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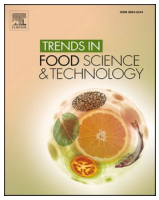

The role of DNA-based biosensors in species identification for food authenticity assessment

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ABSTRACT

Background: The increasing incidence of fraud demands for new robust methods to assess food authenticity. Over the past decades, biosensors have emerged as practical testing devices with exponential growth in diverse research fields. Their numerous advantages have contributed to their implementation in the food sector, with applications ranging from the identification of pathogens, chemical compounds and allergens to spoilage detection and Genetically Modified Organisms (GMO) identification in various food products.

Scope and approach: This review explores the development of DNA-based biosensors for food authenticity assessment with a focus on species identification. The role and versatility of nucleic acids as analytes and bio-recognition elements are discussed, and the available conventional methods are presented. The main transducing principles involved in biosensing, and the use of nanomaterials are briefly introduced. The application of various DNA-based biosensors over the last decade is shown, highlighting the main innovations and how these have contributed to the improvement of their performance. The final section addresses how different technologies can influence biosensor manufacturing and optimization, so these can become established rapid on-site testing devices used to assess food authenticity.

Key findings and conclusions: In the food sector, little research has been carried out in food authenticity regarding biosensors. The development of these devices is mainly aimed at species identification in meat and derived products, although other equally relevant products should be targeted. Several recent technological advances have been successfully integrated into biosensors and must be further explored to promote the establishment of these devices in food authenticity assessment.

1. Introduction

The assessment of food authenticity is a major concern for producers, manufacturers, retailers and inspection bodies who aim to ensure that no deliberate product alterations are made to increase profits. If such alterations are detected, they usually lead to a reduction of consumer confidence and loss of profits for producers (Sotirchos, Georgiou, & Danezis, 2017).

A food product is considered authentic if its composition, processing, and origin comply with the information provided to the consumer (González-Domínguez, 2022). Nowadays, various food products with specific geographical origins and production methods are officially protected by Protected Designation of Origin (PDO) or Protected Geographical Indications (PGI) labels. As these certified products have higher market value, they are more vulnerable to fraudulent practices by unscrupulous producers who buy cheaper raw materials from other species, varieties or regions and illegally sell the product under the same label (Camin et al., 2017).

The best way to guarantee that food products meet the legal standards is to ensure their monitoring throughout the entire process from production to consumption (Ye, Guo, & Sun, 2019). Advances in science and technology and the improvement of global living standards led to the establishment of various methods throughout laboratories to solve food-related issues (Ou, Jin, Fang, Tian, & Zhou, 2020). Currently, the

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most applied analytical methods in food fraud detection are based on polymerase chain reaction (PCR) (Mafra, Ferreira, & Oliveira, 2008), enzyme-linked immunosorbent assay (ELISA) (Asensio, González, García, & Martín, 2008), Fourier transform infrared spectroscopy (FTIR) (Rodriguez-Saona & Allendorf, 2011) and mass spectrometry (MS) (Dou et al., 2023). These are used depending on the characteristics of the targeted analytes (DNA, RNA, proteins, or metabolites) and the type of food samples analyzed. While PCR-based methods are used to detect nucleic acids, ELISA is directed mainly for proteins, FTIR for atoms and small molecules, and MS for metabolites and proteins. FTIR and MS are advanced high throughput techniques that require more complex instrumentation and data analysis than conventional PCR and ELISA. The two aforementioned methods provide fast and reliable results and are the most used for food authentication (Mohamad et al., 2022).

The potential of DNA as target for food authenticity assessment analysis has been uncovered throughout the last decades and is still a research topic of interest in the food field. In the review of Sultana, Azlan, Desa, and Mahyudin (2023), different PCR derived techniques for the identification of microbial and animal species in food products are presented, focusing on conventional DNA-based methods, and their advantages and limitations are also thoroughly discussed. While these traditional laboratory methods remain accurate and highly relevant in the food authenticity field, the time-consuming processes involved may hinder real-time responsiveness. The integration of DNA-based biosensors for onsite applications can offer a proactive, efficient, and cost-effective approach to ensure authenticity throughout the supply chain. It represents a decentralized strategy, aligned with the evolving needs of the food industry, promoting agility, sustainability, and prompt response to emerging challenges in the complex landscape of food fraud. Biosensors allow real-time monitoring at the point of production, processing, distribution, and at the retail level, which is crucial for quick decision-making and implementation of corrective actions if fraud is detected. While reducing the time and logistics associated with sending samples to centralized laboratories, the application of these devices can also reduce overall costs by eliminating the need for sample transportation and laboratory fees. This is especially relevant when routine screening and monitoring are required, for instance, to ensure the authenticity of large product quantities. In addition, biosensors are more user-friendly and require minimal technical expertise, so non-expert individuals such as quality control personnel or even frontline staff, can efficiently perform authenticity evaluations in food products.

Although there is currently a wide variety of published reviews discussing the application of biosensors in food analysis, those in the scope of food authenticity are scarce. In their review, Khalil et al. (2021) explored the DNA-based methods for the authentication of species, and those globally established in laboratories and their specific application in the authentication of meat species are presented. The authors also describe different electrochemical and optical biosensing configurations incorporating nanomaterials, that can be suitable for DNA detection. As practical examples, they present biosensors developed for a broader scope of applications including the detection of pathogenic microorganisms, cancer biomarkers and food allergens. Mohamad et al. (2022) undertook a more directed analytical perspective, and focused on the production and application of DNA fragments to detect different adulterants found in food products, from metabolites, amino acids and proteins to whole cells, pathogens, and chemical compounds. The authors propose potential detection targets that can be used generally in food research, and in sensing platforms to expedite authentication analysis. Xia et al. (2022) explored the application of isothermal nucleic acid amplification in food safety, where the detection of nucleic acid and non-nucleic acid targets is discussed. Among other topics, the authors briefly discuss the implementation of this methodology into biosensing devices developed for authenticity assessment, through the detection of genetic markers present in meat species. In the review published by Melinte, Hosu, Cristea, and Marrazza (2022), the authors summarize the recent progress of electrochemical and optical DNA-based biosensors, developed to detect various contaminants in food products, including pathogens, toxins, allergens, pesticides, and other chemicals/additives.

While these publications shed light on different relevant individual topics, the current literature lacks a unified view on the development of DNA-based biosensors for food authenticity assessment, with focus on species identification. The purpose of this review is to fill the gap on the existing research regarding this field. Our main aim is to merge the current knowledge on the role of DNA as target for species identification strategies, with the potential of biosensors as alternative platforms for authenticity assessment of food products. To provide an overview on the evolution of these devices regarding authenticity, the biosensors described in the literature during the last decade are presented, highlighting the main innovations that were incorporated into their configurations. Different recent technological advancements that can contribute to the enhancement of biosensors are also discussed. Overall, this work can be a starting point for the development of new and improved devices, capable of performing a more broad and practical authenticity evaluation of food products through specific DNA analysis.

2. Targeting DNA in food– authenticity markers detection through conventional methods

DNA analysis is the basis of various methods for species identification and discrimination, a task often performed to assess food authenticity and a relevant research topic in the field (Hellberg, Hernandez, & Hernandez, 2017; Piredda et al., 2022; Verdone, Rao, Coppola, & Corrado, 2018). Compared to proteins, metabolites and other molecules, DNA is more stable and can endure harsh conditions. It is also abundant in all cell types and can be easily extracted from most samples. These properties make DNA an ideal target for the detection and quantification of food fraud and adulteration (Khalil et al., 2021).

Presently, the process of species identification in food is largely based on DNA marker analysis. These allow the identification of genetic variability in a genome and can be very useful in highlighting diversity on an inter and intra-species level (Scarano & Rao, 2014). DNA markers can be identified in genes from the nuclear genome, the mitochondrial genome, and the chloroplast genome. In animal samples and derived products, most applied markers are located in the mitochondrial genome. These include the ATPase subunit 6 and 8 genes (*ATPase6* and *ATPase8*), the cytochrome *b* gene (*cytB*), the cytochrome *c* oxidase subunit I, II and III genes (*COI*, *COII*, and COIII), mitochondrial D-loop region fragments, 12S ribosomal RNA (*12SrRNA*) and 16S ribosomal RNA (*16SrRNA*) genes, tRNA valine (*tRNA-Val*), NADH dehydrogenase 5 (*ND5*) and NADH dehydrogenase 2 (*ND2*) genes (Ali, Razzak, & Hamid, 2014). These genes have a higher copy number in the cells and are suitable for qualitative analysis. Since their copy number varies between species, organisms and even tissues in the same organism, they are not suitable for quantitative assessment. In these cases, markers present on nuclear genes are preferred (Böhme, Calo-Mata, Barros-Velázquez, & Ortea, 2019).

In plant species identification, most applied markers are located in genes of the chloroplast genome, as their mitochondrial genomes are too stable to provide sufficient variation. The main DNA markers used are located in the RuBisCO large subunit (*rbcL*) gene, the maturase K (*matK*) gene, and the intergenic regions trnH-psbA, atpF-atpH and psbK-psbI. Analysis of the ITS region in the nuclear genome has also been reported, but as it is highly conserved, it can only be used to discriminate organisms at the level of orders, classes, and phyla (Nehal, Choudhary, Nagpure, & Gupta, 2021). Other nuclear DNA markers are reported to be able to discriminate between varieties of the same species, mainly simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), for example in olive cultivars (Carvalho et al., 2021; Gomes, Breia, Carvalho, Carnide, & Martins-Lopes, 2018), in grapevine varieties (Gomes, Castro, et al., 2018; Pereira et al., 2017) and in rice varieties (Vieira, Faustino, Lourenço, & Oliveira, 2022; Yuan et al., 2022).

Over the years, new analytical techniques based on DNA marker

analysis have been developed to verify the authenticity of food (Fig. 1). The polymerase chain reaction (PCR) stands as the standard technique for DNA detection. It is based on the amplification of an initial small amount of DNA fragments, whereby several cycles of sequential denaturation, annealing and extension steps result in a much larger amount of a specific target sequence (Böhme et al., 2019). Further methods derived from conventional PCR promptly emerged. These include species-specific PCR, PCR-restriction fragment length polymorphism (PCR-RFLP), random amplified polymorphic DNA (RAPD), multiplex PCR, real-time PCR, digital droplet PCR (ddPCR), High Resolution Melting (HRM), DNA sequencing and DNA barcoding (Dawan & Ahn, 2022; Li, Yu, Xu, Chen, & Han, 2023).

These techniques are known for their selectivity and sensitivity and provide good results in most samples. Despite numerous advances, there are still limitations related to sample complexity. They rely mostly on the analysis of relatively larger DNA fragments, which are very difficult to obtain from highly processed and complex food samples, hampering the success of the analysis (Sultana et al., 2023). Regardless of the chosen method, performance always depends on sample preparation, analyte extraction and purification steps. Extraction buffers, reagents, and other parameters must be optimized according to the type of food sample tested. Another limitation is the instability of the analytes, especially when they are present in processed food items (Mohamad et al., 2022). During food processing, DNA fragments can undergo heating treatments, pH changes and fermentations that can impact their stability and alter their structure (Bogani et al., 2009). This can render the analysis ineffective or lead to false negative results, as the analyte may be present in the sample, but it is simply too altered to be detected. The occurrence of artifacts can also complicate the interpretation of results, as has been reported in real-time PCR analysis of sample extracts collected from processed samples (Ali, Hashim, Mustafa, Che Man, & Yusop, 2011). Other limitations can be pointed out from a more technological perspective. These types of laboratory-based techniques are dependent on specialized technicians, equipment, and infrastructures, as well as complex protocols, resulting in time-consuming and expensive analyses. This can limit the number of samples that can be processed and makes the application of these methods for rapid on-site monitoring of food samples impossible.

3. Biosensors – an alternative approach

Biosensors arose as a promising alternative to established methods in various areas of research (Justino, Duarte, & Rocha-Santos, 2017; Lino et al., 2022; Pohanka, 2019), including the food sector (Barrias, Fernandes, Eiras-Dias, Brazão, & Martins-Lopes, 2019; Griesche & Baeumner, 2020). A biosensor can be defined as an analytical device that combines a biorecognition element with a physicochemical component working that acts as a transducer and generates a measurable signal to detect a biological analyte: target DNA/RNA, enzyme substrate, antigen, whole cells, among others (Perumal & Hashim, 2014). The implementation of these devices depends on the fulfilment of several requirements: high sensitivity, reliability, portability, cost-effectiveness, and rapid response (Labuda et al., 2010; Lehotay & Chen, 2018).

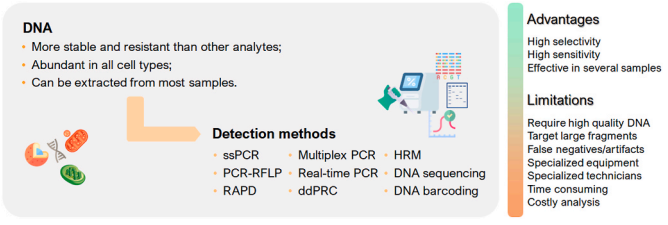

Fig. 1. Food authenticity using conventional DNA-based methods, their advantages and limitations.

There are numerous configurations that can be designed taking advantage of the many materials and biomolecules applicable to biosensor construction. These devices can use different biorecognition elements, such as antibodies, aptamers, enzymes, or cells (Bhalla, Jolly, Formisano, & Estrela, 2016). The binding of the analyte to these molecules causes a physicochemical change in its properties that is detected by the transducer, which converts it into a type of signal that is usually proportional to the extent of the interaction between the analyte and the bioreceptor (Tetyana, Shumbula, & Njengele-Tetyana, 2021). The primary role of a biorecognition element is to capture the specific target analyte that the biosensor is designed to detect. It determines the specificity of the biosensor, where a strong and selective binding between the biorecognition element and the target analyte must occur (Morales & Halpern, 2018).

3.1. Applying nucleic acids as biorecognition element in biosensors

Recent advances in the synthesis and analysis techniques of nucleic acids, along with their enhanced biocompatibility, durability, and flexibility, have made them efficient and desirable biorecognition elements for biosensor development. The convenience of designing and synthesizing their sequence and structure to serve as a probe and match a target analyte, as well as the achievement of high selectivity and sensitivity, are fundamental factors to ensure the efficiency of biosensors. In addition, nucleic acids can be utilized as the basis for different signal amplification strategies, further increasing the sensitivity of DNA-based biosensors (Shalileh, Sabahi, Golbashy, Dadmehr, & Hosseini, 2023). The advantages of DNA-based biosensors in relation to other configurations, such as immunosensors or enzymatic biosensors, are inherent to these advantages of using nucleic acids as biorecognition elements. DNA biosensors are very customizable and allow the detection of a variety of target analytes besides nucleic acids, as proteins, small molecules, and cells. They also have higher thermal stability, longer shelf life and the ability to be regenerated and reused without significantly reducing their functionality (Yu, He, Wang, & Cui, 2023).

During the last decades, a class of single-stranded oligonucleotide sequences, known as aptamers, became increasingly used in biosensing, mainly due to the systematic evolution of ligands by exponential enrichment (SELEX) method (Ellington & Szostak, 1990; Tuerk & Gold, 1990). This technology allows to efficiently generate specific nucleic acid probes against nucleic and non-nucleic acid targets, with high affinity and specificity in a relatively short time. Aptamers also present the advantages of easy scale synthesis, long-term stability, and low production costs, which tend to decrease with the further development of SELEX technology itself (Douaki et al., 2022). They can also be easily modified with a variety of chemical groups (thiol, amine groups, biotin, etc.) to optimize their immobilization in different surfaces (Xing, Sun, et al., 2022). After synthesis and selection of the best design, the specificity of the selected aptamer needs to be validated through different binding experiments before it can be used in biosensing (Mohamad et al., 2022).

Other nucleic acid derivatives obtained through SELEX are deoxyribozymes or simply DNAzymes, synthetic DNA sequences capable of performing catalytic activities. They can be easily synthesized and designed to perform rapid binding with high target affinity and high selectivity towards undesired targets (McConnell et al., 2021). Conventional biosensors have mostly used enzymes (Bollella et al., 2018; Varmira et al., 2018; Zhou et al., 2021) and antibodies (Freitas, Neves, Nouws, & Delerue-Matos, 2021; Melo et al., 2016; Zhang et al., 2019) as biorecognition elements, but their synthesis and optimization for different analytes can be expensive, inconsistent, and difficult to scale up. This can hamper their application in biosensing devices, at a time when there is a great demand for new and improved biosensors for large scale testing on a vast variety of samples. In comparison, DNAzymes provide better stability and detection sensitivity when incorporated into biosensors, and once initially identified, these elements can be easily

produced in large quantities at a fraction of the cost (Khan et al., 2021).

DNA can also be used as a building block to form more complex 2D and 3D nanostructures applied as biorecognition elements. These can be precisely synthesized with personalized size and shape, resulting in various designs, including Y-shaped scaffolds, DNA tetrahedrons, polyhedrons, prisms, DNA dendrimers, DNA hydrogels and DNA origami (Wang et al., 2021). The increasing complexity of these structures results in difficulties and disadvantageous properties, starting with their production. A large number of sequences must be obtained to assemble the nanostructure, increasing the production costs and making manufacturing more time-consuming than synthesizing other DNA-based probes. The process of obtaining reproducible functional nanostructures can also be laborious and greatly dependent on previously acquired experience. In addition, the lack of consensus regarding the stability and nuclease resistance of DNA nanostructures in different media has been discussed (Chandrasekaran, 2021). This can make them unsuitable for the construction of a biosensor for food analysis, due to the nature of most food samples that need to be tested. Nevertheless, DNA probes are still very interesting due to the possibility of using many modifiable sites within the probe sequence, their predictable structure, their high thermal stability, and biocompatibility.

3.2. Transduction principles applied in biosensors

The wide variety of elements available for the development of biosensors extends from the class of biorecognition elements to the class of transducers. According to the transduction principle underlying its functioning, a biosensor can be classified into different categories (Fig. 2), of which electrochemical, optical, and gravimetric are the most common.

The most developed and commercialized biosensors are electrochemical. They are relatively easy to functionalize and do not require expensive manufacturing processes (Huang, Xu, Liu, Wang, & Chen, 2017). These biosensors use several types of inexpensive and easily integrated electrodes to convert biological events into measurable electrical signals and can be further categorized as amperometric, voltammetric, conductometric, impedimetric, potentiometric and field-effect transistor (FET)-based (Thevenot, Tóth, Durst, & Wilson, 1999). Overall, electrochemical biosensors offer a number of advantages, such as low manufacturing cost, portability and miniaturization potential, fast and scalable signal acquisition with low background noise, good selectivity and high sensitivity, and the ability to perform multiple analyte detection. On the other hand, their performance can be affected by electromagnetic interference, so one must consider the environment in which the device is to be used (Campuzano, Montiel, Serafín, Yáñez-Sedeño, & Pingarrón, 2020; Singh et al., 2021).

Optical biosensors are based on the detection of changes in the optical properties of the system, caused by the interaction between the analyte and the biorecognition element. These devices are particularly interesting, mostly due to their sensitivity and selectivity (Qiao, Fu, Lei, & Li, 2020), although their application may be ineffective in opaque or turbid samples, due to naturally present pigments or other fluorescent substances (Shruti, Bage, & Kar, 2024). To surpass this, samples can be diluted, which can be very problematic if a scarce initial amount of analyte is present. Another aspect to consider when developing an optical biosensor is the fact that ambient light also affects detection. Some subtypes of these optical biosensors are based on fluorescence, surface-enhanced Raman scattering (SERS), photonic crystals, guided mode resonance or plasmonic (Chen et al., 2020). Chemiluminescent (CL), electrochemiluminescent (ECL) biosensors (Roda et al., 2016) and colorimetric biosensors can also be counted as optical subtypes (Maddali, Miles, Kohn, & O'Carroll, 2021). The advantages of colorimetric biosensors are naked-eye analyte detection, the simplicity, the low cost, and the fact that no expensive measuring devices are required (Dai, Li, Zhang, Fu, & Li, 2018). Photoelectrochemical biosensors (PEC) combine features of optical and electrochemical sensing; the detection process relies on the irradiation of a photoactive material by a light source, which leads to charge transfer reactions between this material, the analyte and an electrode (Devadoss, Sudhagar, Terashima, Nakata, & Fujishima, 2015). When compared with optical biosensors, analyte detection using PEC biosensors is more rapid, simple, and specific. Moreover, their production is cheaper and have greater miniaturization potential. In comparison to electrochemical biosensors, these devices achieve higher sensitivity due to the reduced background signals (Zhao, Xu, & Chen, 2014).

Gravimetric biosensors are based on the detection of mass exchange between the analyte and the biorecognition element. They are based on the piezoelectric effect that some crystalline classes possess. With the

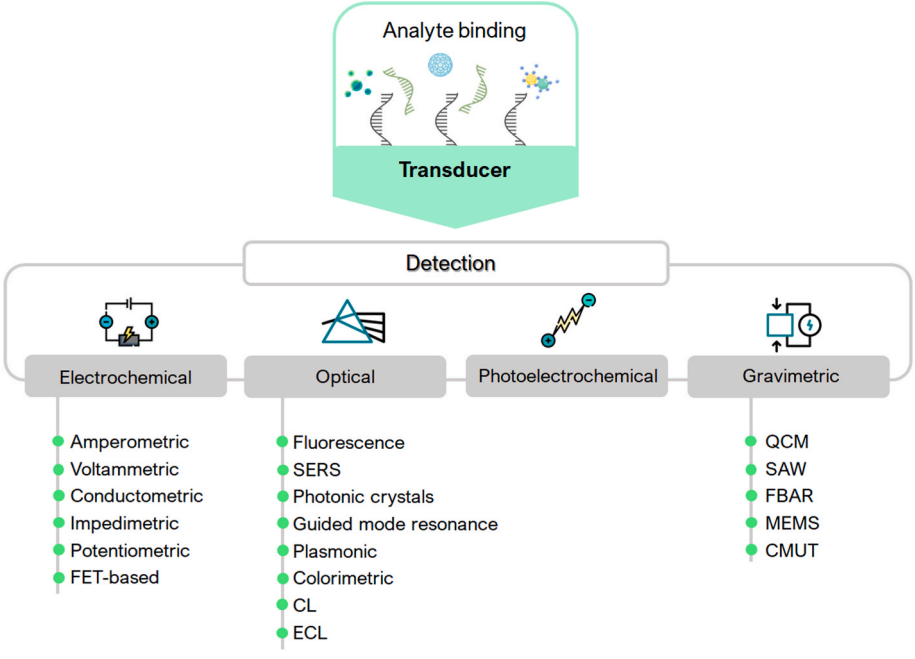

Fig. 2. Biosensor classification according to the transduction principle used for detection upon target analyte binding.

help of this effect, a mechanical resonance oscillation can be triggered in specially shaped crystals by applying a favorably aligned electric field to the crystal (Walton, O'Flaherty, Butler, & Compton, 1993). The most used piezoelectric biosensor platform is the quartz crystal microbalance (QCM), which usually consists of a piezoelectric quartz crystal inserted between two metal electrodes, typically made of gold. Such biosensors measure changes in the resonant frequency of the crystal caused by mass changes at one of the electrodes of the sensor device (Ferreira, da Silva, & Tomé, 2009). The mass changes are caused by the interaction between the bioreceptor immobilized on the electrode surface and the biological analyte (Pohanka, 2018). Other gravimetric biosensor subtypes are based on surface acoustic wave (SAW), film bulk acoustic resonators (FBAR), microelectromechanical systems (MEMS) and capacitive micromachined ultrasonic transducers (CMUT) (Cali, Tuccori, & Persaud, 2020). These sensors allow label-free and real-time detection of biological events, but practical applications can be hindered by the small size and fragility of the crystalline materials employed (Pramanik, Pingguan-Murphy, & Osman, 2013). Since they are based on mass changes, a major limitation of these biosensors is interference from non-targeted molecules present on real samples, capable of binding to the transducer surface, resulting in non-specific adsorption (Tombelli, Minunni, & Mascini, 2005).

Looking at the variety of biorecognition elements and transduction methods available for biosensor development (Fig. 3), the advantages of individual elements compared to others can be easily pointed out. However, the performance superiority of one biosensor configuration over another is not so direct and always depends on the specific application intended for the device, the predicted interaction between the sensing layer and the analyte, and the characteristics of the sample itself. All factors must be considered when choosing the most appropriate platform for food testing.

3.3. Construction of the sensing interface

A critical aspect in biosensor development is the construction of the sensing interface, where the biorecognition and detection processes are carried out. It involves careful optimization that ultimately determines the sensitivity, selectivity, and overall performance of a functional biosensor. Surface modification and DNA probe immobilization are key steps involved in the construction of this interface (Shi et al., 2022).

A sensing interface surface can be modified using physical or chemical techniques. Physical modification changes the surface morphology while avoiding any chemical alteration. It usually involves ultraviolet, plasma, or laser irradiation onto the surface. On the other hand, chemical modification alters the surface chemistry at its most superficial layer, through application of single-layer or multilayer coatings (Roh, Jang, Yoo, & Seong, 2023), which may be composed by different materials such as metals, metal oxides, carbon-based materials, magnetic nanoparticles (MNPs) and polymers (Sonawane & Nimse, 2016). The immobilization of DNA probes and the overall performance of the sensing interface are dependent on surface characteristics that are altered during modification, including hydrophilicity/hydrophobicity, surface charge, surface topography and surface area. These parameters are discussed with more detail by Tjong et al. (2014).

Surface modification can also provide ligands for specific probe immobilization and prevent non-specific interactions by blocking the vacant surface space, inhibiting the binding of untargeted molecules. This non-specific binding can lead to high background signals and interfere with the detection process (Reimhult & Höök, 2015). This is an important aspect in developing biosensors to test complex samples, which is the case for most food samples, that contain multiple different molecules besides the target analyte. Otherwise, these could bind to the sensing surface and compromise biosensor analysis. Depending on substrate material, untargeted molecule binding can be blocked using various molecules, including bovine serum albumin (BSA), dithiothreitol (DTT), 6-mercaptohexanol (MCH) and 3-mercaptopropionic acid (MPA) and polyethylene glycol (PEG) (Campuzano, Pedredo, Yáñez-Sedeño, & Pingarrón, 2019). These can also act as spacers and contribute to a correct orientation of DNA probes during immobilization. Probe density and orientation are two correlated factors that must be controlled during immobilization, to assure that the probes are accessible for target binding. At lower probe densities, the lateral spacing between DNA probes increases and these tend to lie horizontally and flat on the vacant surface by nonspecific adsorption, becoming inaccessible to target binding. If higher probe densities are used during immobilization, the repulsion between the negatively charged phosphate backbone of DNA probes forces them to assume an upright orientation away from the surface. However, if the probe density is too high, the target binding can be inhibited due to steric hindrance and electrostatic forces, resulting in very low or absent detection signals (Ye, Zuo, and Fan (2018).

The immobilization of DNA probes is a fundamental topic that has been thoroughly reviewed by many authors, per example Khan et al. (2021), Thapa, Liu, and Wang (2021) and Zhang and Hu (2014). These publications provide a detailed description of the different immobilization methods, from which we can highlight three main strategies. The most straightforward method relies on the direct immobilization of unmodified DNA probes onto surfaces by physical adsorption. Although direct and simple, the adsorption can be weak and nonspecific, resulting in high background noise, low target binding efficiency and low sensitivity. Another method requires the modification of DNA probe at one end, adding an appropriate functional group such as aldehyde (CHO), amine (NH_2), carboxyl (COOH), or thiol (SH), so they can form a covalent bond with the corresponding functional group present on the surface. This immobilization method is slower and more complex than physical adsorption, but it usually results in higher binding strength and higher probe stability. Other immobilization strategy is based on the bioaffinity interaction between biotin and avidin/streptavidin. In this method, biotinylated probes are immobilized onto an avidin/streptavidin modified surface by a strong non-covalent bond between these

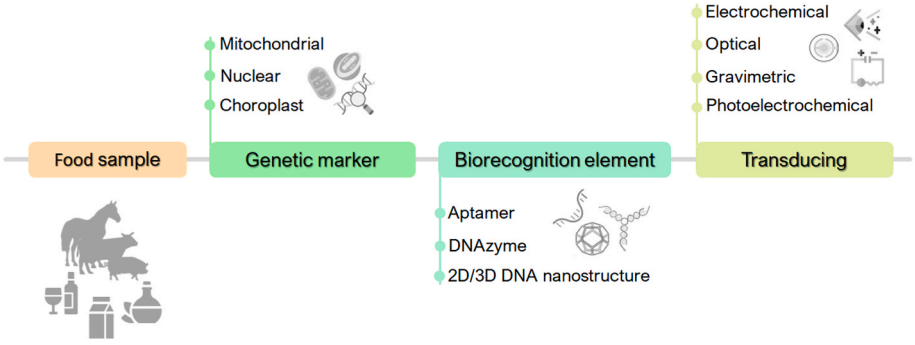

Fig. 3. Different options to consider while designing a DNA-based biosensor aimed at food authenticity assessment.

molecules. This results in an overall stable and highly specific binding, but the process involves multiple steps, which increases the fabrication costs. Given than avidin and streptavidin are proteins, their structure and binding affinity may be affected by storage and assay conditions, hampering the functionality of the sensing interface. Considering the binding stability, cost and complexity of all methods, the immobilization of modified DNA probes by covalent binding may be the most well-suited approach to take in the assembly of a biosensor to analyze most food samples.

3.4. Enhancement of biosensor performance: application of nanomaterials

To ensure the quality and reliability of the results obtained through biosensor analysis, the analytical performance of the device must be evaluated and validated. This is achieved by defining their figures of merit, e.g. sensitivity, selectivity, limit of detection (LOD), and limit of quantification (LOQ) (Justino, Rocha-Santos, & Duarte, 2010). These parameters can be enhanced using a variety of nanomaterials to modify

Table 1
Developed DNA-based biosensors for food authentication through species identification.

Biosensor classification	Gene marker	Biorecognition Element	Tested samples	DNA extraction	PCR	LOD	Reference
Fluorescent	*cytB*	DNA aptamer	Pork meat samples, processed meat samples and cooked meatballs.	Yes (commercial kit)	No	1% adulteration	Ali et al., 2011; Ali, Hashim et al., 2014
Colorimetric	*Accg8, Sad, Ivr1, Lectin, Arah2,* Helianthinin, *Ses i 1, MT3-B.*	DNA aptamer	Peanut, cotton, palm, sesame, maize, sunflower, soybean and rape leaves and oil samples	Yes (optimized protocols)	Yes	0.1 fmol	Bai et al. (2011)
Colorimetric	*GAG56D, SPS, Ivr1, Lectin, legS, ST-LS1*	DNA aptamer	Wheat, corn, potato, pea, soybean and rice flour	Yes (optimized protocol)	Yes	0.1 fmol	Bai et al. (2013)
Colorimetric	D-loop region, *cytB*	DNA aptamer	Deer, rabbit, duck, chicken, beef, horse, sheep, and pork meat samples, processed meat products.	Yes (optimized protocol)	Yes	0.001% adulteration	Wang, Zhu, Chen, Xu, and Zhou (2015)
Colorimetric	*COI, ITS2*	DNA aptamer	Perch and saffron samples.	Yes (commercial kit)	Yes	1% adulteration	Valentini et al. (2017)
Amperometric	D-loop region	RNA aptamer	Beef, horse, turkey, chicken, and pork meat samples, mixed meat samples	No	No	0.5% adulteration	Montiel et al. (2017)
CL	12SrRNA	DNA aptamer	Chicken, beef, lamb, turkey, pork and beef meat samples, mixed meat samples	Yes (optimized protocol)	No	1% adulteration	Torelli et al. (2017)
Colorimetric	*Gcg*	DNA aptamer	Raw meat from 22 animal samples, processed meat products	Yes (commercial kit)	No	10 pg	Xu et al. (2017)
ECL	*cytB*	DNA aptamer	Sheep, ostrich, turkey, goat, buffalo, duck, horse, pig and wild boar, processed food samples, meat mixtures.	Yes (commercial kit)	No	0.1 pg/μL	Azam et al. (2018)
Colorimetric	Prolactin receptor gene, *GHR, β-actin*	DNA aptamer	Sheep, pig, horse, beef meat samples, mixed meat samples	Yes (commercial kit)	Yes	0.01% adulteration	Magiati, Myridaki, Christopoulos, and Kalogianni (2019)
Optical LPG	*F3H*	DNA aptamer	Grapevine leaf, must and wine samples	Yes (optimized protocol)	No	–	Barrias et al. (2019)
Voltammetric	*cytB*	DNA aptamer	Pork meat samples, cooked meatballs	Yes (commercial kit)	No	0.58 μg/mL 0.135 μg/mL	Hartati et al., 2019; Hartati et al., 2023
Colorimetric	Species specific nuclear DNA fragments	DNA aptamers	Cow, sheep and goat yogurt and mixed yogurt samples	Yes (commercial kit)	Yes	0.01% adulteration	Bougadi and Kalogianni (2020)
Optical SPR	Donkey DNA marker	DNA aptamer	Cooked sausages	Yes (commercial kit)	No	1% adulteration	Mansouri et al. (2020)
Voltammetric	Bovine specific DNA sequence	DNA aptamer	Cattle muscle samples	Yes (optimized protocol)	No	8.2 fM	Zhang, Wang, Lin, Liu, and Zhou (2020)
SERS	*cytB*	DNA aptamers	Pork meat samples	Yes (commercial kit)	No	1 fM	Khalil et al. (2020)
Voltammetric	*cytB*	DNA aptamer	Pork and beef meat samples, mixed samples	No	No	1% adulteration	Flauzino et al. (2021)
Impedimetric	*cytB*	DNA aptamer	Pork and beef meat samples, mixed samples	No	No	9% adulteration	Flauzino, Nguyen et al., 2022
SERS/ colorimetric	*ND2*	crRNA fragment	Lamb roll, pork, beef, mutton, steak, duck meat samples, mixed meat samples.	Yes (commercial kit)	No	0.05%/0.1% adulteration	Liu et al. (2021)
SERS	*cytB*	crRNA fragment	Goat milk products	Yes (commercial kit)	No	224 aM	Pan et al. (2022)
FET-based	*F3H*	DNA aptamer	Grape and wine samples	Yes (commercial kits)	Yes	0.19 aM	Purwidyantri et al. (2022)

Table 1 abbreviations: CL – chemiluminescent; ECL – electrochemiluminescent; FET – Field effect transistor; LPG – Long period grating; SERS – Surface-enhanced Raman spectroscopy; SPR – Surface plasmon resonance.

the surface of the transducer.

The development of DNA-based biosensors in which nanomaterials are incorporated into the configuration is currently a hot topic for researchers and industry. They present remarkable selectivity, sensitivity, low cost, miniaturization power and on-site application potential (Vikrant, Bhardwaj, Bhardwaj, Kim, & Deep, 2019). Nanomaterials have various optical, electromagnetic, and structural properties that can greatly improve the performance of the transducer surface. They can be applied in the immobilization of biorecognition elements, in the labelling of biomolecules, as catalytic agents in electrochemical reactions and to enhance electron transfer at the transducer surface (Khalil et al., 2021). The progress achieved in material characterization led to the emergence of different types of nanomaterials: nanoparticles (metal, metal oxide and magnetic); carbon nanotubes (single-walled carbon nanotubes – SWCNTs, multi-walled carbon nanotubes - MWCNTs); graphene nanostructures (graphene oxide – GOx, graphene quantum dots - GQDs); polymer nanocomposites – PNCs, and fullerenes (Malik, Gupta, Malik, & Ameta, 2021). Besides nanomaterials, metal–organic frameworks (MOFs) and covalent organic frameworks (COFs) have also gathered a lot of attention in biosensor development. These are porous crystalline materials with large surface area, variable structures, and an abundance of functional sites (Yuan, Li, & He, 2021).

Overall, the implementation of these materials aims to improve biosensor sensitivity, while lowering the LOD in a minimal response time.

4. DNA biosensors developed for species identification in food products

The development of DNA-based biosensing devices has increased substantially in the last decade with a great impact in the food sector. Their applications have been thoroughly discussed in numerous reviews, and include the detection of pathogens, pesticides, heavy metals, allergens and genetically modified organisms (GMO). In comparison, the development of such devices for food authentication remains less pursued, even considering their potential in identifying the species present in food products through the analysis of DNA samples. In Table 1 are shown the different DNA-based biosensors developed for species identification in several food products, some of which are discussed in more detail on the following topics. In Fig. 4 are presented the different parameters and alternatives that are usually considered in the development of the most typical biosensor configurations for food authenticity.

4.1. Meat samples and derived products

The meat industry represents an extremely valuable market that is also very susceptible to fraudulent practices. The addition or substitution of lower quality and lower value meat from undeclared animal species, in order to increase profits is a common occurrence. This can have serious implications to the consumers, either from a religious and cultural perspective, (noncompliance with halal and kosher standards) or from a health standpoint (appearance of new allergies to different meat proteins) (Flauzino, Alves, Rodovalho, Madurro, & Brito-Madurro, 2022). Therefore, species identification in meat products has become the main objective of a wide range of authenticity assessment methods, and this trend can also be observed in biosensor development, with most reported biosensors being used for this particular task.

Ali et al. (2011) developed a fluorescent biosensor to detect and quantify pork DNA in raw meat samples and products subjected to autoclave conditions. They immobilized a DNA aptamer complementary to a *cytB* gene fragment on the surface of citrate-coated gold nanoparticles (AuNPs). Upon hybridization between the aptamer and the targeted DNA resultant of target binding, changes in the structural conformation of the functionalized nanoparticles generated a fluorescent signal that was sensitive and specific to the hybridization. Three years later, the same group used the same biosensor configuration to detect and quantify pork DNA in raw and cooked meatballs to further prove the applicability of the device in more processed and complex meat samples. The detection process was dependent on DNA extraction but did not require PCR amplification, and the biosensor was able to detect up to 1 % pork adulteration in beef meatball preparations (Ali, Hashim, et al., 2014). In 2015, Wang et al. used a colorimetric biosensor to detect DNA from eight animal species in meat samples and processed commercial food products. They coupled eight DNA aptamers targeting different fragments of mitochondrial DNA (mitochondrial DNA D-loop

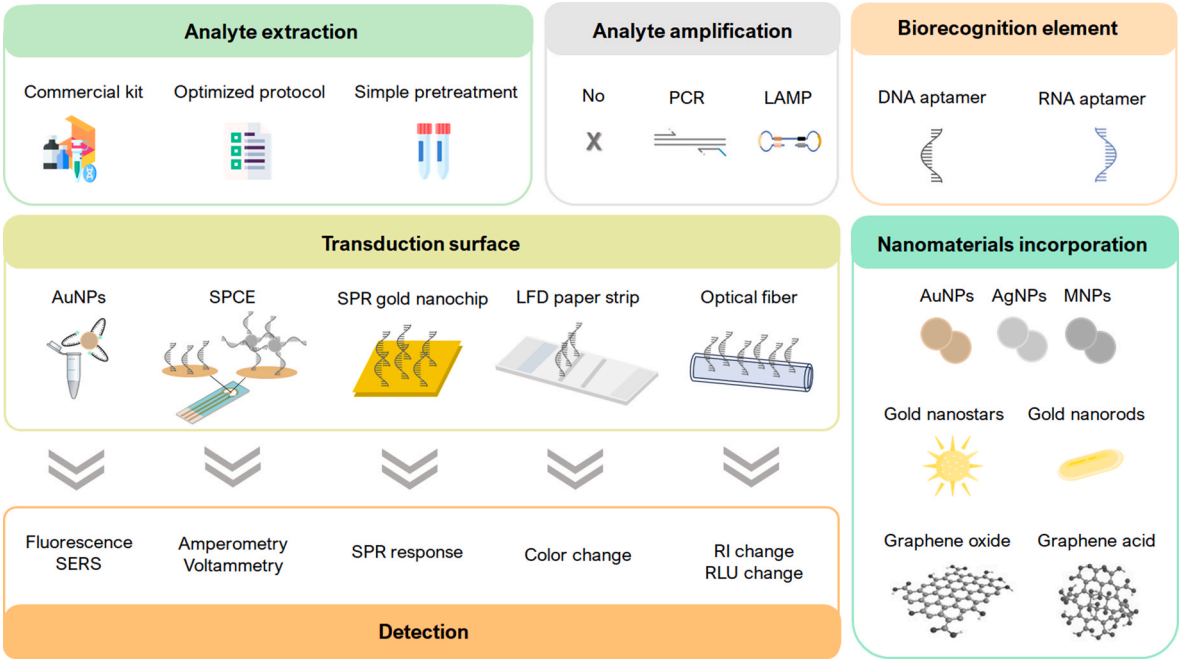

Fig. 4. Overview of the parameters considered in the development of most typical biosensor configurations for food authenticity discussed in the review. RI – refractive index; RLU – relative light units.

region from duck and *cytB* gene from the other species) to a silicon-based optical thin-film biosensor, allowing simultaneous detection of different species in a single sample. In addition, the detection limit of 0,001 % (w/w) achieved for beef/deer meat in mixtures shows the high sensitivity of the device as well as its multiplexing potential.

Montiel et al. (2017) developed an amperometric biosensor to detect adulteration with horsemeat in meat samples. Unlike other biosensors, this configuration consisted of an RNA aptamer coupled to magnetic microbeads, targeting a fragment of the equine mitochondrial DNA D-loop region, and a screen-printed carbon electrode (SPCE) as transducer. This biosensor represents a significant advance over other platforms. No PCR amplification is required, and the system is capable of detecting horse DNA in mitochondrial lysates – completely eliminating the requirement of DNA extraction protocols or kits - with a low detection limit of 0.5 % (w/w) horse meat in spiked beef samples. The simplification of DNA isolation and the elimination of PCR steps really streamlines the detection process and makes the biosensor ideal for rapid on-site analysis. This was also a concern of Torelli, Manzano, and Marks (2017), who developed a chemiluminescent optical fiber biosensor for the detection of porcine DNA in meat mixtures, using DNA aptamers targeting a fragment of mitochondrial 12S rRNA. The authors focused on establishing an improved DNA extraction protocol to reduce the total time of analysis to 2.5 h, which is considerably faster than the application of commercial kits for the same purpose, that require several hours just for DNA extraction.

If for some reason DNA amplification cannot be excluded from the biosensing process, there are currently other methods that can replace PCR. Xu et al. (2017) developed a colorimetric biosensor to detect mammalian DNA in processed food and meat mixtures. They used a DNA aptamer complementary to the *Gcg* gene, a reference gene specific to mammals, and AuNPs deposited on the membrane of a lateral flow device (LFD). The novelty of this device was the application of loop-mediated isothermal amplification (LAMP) instead of conventional PCR. LAMP is one of the most used isothermal nucleic acid amplification methods, a class of alternative, low-cost approaches to PCR that are capable of amplifying DNA/RNA in a short period of time at a constant temperature and with simple equipment. The reaction takes place without the need for temperature cycles or thermal cyclers and results in high amplification and highly sensitive detection, starting from small amounts of nucleic acids. When implemented to biosensor development, LAMP can be used for signal amplification resulting from an initial recognition event set by aptamers or other derived structures (Xia et al., 2022). This increases the sensitivity and overall performance of the final device, which can be of great value in food authenticity assessment where very low concentrations of analytes are typically found.

Azam, Roy, Lim, and Uddin Ahmed (2018) also applied LAMP to develop an electrochemiluminescent biosensor to detect pig extracted DNA in meat samples from nine animal species and processed foods. In similarity to other configurations, the DNA aptamer used was specific to a fragment of the porcine *cytB* gene in pig, and the detection was based on the interaction between the LAMP products, reporter molecules and the surface of an SPCE. For this biosensor, the authors report a very short detection time of 5 min after DNA extraction and a high specificity and sensitivity with a low detection limit of 0.1 pg/μL in binary meat mixtures.

It is noticeable that the development of biosensors has accompanied the technological advances through time. With the advent of new techniques and materials, more intricate configurations have been described to improve the performance of biosensors in food testing, and these developments have exploded in recent years, as shown below. For example, Hartati, Suryani, Agustina, Gaffar, and Anggraeni (2019) developed a voltammetric biosensor for the detection of porcine DNA in raw and processed meat samples using an SPCE in which a bioconjugate was immobilized, composed by AuNPs and a DNA aptamer. The authors propose the use of bioconjugates containing nanoparticles to shorten the detection time while increasing the sensitivity of the biosensor, as they suggest that these bioconjugates amplify the detection signal and increase the signal-to-noise ratio. In fact, the detection limit of this biosensor was set at 0.58 μg/mL, which is lower than many of the previously reported biosensors for the same application.

In 2020, Mansouri et al. proposed an optical surface plasmon resonance biosensor for the detection of donkey DNA in meat samples and cooked sausages. They used a DNA aptamer coupled to a gold chip and gold nanostars to enhance signal transduction. Using extracted DNA, this device was able to detect 1 % donkey meat adulteration in mixed sausages and achieved a limit of quantification of 1 nM using synthetic targets, without the need for PCR or other amplification strategy. Khalil et al. (2020) developed a SERS biosensor to detect porcine DNA in pork meat samples, also using DNA aptamers in conjugation with different nanomaterials - graphene oxide, gold nanorods and AuNPs. In the presence of hybridization between the aptamers and target DNA, these nanomaterials interacted between themselves, enhancing the SERS signal emitted by the device. The biosensor was able to discriminate between DNA sequences from other non-target species (goat, rat, sheep, cow, horse and buffalo) and a mismatched DNA sequence by one SNP. When tested against DNA extracted from pork samples, a remarkably low LOD value of 1 fM was registered.

Also applying nanomaterials, Flauzino, Pimentel, Alves, Madurro, and Brito-Madurro (2021) developed a voltammetric biosensor to detect bovine DNA in beef, pork and mixed meat samples, again using a DNA aptamer coupled to a graphene electrode modified with a graphene oxide nanocomposite. Besides its high sensitivity, their approach involved a simple preparation step for retrieving DNA from lysates, instead of extensive extraction protocols and showed good storage stability (six weeks) and reusability (five uses without loss of signal loss). In 2022, the same group proposed an electrochemical impedimetric biosensor with a similar application composed by a cytB DNA aptamer immobilized on a graphene acid modified SPCE (Flauzino, Nguyen, et al., 2022). The modification of the electrode surface with this type of carbon nanomaterial is advantageous as it improves the sensing properties of the electrodes and facilitates the immobilization of biomolecules on its surface.

One of the latest and most exciting technologies to enter biosensor development is the CRISPR/Cas system. In 2021, Liu et al. developed a dual-mode optical SERS/colorimetric biosensor for the detection of duck DNA (fragment of mitochondrial *ND2* gene) in a variety of samples. This approach combines a LAMP reaction, the CRISPR/Cas12a reaction system, liposomes encapsulating reporter molecules, and AuNPs to provide dual signal readout: target recognition triggering both SERS signal intensity and color change. While LAMP replaces conventional PCR, the CRISPR/Cas12a reaction provides intrinsic signal amplification along with ultra-sensitive and highly specific detection capacity. The CRISPR/Cas mechanism has revolutionized the fields of genome editing and nucleic acid identification (Jinek et al., 2012). Since the discovery of the first CRISPR/Cas system, other variations have been found and applied in different research areas. The CRISPR/Cas12a is composed of a single-stranded crRNA that is responsible for recognizing and binding to the target DNA. Upon binding, the Cas12a enzyme is activated, triggering its non-specific cleavage activity of single-stranded DNA (ssDNA) (Mao et al., 2022). In the biosensor proposed by Liu et al. (2021) ssDNA linkers are used to capture liposomes encapsulating reporter molecules. In the presence of target DNA, the linkers are cleaved by Cas12a and can no longer capture the liposomes. Consequently, the reporter molecules are not released and do not interact with the transduction element in either of the two transduction methods employed in this device. Taking advantage of this signal amplification strategy, the biosensor achieved a LOD of 100 aM and 10 pM for the SERS and the colorimetric method, respectively.

In 2022, Pan et al. also combined LAMP and CRISPR/Cas12a to develop an optical SERS biosensor for the detection of bovine DNA in several commercialized goat milk products. Instead of using reporter molecules encapsulated in liposomes and AuNPs, the authors used

nanolabel-tagged ssDNA and bimetallic Au@Ag nanoparticles. These bimetallic nanoparticles combine the remarkable amplification capacity of Ag nanomaterials with the biocompatibility of Au nanomaterials. They present enhanced performance as SERS substrates, better stability and dispersibility than those consisting of a single noble metal component (Awiaz, Lin, & Wu, 2023). The combination of these technologies and nanomaterials resulted in a biosensor exhibiting an ultra-low background noise and a low LOD value of 224 aM.

4.2. Dairy products, flour, edible oils and wine

The dairy industry is another sector where fraudulent partial or total substitution of milk from other lower value species has been globally reported (Mafra, Honrado, & Amaral, 2022). Considering the high demand for dairy products and their broad presence in the diet of consumers, the development of biosensors for their monitoring is relevant and should be pursued. Nonetheless, descriptions of DNA biosensors for milk testing aimed at species identification are rare. In 2020, Bougadi & Kalogianni proposed a colorimetric biosensor to detect cow, sheep and goat DNA in yogurt samples and binary yogurt mixtures. The device was developed using three DNA aptamers complementary to nuclear genes specific of the three animal species, and AuNPs to allow naked-eye detection. Following DNA extraction and PCR amplification, detection was achieved, and the biosensor achieved a LOD value of 0.01 % adulteration of sheep and goat yogurt with cow milk, which according to the authors, is much lower than other methods available for milk authenticity testing. The success of this biosensor proves the applicability and potential of these devices for milk testing, so it is not clear why their development is not at the level of biosensors for meat authenticity.

The identification of species through biosensor detection is also pertinent in food products of high added value. This can be the case for olive oils, which have both economic and nutritional benefits. Extra virgin olive oil (EVOO) is typically the most high-priced edible oil, due to its excellent organoleptic properties and high content in antioxidants and vitamins and plays an important role in the economy of many producing countries (Salah & Nofal, 2021). Although no DNA-based biosensor has been reported for species or cultivar identification in olive oil, a biosensor has been proposed for testing the composition of other edible oils, proving their potential in handling this type of food matrix. Bai et al. (2011) developed a colorimetric thin-film biosensor coupled with DNA aptamers, capable of detecting eight plant species-specific nuclear genes, to identify the crop species present in vegetable oil samples. They applied the biosensor to PCR amplicons obtained after amplification of DNA extracted from leaves of the eight plant species (peanut, cotton, palm, sesame, maize, sunflower, soybean and rapeseed) and also from the corresponding oils, having obtained similar results with both sample types (although the results of oil samples analysis are not shown). The authors also state that the biosensor is more sensitive than other previously reported PCR-based methods, with a low detection limit of 0.1 fmol. They later applied a similar biosensor to determine the plant species present in six common crop flours (wheat, maize, potato, pea, soybean and rice). Again, DNA aptamers targeting specific fragments of nuclear genes from the six crop species were used as capture probes and a similar detection limit was reported (Bai et al., 2013).

Another great opportunity to explore the value of biosensors in authentication lies in analyzing highly quoted wines, which belong to a billion-euro sector and are also preferred for fraudulent practices (Pereira et al., 2018). Barrias et al. (2019) developed an optical long-period grating biosensor to identify grapevine varieties in different stages of the wine production chain. A DNA aptamer complementary to a region of the *F3H* gene was used, in which three SNP markers were identified as being appropriated to discriminate the tested varieties. The probe was immobilized on the surface of an optical fiber and several types of extracted DNA were tested, namely leaf, must and wine DNA. In this case, no PCR amplification was required, and the device was able to discriminate within the specie (at the variety level), which is a step further than other reported devices. Even though this was a preliminary study, the results obtained through the detection of DNA extracted from wine, a highly complex matrix, reveals that the further optimization of the biosensor is worth pursuing.

In 2022, Purwidyantri et al. developed an FET-based electrochemical biosensor to discriminate grapevine varieties present in wine samples. They also used a DNA aptamer targeting a fragment of the *F3H* gene to functionalize a FET chip modified with graphene. In similarity to the device proposed by Barrias et al. (2019), the aim of this biosensor goes a step further than species identification, as it is designed to discriminate varieties within the same species. The authors tested the platform in DNA extracted from grape and wine samples, using different commercial extraction kits and purification steps, as well as PCR amplification of these samples. In comparison to other reported biosensors for food analysis, this seems to be more time consuming due to all the required sample preparation steps preceding the biosensor detection. Nevertheless, the biosensor showed a very low LOD of 0.19 aM, which is quite remarkable considering the complexity of the tested samples.

5. A brief look at the detection of non-nucleic acid adulterants

As previously mentioned in this review, the synthesis of aptamers targeting nucleic acids and other targets can be achieved through SELEX methods, as a mean to generate DNA/RNA biorecognition elements used in DNA-based biosensing. This allows for new aptamer applications that go beyond the detection of nucleic acids for species identification, to the detection of other adulterants.

A wide variety of analytes present in both raw and processed products can be used to produce specific aptamers, from simple molecules to more complex compounds: amino acid, peptides, proteins, fatty acids, volatile chemicals, and other derivatives (Mohamad et al., 2022). In 2015, Kumar et al. developed a dual-mode readout colorimetric/fluorescent biosensor for the detection of urea in processed milk samples, with a detection limit of 20 nM. Urea is a common adulterant used to misleadingly increase protein content in milk. Through the SELEX method, the authors obtained a urea-specific DNA aptamer that they coupled to AuNPs. This was the first reported application of an aptamer as the biorecognition element of urea, as until then its capture was mainly performed by the enzyme urease. Regarding the results, the authors concluded that the performance of the biosensor is comparable to commercially available urea detection kits, with the advantage of retrieving results in a direct and simpler manner.

Xing, Zheng, et al. (2022) developed an optical SERS biosensor to detect melamine in raw milk samples. Like urea, melamine is another adulterant found in milk and infant formula that is added to increase the protein content in these products. Ingestion of melamine in high concentrations leads to many health problems and after a major incident in 2008 in which 300,000 children were poisoned and six died (Shalileh et al., 2023), the development of new effective methods to detect melamine has become a top priority for researchers and food inspection authorities. Regarding this biosensor, the authors developed a melamine-specific aptamer able to form a complex with silver nanoparticles (AgNPs) to detect melamine through Raman peak intensity comparison. The authors reported a detection time of approximately 20 min, with a LOD of 43.5 ppb.

In 2023, Qin et al. reported a dual-mode readout colorimetric/fluorescent biosensor for the detection of capsaicin, a compound frequently found in adulterated edible oils. Using the SELEX method, the authors selected a capsaicin-specific aptamer, that was immobilized on a graphene oxide/AuNPs nanocomposite, previously deposited onto a paper test disk. They tested the device in commercial pretreated edible oil samples spiked with capsaicin and reported a LOD value of 0.14 ng/mL and a detection time of only 1 h.

6. Future trends

Recently, there have been several technological advances that can contribute to the production and optimization of improved DNA biosensors for food authentication, some of which are shown in Fig. 5. Although several biosensors have been proposed in the last decade, there is still a massive gap between the development of DNA-based biosensors for food authenticity and biosensors developed for other applications. This can be mainly related to the difficulty in handling samples from complex food sources. Regardless of the biosensor configuration, the target analyte must always be available for detection, which is why special attention must be directed to sample preparation. Species identification strategies rely on the detection of nucleic acids, predominantly DNA, so an extraction protocol or kit is usually required to obtain these DNA fragments. To make biosensors competitive with other conventional technologies, lengthy DNA extraction protocols should be avoided and replaced by simpler sample processing steps, such as the preparation of lysates. In cases where DNA extraction cannot be avoided, great efforts should be put into establishing the most rapid and simple protocol that can guarantee a sufficient amount of analyte for testing.

If the initial amount of analyte is still too small and an increased quantity is required, various methods of isothermal nucleic acid amplification are currently available, such as LAMP, which are more convenient than performing target amplification by PCR. These alternative amplification methods also have the potential to be miniaturized and integrated into small microfluidic chips (Jiang et al., 2023), which is also interesting in biosensor fabrication.

During biosensor planning, it is important to consider the biorecognition element to apply. The promising 2D and 3D DNA nanostructures may seem attractive, but as versatile and innovative as they are, at this point their stability still needs to be improved before being broadly applied in biosensors for food testing. If further developments are achieved to simplify their synthesis, resulting in more reproducible and cost-effective DNA nanostructures, their application as capture elements could become more pursued. Until then, the use of aptamers in food biosensing will continue to be preferred.

One new technology that can really improve performance and take biosensors to the next level is the CRISPR/Cas12a system. When applied in biosensor design, its underlying mechanism can be used as a simple and rapid signal amplification strategy that increases the specificity and sensitivity of the final device. Currently, the implementation of CRISPR/Cas systems in biosensors is still at a very early stage and some constraints still need to be surpassed. For instance, the crRNA molecule and the Cas12a protein can be affected by the reaction temperature, the concentration of divalent ions and the presence of RNases (Shi et al., 2021). Nevertheless, currently there is a lot of research focused on improving CRISPR/Cas technology for biosensing, considering the increasing number of recent publications on the topic, so new breakthroughs can be expected soon.

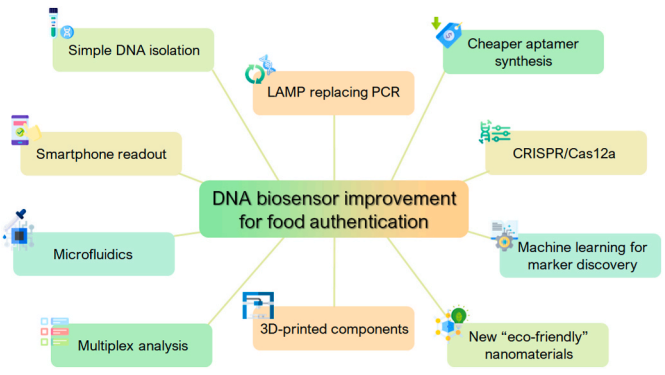

Fig. 5. Different strategies and technologies that can contribute to the fabrication of newly improved DNA biosensors applicable in food authentication.

Another technological advance that can be applied to biosensor technology is the use of machine learning algorithms (Mohamad et al., 2022). These can help to predict and classify new authenticity biomarkers before specific aptamers are selected, and lead to the discovery of markers in species and food products where these have not yet been found. The newly obtained data can be regularly uploaded onto databases, to improve the accuracy of the algorithms. The feasibility of this strategy was reported in a study by Erban et al. (2019), where new metabolic seed markers were identified, but the combination with other data analysis methods was advised until further testing, which highlights its early-stage state.

Regarding transducer performance and new configurations, the advances in the fabrication of new nanomaterials have greatly promoted the growth of new biosensors in numerous application fields (Zhou et al., 2020). Nanomaterial research is thriving, rapidly evolving and directed towards the current trends in establishing "green" synthesis methods, that guarantee sustainability and environmentally friendly applicability (García-Guzmán, López-Iglesias, Bellido-Milla, Palacios-Santander, & Cubillana-Aguilera, 2020). These are also current concerns in biosensor development, so an exponential integration of new and improved nanomaterials in biosensors for food authenticity testing should be expected.

An additional aspect to consider when developing a biosensor is the possibility of using 3D-printed components for its layout. This is a simple, reproducible, and reasonably inexpensive way to produce versatile and customized parts, that can be used in user-friendly portable devices applied in food analysis (dos Santos et al., 2022). Other features can be combined during biosensors development to allow their application as real-time testing devices in field conditions. The ability to perform multiplex target detection in the same response time using a single sample can be interesting, especially when dealing with food samples of scarce quantity and difficult to obtain. The integration of miniaturized microfluidic modules is also favorable, as it reduces the detection time and the volume of reagents needed for the reactions, while contributing to the portability and convenience of biosensors (Weng & Neethirajan, 2017). Lastly, biosensors can greatly benefit from incorporating smartphone-based signal readout, leading to simpler and more direct results interpretation in different testing scenarios.

In summary, there are many ways in which all the technologies and new materials can support the development of biosensors in any part of the food sector, including food authenticity. They can contribute to reduce manufacturing costs, achieve more sensitive and specific detection, and make biosensors overall more competitive with commercial alternatives for food authenticity assessment, so that they can transition from the labs onto the market.

7. Concluding remarks

The increasing demand for food and its susceptibility to fraudulent practices seeking profit is dictating the necessity of implementing new alternatives to the conventional methodologies for food authenticity assessment. DNA-based biosensors are practical and efficient devices that can be applied in continuous monitoring and practical on-site testing for species identification, a key parameter in food origin assessment. This review highlights, to this point, how the development of these devices has been mostly aimed at the identification of animal species in meat samples and derived processed products. A massive discrepancy can be observed in the introduction of DNA-based biosensors for other food products that are also in demand. Some of these are highly priced, so the application of biosensors would be particularly attractive in such products.

When developing a DNA-based biosensor for food authenticity, the biorecognition element and transducer choice will depend on several factors: the general characteristics of the food sample to be analyzed; the requirement and complexity of DNA extraction or other analyte

isolation protocols; the desired sensitivity and/or selectivity; the need for reusability and the available economic and technical resources. In view of the DNA-based biosensors discussed in this review, we can point out some general considerations. The colorimetric biosensors can perform rapid detection but may face sensitivity challenges. Although their sensitivity can be enhanced by performing PCR amplification of the target sequence, the complexity of the analysis and overall detection time will increase. These biosensors can be suitable for the analysis of food products with more permissible legal thresholds that do not require ultra-low detection limits, and in low-resource settings where simple and rapid monitoring is required. On the other hand, electrochemical biosensors usually involve longer detection times but have higher sensitivity. The simultaneous integration of various nanomaterials in electrochemical biosensors can be easily achieved, so their performance can be significantly improved. This makes them particularly efficient at detecting extremely low trace amounts of target sequences/molecules. SERS biosensors combine high sensitivity with quick detection, due to the implementation of diverse signal amplification strategies: LAMP, CRISPR/Cas12a and nanomaterials incorporation as excellent SERS substrates. Nevertheless, complex matrices in food samples can interfere with SERS signals and hamper the analysis, so their efficiency usually depends on DNA extraction. They also have a more complex instrumentation, which, in comparison to other biosensors, can somewhat limit their application in routine analysis. After reviewing the variety of biosensor components available, it is extremely difficult to point out a perfect universal configuration for food authenticity. The establishment of the final product will result from balancing several factors to obtain an adequate biosensing platform for the intended aim.

An expansion of DNA-based biosensors for food authenticity assessment is expected as the methods for nucleic acid probe synthesis become more affordable and simpler, and new methods for marker discovery are being developed. In addition, various other technologies are progressing, resulting in new materials, and manufacturing techniques becoming available for the establishment of innovative configurations with improved sensitivity and selectivity, capable to surpass the constraints related to food sample complexity.

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.

Data availability

Data will be made available on request.

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References

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., Adam, T., & Humayun, Q. (2014). Nanobiosensor for the detection and quantification of pork adulteration in meatball formulation. *Journal of Experimental Nanoscience, 9*(2), 152–160. https://doi.org/10.1080/17458080.2011.640946

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., & Yusop, M. H. M. (2011). Nanobiosensor for the detection and quantification of specific DNA sequences in degraded biological samples. In *IFMBE proceedings* (Vol. 35, pp. 384–387). https://doi.org/10.1007/978-3-642-21729-6_99. IFMBE.

Ali, M. E., Razzak, M. A., & Hamid, S. B. A. (2014). Multiplex PCR in species authentication: Probability and prospects—a review. *Food Analytical Methods, 7*(10), 1933–1949. https://doi.org/10.1007/s12161-014-9844-4

Asensio, L., González, I., García, T., & Martín, R. (2008). Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). *Food Control, 19*(1), 1–8. https://doi.org/10.1016/j.foodcont.2007.02.010

Awiaz, G., Lin, J., & Wu, A. (2023). Recent advances of Au@Ag core–shell SERS-based biosensors. *Explorations, 3*(1). https://doi.org/10.1002/EXP.20220072

Azam, N. F. N., Roy, S., Lim, S. A., & Uddin Ahmed, M. (2018). Meat species identification using DNA-luminol interaction and their slow diffusion onto the biochip surface. *Food Chemistry, 248*, 29–36. https://doi.org/10.1016/j.foodchem.2017.12.046

Bai, S., Li, S., Yao, T., Hu, Y., Bao, F., Zhang, J., et al. (2011). Rapid detection of eight vegetable oils on optical thin-film biosensor chips. *Food Control, 22*(10), 1624–1628. https://doi.org/10.1016/j.foodcont.2011.03.019

Bai, S., Xu, L., Wang, Y., Yang, X., Zhu, F., Chen, Y., et al. (2013). A simple and reliable qualitative detection of six foodstuff powders using optical thin-film biosensor chips. *European Food Research and Technology, 236*(5), 899–904. https://doi.org/10.1007/s00217-013-1940-y

Barrias, S., Fernandes, J. R., Eiras-Dias, J. E., Brazão, J., & Martins-Lopes, P. (2019). Label free DNA-based optical biosensor as a potential system for wine authenticity. *Food Chemistry, 270*, 299–304. https://doi.org/10.1016/J.FOODCHEM.2018.07.058

Bhalla, N., Jolly, P., Formisano, N., & Estrela, P. (2016). Introduction to biosensors. *Essays in Biochemistry, 60*(1), 1–8. https://doi.org/10.1042/EBC20150001

Bogani, P., Minunni, M., Spiriti, M. M., Zavaglia, M., Tombelli, S., Buiatti, M., et al. (2009). Transgenes monitoring in an industrial soybean processing chain by DNA-based conventional approaches and biosensors. *Food Chemistry, 113*(2), 658–664. https://doi.org/10.1016/j.foodchem.2008.07.056

Böhme, K., Calo-Mata, P., Barros-Velázquez, J., & Ortea, I. (2019). Review of recent DNA-based methods for main food-authentication topics. *Journal of Agricultural and Food Chemistry, 67*(14), 3854–3864. https://doi.org/10.1021/acs.jafc.8b07016

Bollella, P., Fusco, G., Stevar, D., Gorton, L., Ludwig, R., Ma, S., et al. (2018). A glucose/oxygen enzymatic fuel cell based on gold nanoparticles modified graphene screen-printed electrode. Proof-of-concept in human saliva. *Sensors and Actuators B: Chemical, 256*, 921–930. https://doi.org/10.1016/j.snb.2017.10.025

Bougadi, E. T., & Kalogianni, D. P. (2020). Paper-based DNA biosensor for food authenticity testing. *Food Chemistry, 322*, Article 126758. https://doi.org/10.1016/j.foodchem.2020.126758

Cali, K., Tuccori, E., & Persaud, K. C. (2020). Gravimetric biosensors. In P. Pelosi, & W. Knoll (Eds.), *Methods in enzymology* (1st ed., Vol. 642, pp. 435–468). Elsevier Inc. https://doi.org/10.1016/bs.mie.2020.05.010.

Camin, F., Boner, M., Bontempo, L., Fauhl-Hassek, C., Kelly, S. D., Riedl, J., et al. (2017). Stable isotope techniques for verifying the declared geographical origin of food in legal cases. *Trends in Food Science and Technology, 61*, 176–187. https://doi.org/10.1016/j.tifs.2016.12.007

Campuzano, S., Montiel, V. R.-V., Serafín, V., Yáñez-Sedeño, P., & Pingarrón, J. M. (2020). Cutting-edge advances in electrochemical affinity biosensing at different molecular level of emerging food allergens and adulterants. *Biosensors, 10*(2), 10. https://doi.org/10.3390/bios10020010

Campuzano, S., Pedredo, M., Yáñez-Sedeño, P., & Pingarrón, J. M. (2019). Antifouling (bio)materials for electrochemical (bio)sensing. *International Journal of Molecular Sciences, 20*(2), 423. https://doi.org/10.3390/ijms20020423

Carvalho, J., Yadav, S., Garrido-Maestu, A., Azinheiro, S., Trujillo, I., Barros-Velázquez, J., et al. (2021). Evaluation of simple sequence repeats (SSR) and single nucleotide polymorphism (SNP)-based methods in olive varieties from the Northwest of Spain and potential for miniaturization. *Food Chemistry: Molecular Sciences, 3*, Article 100038. https://doi.org/10.1016/j.fochms.2021.100038

Chandrasekaran, A. R. (2021). Nuclease resistance of DNA nanostructures. *Nature Reviews Chemistry, 5*(4), 225–239. https://doi.org/10.1038/s41570-021-00251-y

Chen, Y.-T., Lee, Y.-C., Lai, Y.-H., Lim, J.-C., Huang, N.-T., Lin, C.-T., et al. (2020). Review of integrated optical biosensors for point-of-care applications. *Biosensors, 10*(12), 209. https://doi.org/10.3390/bios10120209

Dai, H., Li, Y., Zhang, Q., Fu, Y., & Li, Y. (2018). A colorimetric biosensor based on enzyme-catalysis-induced production of inorganic nanoparticles for sensitive detection of glucose in white grape wine. *RSC Advances, 8*(59), 33960–33967. https://doi.org/10.1039/C8RA06347H

Dawan, J., & Ahn, J. (2022). Application of DNA barcoding for ensuring food safety and quality. *Food Science and Biotechnology, 31*(11), 1355–1364. https://doi.org/10.1007/s10068-022-01143-7

Devadoss, A., Sudhagar, P., Terashima, C., Nakata, K., & Fujishima, A. (2015). Photoelectrochemical biosensors: New insights into promising photoelectrodes and signal amplification strategies. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 24*, 43–63. https://doi.org/10.1016/j.jphotochemrev.2015.06.002

dosSantos, D. M., Cardoso, R. M., Migliorini, F. L., Facure, M. H. M., Mercante, L. A., Mattoso, L. H. C., et al. (2022). Advances in 3D printed sensors for food analysis. *TrAC, Trends in Analytical Chemistry, 154*. https://doi.org/10.1016/j.trac.2022.116672

Dou, X., Zhang, L., Yang, R., Wang, X., Yu, L., Yue, X., et al. (2023). Mass spectrometry in food authentication and origin traceability. *Mass Spectrometry Reviews, 42*(5), 1772–1807. https://doi.org/10.1002/mas.21779

Douaki, A., Garoli, D., Inam, A. K. M. S., Angeli, M. A. C., Cantarella, G., Rocchia, W., et al. (2022). Smart approach for the design of highly selective aptamer-based biosensors. *Biosensors, 12*(8), 574. https://doi.org/10.3390/bios12080574

Ellington, A. D., & Szostak, J. W. (1990). In vitro selection of RNA molecules that bind specific ligands. *Nature, 346*(6287), 818–822. https://doi.org/10.1038/346818a0

Erban, A., Fehrle, I., Martinez-Seidel, F., Brigante, F., Más, A. L., Baroni, V., et al. (2019). Discovery of food identity markers by metabolomics and machine learning technology. *Scientific Reports, 9*(1), 9697. https://doi.org/10.1038/s41598-019-46113-y

Ferreira, G. N. M., da Silva, A. C., & Tomé, B. (2009). Acoustic wave biosensors: Physical models and biological applications of quartz crystal microbalance. *Trends in Biotechnology, 27*(12), 689–697. https://doi.org/10.1016/j.tibtech.2009.09.003

Flauzino, J. M. R., Alves, L. M., Rodovalho, V. R., Madurro, J. M., & Brito-Madurro, A. G. (2022). Application of biosensors for detection of meat species: A short review. *Food Control, 142*, Article 109214. https://doi.org/10.1016/j.foodcont.2022.109214

Flauzino, J. M. R., Nguyen, E. P., Yang, Q., Rosati, G., Panáček, D., Brito-Madurro, A. G., et al. (2022). Label-free and reagentless electrochemical genosensor based on graphene acid for meat adulteration detection. *Biosensors and Bioelectronics, 195*, Article 113628. https://doi.org/10.1016/j.bios.2021.113628

Flauzino, J. M. R., Pimentel, E. L., Alves, L. M., Madurro, J. M., & Brito-Madurro, A. G. (2021). A novel and reusable electrochemical genosensor for detection of beef adulteration. *Electroanalysis, 33*(2), 296–303. https://doi.org/10.1002/elan.202060029

Freitas, M., Neves, M. M. P. S., Nouws, H. P. A., & Delerue-Matos, C. (2021). Electrochemical immunosensor for the simultaneous determination of two main peanut allergenic proteins (Ara h 1 and Ara h 6) in food matrices. *Foods, 10*(8). https://doi.org/10.3390/foods10081718

García-Guzmán, J. J., López-Iglesias, D., Bellido-Milla, D., Palacios-Santander, J. M., & Cubillana-Aguilera, L. (2020). Green synthesis of nanoMaterials for biosensing. In Inamuddin, & A. M. Asiri (Eds.), *Nanotechnology in the life sciences* (1st ed., pp. 135–217). Cham: Springer. https://doi.org/10.1007/978-3-030-45116-5_7.

Gomes, S., Breia, R., Carvalho, T., Carnide, V., & Martins-Lopes, P. (2018). Microsatellite high-resolution melting (SSR-HRM) to track olive genotypes: From field to olive oil. *Journal of Food Science, 83*(10), 2415–2423. https://doi.org/10.1111/1750-3841.14333

Gomes, S., Castro, C., Barrias, S., Pereira, L., Jorge, P., Fernandes, J. R., et al. (2018). Alternative SNP detection platforms, HRM and biosensors, for varietal identification in *Vitis vinifera* L. using *F3H* and *LDOX* genes. *Scientific Reports, 8*(1), 5850. https://doi.org/10.1038/s41598-018-24158-9

González-Domínguez, R. (2022). Food authentication: Techniques, trends and emerging approaches (second issue). *Foods, 11*(13), 1926. https://doi.org/10.3390/foods11131926

Griesche, C., & Baeumner, A. J. (2020). Biosensors to support sustainable agriculture and food safety. *TrAC, Trends in Analytical Chemistry, 128*, Article 115906. https://doi.org/10.1016/j.trac.2020.115906

Hartati, Y. W., Irkham, I., Sumiati, I., Wyantuti, S., Gaffar, S., Zakiyyah, S. N., et al. (2023). The optimization of a label-free electrochemical DNA biosensor for detection of *Sus scrofa* mtDNA as food adulterations. *Biosensors, 13*(6), 657. https://doi.org/10.3390/bios13060657

Hartati, Y. W., Suryani, A. A., Agustina, M., Gaffar, S., & Anggraeni, A. (2019). A gold nanoparticle–DNA bioconjugate–based electrochemical biosensor for detection of *Sus scrofa* mtDNA in raw and processed meat. *Food Analytical Methods, 12*(11), 2591–2600. https://doi.org/10.1007/s12161-019-01593-6

Hellberg, R. S., Hernandez, B. C., & Hernandez, E. L. (2017). Identification of meat and poultry species in food products using DNA barcoding. *Food Control, 80*, 23–28. https://doi.org/10.1016/j.foodcont.2017.04.025

Huang, Y., Xu, J., Liu, J., Wang, X., & Chen, B. (2017). Disease-related detection with electrochemical biosensors: A review. *Sensors, 17*(10), 2375. https://doi.org/10.3390/s17102375

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science, 337*(6096), 816–821. https://doi.org/10.1126/science.1225829

Justino, C., Duarte, A., & Rocha-Santos, T. (2017). Recent progress in biosensors for environmental monitoring: A review. *Sensors, 17*(12), 2918. https://doi.org/10.3390/s17122918

Justino, C., Rocha-Santos, T., & Duarte, A. (2010). Review of analytical figures of merit of sensors and biosensors in clinical applications. *TrAC, Trends in Analytical Chemistry, 29*(10), 1172–1183. https://doi.org/10.1016/j.trac.2010.07.008

Khalil, I., Hashem, A., Nath, A. R., Julkapli, N. M., Yehye, W. A., & Basirun, W. J. (2021). DNA/Nano based advanced genetic detection tools for authentication of species: Strategies, prospects and limitations. *Molecular and Cellular Probes, 59*, Article 101758. https://doi.org/10.1016/j.mcp.2021.101758

Khalil, I., Yehye, W. A., Julkapli, N. M., Sina, A. A. I., Rahmati, S., Basirun, W. J., et al. (2020). Dual platform based sandwich assay surface-enhanced Raman scattering DNA biosensor for the sensitive detection of food adulteration. *The Analyst, 145*(4), 1414–1426. https://doi.org/10.1039/C9AN02106J

Khan, S., Burciu, B., Filipe, C. D. M., Li, Y., Dellinger, K., & Didar, T. F. (2021). DNAzyme-based biosensors: Immobilization strategies, applications, and future prospective. *ACS Nano, 15*(9), 13943–13969. https://doi.org/10.1021/acsnano.1c04327

Kumar, P., Lambadi, P. R., & Navani, N. K. (2015). Non-enzymatic detection of urea using unmodified gold nanoparticles based aptasensor. *Biosensors and Bioelectronics, 72*, 340–347. https://doi.org/10.1016/j.bios.2015.05.029

Labuda, J., Brett, A. M. O., Evtugyn, G., Fojta, M., Mascini, M., Ozsoz, M., et al. (2010). Electrochemical nucleic acid-based biosensors: Concepts, terms, and methodology (IUPAC Technical Report). *Pure and Applied Chemistry, 82*(5), 1161–1187. https://doi.org/10.1351/PAC-REP-09-08-16

Lehotay, S. J., & Chen, Y. (2018). Hits and misses in research trends to monitor contaminants in foods. *Analytical and Bioanalytical Chemistry, 410*(22), 5331–5351. https://doi.org/10.1007/s00216-018-1195-3

Li, B., Yu, M., Xu, W., Chen, L., & Han, J. (2023). Comparison of PCR techniques in adulteration identification of dairy products. *Agriculture, 13*(7), 1450. https://doi.org/10.3390/agriculture13071450

Lino, C., Barrias, S., Chaves, R., Adega, F., Martins-Lopes, P., & Fernandes, J. R. (2022). Biosensors as diagnostic tools in clinical applications. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1877*(3), Article 188726. https://doi.org/10.1016/j.bbcan.2022.188726

Liu, J., Chen, J., Wu, D., Huang, M., Chen, J., Pan, R., et al. (2021). CRISPR-/Cas12a-mediated liposome-amplified strategy for the surface-enhanced Raman scattering and naked-eye detection of nucleic acid and application to food authenticity screening. *Analytical Chemistry, 93*(29), 10167–10174. https://doi.org/10.1021/acs.analchem.1c01163

Maddali, H., Miles, C. E., Kohn, J., & O'Carroll, D. M. (2021). Optical biosensors for virus detection: Prospects for SARS-CoV-2/COVID-19. *ChemBioChem, 22*(7), 1176–1189. https://doi.org/10.1002/cbic.202000744

Mafra, I., Ferreira, I., & Oliveira, M. (2008). Food authentication by PCR-based methods. *European Food Research and Technology, 227*(3), 649–665. https://doi.org/10.1007/s00217-007-0782-x

Mafra, I., Honrado, M., & Amaral, J. S. (2022). Animal species authentication in dairy products. *Foods, 11*(8), 1124. https://doi.org/10.3390/foods11081124

Magiati, M., Myridaki, V. M., Christopoulos, T. K., & Kalogianni, D. P. (2019). Lateral flow test for meat authentication with visual detection. *Food Chemistry, 274*, 803–807. https://doi.org/10.1016/j.foodchem.2018.09.063

Malik, P., Gupta, R., Malik, V., & Ameta, R. K. (2021). Emerging nanomaterials for improved biosensing. *Measurement: Sensors, 16*, Article 100050. https://doi.org/10.1016/j.measen.2021.100050

Mansouri, M., Fathi, F., Jalili, R., Shoeibie, S., Dastmalchi, S., Khataee, A., et al. (2020). SPR enhanced DNA biosensor for sensitive detection of donkey meat adulteration. *Food Chemistry, 331*, Article 127163. https://doi.org/10.1016/j.foodchem.2020.127163

Mao, Z., Chen, R., Wang, X., Zhou, Z., Peng, Y., Li, S., et al. (2022). CRISPR/Cas12a-based technology: A powerful tool for biosensing in food safety. *Trends in Food Science & Technology, 122*, 211–222. https://doi.org/10.1016/j.tifs.2022.02.030

McConnell, E. M., Cozma, I., Mou, Q., Brennan, J. D., Lu, Y., & Li, Y. (2021). Biosensing with DNAzymes. *Chemical Society Reviews, 50*(16), 8954–8994. https://doi.org/10.1039/D1CS00240F

Melinte, G., Hosu, O., Cristea, C., & Marrazza, G. (2022). DNA sensing technology a useful food scanning tool. *TrAC, Trends in Analytical Chemistry, 154*, Article 116679. https://doi.org/10.1016/j.trac.2022.116679

Melo, A. M. A., Alexandre, D. L., Furtado, R. F., Borges, M. F., Figueiredo, E. A. T., Biswas, A., et al. (2016). Electrochemical immunosensors for *Salmonella* detection in food. *Applied Microbiology and Biotechnology, 100*(12), 5301–5312. https://doi.org/10.1007/s00253-016-7548-y

Mohamad, N., Azizan, N. I., Mokhtar, N. F. K., Mustafa, S., Mohd Desa, M. N., & Hashim, A. M. (2022). Future perspectives on aptamer for application in food authentication. *Analytical Biochemistry, 656*. https://doi.org/10.1016/j.ab.2022.114861

Montiel, V. R.-V., Gutiérrez, M. L., Torrente-Rodríguez, R. M., Povedano, E., Vargas, E., Reviejo, Á. J., et al. (2017). Disposable amperometric polymerase chain reaction-free biosensor for direct detection of adulteration with horsemeat in raw lysates targeting mitochondrial DNA. *Analytical Chemistry, 89*(17), 9474–9482. https://doi.org/10.1021/acs.analchem.7b02412

Morales, M. A., & Halpern, J. M. (2018). Guide to selecting a biorecognition element for biosensors. *Bioconjugate Chemistry, 29*(10), 3231–3239. https://doi.org/10.1021/acs.bioconjchem.8b00592

Nehal, N., Choudhary, B., Nagpure, A., & Gupta, R. K. (2021). DNA barcoding: A modern age tool for detection of adulteration in food. *Critical Reviews in Biotechnology, 41*(5), 767–791. https://doi.org/10.1080/07388551.2021.1874279

Ou, Y., Jin, X., Fang, J., Tian, Y., & Zhou, N. (2020). Multi-cycle signal-amplified colorimetric detection of tobramycin based on dual-strand displacement and three-way DNA junction. *Microchemical Journal, 156*, Article 104823. https://doi.org/10.1016/j.microc.2020.104823

Pan, R., Liu, J., Wang, P., Wu, D., Chen, J., Wu, Y., et al. (2022). Ultrasensitive CRISPR/Cas12a-Driven SERS biosensor for onsite nucleic acid detection and its application to milk authenticity testing. *Journal of Agricultural and Food Chemistry, 70*(14), 4484–4491. https://doi.org/10.1021/acs.jafc.1c08262

Pereira, L., Gomes, S., Barrias, S., Gomes, E. P., Baleiras-Couto, M., Fernandes, J. R., et al. (2018). From the field to the bottle — an integrated strategy for wine authenticity. *Beverages, 4*(4), 1–21. https://doi.org/10.3390/beverages4040071

Pereira, L., Gomes, S., Castro, C., Eiras-Dias, J. E., Brazão, J., Graça, A., et al. (2017). High Resolution Melting (HRM) applied to wine authenticity. *Food Chemistry, 216*, 80–86. https://doi.org/10.1016/j.foodchem.2016.07.185

Perumal, V., & Hashim, U. (2014). Advances in biosensors: Principle, architecture and applications. *Journal of Applied Biomedicine, 12*(1), 1–15. https://doi.org/10.1016/j.jab.2013.02.001

Piredda, R., Mottola, A., Cipriano, G., Carlucci, R., Ciccarese, G., & Di Pinto, A. (2022). Next Generation Sequencing (NGS) approach applied to species identification in mixed processed seafood products. *Food Control, 133*, Article 108590. https://doi.org/10.1016/j.foodcont.2021.108590

Pohanka, M. (2018). Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications. *Materials, 11*(3), 448. https://doi.org/10.3390/ma11030448

Pohanka, M. (2019). Current trends in the biosensors for biological warfare agents assay. *Materials, 12*(14), 2303. https://doi.org/10.3390/ma12142303

Pramanik, S., Pingguan-Murphy, B., & Osman, N. A. A. (2013). Developments of immobilized surface modified piezoelectric crystal biosensors for advanced

applications. *International Journal of Electrochemical Science, 8*(6), 8863–8892. https://doi.org/10.1016/S1452-3981(23)12933-6
Purwidyantri, A., Ipatov, A., Domingues, T., Borme, J., Martins, M., Alpuim, P., et al. (2022). Programmable graphene-based microfluidic sensor for DNA detection. *Sensors and Actuators B: Chemical, 367*, Article 132044. https://doi.org/10.1016/j.snb.2022.132044
Qiao, Z., Fu, Y., Lei, C., & Li, Y. (2020). Advances in antimicrobial peptides-based biosensing methods for detection of foodborne pathogens: A review. *Food Control, 112*, Article 107116. https://doi.org/10.1016/j.foodcont.2020.107116
Qin, M., Khan, I. M., Ding, N., Sun, Y., Qi, S., Dong, X., et al. (2023). Aptamers selected and identified as a colorimetric dual-mode sensing platform for selective and sensitive detection of capsaicin. *Sensors and Actuators B: Chemical, 396*, Article 134638. https://doi.org/10.1016/j.snb.2023.134638
Reimhult, E., & Höök, F. (2015). Design of surface modifications for nanoscale sensor applications. *Sensors, 15*(1), 1635–1675. https://doi.org/10.3390/s150101635
Roda, A., Mirasoli, M., Michelini, E., Di Fusco, M., Zangheri, M., Cevenini, L., et al. (2016). Progress in chemical luminescence-based biosensors: A critical review. *Biosensors and Bioelectronics, 76*, 164–179. https://doi.org/10.1016/j.bios.2015.06.017
Rodriguez-Saona, L. E., & Allendorf, M. E. (2011). Use of FTIR for rapid authentication and detection of adulteration of food. *Annual Review of Food Science and Technology, 2*(1), 467–483. https://doi.org/10.1146/annurev-food-022510-133750
Roh, S., Jang, Y., Yoo, J., & Seong, H. (2023). Surface modification strategies for biomedical applications: Enhancing cell–biomaterial interfaces and biochip performances. *BioChip Journal, 17*(1), 174–191. https://doi.org/10.1007/s13206-023-00104-4
Salah, W. A., & Nofal, M. (2021). Review of some adulteration detection techniques of edible oils. *Journal of the Science of Food and Agriculture, 101*(3), 811–819. https://doi.org/10.1002/jsfa.10750
Scarano, D., & Rao, R. (2014). DNA markers for food products authentication. *Diversity, 6*(3), 579–596. https://doi.org/10.3390/d6030579
Shalileh, F., Sabahi, H., Golbashy, M., Dadmehr, M., & Hosseini, M. (2023). Recent developments in DNA nanostructure-based biosensors for the detection of melamine adulteration in milk. *Microchemical Journal, 195*, Article 109316. https://doi.org/10.1016/j.microc.2023.109316
Shi, S., Chen, J., Wang, X., Xiao, M., Chandrasekaran, A. R., Li, L., et al. (2022). Biointerface engineering with nucleic acid materials for biosensing applications. *Advanced Functional Materials, 32*(37), Article 2201069. https://doi.org/10.1002/adfm.202201069
Shi, Y., Fu, X., Yin, Y., Peng, F., Yin, X., Ke, G., et al. (2021). CRISPR-Cas12a system for biosensing and gene regulation. *Chemistry - An Asian Journal, 16*(8), 857–867. https://doi.org/10.1002/asia.202100043
Shruti, A., Bage, N., & Kar, P. (2024). Nanomaterials based sensors for analysis of food safety. *Food Chemistry, 433*, Article 137284. https://doi.org/10.1016/j.foodchem.2023.137284
Singh, A., Sharma, A., Ahmed, A., Sundramoorthy, A. K., Furukawa, H., Arya, S., et al. (2021). Recent advances in electrochemical biosensors: Applications, challenges, and future scope. *Biosensors, 11*(9), 336. https://doi.org/10.3390/bios11090336
Sonawane, M. D., & Nimse, S. B. (2016). Surface modification chemistries of materials used in diagnostic platforms with biomolecules. *Journal of Chemistry, 2016*, Article 9241378. https://doi.org/10.1155/2016/9241378
Sotirchos, D. G., Georgiou, C. A., & Danezis, G. P. (2017). Introduction, definitions and legislation. In C. A. Georgiou, & G. P. Danezis (Eds.), *Food authentication: Management, analysis and regulation* (1st ed., pp. 1–18). Wiley. https://doi.org/10.1002/9781118810224.ch1.
Sultana, S., Azlan, A., Desa, M. N. M., & Mahyudin, N. A. (2023). Multiplex platforms in biosensor based analytical approaches: Opportunities and challenges for the speciation of animal species in food chain. *Food Control, 149*. https://doi.org/10.1016/j.foodcont.2023.109727
Tetyana, P., Shumbula, P. M., & Njengele-Tetyana, Z. (2021). Biosensors: Design, development and applications. In S. Ameen, M. Akhtar, & H.-S. Shin (Eds.), *Nanopores*. IntechOpen. https://doi.org/10.5772/intechopen.97576.
Thapa, K., Liu, W., & Wang, R. (2021). Nucleic acid-based electrochemical biosensor: Recent advances in probe immobilization and signal amplification strategies. *WIREs Nanomedicine and Nanobiotechnology, 14*(1), e1765. https://doi.org/10.1002/wnan.1765
Thevenot, D. R., Tóth, K., Durst, R. A., & Wilson, G. S. (1999). Electrochemical biosensors: Recommended definitions and classification. *Pure and Applied Chemistry, 71*(12), 2333–2348. https://doi.org/10.1351/pac199971122333
Tjon, V., Tang, L., Zauscher, S., & Chilkoti, A. (2014). "Smart" DNA interfaces. *Chemical Society Reviews, 5*(43), 1612–1626. https://doi.org/10.1039/C3CS60331H
Tombelli, S., Minunni, M., & Mascini, M. (2005). Piezoelectric biosensors: Strategies for coupling nucleic acids to piezoelectric devices. *Methods, 37*(1), 48–56. https://doi.org/10.1016/j.ymeth.2005.05.005
Torelli, E., Manzano, M., & Marks, R. S. (2017). Chemiluminescent optical fibre genosensor for porcine meat detection. *Sensors and Actuators B: Chemical, 247*, 868–874. https://doi.org/10.1016/j.snb.2017.03.064
Tuerk, C., & Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science, 249*(4968), 505–510. https://doi.org/10.1126/science.2200121
Valentini, P., Galimberti, A., Mezzasalma, V., De Mattia, F., Casiraghi, M., Labra, M., et al. (2017). DNA Barcoding Meets Nanotechnology: Development of a universal colorimetric test for food authentication. *Angewandte Chemie International Edition, 56*(28), 8094–8098. https://doi.org/10.1002/anie.201702120
Varmira, K., Mohammadi, G., Mahmoudi, M., Khodarahmi, R., Rashidi, K., Hedayati, M., et al. (2018). Fabrication of a novel enzymatic electrochemical biosensor for determination of tyrosine in some food samples. *Talanta, 183*, 1–10. https://doi.org/10.1016/j.talanta.2018.02.053
Verdone, M., Rao, R., Coppola, M., & Corrado, G. (2018). Identification of zucchini varieties in commercial food products by DNA typing. *Food Control, 84*, 197–204. https://doi.org/10.1016/j.foodcont.2017.07.039
Vieira, M. B., Faustino, M. V., Lourenço, T. F., & Oliveira, M. M. (2022). DNA-based tools to certify authenticity of rice varieties—an overview. *Foods, 11*(3), 258. https://doi.org/10.3390/foods11030258
Vikrant, K., Bhardwaj, N., Bhardwaj, S. K., Kim, K.-H., & Deep, A. (2019). Nanomaterials as efficient platforms for sensing DNA. *Biomaterials, 214*, Article 119215. https://doi.org/10.1016/j.biomaterials.2019.05.026
Walton, P. W., O'Flaherty, M. R., Butler, M. E., & Compton, P. (1993). Gravimetric biosensors based on acoustic waves in thin polymer films. *Biosensors and Bioelectronics, 8*(9–10), 401–407. https://doi.org/10.1016/0956-5663(93)80024-J
Wang, D.-X., Wang, J., Wang, Y.-X., Du, Y.-C., Huang, Y., Tang, A.-N., et al. (2021). DNA nanostructure-based nucleic acid probes: Construction and biological applications. *Chemical Science, 12*(22), 7602–7622. https://doi.org/10.1039/D1SC00587A
Wang, W., Zhu, Y., Chen, Y., Xu, X., & Zhou, G. (2015). Rapid visual detection of eight meat species using optical thin-film biosensor chips. *Journal of AOAC International, 98*(2), 410–414. https://doi.org/10.5740/jaoacint.14-230
Weng, X., & Neethirajan, S. (2017). Ensuring food safety: Quality monitoring using microfluidics. *Trends in Food Science & Technology, 65*, 10–22. https://doi.org/10.1016/j.tifs.2017.04.015
Xia, X., Yang, H., Cao, J., Zhang, J., He, Q., & Deng, R. (2022). Isothermal nucleic acid amplification for food safety analysis. *TrAC, Trends in Analytical Chemistry, 153*, Article 116641. https://doi.org/10.1016/j.trac.2022.116641
Xing, G., Sun, X., Li, N., Li, X., Wu, T., & Wang, F. (2022). New advances in lateral flow immunoassay (LFI) technology for food safety detection. *Molecules, 27*(19), 6596. https://doi.org/10.3390/molecules27196596
Xing, H., Zheng, B., Li, X., Dang, X., Zhang, H., Tian, F., et al. (2022). Sensitive SERS detection of melamine and cyromazine in raw milk using aptamer-based *in situ* silver nanoparticles synthesis. *Results in Chemistry, 4*, Article 100266. https://doi.org/10.1016/j.rechem.2021.100266
Xu, Y., Xiang, W., Wang, Q., Cheng, N., Zhang, L., Huang, K., et al. (2017). A smart sealed nucleic acid biosensor based on endogenous reference gene detection to screen and identify mammals on site. *Scientific Reports, 7*(1), Article 43453. https://doi.org/10.1038/srep43453
Ye, Y., Guo, H., & Sun, X. (2019). Recent progress on cell-based biosensors for analysis of food safety and quality control. *Biosensors and Bioelectronics, 126*, 389–404. https://doi.org/10.1016/j.bios.2018.10.039
Ye, D., Zuo, X., & Fan, C. (2018). DNA nanotechnology-enabled interfacial engineering for biosensor development. *Annual Review of Analytical Chemistry, 11*(1), 171–195. https://doi.org/10.1146/annurev-anchem-061417-010007
Yu, M., He, T., Wang, Q., & Cui, C. (2023). Unraveling the possibilities: Recent progress in DNA biosensing. *Biosensors, 13*(9), 899. https://doi.org/10.3390/bios13090889
Yuan, R., Li, H.-K., & He, H. (2021). Recent advances in metal/covalent organic framework-based electrochemical aptasensors for biosensing applications. *Dalton Transactions, 50*(40), 14091–14104. https://doi.org/10.1039/D1DT02360H
Yuan, X., Li, Z., Xiong, L., Song, S., Zheng, X., Tang, Z., et al. (2022). Effective identification of varieties by nucleotide polymorphisms and its application for essentially derived variety identification in rice. *BMC Bioinformatics, 23*(1), 30. https://doi.org/10.1186/s12859-022-04562-9
Zhang, L., Du, X., Chen, Z., Chen, C., Gong, N., Song, Y., et al. (2019). Instrument-free and visual detection of *Salmonella* based on magnetic nanoparticles and an antibody probe immunosensor. *International Journal of Molecular Sciences, 20*(18). https://doi.org/10.3390/ijms20184645
Zhang, X., & Hu, H. (2014). DNA molecules site-specific immobilization and their applications. *Open Chemistry, 12*(10), 977–993. https://doi.org/10.2478/s11532-014-0557-8
Zhang, Y., Wang, W., Lin, Z., Liu, B., & Zhou, X. (2020). Dual-output toehold-mediated strand displacement amplification for sensitive homogeneous electrochemical detection of specie-specific DNA sequences for species identification. *Biosensors and Bioelectronics, 161*, Article 112256. https://doi.org/10.1016/j.bios.2020.112256
Zhao, W.-W., Xu, J.-J., & Chen, H.-Y. (2014). Photoelectrochemical DNA biosensors. *Chemical Reviews, 114*(15), 7421–7441. https://doi.org/10.1021/cr500100j
Zhou, R., Zhao, L., Wang, Y., Hameed, S., Ping, J., Xie, L., et al. (2020). Recent advances in food-derived nanomaterials applied to biosensing. *TrAC, Trends in Analytical Chemistry, 127*, Article 115884. https://doi.org/10.1016/j.trac.2020.115884
Zhou, L., Zhou, X., Zhao, C., Liu, Y., Li, Y., Ma, L., et al. (2021). N-Doped porous molybdenum carbide nanoflowers: A novel sensing platform for organophosphorus pesticides detecting. *Microchemical Journal, 165*, Article 106169. https://doi.org/10.1016/j.microc.2021.106169