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Fluorescence-based quantitative platform for ultrasensitive food allergen detection: From immunoassays to DNA sensors

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Abstract

Food allergies are global health issue with an increasing prevalence that affect food safety; hence, food allergen detection, labeling, and management are considered to be important priorities in the food industry. In this critical review, we provide a comprehensive overview of several fluorescence-based platforms based on different biorecognition ligands, such as antibodies, DNA, aptamers, and cells, for food allergen quantification. Traditional analytical methods are generally unsuitable for food manufacturers to accomplish the real-time identification of food allergens in food products. Therefore, it is important to develop simple, rapid, inexpensive, accurate, and sensitive methods to improve user accessibility. A fluorescence-based quantitative platform provides an excellent detection platform for food allergens because of its high sensitivity. This review summarizes the traditional antibody-based fluorescent techniques for food allergen detection, such as the time-resolved fluoroimmunoassay , immunofluorescence imaging, fluorescence enzyme-linked immune sorbent assay, flow injection fluoroimmunoassay, and fluorescence immunosensors. However, these methods suffer from disadvantages such as the significant rate of false-positive and false-negative results due to antibody cross-reactivity with nontarget food components in the complex food matrix and epitope degradation during food processing. Hence, different types of fluorescence-based immunoassays are suitable for standardization and quantification of allergens in fresh foods. In addition, we summarize new fluorescence-based quantitative platforms, including fluorescence genosensors, fluorescence cell sensors, and fluorescence aptamer sensors. With the advantages of high sensitivity and simple operation, fluorescence biosensors will have great potential in the future and could provide portable methods for multiallergen real-time detection in complex food systems.

KEYWORDS
fluorescence biosensor, fluorescence immunoassay, fluorescence-based quantitative platform, food allergen

1 | INTRODUCTION

Currently, food allergies are recognized as a crucial public health problem and a severe food safety issue, which affect the quality of life of allergic people. Reactions caused by food allergies affect approximately 2% of adults and 6 to 8% of young children (Roberts et al., 2019). Food allergy prevalence in infants is estimated to be 1 to 5% in European countries and the United States, 7% in China, and over 10% in Australia (Tang & Mullins, 2017). Food allergies can initiate immunoglobulin E (IgE)-mediated hypersensitivity to provoke severe acute systemic anaphylaxis, such as asthma, urticaria, gastrointestinal spasm, hypotension, and even shock (Renz et al., 2018). Generally, more than 989 proteins have been registered as food allergens by the World Health Organization and the International Union of Immunological Societies (www.allergen.org). However, 90% of food allergenic reactions are caused by the top eight food allergens: milk, egg, crustacean/shellfish, fish, tree nut, soybean, peanut, and wheat (Nwaru et al., 2014).

Thus far, avoiding the ingestion of foods containing one or more allergens is the only effective way to protect the susceptible population due to the lack of a complete and effective treatment method. According to the Regulation (EU) No 1169/2011, a list of 14 food allergens has been declared to be mandatory for food labeling (European Parliament and European Council, 2011). More importantly, traces of food allergens may be present in processed food products due to cross-contact or cross-contamination. Therefore, to cope with this global challenge and provide more comprehensive information for food allergies patients, it is urgent to develop reliable, feasible, specific, and highly sensitive methods to trace any hidden allergens in food products.

Currently, the most commercially available methods for food industries to monitor food allergens are enzyme-linked immune sorbent assay (ELISA), lateral flow immunoassay (LFIA), and polymerase chain reaction (PCR) (Holzhauser, 2018). ELISA and LFIA rely on monoclonal or polyclonal antibody recognition of target allergen, while PCR is suitable for tracing the presence of the DNA sequences of allergenic foods. Various kits based on ELISA and PCR and LFIA strips have been applied for specific detection of food allergens, offering simple and rapid approaches with suitable sensitivity. However, they also encounter several limitations that lowered the credibility of results. Among them, cross-reactivity with nontarget food components could lead to false positives. Besides, denaturation or degradation of proteins and DNA during food processing could lead to false negatives. In addition, liquid chromatography-mass spectrometry (LC-MS) has been developed as a benchmark technique to unequivocally identify allergens and quantify multiple food allergens from complex matrices in food safety validation and cleaning validation (Marzano et al., 2020). Alternatively, biosensors provide important tools for quantification of food allergens, offering good sensitivity and selectivity, which include optical biosensor [surface-enhanced Raman spectroscopy (Gezer, Liu, & Kokini, 2016) and surface plasmon resonance (Zhou et al., 2019)], electrochemical biosensor [voltammetric biosensor (Alves et al., 2015)], and so forth. However, the application of LC-MS and biosensors in food allergen detection requires further studies, and the lack of on-site testing in food-manufacturing plants and the need for trained lab technicians may limit its wide application in food industry. Generally, they all produce relatively accurate results but are limited in ultrasensitive detection of allergens in processed food products.

In order to overcome such limitations, fluorescence-based technologies have gained increasing attention in the past decade for food allergen detection due to their advantages of nonradioactivity, operator simplicity, high throughput, high sensitivity, and small sample size (Schaferling, 2012). Particularly, with the development of fluorophores and fluorescent technologies, the sensitivity has been dramatically improved, like the level of isotope detection, offering the advantages of safety, convenience, and normal radioactive pollution which is safer than radioactive labels. In addition, many amplification mechanisms, such as quantum dots (QDs), nanoparticles, and cascade amplification strategy, are employed to improve signal-to-noise ratio and achieve higher sensitivity of fluorescence-based methods. Hence, fluorescence-based technologies are propitious to detect single molecule (Suzuki & Yokoyama, 2015), with a sensitivity that is usually two to three orders of magnitude higher than the sensitivity of photometers. Currently, several fluorescence-based quantitative platforms have provided new methods for detecting food allergens using specific fluorescence recognition elements and signal transduction modalities (Sanford & Palmer, 2017), including the fluorescence immunoassay, fluorescence polarization, and fluorescence chemical sensor. However, the fluorescence-based quantitative platform for the determination of food allergens in foods remains challenging due to the complex matrices of food samples and the inherent differences between absorbing materials related to the choice of the appropriate excitation wavelength and fluorescence measurement wavelength.

In this review, we provide a comprehensive discussion of recent developments in fluorescence-based quantitative platforms for allergen detection in food samples and describe the most prospective strategies. We provide an overview of the principles of the different fluorescence-based quantitative platforms and their specific application in the detection for food allergens. We also list such

fluorescent techniques in detail, including the fluoroimmunoassay, real-time fluorescence quantitative PCR, and the fluorescence sensors.

2 | PRINCIPLES OF THE FLUORESCENCE-BASED QUANTITATIVE PLATFORM

Fluorescence is the luminescence phenomenon accompanying the transition of the electron from the first electron excitation to the ground state. Luminescence is a spontaneous emission of light by any substance and occurs from electronically excited states. As a substance absorbs incoming light, the energy of the photon is transferred to the molecule. Once excited, the electrons of molecules are caused to jump from a lower energy level (ground state) to a higher one (excited states), which include excited single states and excited triple states. Molecules in the excited state are very unstable and must be released to return to the ground state by way of radiation transfer or nonradiation transfer. The energy in the radiative transition process is released in the form of photons (Ishikawa-Ankerhold, Ankerhold, & Drummen, 2012). In general, the excitation life of an electron is much shorter than that of a triple state. Hence, fluorescence has a much shorter lifespan, which is on the order of milliseconds (10^{-9}). Fluorescence spectroscopy is considered to be an invaluable and ever more powerful tool in biochemistry and biophysics. Fluorescence spectroscopy is generally presented as emission and excitation spectra. An emission spectrum is the spectrum of fluorescence intensity and emission wavelength measured at a single constant excitation wavelength. On the contrary, an excitation spectrum is dependent on the emission intensity, measured and recorded at the constantly changing excitation wavelength. Due to the different selectivity to absorb light by the molecules, the incident light of different wavelengths has different excitation efficiencies. Some typical fluorescent substances (fluorophores) are encountered in biochemistry. Generally, fluorophores can be divided into intrinsic and extrinsic. Intrinsic fluorophores are those that occur naturally, including the flavins, aromatic amino acids, NADH, chlorophyll, and derivatives of pyridoxyl. Extrinsic fluorophores can provide fluorescence or change the spectral properties of the sample when added, including dansyl, rhodamine, fluorescein, and numerous other substances. As fluorescence probes, intrinsic or extrinsic fluorophores are the most significant area of fluorescence spectroscopy to investigate a large number of interactions at both steady-state and kinetic levels (Table 1). Currently, several fluorescent technologies have been developed based on the measurement of fluorophore characteristics, including fluorescence lifetime, fluorescence quantum yield, and fluorescence polarization.

3 | FLUORESCENCE LIFETIME

Fluorescence emission spectra is a prevalent tool in analytical and bioanalytical chemistry, which is obtained by measuring fluorescence parameters (Krishnamoorthy, 2018). Among them, the fluorescence lifetime is a parameter that plays an important role in (bio)analytical applications.

The fluorescence lifetime is defined as the average time that the fluorescence intensity of the molecule decays to $1/e$ of the original intensity after the excitation light is terminated, which is typically in the scale of nanoseconds (Berezin & Achilefu, 2010). The lifetime of a fluorophore is sensitive to its local microenvironment, such as temperature, pH, ion concentration, and interactions with other molecules. Hence, protein–protein interactions can be measured accurately by using fluorescence lifetime-based technologies (Sun & Periasamy, 2014). For fluorescence lifetime measurements, current methods include sensors, spectroscopy, microscopy, and optical fiber-coupled fluorescence lifetime measurements (Hanulia, Inami, Ono, & Kawata, 2018; Zareanborji, Yang, Town, Luo, & Peng, 2016).

Depending on the measurement of fluorescence lifetime, the time-resolved fluoroimmunoassay (TRFIA) has been successfully developed in food allergen detection. TRFIA assays for food allergen generally utilize earth chelate to label antibody as probes for specific recognition of allergenic proteins. The rare earth chelate probe is mainly a chelate formed by rare earth elements such as Sm^{3+}, Eu^{3+}, and Tb^{3+}, especially Eu^{3+} with some organic compounds such as diketone compound and salicylic acid compound. These chelates have the characteristics of an excitation spectrum bandwidth and a narrow emission spectrum band, which can improve the detection resolution. The fluorescence of rare earth chelates can be measured by coupling with allergen to eliminate the interference of protein background fluorescence.

4 | FLUORESCENCE QUANTUM YIELD

The quantum yield is one of the most significant characteristics of a fluorophore, which is defined as the ratio of the number of photons emitted by fluorescence to the number of photons absorbed. The value of fluorescence quantum yield mainly depends on the structure and property of the compound, as well as the environmental factors of the compound. The larger the value of quantum yield, the stronger the fluorescence of the compound. In general, certain compounds can be fluorescence quenchers

TABLE 1 Introduction of fluorescent dyes for food allergen detection

Dye	Application	Emission maximum max (nm)
SYBR Green I	Highly sensitive DNA fluorescent dye for detection of dsDNA	520
LC Green	Highly sensitive DNA fluorescent dye for detection of dsDNA	470 to 520
EvaGreen	Highly sensitive DNA fluorescent dye for detection of dsDNA	520
Cy3	Markers for antibody and ssDNA	566
Cy5	Markers for antibody and ssDNA	669
Cy3.5	Markers for antibody and ssDNA	596
Cy5.5	Markers for antibody and ssDNA	694
Texas Red	Markers for antibody and ssDNA	616
FAM	Markers for ssDNA	518
TET	Markers for ssDNA	536
JOE	Markers for ssDNA	555
HEX	Markers for ssDNA	556
VIC	Markers for ssDNA	554
TAMRA	Markers for ssDNA	583
NED	Markers for ssDNA	575
Fluorescein isothiocyanate	Immunofluorescence analysis	530
Europium chelates	Markers for antibody	337
Oregon Green 488	Markers for antibody	524
Nile blue	Markers for antibody	680
OliGreen	Highly sensitive DNA fluorescent dye for detection of ssDNA	518
Green fluorescent protein	Markers for cell	509

to reduce the quantum yield or fluorescence intensity of fluorescent substances via dynamic quenching and/or static quenching (Zu et al., 2017). The dynamic quenching is the interaction between the quencher and the fluorescence substance through the collision of two excited states, making fluorescence resonance energy transfer (FRET). The ultrahigh fluorescence quenching ability of quenchers can decrease background noise, thereby significantly increase the signal-to-noise ratio of a FRET system. Based on FRET technology, various fluorescence biosensors are established using molecular beacons constructing by fluorophores and quenchers for quantifying food allergens.

Graphene oxide (GO) is an efficient quencher for various fluorophores (e.g., Cy fluorescent dye, QDs, FAM) due to the nonradioactive electronic excitation energy transfer between the fluorophore and GO. GO can be self-assembled with fluorophores through specific π–π interactions, leading to fluorescence quenching of fluorophores via the FRET process (Wang, Wang, Lv, & Shen, 2018). Cy dyes, including Cy3, Cy3.5, Cy5, and Cy5.5 (Moreira, You, & Owczarzy, 2015; Saha et al., 2019), can be used to label target allergen DNA fragments and antibodies with good photostability. Carboxyfluorescein (FAM), an organic fluorophore, is one of the most widely used dyes for modifying oligonucleotide as DNA probe to hybridize with target allergen DNA fragments (Rao Singuru, Sun, & Chuang, 2020). In addition, QDs are another important fluorescent nanomarkers in immunoassays, fluorescent biosensing, and imaging applications. Particularly, QDs present unique benefits for FRET because of their stability, broad excitation spectra, and narrow emission spectra. QDs are nanocrystals made of semiconductor materials, which can produce different colored fluorescence depending upon the size of the QDs at 2 up to 10 nm (Bonilla, Bozkurt, Ansari, Sozer, & Kokini, 2016). QDs can be successfully conjugated to many biomolecules as biorecognition element for food allergen detection, such as proteins, peptides, and aptamers.

5 | FLUORESCENCE POLARIZATION AND ANISOTROPY

Fluorescence polarization and anisotropy are different representations of the same luminescence property of fluorescence, which are single-plane polarized fluorescence emitted by fluorescent materials after excitation by a single-plane polarized light. The polarization emission is caused by the selection of the excitation photon orientation by fluorescent molecules and the emission photon orientation. During fluorescence polarization and anisotropy, the fluorescence molecule is called as an oscillating dipole, which has an intrinsic absorption dipole moment and emission dipole moment. Due to the differences in electron ground state and electron excited state of fluorescent molecules, the absorption and emission dipoles of fluorescent molecules are usually noncollinear. The angle between the absorbing dipole moment and the emitting dipole moment is determined by the molecular structure of each fluorescent molecule. A system of random orientation of fluorescent molecules excited by polarized light will preferentially excite those fluorescent molecules that absorb the dipole moment parallel to the photonic electric vector.

The anisotropy and polarization are related to the rate and extent of rotational diffusion of fluorescent molecules, which is influenced by both intrinsic properties of the molecules and environmental factors (Zhao et al., 2019). Hence, measuring changes in polarization and anisotropy allow for characterizing macromolecular associations and can provide determinations of protein–DNA and protein–protein interactions (Wu, Pei, Lin, Liu, & Li, 2017). Fluorescence polarization and anisotropy techniques for food allergens can employ fluorescence-labeled aptamers as bioprobes. Anisotropy signal changes generate through bioprobes binding to allergenic proteins. More importantly, the aptamers cannot be too large, otherwise binding will not induce rotational diffusion enough to produce anisotropy signal changes which can be clearly observed.

6 | ANTIBODY-BASED FLUORESCENT TECHNOLOGY

Currently, it is a critical challenge to accurately quantify food allergens from complex food matrices at every stage of food processing. Other proteins in food matrices may bind nonspecifically to the fluorescent probes, obscuring the subsequent quantitative detection results. The most common strategy used to solve this issue is based on the specific and high-affinity interactions between the antibody and target food allergen. Immunoassays with fluorescent probes have been widely used in allergen detection due to many advantages of high sensitivity, a wide dynamic range, marker stabilization, and multiparameter simultaneous detection. Historically, the most common antibody-based fluorescent techniques for food allergen quantification included the TRFIA, immunofluorescence imaging, fluorescence ELISA, flow injection fluoroimmunoassay, and fluorescence immunosensors.

7 | TIME-RESOLVED FLUOROIMMUNOASSAY

The TRFIA is a very effective method for analyte detection (Jameson, Vetromile, & James, 2013), which can effectively reduce and eliminate the interference of background fluorescence and scattered light to improve sensitivity.

7.1 | Basis of TRFIA

In TRFIA assays, long-lived rare-earth ion chelates (lanthanide complexes) are generally selected as the fluorescence labels in bioanalytical applications, since exciting sample with the ultrashort pulse of excitation light can result in short-lived background noises with a luminescence lifetime of nanoseconds to a few microseconds, and the lifetime of the label is relatively longer. The counting time only measures the signal from long-lived lanthanide labels after the luminescence from all the molecules and instruments begins to decay. Generally, the measurement is cycled many times for one cuvette, which further increases the signal-to-noise ratio by repeated experiments. The principle of microsecond time-resolved fluorometric measurements is shown in Figure 1a. In the field of TRFIA assays, microtiter plates are the most common solid phases with different plate readers, which differ in terms of the type of excitation sources, filters, and/or monochromators, time gating, and focus setups used. Particularly, among various excitation sources, the strong 337-nm emission of a nitrogen laser is propitious to most europium chelates (Hagan & Zuchner, 2011).

7.2 | Detection of food allergens by TRFIA

Currently, TRFIA has been increasingly employed in quantitative detection of food allergens. Generally, food allergens are directly captured by an appropriate antibody bound on a solid phase, and this target allergen can then be detected after binding a europium chelate-labeled antibody and measuring the luminescence signal (Figure 1b). Faeste, Holden, Plassen, and Almli (2006) developed and validated a new sensitive TRFIA for the quantification

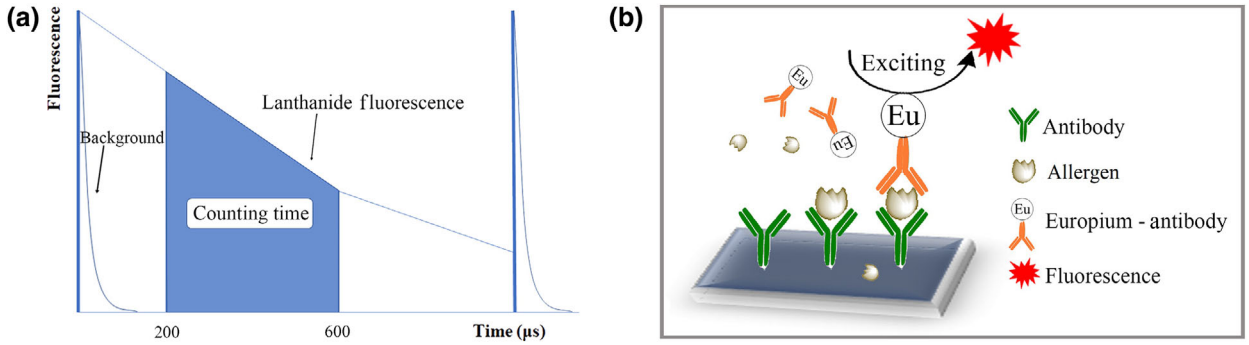

FIGURE 1 Schematic diagram of time-resolved fluorescence. (a) Schematic diagram of time-resolved fluorescence determination principle. (b) In the time-resolved fluorescence assay, food allergens are determined by the measurement of chelate fluorescence, in which the chelate is one-step labeled with the allergen

of hazelnut corylin in food, while evaluating the labeling practices with regard to hazelnut. In this work, the fluorescence characteristics of europium chelate were utilized to improve the signal-to-noise ratio and led to an enhanced sensitivity of 0.1 mg/kg and the limit of quantitation reaching 0.33 mg/kg hazelnut protein. The method can quantitatively detect hazelnut in different food products such as chocolate, cookies, and cereals. Sletten, Løvberg, Moen, Skarpeid, and Egaas (2007) established two different formats of the TRFIA methods to detect the content of casein in different foods (Table 2). The first employed europium-conjugated antibody (direct TRFIA), while the other used biotinylated antibody with europium-conjugated streptavidin (indirect TRFIA). The two methods allowed to quantify milk allergen casein in food samples (spice-mix, packet soup, flour mix, instant potato) at 1.3 mg/kg (direct TRFIA) and 1.5 mg/kg (indirect TRFIA).

8 | IMMUNOFLUORESCENCE IMAGING

Immunofluorescence imaging provides a unique and efficient method for detecting food allergens due to its better specificity and higher sensitivity than histology and optical microscopy.

8.1 | Basis of immunofluorescence imaging

Immunofluorescence imaging is a method that can recognize the fluorescence of labeled molecules in biological samples and infer their intensity and emission spectrum from images or photometric data. Immunofluorescence imaging utilizes ultraviolet rays as the light source to illuminate the fluorophore to emit fluorescence, and then observe the shape and position of the fluorescently labeled object under the microscope, for example, electron microscopy. The application of immunofluorescence imaging technology enables the study of antigens and antibodies to enter the subcellular level and reach the ultrastructural level. Monoclonal antibodies are widely used in immunofluorescence imaging as affinity probes for the location of their target antigens in tissues by transmission electron microscopy and fluorescence microscopy.

8.2 | Detection of food allergens by immunofluorescence imaging

Immunofluorescence imaging has been established to detect food allergens (Table 2). Petrášová, Pospiech, Tremlová, and Randulová (2015) selected immunofluorescence as an examination method to confirm the content of milk proteins in 20 meat products, while comparing the results with the information about milk proteins on the packaging of the product. According to the results, milk protein can be detected in 84.62% of samples in which the manufacturer declared the presence of milk or cheese, which indicates that the immunofluorescence method is effective and reliable for milk protein detection in meat products. In this study, fluorescence microscopy was utilized to examine cryosections of each sample with Texas Red as the fluorochrome due to minimal background fluorescence. Texas Red–labeled antibodies can provide an improved separation of green and red fluorescence. However, it is necessary to further validate this method using more parameters, such as repeatability and reproducibility.

The aim of research conducted by Solas et al. (2008) was to detect allergens in fish tissues produced by fish parasitized with Anisakis larvae. In this study, fish muscle tissue was examined by transmission electron microscopy to observe the fluorescence labeled on the allergenic protein. Enstone, Peterson, and Gijzen (2015) used Oregon Green

TABLE 2 Examples of target using different capturing ligands

Detection principle	Allergenic Ingredient	Target	Capturing ligand	Limit of detection (LOD)	Reference
TRFIA	Hazelnut	Corylin	Antibodies	0.1 mg/kg	Faeste et al. (2006)
Direct TRFIA Indirect TRFIA	Milk	Casein	Antibodies	1.3 mg/kg1.5 m	Sletten et al. (2007)
Immunofluorescence imaging	Milk	β-Casein	Antibodies	–	Petrášová et al. (2015)
Immunofluorescence imaging	Fish	Ani s 4	Rabbit antisera	–	Solas et al. (2008)
Immunofluorescence imaging	Soybean	Hydrophobic protein	Antibodies	–	Enstone et al. (2015)
Fluorescence ELISA	Fish	Paramyosin	Serum	–	Suzuki et al. (2010)
Fluorescence ELISA	Milk	α-Lactalbumin	Antibodies	0.1 ng/mL	Yang et al. (2014)
Fluoroimmunoassay	Soybean	Soybean protein	Antibodies	0.05 mg/L	Godoy-Navajas et al. (2011)
Fluorescence immunosensors	Shrimp	Tropomyosin	Rabbit antisera	0.054 mg/kg	Li et al. (2010)
Fluorescence immunosensors	Egg	Ovalbumin	Antibodies	153 ng/mL	(Fu et al., 2018)
LFIA	Crustacean	Tropomyosin	Antibodies	0.05 μg/mL	Wang et al. (2019)
Flow injection fluoroimmunoassay	Milk	BSA	Antibodies	0.01 mg/mL	Lim et al. (1997)
qPCR	Shrimp	DNA target	TaqMan probes	0.05%	Cao et al. (2011)
qPCR	Lobster	Mitochondrial genome	TaqMan probes	0.1 μg/mL	Eischeid (2016)
qPCR	Crustacean	16S rRNA gene	TaqMan probes	0.1 pg	Fernandes et al. (2018)
qPCR	Fish	*Hoxc*13 gene	TaqMan probes	0.2 pg	Tetzlaff et al. (2016)
qPCR	Fish	18S RNA gene	TaqMan probes	0.05 ng	Herrero et al. (2014)
qPCR	Wheat	Tri a 18	Fluorescent EvaGreen dye	20 mg/kg	Martín-Fernández et al. (2016)
qPCR	Soybean, celery, white and brownmustard	Mannitol dehydrogenase, mRNA	TaqMan probes	–	Luber et al. (2015)
qPCR	Walnut	Jug r 1, Jug r 3 and Jug r 4	SYBR Green fluorescent dye	100 mg/kg	Linacero et al. (2016)
qPCR	Almond	Pru du 5	Fluorescent EvaGreen dye	50 mg/kg	Costa et al. (2012)
Nested qPCR	Almond	Pru du 6	TaqMan probes	50 mg/kg	Costa et al. (2013a)
Nested qPCR	Walnut	Jug r 3	TaqMan probes	50 mg/kg	Costa et al. (2013b)
qPCR	Chestnut	Cas s 9	TaqMan probes	100 μg/mL	Sanchiz et al. (2020)
qPCR	Milk	Mitochondrial 12S rRNA gene	SYBR Green fluorescent dye	0.1%	Liao et al. (2017)
qPCR	Milk	α-Lactalbumin	TaqMan probes	0.05 ng	Xiao et al. (2016)
qPCR	Milk	12S rRNA gene	TaqMan probes	0.05%	Villa et al. (2020)
Digital PCR	Peanut, soybean	Ara h 2, Gly m 5	TaqMan probes	0.003%0.001%	Pierboni et al. (2018)
Fluorescence genosensor	Shrimp, prawn, crab	*Pen* gene	Fluorescence beta-pyrroridinyl peptide nucleic acid probe	–	Charoenchon et al. (2011)

(Continues)

TABLE 2 (Continued)

Detection principle	Allergenic Ingredient	Target	Capturing ligand	Limit of detection (LOD)	Reference
Fluorescence genosensor	Hazelnut, peanut, walnut	Specific DNA sequence	Specific oligonucleotide probes	0.01%	Christopoulou et al. (2017)
Fluorescence genosensor	Peanut	Ara h 1	Specific oligonucleotide probes	4.7×10^{-23} M	Li et al. (2018)
Fluorescence genosensor	Peanut, soybean	Ara h 6, Gly m Bd 28 K	Specific oligonucleotide probes	1 nM	Yuan et al. (2019)
Fluorescence cell sensor	Shrimp, fish	Tropomyosin, Parvalbumin	Mast cell	3.3×10^{-4} ng/mL	Jiang et al. (2015)
Fluorescence cell sensor	Fish	Parvalbumin	Rat basophilic leukemia cell	0.35 ng/mL	Jiang et al. (2014)
Fluorescent Aptamer Sensor	Shellfish	Tropomyosin	Aptamer	0.5 ng/mL	Zhang et al. (2016)
Fluorescent Aptamer Sensor	Shellfish	Tropomyosin	Aptamer	2 nM	Chinnappan et al. (2020)
Fluorescent Aptamer Sensor	Peanut	Ara h 1	Aptamer	56 ng/mL	Weng et al. (2016)

–Not indicated in the literature.

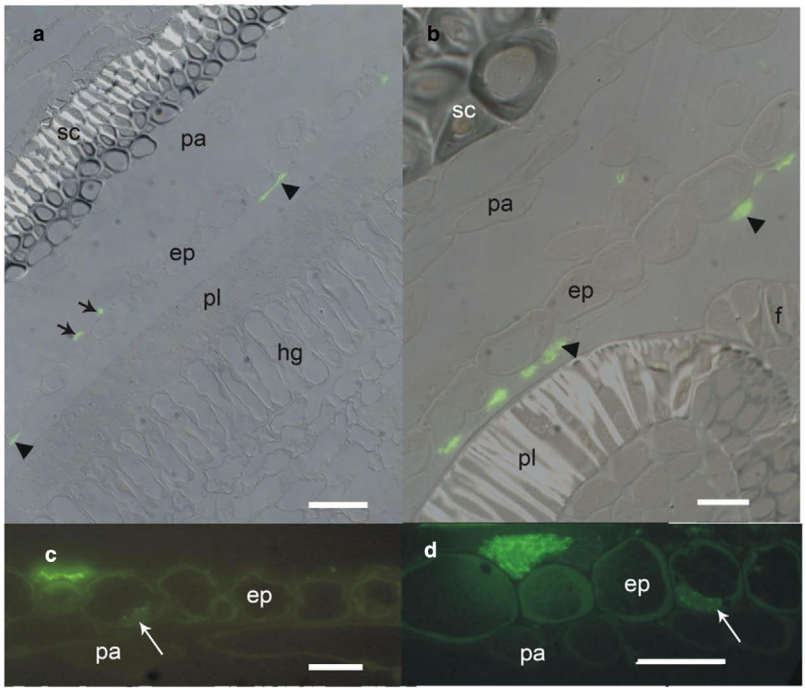

FIGURE 2 Samples from soybean pods and seeds test positive for the presence of HPS by immunofluorescence imaging. All sections are shown after probing with monoclonal antibody to HPS and Oregon Green reporter conjugate (Enstone et al., 2015)

488–labeled monoclonal antibodies against hydrophobic protein from soybean (HPS), an allergen (Gly m 1) from soybean (Figure 2). When they examined the protein with fluorescence and transmission electron microscopy, they found some secretions, which appears HPS, were unevenly distributed on the seed surface and endocarp epidermis.

9 | OTHER FLUOROIMMUNOASSAYS

Currently, other fluoroimmunoassays can be used to detect food allergens in food products. The main principles of these methods rely on the combination of the antigen–antibody immune response and fluorescence detection and analysis.

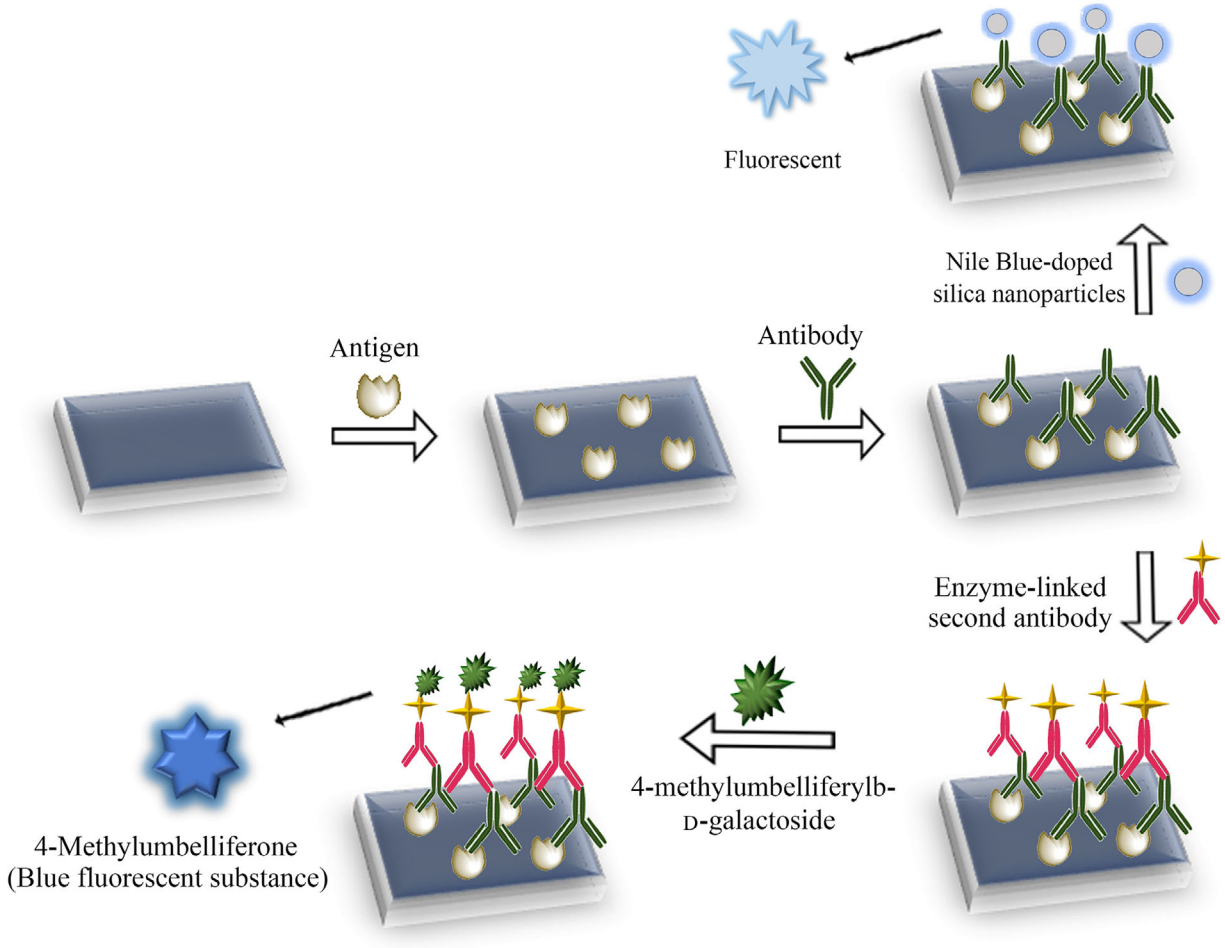

FIGURE 3 Schematic diagram of the fluorescence immunoassay. In the fluorescence immunoassay, fluorescence magnetic beads are directly coupled to antibodies, while 4-methylumbelliferyl-β-D-galactoside is employed to activate the enzyme to produce fluorescence

9.1 | Fluorescence ELISA

ELISA-based methods have been extensively applied to detect food allergens in different sample matrices. In ELISA-based methods, chromogenic substrates react with enzymes to produce colored soluble product to assess the amount of target allergens in the sample extract, such as 3,3′,5,5′-tetramethylbenzidine (TMB)/horseradish peroxidase (HRP). However, in some cases, ELISA may have low sensitivity due to low color change and poor enzyme stability. Fluorescence, in particular, has been exploited to enhance detection sensitivity in ELISA assays. Recently, nanomaterials were implemented as a solid surface in fluorescence ELISA to improve sensitivity and efficiency (Table 2). Figure 3 shows two principles of the fluorescence ELISA for allergen capture in food samples. Suzuki, Kobayashi, Hiraki, Nakata, and Shiomi (2010) described a novel fluorescence ELISA used to identify a 100-kDa protein as a new allergen in the disc abalone *Haliotis discus*, which is called paramyosin. Since the combination of the fluoroimmunoassay with the nanomagnetosphere or high-efficiency separation technology, the immunoassay has a higher efficiency and requires less time than normal specific antigen–antibody reactions. Godoy-Navajas, Aguilar Caballos, and Gomez-Hens (2011) first reported a long-wavelength fluoroimmunoassay for the determination of soybean protein. The method can be used to analyze soybean protein with anti-soybean protein antibodies coupled silica nanoparticles which doped with Nile blue dye. The immunoassay was performed in 96-well microplates using a competitive format, and the fluorescence was measured on the solid surface at ex620 nm and em680 nm. The limit of detection (LOD) is 0.05 mg/L, and the obtained results were not statistically different from those provided by a commercial ELISA kit. Yang et al. (2014) developed a competitive fluorescence ELISA for specifically quantification of bovine α-lactalbumin (α-La) in dairy products. In this study, CdSe/ZnS QDs were designed to covalently conjugate with anti-α-La monoclonal antibods (mAbs), which were helpful for measuring allergens at low concentration. This method based on QD-mAb conjugates can specifically recognize and

quantify α-La, exhibiting high sensitivity with a LOD of 0.1 ng/mL.

9.2 | Flow injection fluoroimmunoassay

Flow injection fluoroimmunoassay has emerged in recent 20 years. The flow injection immunoassay allows continuous semi- or fully automatic measurement of analyte concentration (Soh et al., 2011). The flow injection immunoassay is becoming a powerful technique because of its ability to achieve specific separation to continuously identify the antigen–antibody complex. The flow injection system generally consists of lab-on-valve module, flow cell, spectrophotometer, and light source. Compared with the solid phase immunoassay, this system is rapid and requires only a minimal sample volume. The flow injection fluoroimmunoassay has been widely used to detect food allergens using a cation exchange column. In this work (Lim, Nakamura, & Matsunaga, 1997), the milk allergen bovine serum albumin (BSA) and fluorescein isothiocyanate (FITC)-conjugated antibody complexes can be distinguished from the free FITC-conjugated IgE antibody, with the detection range of 0.01 ± 2.0 mg/mL (Table 2). Hence, this simple and rapid method based on the fluoroimmunoassay can be used for the continuous detection of food allergens.

9.3 | Fluorescence immunosensor

Fluorescence immunosensors use the specifically binding of allergenic proteins and antibodies and measure the fluorescence intensity by different types of transducers. The principles are similar to classic immunoassays. Fluorescence immunosensors are used to trace the presence of allergenic proteins in food (Table 2). In the present study (Li, Zhang, Lin, Haider, & Jiang, 2010), they established a sandwich-type method based on a protein chip for the determination of the major allergen tropomyosin (TM) in shrimp. In this method, the commercial streptavidin-conjugated Cy3 fluorescence substance was labeled on monoclonal antibody, and the compound was used as the detection reagent to bind on the rabbit antiserum. The content of TM can be quantified by the detection of fluorescence, and the LOD of detection is 0.054 mg/kg. This method represents high specificity and reliability for detecting shrimp allergens. Moreover, different monoclonal antibodies can be used to construct protein chips to detect other food allergens. In another study, a universal sensor was established based on the FRET technology for the detection of egg allergen ovalbumin (OVA) with a LOD of 153 ng/mL using nitrogen, oxygen, phosphoric codoped carbon dot (NOP-CD), and GO (Fu et al., 2018). In this FRET system, the bright fluorescence from the NOP-CD-anti-OVA was quenched by absorbing on the GO surface through electrostatic attraction. With the addition of OVA in sample, NOP-CD-anti-OVA were detached from GO surface and attached to OVA, creating a "turn-on" state and leading to the recovery of fluorescence intensity.

9.4 | Fluorescent lateral flow immunoassay

Fluorescent LFIA can improve applications for large-scale screening or point-of-care detection of food allergens. One of the LFIA strips based on a competitive format has been constructed for detection of major crustacean allergen TM (Wang et al., 2019). This strip utilizes CdSe/ZnS core–shell QD instead of the organic fluorophores to label the anti-TM Ab for enhancing the stability and sensitivity. Free TM in sample competes with immobilized TM on the test-line for binding QD-Ab, leading to a clear fluorescent signal in the test-line. The detection results can be obtained within 30 min with the visual LOD of 0.5 μg/mL and instrumental LOD of 0.05 μg/mL.

As a quantitative tool for food allergens, antibody-based fluorescent technology has been accepted for the identification of food allergens in matrices as a protein-based confirmatory technique with the advantages of simple operation, good stability, and high sensitivity. However, due to the antibody cross-reactivity with nontarget food components and degradation of epitopes during food processing, antibody-based fluorescent technology also suffers from practical limitations: (1) it is difficult to monitor allergenic ingredients in food products during industrial processes; (2) it provides a significant rate of false-positive results due to cross-reactivity between antibodies with nontarget food components; (3) it provides a significant rate of false-negative results because of the denaturation of allergenic proteins during food processing.

9.5 | Fluorescent technology based on DNA, aptamers, and cells

This procedure is particularly significant in the field of food allergen analysis, in which analytical methods must be specific, accurate, sensitive, and customer friendly, so that food manufacturers can apply the technique to manage food allergens in the food matrices during food processing. Generally speaking, several different types of biorecognition molecules, such as DNA, aptamers, or cells, can be used in fluorescent technologies for efficient food allergen capture.

10 | REAL-TIME FLUORESCENCE QUANTIFICATION PCR

Real-time fluorescence quantification PCR (qPCR) is one of the most common methods in fluorescent technology, which has been gradually applied in the determination of food allergens.

10.1 | Basis of qPCR

In conventional PCR, it is difficult to quantify DNA because the content of PCR products is not equal to the DNA template molecules. Based on conventional PCR, qPCR was developed for the quantitative detection of DNA (Kang, 2019). In qPCR, fluorophores are added to the reaction system, and the specific DNA is analyzed by monitoring the accumulation of the fluorescent signal in real time. Qualitative and quantitative analysis of the PCR products in unknown samples is achieved by melting curve analysis through the relationship between fluorescence dye intensity and temperature. (Miao et al., 2020). The amount of DNA template molecules (specific DNA) can be determined by the cycle threshold (Ct), which is related to the number of cycles where the fluorescence increases above the background level. Based on melting curve analysis, high-resolution melting (HRM) analysis was developed to identify specific DNA of allergenic foods based on their melting temperature (T_m) at which 50% of the DNA is single stranded (Martín-Fernández et al., 2016). In addition, nested qPCR was successfully combined with qPCR for direct monitoring PCR productions in the second phase of the reaction (Costa, Oliveira, & Mafra, 2013b).

Thus far, the number of specific fluorescent dyes and fluorescent-labeling probes have been designed as fluorescent probes to monitor PCRs in real time to increase specificity and to eliminate the need for post-PCR confirmation of the results. A fluorescent dye such as SYBR Green represents a kind of unsaturated cyanide fluorescein, which can emit fluorescence only when they bound to the dsDNA in a nonspecifically way (Baris, Etlik, Koksal, Ocak, & Baris, 2013). The fluorescence intensity of the dye is directly related to the amount of accumulated PCR products (Pafundo, Gulli, & Marmiroli, 2010). In comparison to fluorescent-labeling probe, the method using the SYBR Green dye is nontoxic, user-friendly, and cost-effective without requiring an intricate probe design and prioritization scheme (Fajardo et al., 2008). However, the SYBR Green dye provides lower specificity compared with probes since it can be combined with any dsDNA (Martín et al., 2009). Hence, the SYBR Green dye is not suitable for multiplex qPCR assays. LC Green, a new type of dsDNA binding dye, is a saturated binding dye compared with SYBR Green, and a high concentration will not inhibit the PCR, offering a higher accuracy in multiplex qPCR assays (Wittwer, 2003). Additionally, other detectors, such as the TaqMan probe and molecular beacon (Yusop, Mustafa, Che Man, Omar, & Mokhtar, 2011), are designed as short oligonucleotides that bind to both the fluorophore and quencher, which can absorb the reporter gene fluorescence (Lopez-Andreo, Lugo, Garrido-Pertierra, Prieto, & Puyet, 2005). Several fluorophores, such as tetrachlorofluorescein (TET), 2, 7-dimethyl-4, 5-dichloro-6-carboxyfluorescein (JOE), hexachlorofluorescein (HEX), VIC, tetramethylrhodamine (TAMRA), NED, and Texas Red, are widely used in TaqMan probes. The fluorescent probe is hydrolyzed by Taq polymerase at each cycle, releasing the fluorescent dye from the quencher and achieving quantitative detection of its high signal, as well as good discrimination ability. Compared with other conventional oligonucleotide probes, the molecular beacon probe has a stem-and-loop structure that shows remarkable specificity for detecting target DNA. The molecular beacon probe can reduce the initial fluorescence background since the stem-and-loop structure can provide more effective quenching (Mhlanga & Malmberg, 2001).

10.2 | Detection of food allergens by qPCR

qPCR is the most popular fluorescent technology to identify allergenic foods using the DNA-based food allergen detection technology. For example, major crustacean allergens, such as TM and arginine kinase, contain relatively high cross-immunity, and the protein source cannot be determined by antibody-based fluorescent technology. Conversely, qPCR can be employed to identify crustacean allergens from different crustaceans. Cao et al. (2011) designed specific primers and TaqMan probes to successfully identify shrimp-derived components using qPCR, depending on a large number of nucleic acid sequences from different species of shrimp. In total, 14 kinds of shrimp could be accurately identified with high specificity and sensitivity, including prawns, *Crangon crangon*, and septemspinosa. Eischeid (2016) developed a qPCR assay to detect lobster meat in different foods under different production conditions (high temperature and high pressure) with LOD of 0.1 to 1 μg/mL. This method was applied to detect crustaceans in 60 food products which contained crustaceans as an ingredient or carried precautionary labeling with crustacean allergens, indicating that crab can be effectively differentiated from other crustaceans (Eischeid, 2018). Besides, another qPCR system was developed to trace crustaceans through targeting 16S rRNA mitochondrial gene in foods, allowing the detection and quantification down to 0.1 pg and 0.0001%

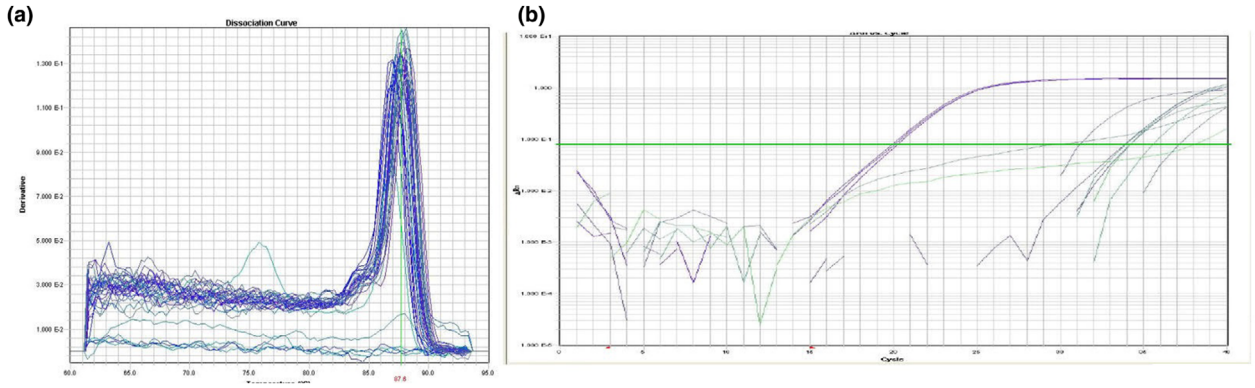

FIGURE 4 Real-time PCR amplification with SYBR Green dye target Jug r 3 gene of walnut. (a) Melting curve. (b) Ct values, the walnut cultivars showed Ct values around 20 and the other species hazelnut, almond, peanut, wheat, barley, rye ranged from 33.88 to 37.10 (Linacero et al., 2016)

(w/w) of shrimp DNA and shrimp in model mixtures (Fernandes, Joana, Oliveira, & Isabel, 2018). Tetzlaff et al. (2016) reported a qPCR method with an ability to indicate the presence of organisms belonging to Teleostei in food. A cost-effective qPCR scheme was established by Herrero, Vieites, and Espineira (2014) to confirm the presence of fish. This in-house fast qPCR can trace fish without cross-reactivity with some species of bivalves, cephalopods, crustacean, meats, spices, and vegetables within 43 min. A qPCR approach based on HRM analysis was also proposed to detect wheat (Tri a 18), allowing detecting and quantifying wheat down to 20 mg/kg in rice flour (Martín-Fernández et al., 2016).

Additionally, qPCR has been widely applied to detect tree nut in dairy processed food products (Linacero, Sanchiz, Ballesteros, & Cuadrado, 2019). Linacero et al. (2016) established the walnut SYBR Green qPCR detection method according to the coding genes of walnut Jug r 1, Jug r 3, and Jug r 4, which is sensitive to 100 mg/kg of raw walnut (Figure 4). They also analyzed the impact of different processing methods on the detection results using the qPCR method, demonstrating that high-pressure sterilization methods could destroy the DNA integrity and extraction content, thus reducing the amplification efficiency, while ultra-high-pressure processing had no impact on DNA amplification. Costa, Mafra, and Oliveira (2012) proposed an HRM approach to detect almond, with designed primers targeting the specific DNA region encoding the Pru du 5. It can distinguish the almond from other food components because the unspecific PCR products is different. This method can trace the presence of almond (50 mg/kg) in almond/walnut mixtures and has been successfully applied to processed foods. A single tube–nested qPCR system was developed to trace almond (Costa, Oliveira, & Mafra, 2013a) and walnut (Costa et al., 2013b) in processed foods. This system used two sets of primers and a hydrolysis probe to amplify the specific DNA fragments of Pru du 6 and Jug r 3 and monitor the PCR production throughout the entire second phase of the reaction. The PCR productions in the first phase were functioned as DNA template in the second phase of the reaction. The system presented a LOD of 50 mg/kg almond spiked in walnut and 50 mg/kg of walnut in both batter and sponge cakes. Sanchiz et al. (2020) described a qPCR assay for chestnut detection with a TaqMan probe and primers for Cas s 9, providing a LOD of 100 ppm in binary mixtures. This assay was applied for analysis of chestnut in several commercial food products, indicating that amplification efficiency of Cas s 9 was importantly affected when heat and pressure were applied. In another study, a tetraplex qPCR method was designed to simultaneously trace soybean, celery, white, and brown mustard in food products (Luber, Demmel, Pankofer, Busch, & Engel, 2015).

As for milk detection, two qPCR systems targeting the mitochondrial 12S rRNA gene (Liao, Liu, Ku, Liu, & Huang, 2017) and α-lactalbumin-encoding gene (Xiao, Qin, Wenju, & Qin, 2016) were able to detect 0.1% of cow's milk and 0.05 ng of bovine DNA, respectively. In another study, a qPCR system targeting the 12S rRNA gene was applied to quantify traces of milk in foods, providing a sensitivity down to 0.05% of cow's milk in raw meat and cooked ham (Villa, Costa, Oliveira, & Mafra, 2020).

Futhermore, digital PCR, the last generation of PCR, was applied for the detection of peanut and soybean in foods (Pierboni et al., 2018). This method is based on fractioned PCR through partitioning a sample into many droplets or cavity for separate reactions and partitioned counting positive and negative. In this study, the nonfluorescent quenchers as MGB (minor groove binder) was labeled at the 3′ end of probes targeting peanut Ara h 2 and soybean Gly m 5, reducing cross-talk signals, providing LODs of 0.003% for peanut and 0.001% fot soybean. More importantly, digital PCR can be

further developed for simultaneous quantification of target DNA of multiple allergens without the need of a standard curve.

11 | FLUORESCENCE SENSORS

Fluorescence sensors are a kind of device constituted by a sensitive element and a signal transduction element connected to a data-processing system that can detect and acquire a fluorescent signal (Rosa, Linda, & Angelo, 2013). The specific quantitative analytical information is obtained by measuring and converting signal into a quantifiable one according to certain rules during the process of being used. The principle of analyte quantification is based on the measurement of the fluorescence emission of either the intrinsic fluorescence of the analytes or the fluorescently labeled material. Fluorescence sensors are being increasingly developed for the quantitative allergen detection due to their ability to miniaturize the device, undergo real-time analysis and high speed of execution (Ross, Bremer, & Nielen, 2018).

Recently, more innovations are developed by combining biosensors with various biorecognition ligands to exploit point of care devices to monitor food allergens, such as genosensors and cell assays (Neethirajan, Weng, Tah, Cordero, & Ragavan, 2018). The fluorescence sensor can be divided into many types according to different recognition elements in the sensing phase. In this method, the receptor can be an ssDNA molecule which can hybridize with specific DNA fragment of the allergen, an antibody capable of specifically recognizing allergen, or an aptamer capable of binding the target allergen directly. A variety of fluorescence sensors can be constructed through transforming either the biological recognition component or the type of transducer for food allergen detection (Liu, Ding, Zhang, & Ding, 2019). However, one of the greatest challenges ahead of sensor analysis is to extract the allergen or corresponding DNA successfully from a food product. Extraction and isolation of allergenic proteins and genomic DNA from food ingredients or food products is regarded as the crucial step for successful allergen detection via different fluorescence-based detection methods. In previous studies, numerous cost-effective and high-throughput methods have been developed for the extraction of proteins and DNA from foods, including commercial kits and in-house methods (Faisal, Vasiljevic, & Donkor, 2019; Yalçınkaya, Yumbul, Mozioğlu, & Akgoz, 2017). In terms of the quantity and integrity, the most suitable method is strongly dependent on the types of food matrices; thus, it is hard to obtain high values of extract from highly processed foods.

12 | FLUORESCENCE GENOSENSORS

12.1 | Basis of fluorescence genosensors

Genosensors (Martin-Fernandez, Manzanares-Palenzuela, Sanchez-Paniagua Lopez, de-Los-Santos-Alvarez, & Lopez-Ruiz, 2017), also known as DNA biosensors, depend on a DNA hybridization recognition reaction between the target and the probe, which is considered as a recognition component. DNA hybridization is commonly used for the analysis of specific gene sequences due to its simplicity and high specificity. These sensors convert the reaction between the target and probe into a measurable signal through incorporating a reporter molecule such as a fluorescent probe. Besides, such fluorescent probes can be designed for the detection of hybridization to provide very high sensitivity and high selectivity because the probes are activated for emission only by the presence of hybridized nucleic acid. Figure 5a displays one of the principles of fluorescence genosensors to identify allergenic foods. The target DNA can be identified by a fluorescent-labeled ssDNA probe, which can form a double-stranded hybrid with its complementary strand.

12.2 | Detection of food allergens by fluorescence genosensors

Charoenchon et al. (2011) have developed a method based on hybridizing DNA competitively to detect shrimp, prawn, and crab residues within 2 hr in food products. They designed the fluorescent beta-pyrolidinyl peptide nucleic acid probe and primers to a specific gene of crustacean. In this method, the target gene was first amplified via isothermal DNA amplification, then the probe and corresponding quencher competitively hybridized DNA products, as well as the target gene can be detected via fluorescence visualization on UV light source. Christopoulou, Karaiskou, and Kalogianni (2017) developed a bead array-based sensor constituting fluorescent polystyrene microbeads for the determination of three nut allergenic foods (walnut, peanut, and hazelnut) within 3.5 to 4 hr in processed food (cookies) by identifying specific DNA sequences. With a LOD of 1.4 pM of hazelnut and 4.1 pM of peanut and walnut, 0.01% content of peanut, hazelnut, and walnut can be traced in cookies. Li, Liu, Zhu, and Li (2018) synthesize a highly sensitive sensing material, gold–palladium nanowaxberries (AuPd NWs)/dodecylamine functionalized graphene quantum dots (D-GQDs)-graphene microaerogel (GMA), for establishing a genosensor to detect peanut Ara h 1. In this study, probe was attached to the AuPd NW on the electrode

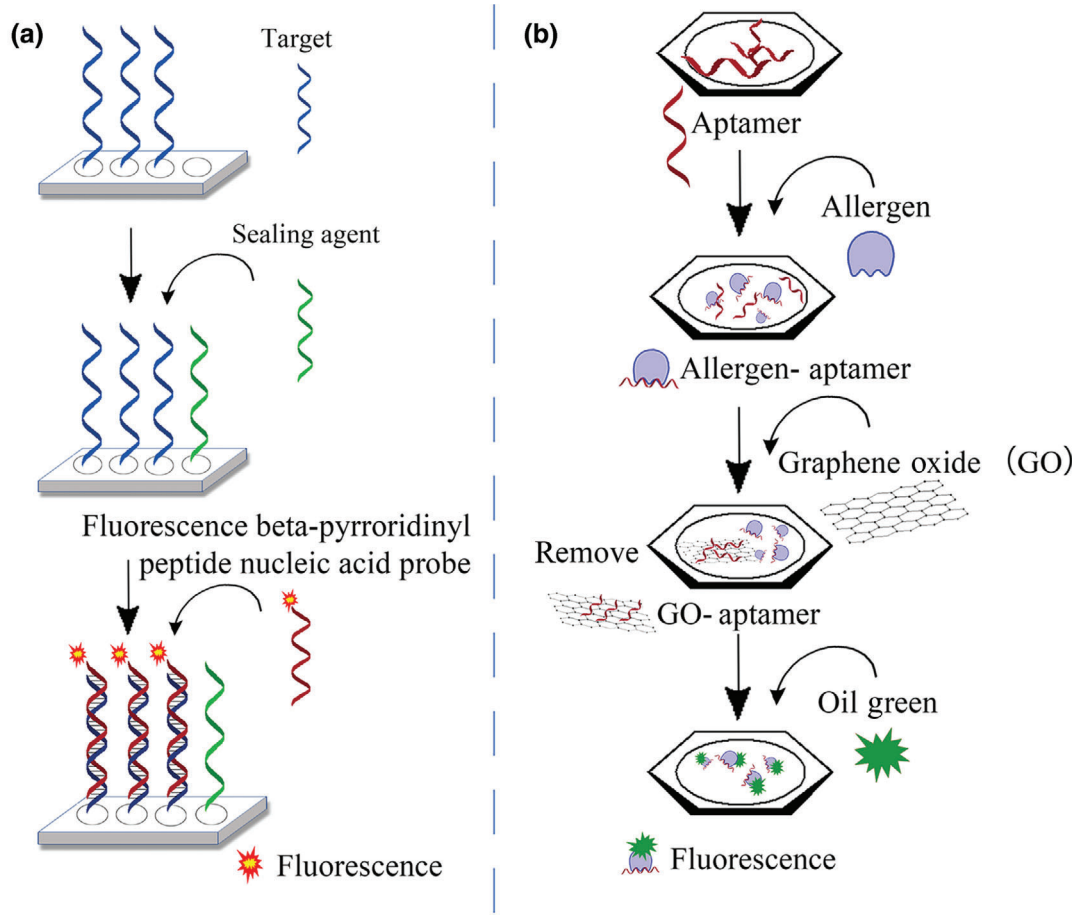

FIGURE 5 Schematic diagram of the fluorescence genosensor and fluorescent aptamer-based GO sensor. (a) Schematic diagram of the fluorescence genosensor. The fluorescent beta-pyrroridinyl peptide nucleic acid probe is specifically combined with allergen genes, and the allergen is measured by detection of the relative fluorescence intensity. (b) Schematic overview of the fluorescent aptamer-based GO sensor. Food allergens are analyzed by measuring the fluorescence intensity of the fluorescent dye, which is bound to the specific aptamer, while the unbound aptamers are removed by GO

surface, forming a stem-loop structure by self-assembling via gold–thiol bonding. Once the probe hybridized with the target DNA, the conformation changed to an "open" structure and finally form a thermodynamically stable dsDNA product, which can interact with the sensing material to form stable bioconjugate and trigger the DNA conductivity, leading to an enhanced differential pulse voltammetric response towards $Fe(CN)_6^{4-}$. More importantly, AuPd NWs/DGQDs-GMA was designed as an architecture of conductor/semiconductor/conductor, which can bring an enhanced electrochemical response towards $Fe(CN)_6^{4-}$. Hence, this genosensor exhibits better sensitivity for the electrochemical detection of peanut Ara h 1 with a LOD of 4.7×10^{-23} M. Yuan, Kong, Fang, and Chen (2019) designed a FRET-based GO paper chip to simultaneously detect DNA targets of peanut and soybean with the use of a combination of Cy3 and FAM-label hairpin probes of peanut and soybean. The peanut and soybean DNA in a sample can be traced by measuring red and green fluorescence intensity since long dsDNA products produced by hybridization and target DNA can be detached from the GO, resulting in fluorescence recovery. This method provided LODs of both peanut and soybean DNA at 1 nM, indicating high sensitivity.

13 | FLUORESCENCE CELL SENSORS

With the development of in vitro cell culture technology and microelectronics technology, cell sensor has emerged as a new direction of research in the field of biosensing (Banerjee, Franz, & Bhunia, 2010). Cell biosensors are widely used as powerful portable devices for food safety and quality analyses (Table 2). This biosensor is suitable for an initial screening of food by providing the biological activity of the analytes (Ye, Guo, & Sun, 2018).

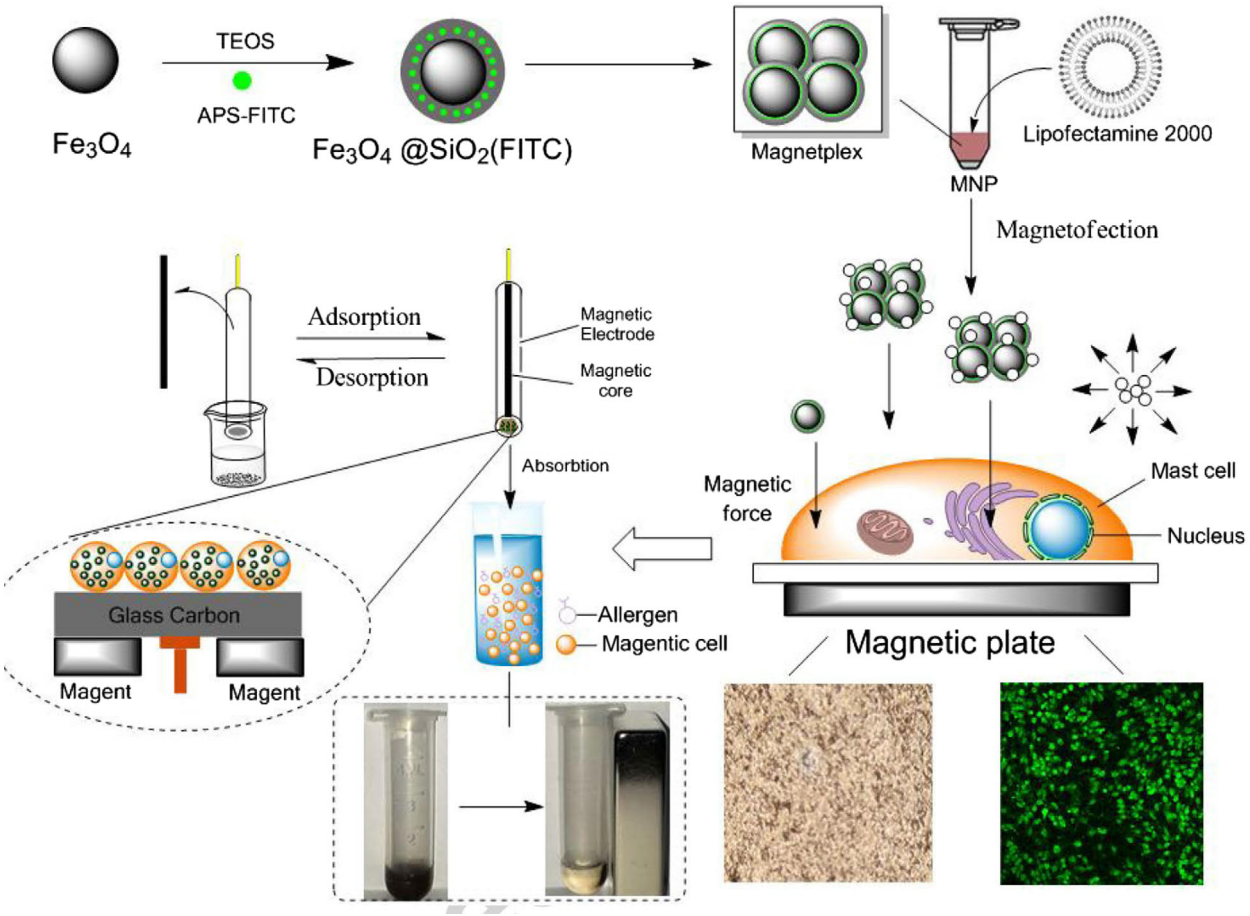

FIGURE 6 Schematic illustration of the preparation of magnetic RBL-2H3 cell sensor and process of allergen detection (Jiang et al., 2015)

13.1 | Basis of fluorescence cell sensors

With cell sensors, cells are designed as sensing elements, and the characteristics of highly sensitive cells are employed to quickly produce changes in the external environment to achieve sample detection. Among several types of cell sensors, optical cell sensors as an emerging cell sensor can monitor the physiological activities of cells without contacting them through real-time chemiluminescence (Grist, Chrostowski, & Cheung, 2010). In this class of cell sensors, food allergens are added to stimulate cells cultured on a chip to produce inflammatory cytokines, which is detected by an optical device. This method has the advantages of strong specificity, stability, and sensitivity; however, how to achieve the on-site online detection of food allergens is still an urgent problem that remains to be solved.

13.2 | Detection of food allergens by fluorescence cell sensors

Jiang et al. (2015) reported a novel electrochemical cell sensor based on fluorescent magnetic beads to detect and evaluate different allergens in food products (Figure 6). In this study, fluorescent magnetic beads fusing the FITC were encapsulated with lipidosometo for mast cell magnetofection. According to the results, the sensor could accurately detect the shrimp allergen TM and fish allergen parvalbumin. Additionally, a cell fluorescence sensor based on rat basophilic leukemia cells was also established to detect and identify parvalbumin at the nanogram level (Jiang et al., 2014). They constructed and transfected a plasmid which contained a CD63-enhanced green fluorescent protein (EGFP) into rat basophilic leukemia (RBL) cells. After the interaction of specific allergen and

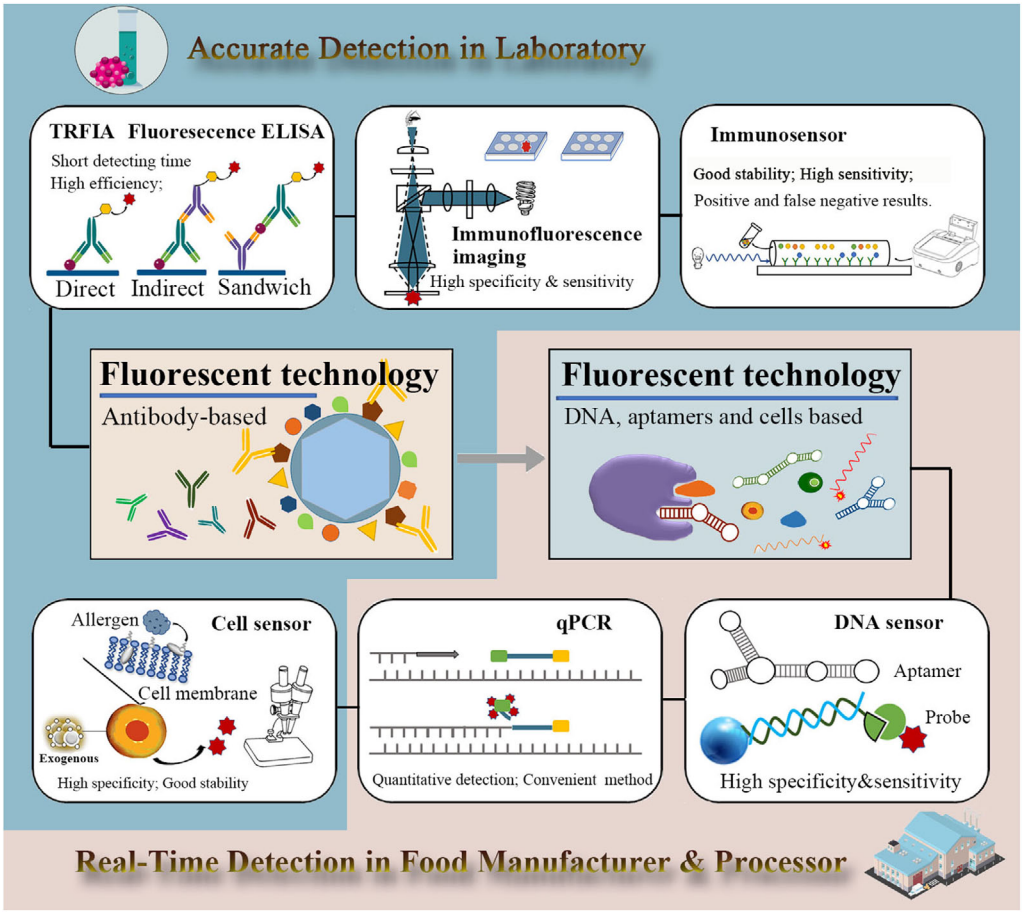

FIGURE 7 Strengths and weaknesses of current evaluating fluorescence-based quantitative methods for food allergen detection

antibody-presensitized mast cells, the mast cell plasma membrane fused with the cytoplasmic granules and then the CD63 and EGFP are triggered on the cell surface. Thus, food allergen can be identified via the increase of fluorescence signal in RBL cells.

14 | FLUORESCENCE APTAMER SENSORS

14.1 | Basis of fluorescence aptamer sensors

Aptamers are a kind of short-chain nucleotide that can recognize target allergens. Aptamers can fold into various stable secondary structures (stem, bugle, loop, and G-quadruplex) and form unique three-dimensional structures with target allergens through the pairing of complementary bases (Yan et al., 2019) and Electrostatic and hydrogen bonding. Currently, aptamers attracted a great attention and several aptasensing detection strategies have been investigated for food allergen detection because of their small size, low cost, and ease of manufacturing (Khedri, Ramezani, Rafatpanah, & Abnous, 2018). Aptamers are designed as probes in fluorescence sensors, while QDs and nanomagnetic bead are usually employed to embellish the probe. Fluorescent aptamer sensors can be used to detect small molecules (Duan, Ning, Song, & Deng, 2014; Zhao et al., 2013), such as microorganisms, toxins, and large molecules (Table 2).

14.2 | Detection of food allergens by fluorescence aptamer sensors

GO, an unconventional type of flexible material with properties of polymers, colloids, thin films, and amphoteric molecules, has been extensively applied as a quencher in the development of numerous biosensors based on FRET technology (Song et al., 2013). Figure 5b briefly describes the procedure used for fluorescent aptamer-based GO sensors to analyze food allergens. Zhang, Wu, Wei, Zhang, and Mo (2016) investigated the selected aptamers as sensing probe for shellfish allergen TM and established a label-free fluorescent GO-sensing platform. By determining the critical amount of GO and ssDNA aptamer under

TABLE 3 The summary of advantages and disadvantages of detection methods

Detection mode	Advantages	Disadvantages
Time-resolved fluoroimmunoassay	• Eliminate the interference of background fluorescence	• Laboratory application
	• Sensitivity.	• Complicated operation
	• Highly signal to noise ratio	• Requires trained personnel
Immunofluorescence imaging	• Simplicity of operator	• Expensive apparatuses.
	• Direct viewing	• No quantitative analysis
	• High specificity	• Preprocessing complexity
	• Sensitivity	
Fluorescence ELISA	• Desirable specificity	• Cross-reactivity
	• High efficiency	• Low sensitivity
	• Short detecting time	• False negative results
		• Requirement of antibodies or
		• antigens labeling
Fluoroimmunoassay	• Desirable specificity	• Requirement of antibodies or
	• High efficiency	• antigens labeling
Fluorescence immunosensors	• Good stability	• False negative results
	• High sensitivity	• Positive and false negative results
Real-time fluorescence quantification PCR	• Quantitative detection	• Relative quantitative detection
	• Convenient method	
Fluorescence genosensor	• High sensitivity	• High cost
	• Real-time detection	• Interference with food
	• Label-free detection	
Fluorescence cell sensor	• High specificity	• Complicated operation
	• Good stability	
Fluorescent aptamer sensor	• High specificity	• Requirement of aptamer labeling
	• Good stability	

different concentrations of TM, a calibration curve was obtained by plotting the fluorescence increment against the TM concentrations. This sensor highlighted the viability of aptamer-based sensors with high selectivity and sensitivity for TM detection in food samples by constructing a novel signal amplification strategy. In another study, fluorescein-labeled anti-TM aptamers were first bound on the GO surface. After the addition of TM, fluorescein-labeled aptamers can be released from the GO surface and form aptamers-TM complex with the fluorescence recovery. Similarly, the TM concentration was analyzed through the fluorescence intensity with a LOD of 2 nM in 30 min (Chinnappan et al., 2020). Weng et al. (2016) established a microfluidic system incorporated with a QDs-aptamer functionalized GO nanobiosensor to detect the major allergen within 10 min in peanuts. The QDs-aptamer-GO

complexes worked as probes to combine with the allergen, resulting in a change in fluorescence owing to the fluorescence properties of GO, the fluorescence can be effectively quenched by FRET. They observed the fluorescence signal by adsorption or dissociation of aptamer-conjugated QDs, suggesting that the method had remarkable sensitivity and selectivity and could also be applicable to the determination of other allergens with corresponding specific binding aptamers.

Furthermore, DNA aptamers can be covalently bound to the surface of magnetic nanoparticles (MNPs) playing a role of chemical coupling. The aptamer–MNP complex will eliminate matrix interferences and is easy to operate (Tram, Kanda, Salena, Huan, & Li, 2014). In addition, a new sensor was reported to quantitatively detect TM by observing the signal of fluorescence enhancement, with the design of functionalized MNPs as separation material and the OliGreen dye as the signal probe (Zhang et al., 2018). First, the detection probe was formed through binding aptamer on the surface of MNPs, and the aptamer was hybridized with the capture probe (complementary DNA sequence). And then, TM in samples combined with aptamer, leads to the aptamer–TM complex releasing to a supernatant, thereby resulting in a strong fluorescence response produced by the OliGreen dye based on its specific fluorescence enhancement upon binding to an aptamer.

15 | SUMMARY

Food allergies represent a significant worldwide problem in the field of food safety, and therefore efficient allergen quantitation is considered as a priority in the food industry. Currently, the popularity of using fluorescence-based quantitative platform has greatly improved in food allergen quantitation due to the high sensitivity and good efficiency of the technique (Figure 7). The food allergens can be determined and evaluated by the above-mentioned fluorescence-based quantitative platforms. Table 2 summarizes the applications of these techniques for quantitative allergen detection in food matrices. Table 3 lists the main advantages and limitations of various fluorescence-based quantitative platforms applied for food allergen detection. Currently, antibodies are the most wildly used biorecognition ligands for identifying and quantifying food allergens in fluorescence-based quantitative platforms such as TRFIA, immunofluorescence imaging, fluorescence ELISA, flow injection fluoroimmunoassay, and fluorescence immunosensor. However, the challenge is that the characteristics of food proteins may be affected by food-processing treatments. Hence, there is an urgent need to develop simple, rapid, accurate, inexpensive, real-time methods for food manufacturers to manage allergens in food matrices.

In response to the demands, great progress has been achieved in the development of fluorescence biosensors with DNA, cells, and aptamers as biorecognition ligands for a simple, fast, and reliable analysis of food allergen. Fluorescence biosensors have emerged as innovative and efficient devices that may allow easy detection of allergens in real-time owing to the ability of integrating with inexpensive and portable devices. For food manufacturers, portable devices with suitable sensitivity are the best choices for food allergen detection to manage the cross-contamination of allergens in food processing. Moreover, fluorescence biosensors allow multiallergen capture in food samples for high-throughput multiplex analysis of by changing the different elements or adding specific combinations with the target allergens, which will be employed for accurate determination to manage allergen labels on food product packages. However, there are still some challenges to overcome for high-throughput multiplex analysis of allergens in food products. For example, the sensitivity and detection efficiency of fluorescence biosensors largely depend on the food sample preparation procedure. To achieve these fluorescence biosensors in the practical application, future efforts should be made on simplifying operation and standardizing sample pretreatment process. We hope fluorescence biosensors could provide a great advantage in monitoring food allergens from ingredients and cross-contamination for food processors, food manufacturers, and food safety control departments.

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AUTHOR CONTRIBUTIONS

Linglin Fu and Yanbo Wang conceptualized the study. Linglin Fu and Yifan Qian prepared the first draft of the manuscript. Jinru Zhou and Lei Zheng wrote the final manuscript.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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