due to its rapid detection and greener approach (Pilařová et al. 2019).

Magnetic extractants are increasingly used for sample preparative steps in food analysis for being economical in operation compared to other sample preparation steps. It is driven by the advancement in the synthesis of magnetic nanomaterials with fascinating properties such as superparamagnetism at room temperature. Magnetic nanomaterials are conjugated with selective adsorbents towards the molecule of interest and separated from the matrix with the combination of complementary shape, charge, and size. Novel chemical and physical functionalization methods boost magnetic extractants' application in food analysis (Li and Shi 2020; Ragavan and Rastogi 2017).

4 Advancement in Recognition Elements and Nanomaterials

From the beginning of twenty-first century, there has been a significant advancement in the synthesis and characterization of nanomaterials and related materials. It is mainly due to the new chemical routes for the synthesis of nanomaterials and progress in the instrumentation techniques to study their structure and properties.

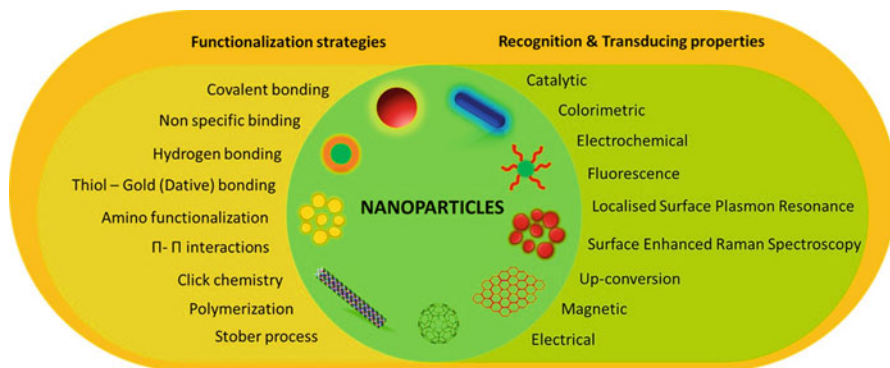


Fig. 11.3 Major types of nanomaterials utilized for the development of novel food sensors and detection methods. Left wing lists different physical and chemical routes followed for functionalization of nanomaterials with other sensor components. Right wing lists the role of these nanomaterials in the developed sensors (1). Permission obtained

As a result, numerous novel applications were reported in almost every possible field. Nanomaterials contributed significantly to food analysis and testing, which is evident from the quantum of research publications reported in the past two decades. Specific property and role of different nanomaterials in terms of their functionality for developing sensors and biosensors for food analysis are listed as a table elsewhere (Ragavan and Neethirajan 2019). Nanomaterial combination with different types of novel recognition elements has resulted in new detection methods with better analytical attributes (Fig. 11.3) (Ragavan et al. 2013a; Sharma et al. 2015). Recognition elements commonly used to integrate with nanomaterials for food analysis are briefly discussed below.

Antibodies Antibodies are the most reliable bio-recognition elements used to produce immunobased assays and kits (Ayyar et al. 2019; Yakes et al. 2016). They offer the advantage of conjugation with a wide range of molecules, including proteins, dyes, and nanoparticles, to develop food detection methods (Abhijith et al. 2013; Selvakumar et al. 2013).

Aptamers Aptamers are single-stranded nucleic acids such as DNA, RNA, or peptide sequences having a strong affinity to bind with different molecules with better affinity than antibodies. As a result, they are increasingly being used as a recognition element in the development of sensors. It also offers excellent functionalization with nanomaterials that improved the sensor attributes (Ragavan et al. 2013b; Sharma et al. 2015).

Enzymes Enzymes exhibit high specificity towards their target molecules through complementary structure and bond formation. The above property was the driving force in fabricating biosensors during the initial stages of biosensor development. It also offers good compatibility in functionalization with other sensor elements such as optical dyes, nanomaterials, and so on (Asal et al. 2018; Vaidya and Annappure 2019).

Nanomaterials Among the exciting properties displayed by nanomaterials, the catalytic property is utilized to design sensors without traditional enzymes, which are comparatively sensitive to environmental factors. The catalytic activity of nanomaterial depends upon the composition, morphology, and size. Detailed discussion on the topic is discussed elsewhere (Lin et al. 2014; Roduner 2006). Some nanomaterials exhibit multiple enzymatic activities, which is advantageous in developing a sensor for multi-analyte detection. Catalytically active nanomaterials are increasingly used as an alternative to enzymes in fabricating sensors for food analysis (Mustafa and Andreescu 2020; Ragavan et al. 2018b).

Molecularly Imprinted Polymers (MIPs) MIPs are synthetic counterparts of enzymes and receptors which mimic their function through complementary structure and chemical interaction brought about by polymers. The inherent nature of MIPs exhibits better electrical properties than enzymes and finds application in the fabrication of electrochemical sensors for food analysis with superlative analytical performance. Simultaneously, they are cost-effective and relatively stable compared to enzymes and other biological recognition elements, which require specific buffers for optimal activity. MIPs being selective towards a particular molecule find application in sample preparation for various analytical methods (Ashley et al. 2017; BelBruno 2019; Rhouati et al. 2019).

Receptors Receptors are highly selective and sensitive biomolecules that are part of signal transduction in living organisms. Purified receptors are used as bio-recognition elements in fabricating sensors for primarily volatile organic compounds. Disadvantages include difficulties in integration with other sensor components, limited receptors, high cost of purification, and less stability compared to its counterparts (Bohbot and Vernick 2020; Wu et al. 2014).

Whole Cells Instead of cellular components such as nucleic acids, enzymes, receptors, peptides, etc., whole bacterial or mammalian cells are used for sensing and screening different types of food components, including toxins and contaminants. Optical and electrochemical detection platforms based on whole cells are reported with the advantage of being stable and low cost. However, it requires specific conditions to culture them, and the present application is limited to very few analytes (Ye et al. 2019; Yu et al. 2017).

In the following section, important nanomaterials and their role in food testing are discussed below (Table 11.1).

4.1 Graphene and Related Materials

Graphene is an atom-thick two-dimensional carbon material known for fascinating optical, mechanical, electrical, and magnetic properties. It offers to tune the properties of graphene through changes in its composition and structure, resulting in graphene-related materials such as graphene oxide, graphene quantum dots, reduced

Table 11.1 Functional role of nanomaterials in various biosensor platforms along with their strengths and weakness

Nano-biosensor platform	Function of nanomaterial	Strengths	Weakness
Chemiluminescence (CL)	Catalyst in oxidation reaction, overcomes the need of enzymes	Highly sensitive (pM – fM), better signal to noise ratio	Not selective, quantum yields of the CL reaction is low
Aptasensor	Acts as an optical transducer (molar extinction coefficient is higher than chemical dyes)	Sensitive (sub nM), doesn't require instrument for interpretation,	Stability of aptamers is a concern, coloured compounds in the sample may interfere with results
Enzyme sensor		Fairly sensitive (μM – nM)	Most of the enzymes are not selective and presence of inhibitors reduces the efficiency of enzymes
Immunosensor		Sensitive (sub nM)	Dissociation constants of polyclonal antibodies are low
Electrochemical	Electrode modifier improves the surface area, electron conductivity of electrodes, sensitivity of the sensor and biocompatible	Highly accurate and sensitive analysis	Sample preparation is required
Fluorescence	Stable to photo bleaching and emission can be tuned	Highly sensitive (nM – fM)	Some of the dyes are highly toxic and prone to photo-bleaching
Microfluidics	Improves the analytical performance of microfluidic chip and device	Miniaturization, multiplex detection, high throughput analysis and less sample and reagent requirement	In nascent stage of growth, understanding the basic properties of fluids is required for better application
Quartz crystal microbalance (QCM)	Improves the sensitivity of the sensor	Possibility of miniaturization	Sensitivity of small molecules are low
Surface plasmon resonance (SPR)	Act as plasmonic substrate	Highly accurate analysis and overcomes the sample preparation steps, label free analysis	Advanced and sophisticated instruments are required
Surface enhanced Raman spectroscopy (SERS)	Nanoparticles enhanced the Raman signals by 10^{10} – 10^{11}	Highly accurate and sensitive technique.	Advanced and sophisticated instruments are required

graphene oxide, graphene aerogels, and graphene-based composites. It is one of the best available materials to serve as an anchor for nanoparticles in the formation of composites while preserving nanomaterial properties (Ragavan and Rastogi 2016, 2017). Graphene acts as a quencher in its two-dimensional morphology and as an emitter of fluorescence signals while in spherical format (graphene quantum dots and carbon quantum dots), which finds huge applications in sensors as an alternative to semiconductor quantum dots (Pan et al. 2020; Ragavan and Neethirajan 2019; Zheng and Wu 2017). Carbon allotropes such as carbon nanotubes (CNTs) and fullerene are known for their electrical properties especially electrical conductivity, which makes them the material of choice for the fabrication of electrochemical sensors (Merkoçi et al. 2005; Taouri et al. 2021). Similar to graphene, other 2D materials such as MoS₂ and other transitional metal dichalcogenides, transitional metal carbides, nitrides hexagonal boron nitrides, carbonitrides, metal oxides, and metal-organic frameworks bring in interesting optical and electronic properties for designing sensors and detection methods meant for food analysis (Boroujerdi et al. 2020; Shavanova et al. 2016).

4.2 Metal and Metal Oxide-Based Nanomaterials

Transition metal and metal oxide nanoparticles such as gold, silver, zinc, copper, Iron, Titanium, etc., are commonly used nanomaterials. Among them, gold, silver, and their alloy in the form of nanoclusters, nanoparticles, nanorods, and other morphologies exhibit the best optical properties compared to other nanomaterials and dyes in terms of extinction coefficient (Ragavan et al. 2013b). Unique catalytic, distance-, and size-dependent optical properties are widely employed in developing colorimetric, fluorescence, chemiluminescence, surface plasmon resonance, and surface-enhanced Raman spectroscopy-based sensors for food analysis (Chen et al. 2018). Recently, gold and silver nanoclusters are being investigated for their optical properties and found applications in detecting food analytes (Hu et al. 2020; Li et al. 2019). Iron oxide nanoparticles exhibit superparamagnetism at room temperature, along with catalytic properties. It is the material of choice for sample preparation in food analysis, and it is conjugated with other nanomaterials to bring in multiple roles (Cao et al. 2012; Li and Shi 2020; Ragavan and Rastogi 2017). In many cases, they help overcome the matrix effect by separating the molecule of interest in relatively simple steps due to its magnetic property. Titanium-based nanomaterials and their hybrids find specific applications in the development of electrochemical sensors for their semiconductor-like electrical properties (Romero-Arcos et al. 2016; Shetti et al. 2019).

4.3 *Miscellaneous Nanomaterials*

Silica-Based Nanomaterials Silica and its nanomaterials for their biocompatibility and less toxicity offer an excellent platform for conjugation of biomolecules. It finds major applications for in-vivo applications. However, their role in sensors for food analysis can't be ignored.

Nanodiamonds Nanodiamonds are an allotrope of carbon, known for their mechanical and electrical properties, to fabricate quartz crystal microbalance-based sensors (Yao and Xue 2015). Doping of nanodiamonds with boron overcomes electrode surface fouling and improves the sensor attributes (Jiang et al. 2021).

Cerium-Based Nanomaterials These nanomaterials are known for their catalytic and electrochemical properties, find application in the development of electrochemical sensors for food analysis (Esmaeili et al. 2019; Yang et al. 2017). The catalytic property of cerium nanoparticles towards different antioxidants in food products resulted in products with different hues, which was utilized to demonstrate cerium nanoparticle-based optical sensors (Sharpe et al. 2013).

Palladium-Based Nanomaterials They are known for their attractive multi catalytic properties used to fabricate paper-based color sensors (Fig. 11.4) (Ragavan et al. 2018b). Palladium nanoparticles are reported to be compatible for conjugation with aptamers for designing fluorescence sensors to detect tetracycline in milk (Ahmed et al. 2021).

Semiconductor QDs They are highly fluorescent nanomaterials with very high quantum yield and also offer the advantage of tuning their emission through composition and size. They are the nanomaterial of choice as optical tags in optical sensors and fluorescence resonance energy transfer (FRET) based sensors (Chern et al. 2019; Freeman et al. 2013; Pedrero et al. 2017).

Upconversion Nanoparticles A unique class of nanomaterials known for their optical property of absorbing low energy electrons and emitting them in high energy or shorter wavelength. These nanoparticles have a larger stokes shift than conventional fluorescent dyes and semiconductor quantum dots. Sensors for detecting antibiotic residues are designed using these upconversion nanomaterials (Peltomaa et al. 2021; Wen et al. 2018).

5 Mobile/Smart Phone-Based Sensors/Biosensors

Consumer access to smartphones across the globe is increasing, and the day is not far when almost every human has a smartphone. It is perceived as a digital companion and an extension of the user (Carolus et al. 2019; Harkin and Kuss 2021). Smartphones are increasingly replacing conventional imaging infrastructure for measuring various parameters, including contact angle (Chen et al. 2018). Similarly,

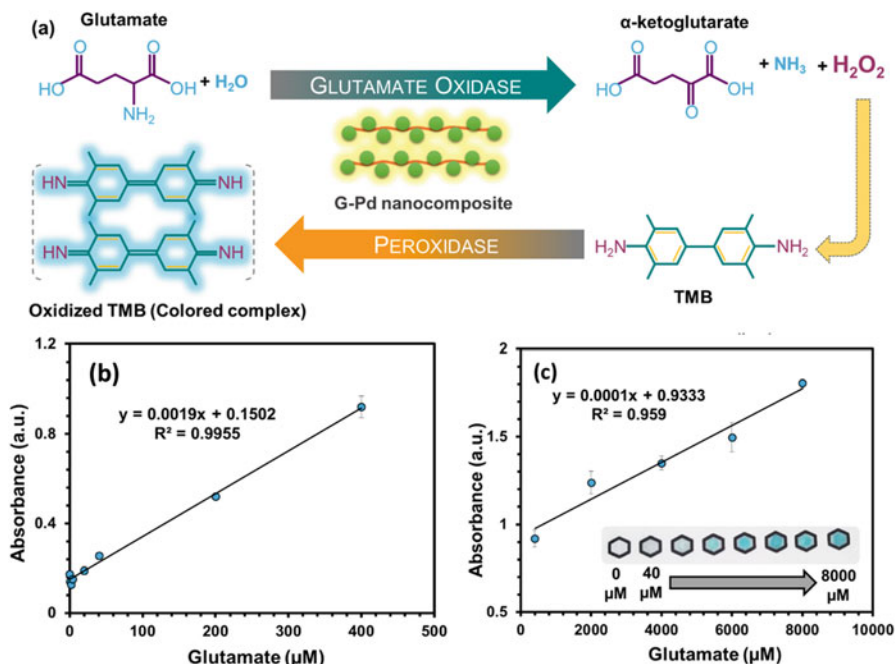


Fig. 11.4 (a) Working principle of graphene-palladium nanocomposite containing paper based colorimetric paper sensor. Graphene-palladium nanocomposite exhibits dual catalytic activity for the detection of glutamate in water samples. (b–c) Response of the developed colorimetric paper sensor towards glutamate (2). (Permission obtained)

interest in using smartphones for point of care and onsite analysis is mainly due to the following advantages. In terms of hardware design, they are compact and easy to operate; also, it provides access to location data and communicates necessary information to concerned stakeholders (Fig. 11.5). Smartphones are mainly integrated with most of the assay types for detecting a wide range of food compounds in solid and liquid matrices (Fig. 11.6) (Lu et al. 2019; Nelis et al. 2020). Smartphone-based sensors are reported to have applications in different types of food matrix with.

5.1 Electrochemical Sensors

Portable electrochemical sensors are developed with the help of a miniaturized potentiostat for generating the required electrical signals. Output from the electrodes can be processed through smartphones which overcome the use of bulky computers. An exclusive app for the quantification of electrochemical signals into a qualitative or quantitative measurement is necessary for the setup. Various types of food

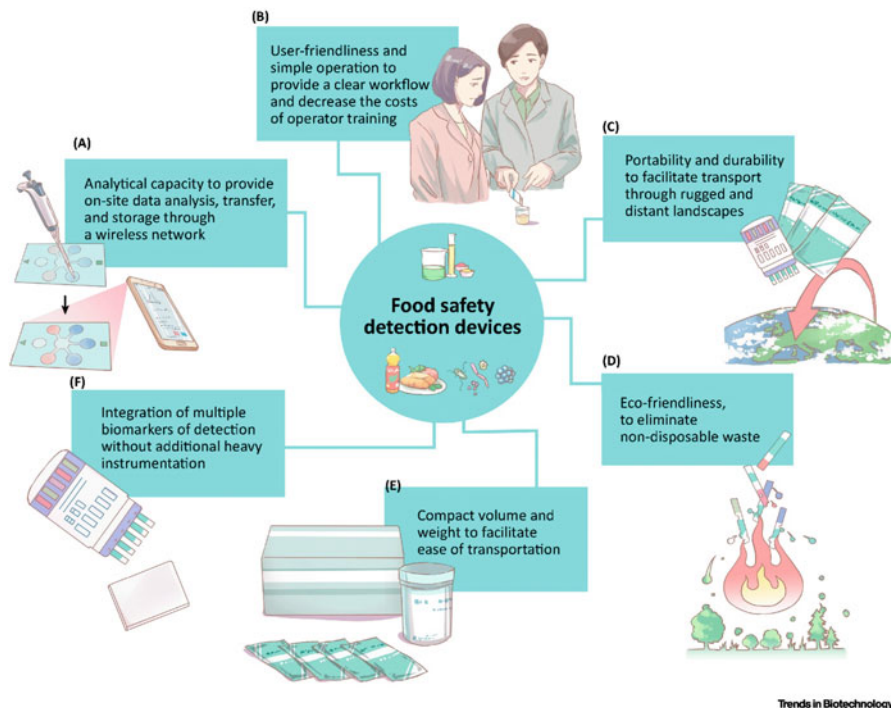


Fig. 11.5 Important features of smartphone based food safety detection devices compared to conventional analytical methods (3). (Permission obtained)

analytes are reported to be detected through smartphone-based electrochemical sensors (Nelis et al. 2020; Seo et al. 2019; Sivakumar and Lee 2021).

5.2 Optical Sensors and Microscopy

Compared to the smartphone-based electrochemical sensors, optical sensor integration requires the smartphone’s optical features and data processing facilities. For the data processing, unlike electrochemical sensors, certain commonly available apps can be used, however transducing the optical signals to an analyte concentration requires specific programs or apps for the smartphone (Fig. 11.7) (Nelis et al. 2020; Seo et al. 2019; Sivakumar and Lee 2021). Smartphone LEDs specifications vary widely from manufacturer to model, however, recent smartphones have advanced LED features such as provision for warm and cold colour temperature, better intensity and illumination. In colorimetric, fluorescence, luminescence, and spectroscopic methods, specific filters, excitation sources, and an external setup are necessary. It includes UV based LEDs in the sensor kit (mobile accessory) to excite

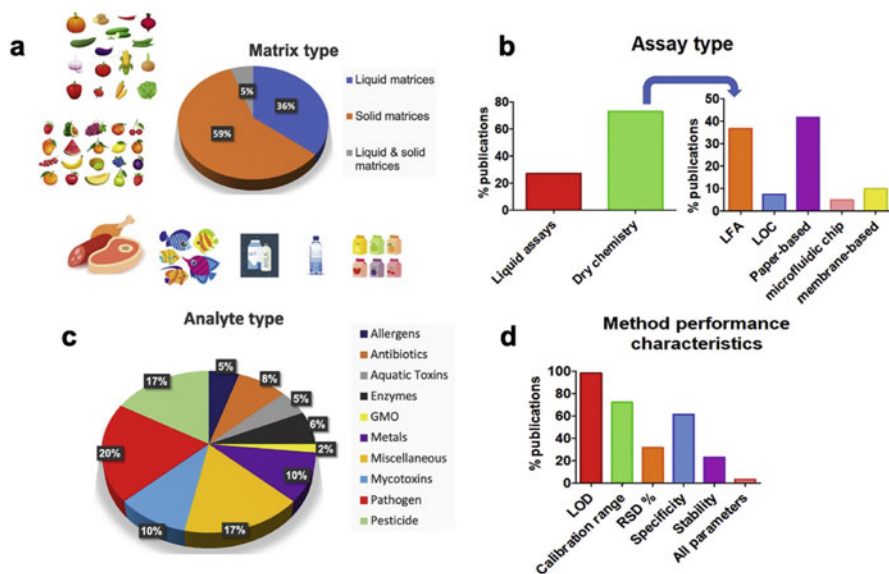


Fig. 11.6 (a) Summary of type of food matrix used for testing the developed smartphone based sensors. (b) Dry chemistry techniques are more often reported compared to liquid based sensing or testing methods. (c) Food analytes, toxins, contaminants against which the sensors are developed. (d) Analytical performance indicators analysed in the reported literature (4). (CC BY 4.0)

fluorophores in the assays (Rateni et al. 2017). Information related to the spectrum, colour temperature, intensity and other relevant information are seldom collected and presented in the literature. Overall, smartphones might serve as an excellent platform to test the quality and safety of food meant for consumption (traceability) as well.

6 Miscellaneous

Numerous types of sensors/biosensors for testing food quality and safety have been reported in the literature, and some of the promising technology/types are discussed below.

6.1 Microfluidics

Microfluidic devices are miniature devices designed through lithography methods predominantly composed of silica-containing compounds that are increasingly used for food testing. In a typical microfluidic device, microcapillary paths/pores are designed to move fluid by inherent capillary forces overcoming the need for an

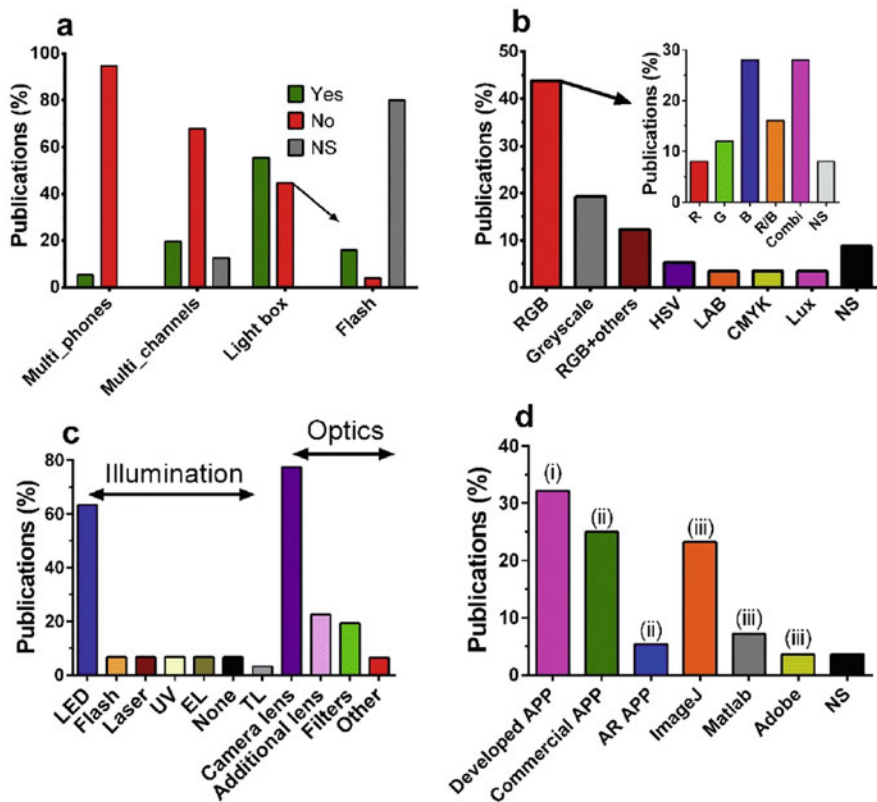


Fig. 11.7 Summary of smartphone based sensor publications (a): comparison of developed method with different phones, channels, light boxes and flashes to evaluate the performance. (b): Different type of color modes/space followed in the publications. (c): Illumination and optics used in the developed sensors. (d): Apps used in the reported literature, in which custom made and commercial apps used predominantly (4). (CC BY 4.0)

external force or pump. They are familiarly known as “lab on a chip” and “μpad” devices, which are further classified into microelectromechanical systems (MEMS) and micro total analysis systems (μTAS). As the name suggests, these devices require less volume of sample, can integrate multiple analysis into a single device, and importantly offers onsite analysis without the requirement of sample transportation. It also offers other advantages such as automation and high-throughput screening at an economical cost compared to conventional analytical methods to estimate food quality and safety parameters (Romao et al. 2017; Weng and Neethirajan 2017; Wu et al. 2017). Lab on a chip are the most promising devices with the potential for onsite detection/analysis. It combines multiple operations to be carried out in a small chip with approximately having an area of 20–30 cm².

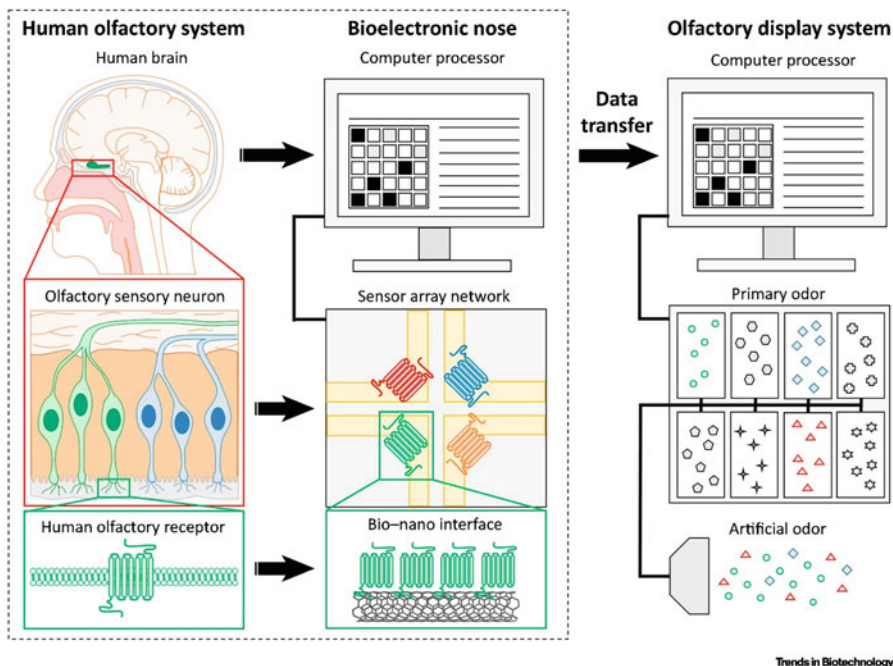
Paper-based microfluidic devices are known as ‘lab on paper’ in which cellulose-based paper is used as a substrate rather than silica-based molecules. Micro 2D and 3D patterns are fabricated to aid the movement of hydrophilic fluid through absorption and capillary action. Paper-based microfluidic devices are practical for one-time usage, low cost, biodegradable compared to silica-based microfluidic devices. Apart from cellulose, other cellulose-containing materials such as lignocellulose, bamboo, and cotton are explored for their suitability to develop sensors to detect food analytes (Malon et al. 2017; Wu et al. 2017).

In an exciting invention, Manu Prakash and his group have fabricated a paper-based centrifuge device named “paperfuge” to overcome sample preparation for onsite detection and diagnosis. It overcomes the need for using an expensive centrifuge for sample preparation at low resource environments for analysis. It is basically a microfluidic device containing a capillary tube utilizing human power to operate it akin to a “whirligig” capable of achieving 125,000 rpm or 30,000 g. Even though it has been demonstrated to separate plasma from the blood to detect malaria parasites (Bhamla et al. 2017). It can be generalized for handling food samples, which might overcome cumbersome sample preparation steps.

6.2 *Bio-electronic Nose or Artificial Nose*

Visual, hearing, and touch are classified into physical senses among the five human senses, whereas odour and taste are chemical senses. Among them, vision, hearing, and touch are standardized to a great extent through colour, sound, and texture estimation. In the case of taste, trained sensory panellist’s responses are universally accepted and followed. However, quantification of olfactory senses through panellists is contradictory, raising the need for a device-based evaluation. Bioelectronic nose or artificial nose or electronic nose (Fig. 11.8) is a complex device fabricated to sense and reconstitute odor as perceived by the human olfactory system (Fitzgerald et al. 2017; Gancarz et al. 2017). Conventionally, volatile organic compounds responsible for odour perception are primarily quantified through gas chromatography coupled with mass spectroscopy (GC-MS). However, in the case of electronic nose, detection/sensing mechanism is classified into three broad groups,

- (i) Electric – change in electrical response (mainly conductivity) in the presence of the analyte. The design includes field-effect transistors coupled with conducting polymers.
- (ii) Gravimetric – change in frequency due to the binding of analyte – Piezoelectric crystals conjugated to receptor binds with the analyte, which leads to change in mass on crystal, dampening the resonant frequency.
- (iii) Optical – Change in signal (fluorescence/chemiluminescence) intensity or shift in absorption or emission of the dyes – interaction between the dye and analyte results in the distinguishable optical signal, quantitatively or qualitatively correlated to the analyte concentration. In recent times, bar-coded resins with



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Fig. 11.8 (a) Comparison of human olfactory mechanism with working principle of bioelectronics nose. Receptor of human olfactory system are mimicked by fabricating a delicate composite of nanomaterial with biomolecules (bio-nano interface). Signals from the interface is transferred and processed with the aid of computers which is reconstituted by combining basic odours as perceived by the human interface in a standard format (5). (Permission obtained)

unique Raman or infra-red spectra (fingerprint) are used for rapid detection of volatiles (Fitzgerald et al. 2017; Sanaeifar et al. 2017; Son et al. 2017). More studies concerning calibration and validation are required to further improve the real-time application of these attractive electronic nose based devices.

DNA metabarcoding helps in the identification of species in a food matrix, which includes microbial, plant or animal origin proposed initially by researchers at the University of Guelph (Hebert et al. 2003). It involves extracting DNA from the food samples, its amplification, sequencing, bioinformatics analysis and species identification to figure out the DNA of different species present in the sample. It is better than DNA barcoding because it identifies multi-species DNA rather than a single species in a single reaction by including multi-species DNA data. Hence, it finds application in food authenticity to validate the ingredients and their source as claimed in the products, to identify the pathogenic/spoilage microbes in contaminated foods and other food quality and safety aspects (Bruno et al. 2019; Grützke et al. 2019).

Apart from the above discussed food testing methods and sensors, there are numerous novel methods and materials reported in the literature for food analysis; however, they need more exploration and studies to grow as a considerable method (Ragavan et al. 2018a; Ye et al. 2019).

7 Perspectives and Conclusion

Global food supply is a complex activity involving multiple parties, the onus to supply safe and quality goods rests with producers, suppliers, and concerned regulatory agencies. Any lapse in the quality or safety of food supplied impacts global trade adversely, which is evident from the past incidents (Hussain and Dawson 2013). Food production is getting better every day due to advancements in food processing methods, equipment design, and understanding of processing parameters and conditions. Most of the processing aims to be less resource-intensive with the least waste generation to comply with environmental and food regulations. Simultaneously, monitoring the process through automation and frequent quality checks results in better quality and safe products sustainably compared to earlier. Comprehensive food testing is necessary to ensure the food meant for consumption is free from hazards and quality as per marketing claims. However, it is affected by one or more following means in some instances, including food fraud, ineffective food laws and regulations, fraudulent science, and miscommunication (Neethirajan et al. 2018). In recent times, following food products and food analytes derived from new and alternative sources (GMOs, insect proteins, amnesic shellfish poisoning, and food processing contaminants) require more attention from the researchers and regulatory agencies to develop food testing methods and regulations, respectively. It is crucial to develop regulations and testing methods for most of the above products to gain consumer acceptability.

Smartphone-based methods offer multiple advantages for onsite food testing. It is relatively new in the market and is constantly evolving, so the developed methods might have a shorter life span than conventional analytical methods. Another important aspect is the plethora of detection strategies has to be communicated in a globally standardized format (Global standards). An additional requirement of sensor accessories/attachments might be a hindrance to its adoption by consumers. Also, the integration of these sensor attachments with different smartphones for hardware and software is a challenge. However, lack of communication and information can be solved with mobile/smartphone-based sensors for real-time monitoring and data sharing. Similarly, microfluidic-based devices, bioelectronics nose, and nanomaterial-based food testing methods are gaining prominence owing to their features. It is expected that they might have an even greater role to play in the coming days. Food matrix being a complex one, interferes with the outcome of the testing method in multiple ways leading to less useful information from the tests. Considerable improvement to overcome matrix effects in food samples is necessary to

render these techniques adopted everywhere. Research towards the development of innovative and straightforward steps for sample preparation without sophisticated instruments and solvents might help to an extent.

It is worth noting that, even though several thousand research articles are published every year with novel detection methods, they seldom move to industry for product development and marketing, evident from very few food testing devices in the market (Luong et al. 2008). Despite these advancements, reported sensors are in the nascent stage in real-life applications, which requires rigorous on-site testing and validation. Moreover, the food matrix is one of the most complexes and challenging due to multiphasic in nature with a plethora of compounds with a wide range of functional properties (Aguilera 2019). Hence, most detection methods are conveniently tested in simple matrices such as water (Nelis et al. 2020). Due to the above-said drawbacks, currently, there is no single method which can be used to detect most of the target compounds related to food quality, food fraud, food adulteration, or food safety. Analytical parameters of the developed method or the food testing device including data analysis need to be rigorously evaluated comprehensively to render them reliable and get approval from regulatory agencies (Wu et al. 2020). Emphasis on validation and calibration of the developed methods by the researchers is one of the solutions to come up with robust food testing methods/devices. Such recognition for food testing devices might increase the usage among consumers and expand the market share (Nelis et al. 2020). It is expected that developed sensors and biosensors to act as reliable food testing devices for the initial screening (a mode for early warning system) of samples for various parameters, which will certainly reduce the burden of overall cost spent for food analysis at the industry and marketplace. From a consumer, it is necessary to have a reliable qualitative food testing device to ensure the food /food product is safe and quality is the same as marketing.

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