Sensing food contaminants: advances in analytical methods and techniques

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It is estimated that approximately 600 million people (equivalent to 1 in 10 people worldwide) succumb annually to foodborne illnesses due to food contamination.[1] Changes in the global environment due to urbanization and climate change add variability in overall crop yield and distribution, leading to both direct and indirect impacts on food safety.[2] These erratic changes affect rainfall patterns, microbial ecology, and the emergence of new plant diseases- all impacting the food production chain.[3] This food production chain, "from farm to table,"[4] presents multiple opportunities for food contamination to occur.[5] Food production, processing, distribution and transport, and preparation, with the added pressure of food globalization, all contribute to more than 200 distinct foodborne diseases and illnesses.[6]

As a direct result of food contaminants, food safety and food security are intimately connected. Food contamination exposure levels in developing countries are much higher than those in the United States or Europe.[7] Lack of proper food storage, which can lead to food contamination, or insufficient access to safely processed foods is linked to malnourishment and hunger in these countries.[8,9,10] According to the World Health Organization (WHO), approximately $100 billion is spent yearly on medical expenses from the consumption of contaminated food in low- and middle- income countries.[1] This food safety market continues to expand drastically and will for the foreseeable future, with the world population projected to

reach 9.8 billion by 2050.[11] While it is difficult to manage the food production chain globally and prevent contamination,[12] generating novel ways to detect food contaminants has the potential to reduce the adverse impacts that food contamination has on the global population.[13]

Food contaminants can generally be categorized into six classifications: (1) chemical contamination, such as pesticides,[14] fertilizers,[15] small molecule toxins,[16] or chemical residue from cleaning products,[17] (2) bacterial/microbial contamination, such as *Salmonella,*[18] *Listeria,*[19] and *E. coli,*[20] (3) viral contamination, such as norovirus[21] and hepatitis A,[22] (4) protein contamination, such as biotoxins[23] (ricin, botulin, shellfish neurotoxins[24]) or allergens[25] (peanut, wheat, etc.), (5) parasite contamination[26] such as tapeworms[27] or *Toxoplasma gondii*[28], and (6) fungal contamination,[29,30] such as mold[31] or yeasts[32], which can also produce other toxic chemicals naturally on the food surface. While there are numerous reviews and articles that go into detail regarding various ways to detect each specific contaminant, this review article will focus on the overall advances made in analytical techniques and methodology for food safety detection in the last four years. Herein, we will discuss advances and technical gaps for the most common techniques used for contaminant detection: UV-visible spectroscopy and other colorimetric techniques, immunoassays and lateral flow assays, chromatography, surface-enhanced Raman spectroscopy, and electrochemical field-effect transistors. While a lot of these techniques yield reasonable limits of detection for various food contaminants, work still needs to be done to address sensing in complex food matrices, multiplex sensing of contaminants, and the ability to perform in-field measurements in real-world and low resource settings.

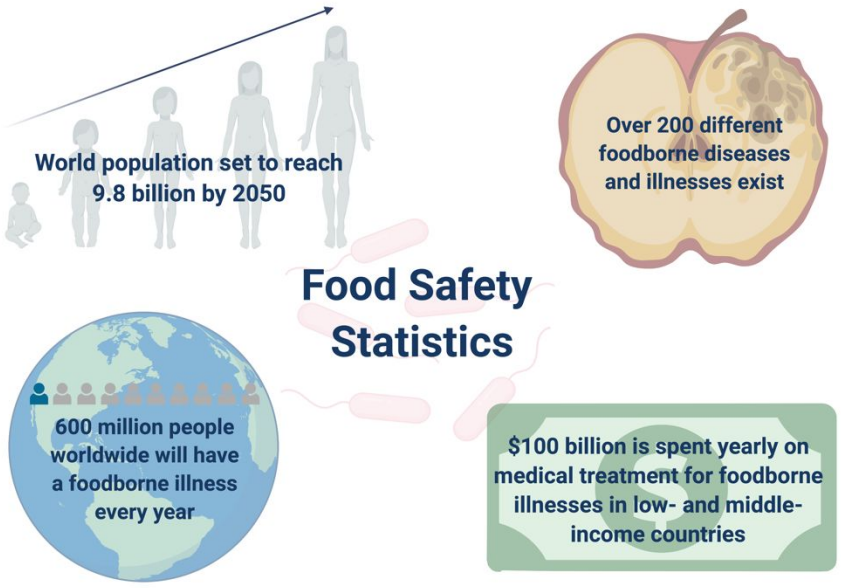

Figure 1. Infographic displaying relevant food safety statistics. Figure created with BioRender.com

UV-visible Spectroscopy/Colorimetric Techniques

Introduction to UV-visible Spectroscopy. Ultraviolet-visible spectroscopy or UV-visible (UV-vis) spectroscopy is a very common absorption spectroscopy technique relevant for food contaminant detection. Generally, molecules interact with light in the ultraviolet or visible regions, exciting electrons within the molecule and absorbing particular wavelengths depending on the molecular structure. The absorbed wavelengths provide specific information about the extent of conjugation and functional groups present in the molecule. This phenomenon is particularly of interest when detecting organic food contaminants, and when combined with the Beer-Lambert law,[33] one can also directly correlate intensity or integrated area of the absorption peak to concentration of the analyte. While UV-vis absorption spectroscopy has been used for many years to perform direct detection of organic and biomolecules, including

those relevant in food contamination,[34,35,36,37] its use in combination with nanoparticles (NPs) is beneficial to the food sensor world as well. UV-vis can be used to infer the sizes of various NPs,[38] and this has been particularly of interest in the sensing field due to NPs ability to facilitate sensing of a wide variety of targets. In fact, it is UV-vis extinction spectroscopy, where extinction is the sum of absorption and scattering, that is measured with NP samples. A theoretically calculated extinction coefficient, derived from the refractive index and dependent on the λ_{max} of the spectrum, can be used to calculate NP diameter or geometry (thus, making it possible to identify varied nanoparticle tags). The UV-vis extinction observation of nanoparticle size is comparable to that observed with transmission electron microscopy (TEM) when an average size is calculated from a large number of imaged NPs;[39] however, UV-vis extinction measurements are much simpler than TEM analysis. Different sizes and shapes of particles can label different food contaminants during sensing.[40,41] Not only does UV-vis extinction spectroscopy provide insight on sizing of nanospheres or other nanostructures, but shifts in the extinction peak can be monitored when specific ligands and/or targets associate with the NPs, changing the local refractive index. Additionally, while there are numerous biological colorimetric assays that reveal the presence (but not the amount) of a food contaminant such as *Listeria*[42] and *Salmonella,*[43] these assays must be combined with quantitative polymerase chain reaction (qPCR) techniques[44,45,46] to amplify and quantify bacterial presence in food. A variety of review articles have been published describing the advantages and disadvantages of qPCR assays in food safety.[47,48,49,50] Here, we will focus on the use of traditional UV-visible spectroscopy for the detection of organic contaminants and leveraging UV-vis for NP-enabled detection schemes.

UV-visible Spectroscopy Detection of Absorbing Molecules. Cyanide (CN^-) is a toxic ion that can cause death in humans at low doses.[51] It is a toxin of interest in food security due to its presence in the pits of fruit, bitter almonds, and plants.[52] These molecules have the ability to leach from the pits of fruit into jams or marmalades during the food production process, and once consumed, CN^- can easily inhibit enzymes in the body.[53] To address this concern, Zhu et al. worked to create a colorimetric sensor to detect cyanide with UV-visible spectroscopy.[54] The authors synthesized four anthraquinone derivatives that can conjugate CN^- with a metallized C-H group via intramolecular hydrogen bonding. Without the presence of CN^-, the anthraquinone solutions appear clear and do not exhibit large absorption peaks in the UV-visible spectrum. Once the CN^- binds to the anthraquinone molecule, the solution appears blue due to an electron transfer, and a large absorption peak is observed at 588 nm. Using this assay, the authors were able to reach a limit of detection (LOD) of 29.48 μM (7.67 ppm) CN^-; this is 10x less than the LD_{50} of cyanide for humans (100-200 ppm). The LD_{50} is a toxicological statistic stating the lethal dose at which 50% of animals or humans would die at the dosage listed.

To ensure specificity, the anthraquinone derivatives were screened against other anions such as fluoride, phosphate, sulfide, and chloride. These anions are not food contaminants themselves, but the authors wanted to confirm that any anions that may be present in food would not bind to the anthraquinone. The UV-visible spectra showed broadened, less intense peaks around 800 nm, and none of the anions masked or affected the absorbance intensity of the cyanide peak at 588 nm in the competitive assay. Additionally, the authors showed the sensor's reversible performance when titrated and deprotonated with trifluoroacetic acid (TFA) as shown in Figure 2A-C. To test sensor viability in food, the anthraquinone was deposited onto

a testing strip to do in-solution analysis of CN^- in food sources. Both cassava flour and bitter almonds were infused into a sodium hydroxide solution and spiked with 20 μM CN^-. New absorption peaks were observed in the presence of both cassava flour and bitter almonds, but they did not overlap nor interfere with the CN^- binding absorption peak (Figure 2D). In conclusion, this work leveraged UV-visible spectroscopy and anthraquinone's ability to act as a chromophore in the presence of CN^- in order to monitor cyanide contamination in a relevant food matrix. Unfortunately, if the target is not able to bind to the sensor in a way that promotes changes in its absorption of light in the UV or visible regime, UV-visible spectroscopy is not a viable option for sensing. For this reason, the intense UV-vis extinction by nanoparticles are often employed to detect food contamination where the nanoparticle and contaminant interaction is mediated with an affinity agent.

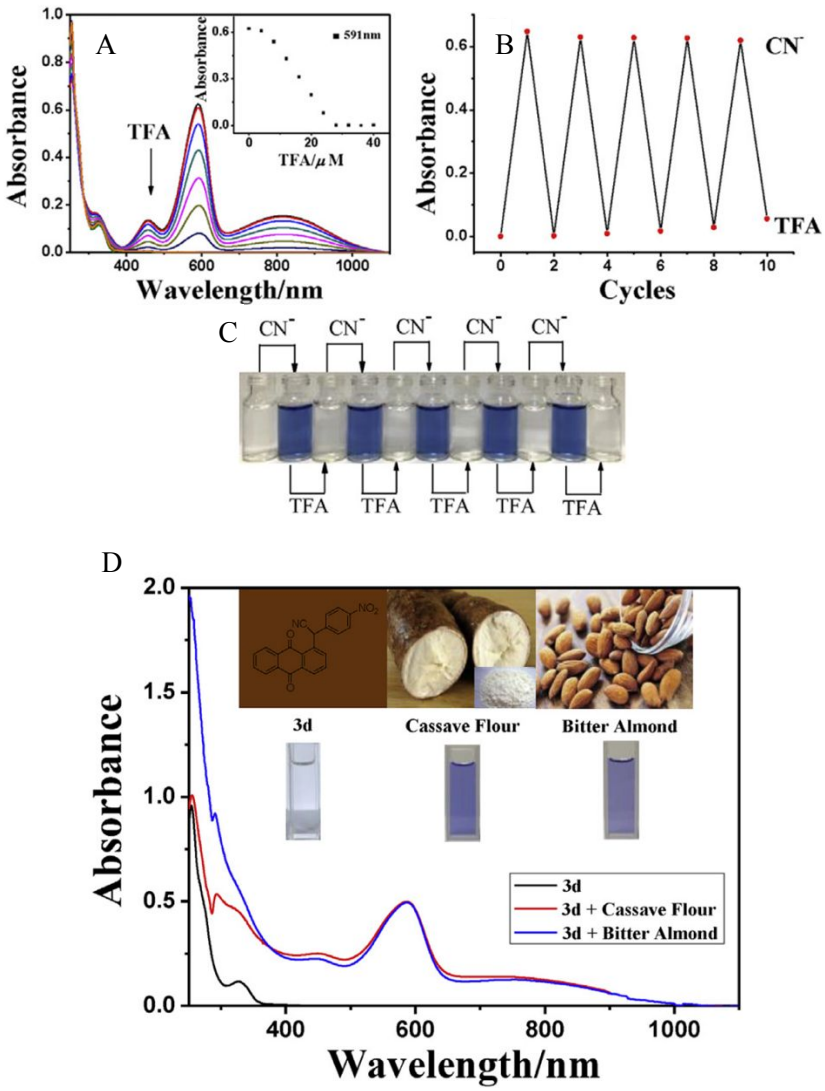

Figure 2. A. UV-Visible spectra of TFA titration cycling in the binding and unbinding of CN^-. B. Absorbance vs. number of cycles before and after titration. C. Image of clear to blue color change in the presence of CN^-. D. UV-visible spectra of CN^- spiked cassava flour and bitter almonds in solution. Reproduced from Zhu, T.; Li, Z.; Fu, C.; Chen, L.; Chen, X.; Gao, C.; Zhang, S.; Liu, C. *Tetrahedron* **2020**, *76* (38), 131479. (ref. 54). Copyright 2020 Elsevier.

As previously mentioned, the UV-vis extinction properties of nanomaterials are frequently exploited for sensing applications. Plasmonic metal NPs, in particular, have extinction properties in the UV-vis that are very sensitive to local refractive index (RI) changes and/or nanoparticle aggregation due to their localized surface plasmon resonance (LSPR).[55] The LSPR is the oscillation of conduction electrons excited by absorption of incident light at a

particular wavelength.[56,57] Plasmonic sensors that exploit this LSPR phenomena can use UV-visible spectroscopy as a signal transduction mechanism.[58] The position of the LSPR peak is very sensitive to changes in the RI near the noble metal NP surface which can be associated with either biological or chemical binding or association.[59]

UV-visible Spectroscopy and Nanotechnology. Recent work done by Loiseau et. al[60] explored the use of core-shell metal NPs (Au@Ag NPs and Ag@Au NPs) to detect staphylococcal enterotoxin A (SEA) based on observed LSPR shifts. SEA is a small protein, only 28 kDa, that is a food toxin causing most staphylococcal-related food contamination.[61] It is known to cause severe gastroenteritis and sometimes even death in humans.[62] Thus, the researchers synthesized these metal NPs and bioconjugated a SEA antibody on the outer shell of the NPs. While nanosensors often rely on very slight shifts in the extinction maximum (1-5 nm), the authors observed large red shifts (100-150 nm) in their UV-visible spectra once the SEA bound to the SEA antibody. These nanosensors had such a distinct shift in the spectra that the NP solutions changed color in a way that could be observed by the naked human eye. The Ag@AuNPs changed from an orange color to a red color in the presence of SEA and the Au@AgNPs changed from red to pink. They were able to reach LODs of 0.4 nM (0.,01 ppm) and 0.2 nM (0.006 ppm), respectively, limited by the background noise in the UV-vis spectrometer. SEA can start showing adverse effects to the human body at around 175 nM (4.9 ppm), so these LODs are more than sufficient.[61] Additionally, their responses were sensitive enough to build a dose-response curve based on the change in the λ_{max} of the extinction peak versus the concentration of SEA added to the nanoprobes.

UV-visible Spectroscopy Sensing Conclusions. While UV-visible spectroscopy is a useful and effective technique for food contaminant detection, many sensors rely on relatively small shifts in the spectra to reveal target detection. The addition of a food matrix may also complicate or mask the observed absorption peak due to other small molecules in solution that are active in the UV or visible regime or larger matrix components that may non-specifically scatter UV-visible light. For this reason, UV-visible spectroscopy is less of a stand-alone technique viable for this type of sensing. UV-visible spectroscopy will continue to serve as an initial screening technique or supplementary technique to much more specific technologies for food contamination detection.

Chromatography Techniques

Introduction to Chromatography Techniques. Chromatographic separation techniques are advantageous for detecting food contaminants and residues, most frequently organic molecules like mycotoxins, toxins naturally produced by fungi, and pesticides. Gas chromatography (GC) and liquid chromatography (LC) are two very established chromatographic techniques used to detect and quantify these small molecule food contaminants due to their ability to separate complex mixtures.[63,64] Both techniques are based on the partitioning of the target analytes into a stationary phase within the column as a mobile phase flows/carries it through. Partitioning occurs due to favorable interactions between the stationary phase and the target analytes; thus, the time required to elute the analytes (retention time) varies, allowing for several compounds to be separated during this process.[65] Numerous types of columns that contain different stationary phases are available for the

detection of various target analytes. In GC, the mobile phase is a chemically inert carrier gas, such as helium or nitrogen, while the stationary phase is a microscopic liquid or polymer layer coated on the inside of the column walls. This technique is often used for the detection of small organic compounds as it requires volatile samples. In contrast, LC involves the use of a liquid mobile phase (commonly water or methanol) with solid adsorbents as the stationary phase, allowing for the analysis of non-volatile compounds. Oftentimes, these chromatographic techniques are coupled with mass spectrometers or other detectors that have extremely low limits of detection for the quantification needed for food contaminants and residues.[66]

Some of the first work using GC for food contamination was done by Coulson et al. in 1959 and focused on detecting pesticides.[67] While this study only proposed a method for detecting vegetable extracts and did not verify the technique in a food matrix, by 1964, GC had become the gold standard at the FDA for detecting chlorinated pesticides, such as DDT, aldrin, and heptachlor, with detection limits around 0.002 ppm.[68] In 1973, high performance (also known as high pressure) LC (HPLC) was proposed as an alternative technique to GC by Eisenbeiss and Sieper for pesticide residue analysis to detect less volatile substances and those that may decompose at higher temperatures.[69] This was successfully carried out in 1976 by Dolphin et al. to detect organochlorine pesticides in milk at the ~0.1 ppm level,[70] and by Lawrence to detect carbamate pesticides in crops at levels ranging from 0.004 – 0.3 ppm.[71]

Around this time, HPLC was increasingly used for mycotoxin detection due to its advantages with speed, resolution, accuracy, and precision. Mycotoxin detection using HPLC was quickly applied to foodstuffs like grain samples,[72] nuts,[73] wine,[74] milk,[75] and cottonseed (grain used for livestock),[76] and was able to detect mycotoxins at concentrations as low as 5

μg/kg (0.005 ppm). By the 1980's, GC and LC had become gold standards for detecting mycotoxin food contamination.[77,78,79,80] Since then, many advances have been made to both techniques to improve LODs and LOQs (limit of quantitation), address issues with sample preparation, and move towards complex matrix and multiplexing for detection of food contaminants. Herein, we will highlight these advancements to the food sensor world.

QuEChERS. The need for thorough sample preparation for chromatography stems from the instrument needing relatively pure samples to properly use the technique. Thus, in highlighting some of the advances made to chromatographic techniques, sample preparation plays a key role in being able to sense contaminants found in complex mixtures. These sample preparation techniques focus mainly on extracting and cleaning the sample for further analysis. By far, one of the greatest advancements in preparatory techniques was the development of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), which revolutionized how multiresidue and multiclass analysis was done. QuEChERS was developed in 2002 by Anastassiades et al.,[81] and verified in 2003 by Schenk et al.[82] Briefly, it uses acetonitrile to extract the target meant to be detected from a complex solvent matrix. This sample is centrifuged to create a liquid-liquid partition to easily extract out the sample, which is then purified by dispersive solid-phase extraction (d-SPE). d-SPE is a sorbent purification technique used to eliminate any remaining contaminants from the sample with anhydrous salts and/or black carbon. This is then extracted once more, leaving the sample clean of any other sample contaminants.[77] QuEChERS and d-SPE can extract food contaminants such as mycotoxins, pesticides, or other organic toxins from their complex food source sample within 20-30 minutes.[83,78,79,80,84] Additionally, these preparatory techniques have drastically increased sample throughput and have proved capable

of adapting to a wide variety of analytes and food matrices while maintaining reasonable analysis times.[77] Despite this, the technique remains a manual procedure that requires a level of technical expertise to conduct and works best for organic contaminants.

Solid-phase microextraction (SPME). Similar to QuEChERS and d-SPE, solid-phase microextraction or SPME is another common sample preparation technique for chromatography. SPME is of particular interest in chromatography for food contaminant detection because it uses little to no organic solvent. First developed in the 1990's, Pawliszyn et al.[85] used this preparation technique to target volatile and nonvolatile compounds in complex samples. Briefly, traditional SPME devices are made of thin, fused silica fibers coated with a type of sorbent material. This material is then placed in a complex sample matrix and allowed to reach equilibrium with the adsorbed targeted compounds.[86] This is then directly injected into the chromatography column. There are three different modes of SPME: direct-insertion SPME (DI-SPME) which is the standard that has been described, head-space SPME (HS-SPME) which is where the device is placed above the liquid sample to allow volatile compounds to adhere to the fibers, and membrane-protected SPME where it follows the same protocol as HS-SPME, but the device has a protective membrane to prevent diffusion of large molecules.[87] Over the past 10 years, there have been many advancements to SPME devices along with changes in the sorbent material, changes in membranes, and various coatings that can promote adherence of particular targets onto the SPME device surface.[88,89] These advancements have been specifically of interest in the food sensing field with the detection of organic volatile and non-volatile toxins in wine, meats, cereals, fruits, and juices - all of which present very complicated matrices and do not require organic solvent for extraction.[90] Both QuEChERS and SPME in particular are

pivotal methods needed to properly use chromatography as a food sensing technique. Herein, we will describe the use of extraction techniques in conjunction with the new standard of chromatography, ultra-performance liquid chromatography, and what appears to be the future of chromatography in food sensing: multi-dimensional chromatography.

Ultra-performance Liquid Chromatography. In 2004, ultra-performance liquid chromatography (UPLC), also referred to as ultra-high-performance liquid chromatography (UHPLC) was first introduced.[91] As the name suggests, this improvement relies on high pressures (up to 1,000 bar) within the column to improve the speed, sensitivity, and peak separation, or resolution, of the separation. These higher pressures can be attained due to the size of the particles used as the stationary phase within the column.[92] Typically, HPLC relies on particles with a diameter of 3 to 5 μm; however, UPLC uses particles with a diameter of less than 2 μm, reducing the distance between the target analytes and the stationary phase to allow for better sensitivity. Today, UPLC is commonly used for the detection of food contaminants due to the advantages that it has to offer over GC and HPLC.[93]

In 2019, Zhang et al. employed UHPLC with tandem mass spectrometry (MS/MS) detection for the simultaneous determination of 58 pesticides in eggs.[94] Pesticides can bioaccumulate through the food chain and pose hazards to humans when consumed in contaminated foods. This study performed a clean-up step using a multi-functional filter that was based on QuEChERS before injecting the sample into the UHPLC. The LOD and LOQ for the 58 pesticides were 0.1-1.0 μg/kg (0.0001-0.001 ppm) and 0.2-5.0 μg/kg (0.0002-0.005 ppm), respectively. Additionally, 70% of the compounds had LODs that were significantly lower than

what can be detected with LC-MS/MS.[95,96] This method was then successfully applied for the determination of pesticides in real egg samples.

Another study performed by Castro et al. utilized solid phase extraction (SPE), similar to SPME, and UPLC-MS/MS to detect 50 pesticides in red and white wines, as there is evidence that pesticides can transfer from grapes to wine during the fermentation process.[97] Wine samples were passed through the SPE sorbent to adsorb the target analytes that were then eluted with an acetonitrile/methanol mixture. Next, the extract was directly injected into the UPLC for analysis. This method provided LODs below 1 ng/mL (0.001 ppm) for 48 out of the 50 tested pesticides and had an analysis time of only 10 minutes. This analysis time is four times less than the time needed for previous methods dealing with a similar number of analytes.[98,99] The method was also applied to 25 wines and found that all samples, except for one, contained residues from at least one pesticide. Both of these methods show the utility of sample preparation techniques and UPLC for the detection of food contaminants along with their advantages over other LC methods.

Multi-dimensional Chromatography. Multi-dimensional chromatography is a way to increase separation performance in complex matrices. The sample passes through two different separation stages accomplished through the use of multiple columns with different stationary phases. This allows for an added degree of separation, which is especially important in food contamination analysis due to significant sample complexity. This can be applied to both GC and LC techniques[100,101] with a schematic of this technique shown in Figure 3.

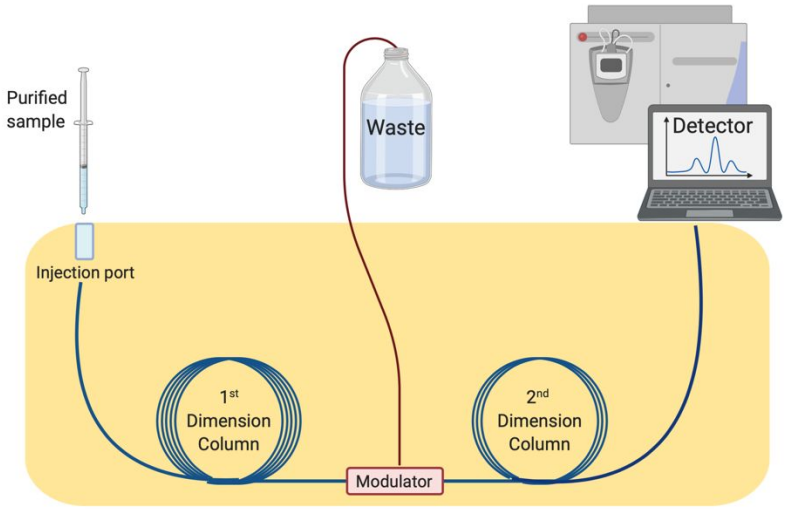

Figure 3. Schematic of multi-dimensional chromatography instrument set up. The purified and prepped sample is injected into the injection port and the sample passes through columns in the first and second dimension with different mobile phases orthogonal to one another. The sample is then detected, often with MS. Figure created with BioRender.com

Recent work by Ruiz del Castillo and coworkers studied the use of SPME alongside multi-dimensional gas chromatography-mass spectrometry (MDGC-MS) to detect pesticides in commercial and homemade strawberry jam.[102] The motivation behind MDGC for pesticide separation is that this technique allows for a wide variety of polarities of molecules to be separated due to varied affinity for the multiple columns. Therefore, their goal was to detect and quantify sixteen different pesticides in strawberry jam. First, the authors determined what peaks in their first-dimension chromatogram were associated with typical flavor and aroma compounds in the jam after SPME, to understand the background matrix. The jams were spiked with all sixteen pesticides, and first- and second-dimension chromatograms were recorded. Although two of the pesticides peak signals overlapped with the background matrix, the added separation allowed these peaks to be resolved in the second dimension to alllow quantitation despite peak overlap.

The LODs for the pesticides were found to be 0.11-0.42 ng/kg ($1.1 \times 10^{-7} – 4.2 \times 10^{-7}$ ppm) in the first-dimension and 0.013-0.093 ng/kg ($1.3 \times 10^{-8} – 9.3 \times 10^{-8}$ ppm) in the second-dimension after the first column's separation. There is no regulation of pesticides in jams, but compared to the regulatory limits on strawberries, MS-detected pesticides eluted from the second column were detected at LODs much lower than required. In fact, ten of the pesticides were able to be identified in the complex matrix based on the MS data, revealing the strength of specialized analysis to do multiplex detection of pesticides in a complex food matrix. Although the measured chromatograms are complicated and rely on efficient sample preparation to distinguish the target analyte from the overall matrix, it does show how multiplexing is possible for organic food contaminants when adding another layer of instrumentation and analysis.

Chromatography Techniques Conclusions. Chromatography techniques have long served as the gold standard for organic compound separation. Paired with other instrumentation, such as MS, fluorescence, or UV-vis absorption, it can be a powerful tool for quantitative analysis of organic food contaminants. However, chromatography is limited to a small range of analytes and is only appropriate when detecting pesticides, mycotoxins, or other small molecule compounds that contaminate food sources, leaving out bacteria, proteins, and other larger food contaminants. While some of the greatest advancements to these techniques are their work in sample preparation, the need for a considerable amount of sample preparation makes these techniques less viable for food contamination detection outside of a laboratory. Multiplexing is possible with multi-dimensional chromatography, but it is important to note that this analysis is complicated and requires specialized personnel to perform analysis,

quantify targets, and maintain these instruments.[103] The added cost makes these methods and instruments most suitable in academic and industrial settings rather than other analytical techniques that enable robust, in-field measurements.

Immunoassays and Lateral Flow Assays

Introduction to Immunoassays. Immunoassays are a bioanalytical method used to measure the presence or concentration of analytes ranging from small molecules to macromolecules. This method relies on the use of an antibody or antigen as a biorecognition agent.[104] There are several types of immunoassays including: radioimmunoassays, chemiluminescence immunoassays, counting immunoassays, enzyme or enzyme-linked immunosorbent assays, and lateral flow immunoassays. While several of these methods and their applications have been reviewed elsewhere,[105-111] this section will mainly focus on lateral flow immunoassays and enzyme-linked immunosorbent assays as these methods are most commonly used for the detection of food contaminants, and they are considered the gold standards in this field.

Introduction to Lateral Flow Immunoassays. Lateral flow immunoassays (LFIA), also known as lateral flow immunochromatography assays, are diagnostic tests intended to detect the presence of a target analyte within a complex liquid sample. This simple technique is widely used for home testing, point of care, and laboratory use due to its rapid detection (5-30 min) at low concentrations.[112] A typical lateral flow device (LFD) consists of four components: sample pad, conjugate pad, nitrocellulose membrane, and an absorbent pad that are assembled to create a continuous flowing channel between the four sections that exploit capillary forces.[113] The sample is initially placed on the sample pad that allows for filtering of large unwanted

particulates before controlling the release of the sample to the conjugate pad. In the conjugate pad, the sample interacts with antibodies that are specific for the target analyte and conjugated to scattering or fluorescent particles (most often colloidal gold or fluorescent latex microspheres).[112] The nitrocellulose membrane then contains a line of immobilized capture antibodies that bind the target analyte at the test line. A control line is also present after the test line to ensure proper liquid flow through the device. Lastly, the absorbent pad wicks up any solution that passes all the way through the device. LFIAs can either be of the sandwich or competitive format. The sandwich format involves "sandwiching" the target analyte between two antibodies, and a positive result produces a colored line. On the other hand, in the competitive format, the analyte blocks the binding site on the antibodies, preventing interactions with the colored conjugated antibody, resulting in no color observed for the sample line in the detection zone. While one of the most common uses of LFIAs is the at-home pregnancy test, they are also utilized for screening harmful contaminants in food production[112] as well as agriculturally relevant proteins and small molecules that may be harmful to humans.[114]

LFIA Food Contaminant Sensing. LFIAs were derived from the latex agglutination assay, a method used to identify certain antibodies or antigens in bodily fluids through the use of latex beads that aggregate in the presence of the target analyte, allowing for visual detection of the grouped latex beads.[108] The latex agglutination assay was developed in 1956, but it was not until 1970 when the LFIA was first described and patented. In the 1970s, LFIAs were developed and mainly used for detection of the human hormone gonadotropin in urine in at-home

pregnancy tests.[111] Some of the first work performed for the detection of food with LFIAs was in 2004 when Goodwin et al. detected the food allergen, gluten, using a sandwich LFIA.[115] Once the sample migrated from the sample pad to the conjugate pad, gluten within the sample bound to gluten antibodies conjugated to bright blue polystyrene latex particles. If gluten was present, a capture antibody bound the sample on the test line producing a visible blue line. In addition to latex particles, gold nanoparticles (AuNPs) have increasingly been incorporated into LFIAs as reporting labels for the colorimetric detection of mycotoxins and other food contaminants.[108,114,116]

While LFIAs often incorporate AuNPs as reporting labels, a number of methods have been used to enhance sensitivity and amplify the signal produced. One way this has been done is using silver enhancement achieved through autocatalytic growth of metallic silver on gold nanoparticles. Yang et al. first performed this method in 2011 using a sandwich LFIA for the detection of the protein abrin-a, a natural poison produced in rosary peas and used as a bioterrorism agent.[117] Water and soybean milk samples that contained abrin-a were placed onto the LFD sample pad, flowed to the conjugate pad where abrin-a in solution bound to the detection antibody labeled with AuNPs. The conjugated complex was then captured by the antibodies at the test line. When color appeared on the control line, indicating proper flow through the device, a pad that contained $AgNO_3$ was placed on top of the test line and control line regions. A reducing pad was then placed on top of the $AgNO_3$ pad and wetting of the pads re-solubilized reagents. Metallic silver coated the AuNPs present on the test and control lines, changing the color of the lines from red to black and enhancing the signal. This enhancement has been attributed to the enlargement of NPs, making them more visible, and to the increased

contrast of black-appearing silver-coated AuNPs compared to the blue absorption and scattering (red-appearing) AuNPs on the white background of the LFD. Similarly, silver enhancement has also been carried out for the detection of the mycotoxin ochratoxin A (OTA) in grapes and wine.[116]

Another method for increasing sensitivity and detection within LFIAs is the use of up-converting phosphor (UCP) NPs; these luminescent NPs emit in the visible or ultraviolet regimes when exposed to low energy radiation.[113] Zhao et al. developed a competitive LFIA using up-converting phosphor technology for the detection of a common crop contaminant aflatoxin B_1 (AFB1) that is produced by fungi.[118] The AFB1 target analyte interacted with UCP NPs conjugated to antibodies on the conjugate pad and then competed against the analyte for the capture antibodies immobilized at the test line. The UCP NP antibody conjugates were washed away; thus, they did not produce a colored test line. A 980 nm laser was then utilized to excite the UCP NPs on the surface of the device to collect the luminescence emitted by the UCPs on the test and control lines. Since it was a competitive LFIA, a positive result was indicated by no signal observed on the test line with signal observed for the control line. The LOD for standard AFB1 solutions was 0.03 ng/mL (0.00003 ppm), showing improved sensitivity over traditional gold NP LFIAs with LODs around 0.25 ng/mL (0.00025 ppm).[119] Additionally, spiked crop samples that included corn, peanuts, rice, soybeans, and others showed LODs ranging from 0.1 to 5 ng/g (0.0001 – 0.005 ppm), making this technology a promising approach for on-site and in-field detection of AFB1.

Compared to other immunoassay techniques, LFIAs are well-suited for multiplex analysis as the nitrocellulose membrane can be equipped with more than one detection site or test line

on a single device. In 2016, Chen et al. developed a competitive LFD for the on-site multiplex detection of three mycotoxins, AFB1, zearalenone (ZEA), and OTA.[120] On the conjugate pad, free mycotoxins interact with antibody AuNP conjugates to form complexes. These complexes then compete with the mycotoxin analytes for the capture antibodies that are specific for each mycotoxin at three different test lines. The visual LOD was 10 μg/kg for AFB1 (0.01 ppm), 50 μg/kg for ZEA (0.05 ppm), and 15 μg/kg for OTA (0.015 ppm). The LODs for quantitative analysis were 0.10 – 0.13 μg/kg for AFB1 (0.0001 – 0.00013 ppm), 0.42 – 0.46 for ZEA (0.00042 – 0.00046 ppm), and 0.19 – 0.24 μg/kg for OTA (0.00019 – 0.00024 ppm), all of which were far below regulatory limits set by the European Commission. Furthermore, this multiplex device was tested with spiked corn, rice, and peanut samples that contained varying concentrations of AFB1, ZEA, and OTA. The results for the spiked corn samples are shown in Figure 4. Due to the competitive format of the LFD, the red lines indicate a negative result while no line indicates a positive result. These results show the success of the device in detecting single mycotoxins in addition to the simultaneous detection of the three mycotoxins, making it a useful method for multiplex detection in the field.

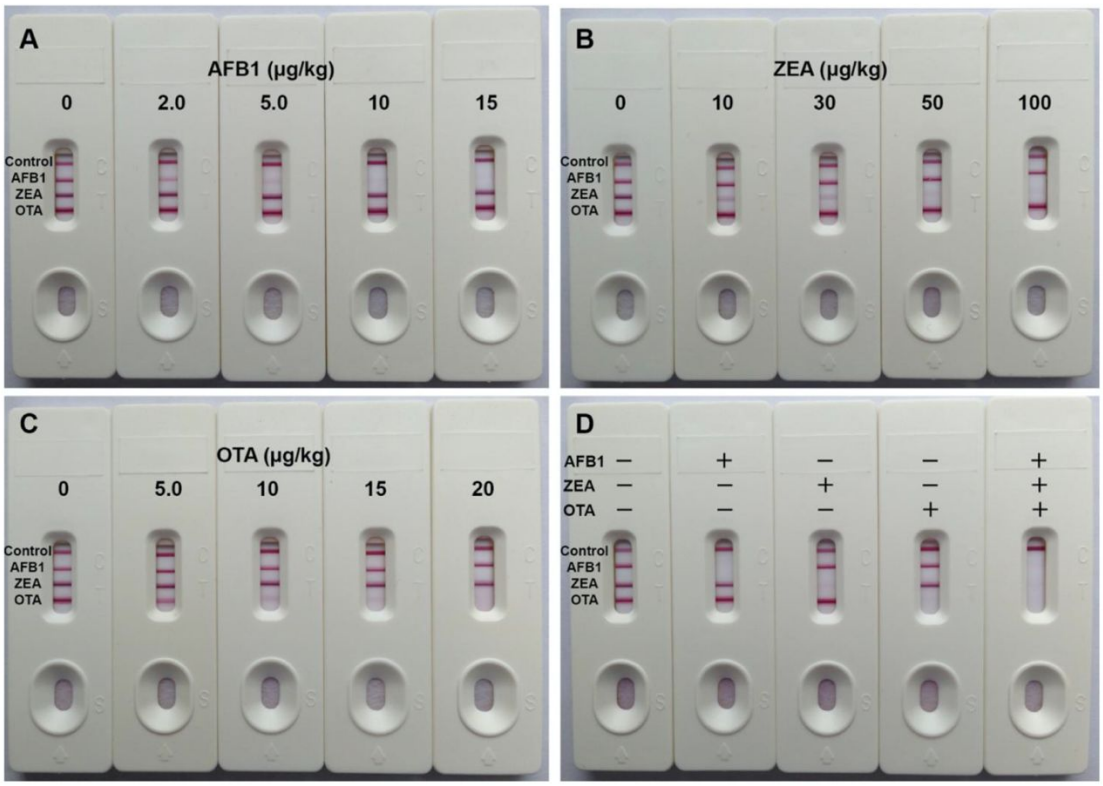

Figure 4. Image of an LFIA for multiplex detection of mycotoxins in corn samples. A. 0 to 15 μ g/kg of AFB1 spiked in corn. B. 0 to 100 μg/kg of ZEA spiked in corn. C. 0 to 20 μg/kg of OTA in corn. D. Positive results for LFIA both in single and multiple mycotoxins. Reproduced from Chen, Y.; Chen, Q.; Han, M.; Zhou, J.; Gong, L.; Niu, Y.; Zhang, Y.; He, L.; Zhang, L. *Food Chemistry.* **2016**, 213, 478–484. (ref 120) Copyright 2016 Elsevier.

Additionally, multiplexing in LFIAs has been carried out for the detection of bacterial food contaminants. Zhao, et al. developed a 10-channel UCP LFIA in the sandwich format for the rapid and simultaneous detection of 10 epidemic foodborne pathogens.[121] This study established 10 single target UPC technology strips that were integrated into a disc that contained 10 channels for the strips. The specificity of each detection channel was assessed individually with the use of the other nine species of foodborne bacteria. For all 10 channels tested, only the target bacterium displayed a strong detection signal with no cross-reactivity present from the other bacteria, confirming the high specificity of this assay. Additionally, 100 food samples were spiked with a single pathogenic bacterium, and the disc correctly identified

88% of the pathogens within the samples with a detection sensitivity around 10 CFU/0.6 mg. Then, 10 food samples were contaminated with all 10 pathogens simultaneously; in this case, at least seven different target pathogens were detected in all samples, but all 10 pathogens were identified in only four samples. It was hypothesized that the interactions between the 10 pathogens in liquid media were complex and the growth of particular bacteria was inhibited.[121] Nevertheless, this device had good sensitivity and specificity for the simultaneous detection of several bacterial pathogens, although more improvements need to be made for successful multiplexing and detection of all 10 contaminants.

LFIA Conclusions. While great strides have been made in multiplexing for the detection of food contaminants in the last decade, no other major advancements have been made for LFIAs in the past two years. These devices have several advantages over other methods for food contamination detection including their ease of use, rapid detection at low concentrations, portability for field testing, and ability for multiplexing. However, LFIAs require proper storage, and improper storage conditions can lead to the degradation of these devices. Furthermore, due to the antibody and antigen components used in LFIAs, they can still lose activity over time. The degradation of the various components can result in false positives, false negatives, or invalid results. This may lead to the need for additional devices for further testing, which can be expensive. Additionally, when used in the field, these devices are only qualitative and cannot provide quantitative data. Finally, there is no opportunity to enhance readouts using enzymes, which is a method that can be exploited when using enzyme-linked immunosorbent assays.

Enzyme-linked Immunosorbent Assays (ELISA)

Introduction to ELISA. Enzyme-linked immunosorbent assays (ELISA) can detect a variety of biological molecules by linking the specific analyte target to antibodies conjugated to enzymes that allow for improved detection. ELISA tests are most commonly performed in a well-plate format that varies depending on the type of ELISA – either direct, indirect, sandwich, or competitive (Figure 5B). Direct ELISA detects antigens, immobilized on the surface of a well-plate, with an antibody that is specific for that antigen and directly conjugated to a tag for detection. Indirect ELISA is similar to direct ELISA; however, it uses a two-step process. Again, the antigen is immobilized on the surface of a well-plate, and a primary antibody specific for that antigen binds. Next, a labeled secondary antibody binds to the primary antibody to facilitate detection. Sandwich ELISA detects an antigen by "sandwiching" it between two antibodies. This type of ELISA requires two antibodies specific for different epitopes (Figure 5A), or recognition/binding sites, of the antigen. A capture antibody is immobilized on the surface and binds the antigen, which is then followed by the conjugation of a second antibody that facilitates detection of the antigen. Lastly, in competitive ELISA, a sample antigen competes with a reference antigen for binding to a specific amount of labeled antibody. The reference antigen is coated on the well plate. The sample antigen is pre-incubated with the labeled antibody before being added to the wells. If the proper antigens are present, the antibodies will bind to the antigens, creating an antigen-antibody complex. These complexes are added to the well plate, interact, and then the well is washed so that any unbound antibodies or complexes are removed. HIV testing in a clinical setting is one of the most common uses of ELISA, although it has proved useful in the detection of food contaminants as well.

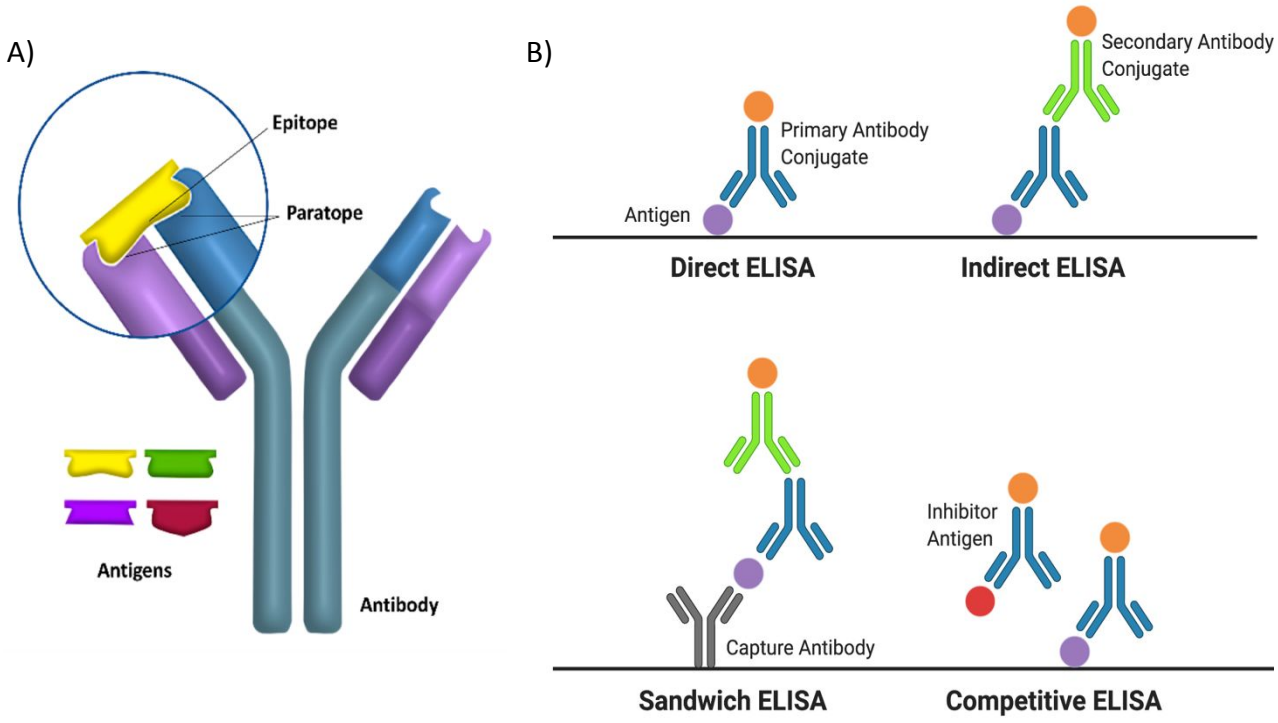

Figure 5. A) Figure of an antibody binding an antigen. Both binding regions are shown. Reproduced from Szlag, V. M.; Rodriguez, R. S.; He, J.; Hudson-Smith, N.; Kang, H.; Le, N.; Reineke, T. M.; Haynes, C. L. *ACS Appl. Mater. Interfaces* **2018**, *10* (38), 31825–31844 (ref 141) Copyright 2018 American Chemical Society. B) The four types of ELISA – direct, indirect, sandwich, and competitive. Figure created with Biorender.com.

ELISA for Food Contamination Sensing. ELISA was first used in 1971 by two different groups, Engvall and Perlman[122] and Van Weemen and Schuurs,[123] as a modification and safer alternative to the radioimmunoassay which used radioactive labels for detection.[105] Engvall and Perlman used the method with enzymes in place of the radioactive labels to determine the levels of immunoglobulin G (IgG) in rabbit serum.[122] Around the same time, Van Weemen and Schuurs performed ELISA to quantify human chorionic gonadotropin, the chemical measured in a pregnancy test, in urine using the enzyme horseradish peroxidase (HRP).[123] ELISA adapted over the years and became a popular technique for detecting food contaminants in the late 1970s

and early 1980s with much of this early work focusing on the detection of mycotoxins. Lawellin et al. performed tube ELISA and detected the mycotoxin AFB1 in serum at concentrations less than 10 pg/mL (0.00001 ppm).[124] Although the detection limit was low, the tube ELISA was cross-reactive, meaning that the antibody had low specificity and could bind antigens of other aflatoxins that have similar recognition sites, potentially resulting in false readouts. Pestka et al. improved upon this method using competitive microplate ELISA by altering the conjugation of the antibody to reduce the amount of non-specific binding.[125] This method resulted in low cross-reactivity with other aflatoxins and could detect 0.5 - 50 ng/mL (0.0005 - 0.05 ppm) of AFB1 per assay. Additionally, Pestka et al. developed an ELISA test to detect the mycotoxins T-2 and OTA with detection levels of 2.5 pg/assay and 25 pg/assay, respectively.[126127] While successful for detection of food contaminants early on, ELISA has been continually adapted over the years to improve detection limits and the selectivity for specific food contaminants.

Plasmonic ELISA. In 2011, plasmonic ELISA (pELISA) combined LSPR refractive index sensing with ELISA.[128] This technique used AuNPs to bind the enzyme HRP which can then catalyze a precipitation reaction at the surface of the NPs. This enzyme reaction causes a dramatic shift in the LSPR extinction wavelength, making it possible to detect the presence of one or more HRP molecules per NP with the use of a spectrometer. This methodology was initially used for the detection of disease biomarkers[129] and has since been expanded to encompass detection of food contaminants.

Pei et al. reported use of pELISA for the detection of the mycotoxin OTA based on the urease-induced silver metallization on the surface of gold nanoflowers.[130] OTA labeled with urease, an enzyme that catalyzes the hydrolysis of urea, was used as the competing antigen to

hydrolyze urea into ammonia. In the presence of the produced ammonia molecules, silver ions in solution were reduced to generate a silver shell on the surface of the gold nanoflowers. Upon generation of the silver shell, the color of the solution changed from blue to a brownish-red hue. The visual LOD observed with the naked eye was 40 pg/mL (0.00004 ppm), while the calculated LOD was 8.205 pg/mL (0.000008 ppm). This calculated LOD was based on the lowest concentration of ammonia that generated a higher signal than the blank, plus three standard deviations, and it was found to be approximately 14-fold lower than LODs obtained using HRP-based ELISA. This method proved to be specific for OTA as it was tested against four other mycotoxins, deoxynivalenol (DON), ZEA, fumonisin B1, and AFB1. pELISA has been successful in detecting mycotoxins and has potential applications for the detection of bacteria that cause foodborne illnesses.

Gao et al. applied pELISA for the detection of *Salmonella enterica Choleraesuis*, a common bacterium found in food and water that is responsible for the bacterial disease salmonellosis that affects the human intestinal tract.[131] While conventional ELISA relies on HRP to catalyze organic dyes, such as 3,3',5,5'-tetramethyl-benzidine, to produce colored products, it produces a relatively low colorimetric signal intensity. The weak signal makes it challenging to detect low concentrations of bacterial contaminants. Therefore, this study, similar to the study performed by Pei et al., used urease-induced silver metallization on the surface of gold nanorods to detect *S. enterica Choleraesuis* in spiked whole milk samples. LOD values were as low as 1.21×10^2 CFU/mL for qualitative detection with the naked eye and 12.1 CFU/mL with quantitative detection. These LOD values are 2-3 orders of magnitude lower than those obtained with conventional HRP-based ELISA.

Over the years, the field of food contaminant detection with ELISA has also moved towards using monoclonal antibodies (mAbs) over polyclonal antibodies. Typically, ELISA has used polyclonal antibodies, a heterogenous mixture of antibodies that can recognize and bind to many different epitopes, sometimes creating poor readouts in addition to specificity issues. One way to overcome these limitations is through the use of mAbs that only recognize a single epitope of an antigen. Brandon et al. employed the use of mAb in sandwich ELISA to detect both ricin and Shiga toxin proteins in milk and ground beef.[132] Ricin is a poisonous toxin that is naturally produced in the seeds of castor oil plants and has previously been used as a bioterrorism agent. Shiga toxins, produced by *Shigella* and *E. coli* bacteria, are also toxins that can cause foodborne illnesses from the consumption of contaminated milk, ground meat, or vegetables. In this study, microwell plates were coated with two different mAbs: one specific for ricin and the other specific for Shiga toxins. Next, the sample antigen was added and bound to the mAbs. A secondary HRP-conjugated antibody was then added and used for detection. The LODs for the ricin and Shiga toxin systems were 0.13 ng/mL in milk (0.00013 ppm), which is 1×10^{-4} lower than the estimated lethal dose of either toxin. In ground beef, the LODs were 0.8 ng/g and 0.7 ng/g (0.0008 and 0.0007 ppm) for the ricin and Shiga toxin systems, respectively. Specificity was tested by cross-reactivity using toxin analogs and heat-denatured toxins. For ricin, the cross-reactivity for the toxin analog *Ricinus communis* agglutinin and the heat-denatured toxin was around 6% and 1%, respectively. For Shiga toxin, there was less than 1% cross-reactivity for both the toxin analog and the heat denatured toxin. The low cross-reactivities indicate good selectivity for the mAB ELISA tests targeting ricin and Shiga toxins.

This suggests that this platform would be useful for any other food contaminants where mABs are readily available.

ELISA Conclusions. Overall, ELISA has developed over the years to make use of nanotechnology and highly specific and selective mAbs. Today, it is considered the gold standard for the detection of small molecule, protein, and bacterial food contaminants as it allows for low detection limits, relatively fast readout times, and the enzymes used are reasonably shelf-stable. However, there are still some drawbacks for the use of ELISA. Specifically, ELISA cannot multiplex, or detect multiple contaminants at the same time without running separate ELISAs or having more than one antibody for binding. Additionally, ELISA requires technical expertise and expensive equipment, making it challenging to perform these tests outside of a laboratory setting. Furthermore, the development of a new ELISA can be expensive and take months due to the time needed for generating, synthesizing, and purifying the desired antigens and antibodies for specific sensing.

Surface-enhanced Raman Spectroscopy (SERS)

SERS Sensing Introduction. Raman spectroscopy is a technique that provides a molecular fingerprint for a specific molecule based on its vibrational frequencies. This technique relies on inelastic light scattering, which is an incredibly rare occurrence (1 in 10^8 photons), thus rendering normal Raman scattering a relatively weak phenomenon and a nonideal analytical technique for trace contaminant sensing applications. In 1974, surface-enhanced Raman scattering (SERS) was first measured for pyridine adsorbed onto an electrochemically

roughened silver substrate.[133] Later work done by Van Duyne et al.[134] discovered that excitation of conduction band electrons within this nanostructured roughness (the LSPR) generates an enhanced electromagnetic field, increasing the observed Raman scattering signal by factors up to 10^{10}-10^{11}. SERS is particularly of interest in target detection due to water being a poor Raman scatterer, allowing for a wide variety of species to be detected within biological systems. Food contaminants must often be sensed in the complex food matrix where they occur, and SERS has the potential to distinguish the contaminant from the matrix based on the inherent vibrational modes of the contaminant itself.

Some of the first work using SERS as an analytical technique for food contaminant detection was done in 1987 by Carrabba and coworkers.[135] Their work aimed to detect organic aromatic contaminants in water such as pyridine, quinoline, and benzothiophene. These small molecule organic compounds are a direct result of industrial, energetic, and nuclear waste and often contaminate food supply for decades after initial contamination. This work was a direct result of a Department of Energy (DOE) study released in 1985[136] stating that a large amount of solid, liquid, and atmospheric byproducts of nuclear and nonnuclear energy waste was being excreted into water sources. This waste was leaching into plant and crop sources and could biomagnify at each step of the food chain. For this reason, the authors aimed to understand the toxicity of these compounds as they are transported within their environment at surface and sub-surface levels of water monitoring. The SERS substrate, a roughened silver wire in an electrochemical cell, allowed the authors to monitor potential transformations of these naturally strong Raman scatterers. These changes may be due to natural transport, immobilization, or redox when in their natural environment. By applying a set potential and

different excitation wavelengths during detection, they were able to monitor charge transfer mechanisms occurring on the substrate surface, leveraging the SERS enhancement field. Their LOD was calculated to be 5 x 10^{-5} M (4 ppm) for pyridine, an aromatic compound known to have serious effects at 1 mg/kg (1 ppm) dosages.[137] Carrabba used this work as a basis to then probe contaminated drinking water and water that leaches into crop sources in later work.[138,139,140] The authors also observed potential-dependent peaks and intensities that could be used for direct identification of those compounds even when mixed with other contaminants before and after potential transformation, although they did not specify if these transformed compounds were also toxic. Since this early article was published, significant work has been done using SERS as a dynamic technique due to its ability to give a "fingerprint" spectrum of the target; in many cases, this target is detected by virtue of a substrate-bound affinity agent that captures the target, holding it in close proximity to the enhancing substrate. An affinity agent, such as an aptamer, antibody, small molecule, or polymer, facilitates detection of targets such as food contaminants.[141]

Biological SERS Sensing. Due to SERS compatibility with water, it is suitable for sensing biological samples such as bacteria; sensing bacteria is important as they are a major source of food contamination.[142] Wang and coworkers synthesized a SERS nanoprobe for bacteria detection using a M13 microphage.[143] This specific microphage was a filamentous bacteriophage composed of single-stranded DNA that works as a virus to infect and kill bacteria as they are replicating.[144] Their work focused specifically on detecting and deactivating *Staphylococcus aureus* (*S. aureus*), a Gram-positive bacterium that can cause food poisoning due to contamination in milk and cheese products. *S. aureus* is a facultative bacterium, allowing

it to survive in a multitude of environments.[145] The authors synthesized a SERS probe that attached to a single bacterium with multiple AuNPs on the surface of the M13. Briefly, multiple M13 phages specifically adhered to the *S. aureus* surface and pVIII proteins were added to act as conjugation ligands for *in situ* growth of multiple AuNPs on the surface of each M13 phage. This was done to ensure an orderly and aligned chain of particles surrounding the bacteria so that the LSPR was optimal for SERS detection. These AuNPs were treated with Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), an efficient Raman scatterer) at the exterior of the nanospheres to generate a large SERS signal. To ensure specificity, this probe was screened in the presence of *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and produced no signal for these bacteria. In an effort to detect this bacterium in a relevant food matrix, capture and detection were done in orange juice, pure milk, and milk beverage. These beverages were spiked with approximately 1000 CFU/mL of bacteria along with the probe for binding. The excess probes were centrifuged out, and the solution was dropped onto filter paper ahead of SERS measurements. The sensors had a spiking recovery of 103.3-110.0%, which insinuates bacterial growth during the sensing process, but the high recovery makes it a viable sensor in complicated food matrices with some additional purification due to a complicated background signal.

SERS and Chemometrics. One of the shortcomings of SERS detection in food, as highlighted by the approach used in the previous paper, is the need for a SERS tag (a naturally strong Raman scatterer bound to the plasmonic NPs). Additionally, further chemometric analysis or other data processing is often needed to distinguish between multiple SERS tags on targets or differentiate

the target from the matrix. Intrinsic SERS, however, allows for the direct detection of the target of interest. Instead of monitoring shifts or vibrational peaks in the spectra relating to the tag, one is monitoring vibrational changes inherent to the specific target.[146] An example of this use of chemometrics and intrinsic SERS can be seen with research done by Li et al. which focused on the synthesis of 3-D cauliflower-inspired SERS substrates for rapid multiplex detection of three mycotoxins: AFB1, DON, and zearalenone (ZON).[147] Multiplex detection of these toxins is of particular interest because multiple food contaminants are often found on a single food source. In this paper, the authors give linear ranges for individual detection of each mycotoxin, 0.005 –1 μg/mL (0.005 – 1 ppm), 0.1 – 50 μg/mL (0.1 – 50 ppm), and 0.05 – 10 μg/mL (0.05 – 10 ppm), respectively, all of which are below the FDA, China, and EU regulatory limits without the need for a SERS tag. They then mixed the mycotoxins together at different concentrations to simultaneously monitor vibrational modes inherent to each toxin (Figure 6).

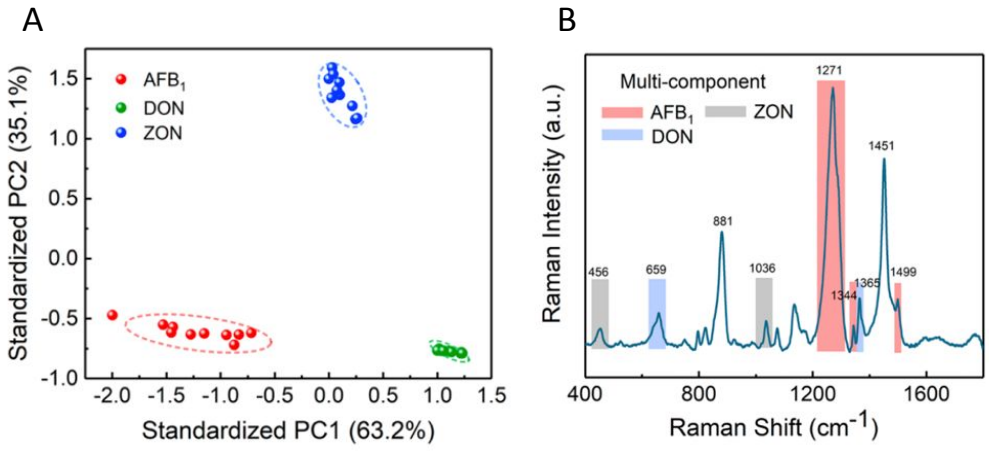

Figure 6. A. Principal component analysis of PC1 and PC2 displaying good separation of SERS data for each mycotoxin. B. SERS spectra of spiked maize solution with AFB1, DON, and ZON character. Reproduced from Li, J.; Yan, H.; Tan, X.; Lu, Z.; Han, H. *Anal. Chem*. **2019**, 91 (6), 3885–3892 (ref 147) Copyright 2019 American Chemical Society.

Although there are distinguishing peaks and features relating to each toxin, there is still significant overlap in their band assignments. For this reason, the authors use principal component analysis (PCA), a common chemometric analysis technique used when analyzing SERS spectra. PCA is a mathematical way to reduce large data sets by displaying the largest changes in variance observed that is inherent to the data.[148] Herein, the largest variance in the data set can be seen through the first principal components where the data can have good or poor separation based on this blind variance (i.e. large or small differences in the data set that are not necessarily known when the analysis is applied). Thus, Li and coworkers successfully distinguished each mycotoxin from the others at low concentrations based on the good separation observed in PCA (Figure 6A). This indicated that combined spectra have distinct enough features to differentiate between the various toxins. They were also able to detect the mycotoxins in maize, a common matrix contaminated by these mycotoxins, with additional maize purification. As expected, the solutions had higher LODs than previously observed when detected alone: 0.01 μg/mL (0.01 ppm), 0.1 μg/mL (0.1 ppm), 0.5 μg/mL (0.5 ppm) for AFB1, DON, and ZON, respectively. This work by Li et al. displayed how a unique SERS substrate, with an enhancement factor of 2.2×10^6 and computer simulations showing a high electric field distribution around the "cauliflower heads," can lead to lower limits of detection than the regulated concentration for food contaminants without the need for a SERS tag, thus, allowing one to directly sense each target when multiplexed together. However, additional need for chemometric analysis adds complication in SERS food detection.

Conclusions to SERS Sensing. As previously stated, multiple food contaminants are oftentimes found on a single food source, meaning simple multiplex detection of these contaminants is vital for practical monitoring of food safety. SERS can serve to detect small molecules, proteins, bacteria, and although not yet done with food contaminants, it can detect viruses as well.[149,150,151,152] Though a powerful and sensitive technique, SERS often requires additional analysis, especially in food matrices where the spectra may be very complicated, making it less desirable for real-world application. Other SERS detection methods combine the use of SERS with other analytical techniques or methodology such as ELISA, electrochemistry, or microfluidic devices.[153,154,155,156] However, this added need for analysis, purification, or instrumentation continues to hold back the ability for SERS to be a stand-alone technique. Therefore, continued work on simplifying SERS spectra for multiplex detection in real-world samples, such as creating a library of spectra or barcoding samples would be a beneficial direction for SERS food safety detection to move towards.

Field-effect Transistors (FETs)

Introduction to FETs. Detection techniques using electrochemical sensors are becoming much more common in the food safety industry as alternatives to more conventional techniques like chromatography and mass spectrometry. Electrochemical sensors provide advantages over these techniques because they require lower sample volumes, yield faster detection times, and entail simple sample preparation.[157] In addition, the many straightforward ways to measure moving electrons and the inherently quantitative nature of electrochemical measurements support the great potential for electrochemical sensors. A wide range of electrochemical

sensors have been developed specifically for the detection of food contaminants, including impedimetric aptasensors,[158,159,160] square-wave voltammetric electrodes,[161,162] and amperometric electrodes.[163,164] In this review, we will focus on field-effect transistor (FET) sensors based on their ability to reach very low limits of detection and their high potential for on-site use as compared to other electrochemical sensors.[165] This technology has been employed to detect five of the six classifications of food contaminants, missing only parasites, which tend to be too large compared to the surface of the FET for the method to be effective.[166–169] FET sensors detect changes in potential or current across a conductive channel between an oxide insulator and a semiconductor. These changes are caused by the binding of analytes in solution to receptors on the oxide layer, which alters the number of charge carriers in the conductive channel. The first sensors using FET technology were ion-sensitive FETs (ISFETs) developed by Bergveld in 1972[170] for taking electrophysiological measurements of ion activities to investigate neural activity. These sensors were modeled after metal-oxide-semiconductor field-effect transistors, with an electrolyte solution replacing the gate metal above the oxide layer (Figure 7).[171]

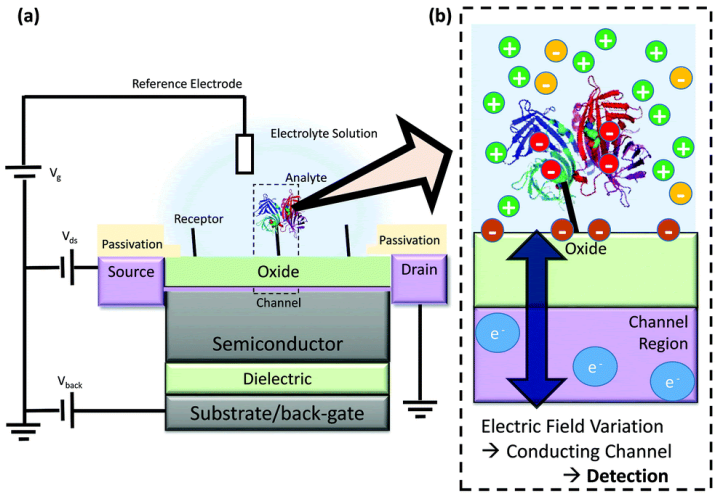

Figure 7. Schematic of ISFET sensor with streptavidin as example analyte. Changes in ion adsorption to the oxide layer due to analyte binding at receptor cause measurable shifts in current and potential in the channel region between the source and drain electrodes. Reproduced from Lowe, B. M.; Sun, K.; Zeimpekis, I.; Skylaris, C. K.; Green, N. G. *Analyst.* **2017**, pp 4173–4200. (ref 171) Copyright 2017 Royal Society of Chemistry under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)..

One of the earliest works using these sensors for detection of food contaminants was the 1980 application of enzyme-coupled FETs (ENFETs) to detect penicillin by Caras and Janata.[172] Penicillin detection is necessary for the dairy industry where milk is checked for antibiotic contamination.[173] The device takes advantage of the fact that the enzyme penicillinase causes the hydrolysis of penicillin into penicilloic acid, lowering the pH at the electrode surface. The ENFETs were constructed with a membrane of albumin and penicillinase enzyme between the oxide layer and the solution of a pH-sensitive ISFET. Thus, the presence of penicillin leads to a measurable shift in potential that corresponds to the concentration of penicillin. They were able to achieve a LOD of 0.1 mM (33 ppm). These ENFETs also provided an advantage over many other enzyme-based detection techniques in that relatively little enzyme is needed due to the small sensing area of 0.5 mm^2 that is functionalized with enzyme.

Biological Sensing with FETs. FETs have increasingly been used in the detection of bacteria such as *Pseudomonas aeruginosa*[174] and *Salmonella infantis*[175] which are known to cause foodborne illnesses. So et al. developed a single-walled carbon-nanotube field-effect transistor (SWCNT-FET) functionalized with RNA aptamers to detect *Escherichia coli.*[176] Binding of *E. coli* to the aptamers, which are specific to *E. coli*, on the FET surface decreased the measured conductance of the channel. In this study, however, detection was not quantitative as it was only used to determine whether samples contained or did not contain the bacteria, not to determine the bacterial concentration. The FETs were also found to be highly selective for *E. coli* and did not respond with a decrease in conductance in the presence of *Salmonella typhimurium*. While FET sensors are highly selective, a major shortcoming of FET sensors in detection of food-contaminating microorganisms is that it can be difficult for them to quantitatively detect bacteria due to uneven distribution of bacterial cells within a sample, especially when only a few microliters of a sample solution is used.[177] In the case of So et al. this problem was overcome by using the most probable number[178] (MPN) method wherein the bacterial concentration is estimated by making three or more dilutions of a sample and taking measurements from at least three aliquots of each dilution. The number of aliquots positive and negative for the bacteria as well as their corresponding dilutions are compared to a standardized MPN table to give an estimate of bacterial concentration. Despite such difficulties, these sensors do allow for much faster detection than traditional bacteria culture-based techniques since they do not require a long incubation time. Though stability is a challenge in some FET work, there are examples of FET sensor designs have also shown high stability over

time, such as an FET functionalized with DNA and highly conductive indium tin oxide nanowires, which retains 96% of original signal response after 5 weeks.[179]

More recently, FETs with an oxide layer of titanium dioxide on molybdenum disulfide have been used for the detection of Gram-positive bacteria, specifically *Staphylococcus aureus*, a common cause of food poisoning.[180] This hybrid oxide layer structure provides greater sensitivity due to weak interlayer bonding in MoS_2 as well as desirable adsorption properties and high stability from TiO_2. Moudgil et al. developed this hybrid FET, which used immobilized vancomycin, an antibiotic, to bind to peptidoglycans on the cell wall of the bacteria.[181] When the *S. aureus* bacteria, which are negatively charged due to anionic lipoteichoic acids and lipopolysaccharides in the cell wall,[182] are captured by vancomycin, the current in the channel decreases due to a reduction in charge carriers. The sensor was highly sensitive, with a LOD of 50 CFU/mL, and was reported to distinguish between samples of live and dead bacteria. This is due to the fact that the peptidoglycan layer of the cell wall in the dead cells is disrupted and vancomycin cannot effectively bind to the bacteria, so minimal change in current is detected in the presence of dead bacteria. Bacterial detection was also successfully performed in fetal bovine serum, which showed that the electrical response is similar in a complex matrix to when performed in buffer.

FET sensors are highly sensitive to analytes that are highly charged, like bacteria, since large changes in charge density near the surface of the FET result in greater changes in conductance and potential in the FET channel. However, when the target of detection is a molecule with little to no charge, many more molecules are needed at the surface of the FET to induce the same amount of change in channel current observed with highly charged molecules

or organisms. FETs suffer from low sensitivity for these low-charge molecules due to the Nernst sensitivity limit of 59 mV/pH as the maximum change in sensor voltage per change in pH.[183] To combat this problem, signal amplification techniques have been developed such as the use of ionic surfactants. Hideshima et al. detected BWp16, a buckwheat allergenic protein, by coupling it to sodium dodecyl sulfate.[184] This increased the protein's charge from -3.6 to -50.6, causing a greater change in potential in the FET to be observed. This method allowed for a LOD of 10 ng/mL (0.01 ppm) of BWp16. Furthermore, signal amplification of 100 times while detecting mycotoxins has been achieved by Ah et al.[185] using AuNPs for signal amplification. They immobilized bovine serum albumin and keyhole limpet hemocyanin as mycotoxin receptors on the surface of the FET oxide layer which bind to mAbs conjugated to the negatively charged AuNPs. Mycotoxins competitively bind to the receptors and cause some of the AuNPs to detach. A gold deposition reaction is then used to grow and increase the negative charge of the remaining AuNPs which causes charge carriers (holes) to accumulate in the FET channel, increasing the measured conductance. When a sample contains a greater concentration of mycotoxins, more AuNPs are detached, leading to a smaller increase in conductance. Concentrations were detected as low as 0.5 ng/mL (0.0005 ppm) for AFB1, ZEA, and OTA. As another example, graphene field-effect transistors (GFETs) are able to enhance signal strength greatly because the channel is a single layer of graphene. This single layer's conductance is much more affected by changes in charge near the surface than for the traditionally-used thicker layers of silicon.[186] Aptamer-functionalized GFETs have been used to reach a LOD of 4 pg/mL (0.000004 ppm) for OTA with a linear response range of 10 pg/mL – 4 ng/mL (0.00001 – 0.004 ppm).[187]

An advantage of field-effect transistor sensors is that they can be used for on-site detection due to their small size, fast response time, and simple operation not requiring advanced technicians. This makes them preferable to other more traditional techniques like HPLC and GC-MS for detecting food contaminants at any point in the food production chain. A GFET was developed by Islam et al. for detecting the pesticide chlorpyrifos, which can cause severe neurological disorders, as a potential method for on-field testing of fruits and vegetables.[188] Antibodies immobilized on the graphene channel were used to bind the chlorpyrifos from the sample solution, causing a decrease in the resistance of the channel. The sensor reached a LOD of 1.8 fM (6.3×10^{-10} ppm), far below the regulatory limit set by the WHO and an order of magnitude lower than earlier FET chlorpyrifos sensors.[189] Other FETs have also been developed for on-site pesticide detection, such as an enzyme-functionalized GFET for detection of the pesticide carbaryl.[190] In this sensor, the hydrolysis of urea by urease releases ions that adsorb onto the graphene surface, which reduces the current. To detect carbaryl, the FET is first exposed to the unknown solution for 30 minutes to allow for any carbaryl present to complex with the active sites of urease, which inhibits the enzyme. The FET is then exposed to a solution of urea while measurements are taken. Carbaryl inhibition of urease leads to fewer ions adsorbing to graphene; therefore, there is a smaller decline in current than with non-inhibited urease. This method attained a LOD of 10^{-8} µg/mL (10^{-8} ppm), and since the inhibition of urease is reversible, the sensors can be regenerated and reused.

FET Sensor Conclusions. While FET sensor technology has made progress in detection accuracy for non-homogeneous samples such as bacterial cultures and in sensitivity for low-charge

molecules, there is still much room for improvement in areas including sensitivity, detection in complex matrices, specificity, and shelf-life. Despite some of their shortcomings, FETs provide numerous advantages over other sensing techniques. They tend to be low-cost with little required sample preparation, allow short detection time, and have no need for highly skilled technicians or expensive and large equipment. These factors and their very small size give FET sensors the potential to be used as highly effective on-site detection units at any point in the food production chain.

Conclusions

The field of food safety and food contamination is constantly evolving. For this reason, this review has aimed to evaluate common analytical techniques along with recent advancements for food contamination sensing. It is important to note that the future of food contamination detection relies heavily on advances in two abilities: multiplex detection and detection in a complex matrix. These capabilities, along with evaluation of ease, cost, and robustness of the overall sensing capacity will dictate how promising any given technology is in helping to achieve food safety and security. Upon comparing these analytical techniques, it is clear that it is possible to increase sensitivity and range of relevant analytes when combining complementary analytical techniques. Of course, using combined or hyphenated techniques expands the technique's utility but adds complexity to resulting analysis. Additionally, food contaminant classes such as parasites, viruses, and fungi are not well represented in this article due to lack of research done to detect these targets with the most common methodology and instrumentation. This are important contaminant classes, and the food sensor world should

expand research to take on these challenges. To help identify complementary methods described in this review, Figure 8 shows a comparison of the techniques considered: UV-visible spectroscopy, chromatography, lateral flow and immunoassays, surface-enhanced Raman spectroscopy, and field-effect transistors.

ANALYTICAL TECHNIQUES FOR FOOD SENSING

Technique	Multiplexing	Sensing in Complex Matrix	Ease of Use and Cost	Analytes	Biggest Advantages and Disadvantages	Notable Advancements
UV-visible Spectroscopy	Yes, only if spectral peaks are distinct	Yes, only if matrix peaks do not overlap with analyte	Easy and relatively inexpensive, robust enough for in-field measurements	Limited to UV-visible absorbing organic molecules or NP binding to bacteria or organic molecules	**Adv:** fast and robust **Disadv:** very small shifts in spectra for detection	Plasmonic NPs with affinity agents to capture various analytes
Chromatography	Yes, but chromatographs can be very complicated	Yes, only with various sample preparation techniques like SPME and QuEChERS	Expensive, special personnel needed for analysis and maintenance	Only small molecules	**Adv:** low LODs **Disadv:** specialized, expensive, does not encompass many analytes	Multi-dimensional analysis for multiplex detection
Lateral Flow and Immunoassays	Yes with multiple affinity agents	Yes, but with sample purification	Relatively inexpensive, robust enough for in-field measurements	Small molecules, bacteria, proteins	**Adv:** fast, gold standard **Disadv:** false +/-, requires proper storage, long fabrication for specificity	Plasmonic ELISA and LFIAs
SERS	Yes, with multiple affinity agents or added chemometrics	Yes, some chemometric analysis may be needed	Somewhat inexpensive, robust enough for in-field measurements	Small molecules, bacteria, proteins, viruses	**Adv:** can distinguish multiple targets from one another **Disadv:** chemometric analysis is complicated	SERS affinity agents for multiplexing, large range of contaminants can be detected
FETs	Yes, with multiple affinity agents, though little work has been attempted to date	No, but there is potential in the future	Synthesis of sensor is complicated, but visual changes in data are relatively straightforward	Small molecules, bacteria, proteins, viruses, fungi, any charged species	**Adv:** small, fast, low LODs **Disadv:** short shelf-life, complicated synthesis	Amplification of uncharged species, targeting of 5 different classes of food contaminants

Figure 8. Comprehensive chart evaluating important characteristics of food sensor technology and techniques. “Adv” are advantages and “disadv” are disadvantages to the techniques. Refer back to individual sections for references related to notable advancements. Figure created with BioRender.com

As previously noted, UV-visible spectroscopy will continue to serve as a supplementary technique for food safety detection. UV-visible spectroscopy is a robust technique that can even use a cell phone camera as a detector,[191] but if the sample’s matrix masks the absorption peak, detection is not possible. While plasmonic NPs have played a large role in expanding the analytes that can be detected with UV-vis extinction spectroscopy, plasmonic extinction shifts

observed upon target association are often very small, making it difficult to obtain reliable data with inexpensive equipment.

Chromatography techniques have long served as the gold standard for food detection due to their low LODs, but the range of analytes are largely restricted to organic compounds, eliminating its potential as a universal sensing technology. The need for multiple types of sample preparation make chromatography less attractive than other techniques considered here; however, its ability to perform multiplex detection in complex media (after sample preparation) make it a viable technique for pesticides and mycotoxins. Multi-dimensional chromatography, able to separate upwards of 16 compounds quantitatively in complex mixtures, helps achieve this best, thus serving as the most promising avenue for continued development.

ELISA and LFIAs currently serve as the gold standard for food contamination detection. The use of plasmonic particles to help amplify signal has attributed to increased success in detecting small molecules, bacteria, and various types of proteins. Once an affinity agent is synthesized, its specificity for its target allows the entire analysis to require less than 30 minutes,[192] some of the fastest food sensing technology that exists. Though they are fast and relatively inexpensive, their false positive or negative test results often result in the need for more than one test, adding to overall cost. Multiplexing in complex media is possible with some sample preparation, but synthesis of the affinity agent for each target have long time scales, making the development in this field much slower. These tests will long serve as ways to quickly screen for food contaminants that have affinity agents well established in literature.

SERS is a unique technique because of all the different targets it can detect. SERS tags amplify a target's signal, but intrinsic SERS best serves food sensing when doing multiplex detection and detection in complex media. Though a lot of multiplexing with SERS requires extra chemometric analyses to distinguish each target within the food matrix, exploration of different types of SERS affinity agents can result in a single affinity agent that can bind to an entire class of targets.[193] By doing so, there is no longer a need for multiple affinity agents which results in a less complicated spectra. This, in turn, yields potential to create simplified spectral libraries of food, perhaps eliminating the need for chemometric analysis of captured data.

A lot of research still needs to be done on the use of FET sensors for food contamination detection. They are the most promising when targeting all classes of food contaminants, observing extremely low LODs, and their small size make them perfect for relatively fast use with very little sample volume needed. However, their complicated fabrication when optimizing the sensors and their very short shelf-life (subject to oxidation) make them unstable for commercial use or in-field measurements. Addressing these needs would make FETs one of the most powerful food safety sensors. Current work is being done to target these needs, moving the field in the right direction.[194,195,196]

Food safety will only increase in importance as the world population increases, globalization continues, and climate change impacts food production. To address safety concerns and continue to advance this field, more work needs to be done in multiplex detection in complex food samples while minimizing time spent on sample preparation. This, alongside relatively fast sensing times and simplified read-out technology, will enhance this

field to create sensing technologies that are robust for in-field measurements to support a safe global food supply.

BIOGRAPHIES

Rebeca S. Rodriguez is a 5th year Ph.D. candidate in Chemistry at the University of Minnesota working under Dr. Christy L. Haynes. Her research is focused on detecting various food contaminants with linear polymer affinity agents and surface-enhanced Raman spectroscopy. She received her B.S. in Chemistry at American University in 2016 and her M.S. in Chemistry at the University of Minnesota in 2018.

Tana L. O'Keefe is a 3rd year Ph.D. candidate in Chemistry at the University of Minnesota in Dr. Christy L. Haynes research group. As part of the NSF Center for Sustainable Nanotechnology, her research is focused on developing novel nanomaterials for use in agricultural applications. She received her B.S. in Chemistry from the University of Minnesota-Duluth in 2018 and her M.S. in Chemistry at the University of Minnesota in 2020.

Clarice E. Froehlich is a 3rd year undergraduate student in the Honors Program at the University of Minnesota studying Chemistry and Chemical Engineering. She is currently working in the lab of Dr. Christy L. Haynes, and her research focuses on the detection of food contaminants using surface-enhanced Raman spectroscopy as well as the optimization of carbon dot synthesis.

Riley E. Lewis is a 2nd year graduate student in Chemistry at the University of Minnesota with Dr. Christy L. Haynes. He received his B.A. in Chemistry from the New College of Florida in 2019. His current research focuses on understanding interactions between nanomaterials and persistent organic pollutants as part of the NSF Center for Sustainable Nanotechnology.

Trever R. Sheldon is a 4th year undergraduate student in Chemistry at the University of Minnesota working under Dr. Christy L. Haynes. His current research focuses on assisting Rebeca Rodriguez in detecting various food contaminants with linear polymer affinity agents and surface-enhanced Raman spectroscopy.

Christy L. Haynes is currently a Distinguished McKnight University Professor in the Department of Chemistry at the University of Minnesota. Haynes completed her undergraduate work at Macalester College in St. Paul, MN (1998), and her doctoral work was done at Northwestern University in Evanston, IL (2003) under the direction of Richard P. Van Duyne. Her doctoral thesis title is "Fundamentals and Applications of Nanoparticle Optics and Surface-Enhanced Raman Scattering." Before arriving at the University of Minnesota to start her independent career, Professor Haynes performed postdoctoral research in the laboratory of R. Mark Wightman at the University of North Carolina, Chapel Hill as an NIH NRSA Postdoctoral Fellow. Her efforts in the Wightman lab focused on applying microelectrode amperometry to probe single cell exocytosis. At the University of Minnesota since 2005, the Haynes research group develops new analytical methods and nanomaterials to approach chemistry problems at the interface of analytical, biological, and environmental, and materials chemistry. Haynes is currently Associate Department Head at the University of Minnesota, Associate Director of the NSF Center for Sustainable Nanotechnology, and an Associate Editor for *Analytical Chemistry*.

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