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Surface Plasmon Resonance Analysis of Food Toxins and Toxicants

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7.1 Introduction

Production of quality food is not a simple task. The control of hazards potentially associated with food is of great importance. Risks often appear due to the infection of food with microorganisms resulting from inappropriate conditions of storage and delivery, which may not always be optimal. Food safety and quality may be further impaired by natural occurring pathogens and contaminants or undesirable fermentation products. Furthermore, with the expanding use of various substances which improve the efficiency of food production, like veterinary drugs, pesticides and growth-promoting hormones, and substances that enable its longevity, the food producers are intermittently exposing their customers to risk. For example, many pesticides are harmful to humans by causing acute or chronic poisoning. These are more frequent in developing countries where they can cause more deaths than infectious diseases (Eddleston *et al*. 2002). The recent study shows that the number of identified and reported foodborne disease multistate outbreaks has increased in 1973–2010 (Nguyen *et al*. 2015).

For the detection of various substances that deteriorate the quality of food and eventually endanger the health of the user, the development of methods that detect the integrity of the food is of extreme importance. Food safety control programs urgently need diagnostic system and test kits which are sensitive, rapid, cost-effective, easy to use and portable. The approach that satisfies these conditions is employing biosensors (Scheller *et al*. 1989). Such devices are made up of a transducer and a biological element that may be an enzyme, antibody, aptamer, microbe, organelle, even living cell or any other biological material. The biological response is converted to a quantifiable electrical signal by a transducer, a sensing device, which can be optical, electrochemical, thermometric or piezoelectric, depending on a method used for the signal transduction. The electrochemical biosensors are the most investigated among the sensors and can be further divided into three classes, amperometric, potentiometric and impendance sensors. Amperometric sensing devices measure the current when a potential is applied to, usually, an antibody coated electrode, whereas potentiometric devices measure the changes in pH and ion concentration when the interaction with the detecting molecule occurs. Thermometric biosensors detect the change in temperature which can occur due to evolution or absorption of heat accompanying some specific reactions. Piezoelectric biosensors exploit the ability of quartz crystals to vibrate under the influence of an electric field. Recently, among more commonly used piezoelectric sensors are quartz crystal microbalance based sensors.

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Optical biosensors exploit the changes in light. One of the optical biosensors that has been implemented into food safety control is surface plasmon resonance (SPR). SPR was introduced in the 1980s as technology for biomolecular interaction analysis (Liedberg *et al*. 1983; Malmqvist, 1999). Since then, this technology has grown rapidly and resulted in a vast array of commercial platforms as well as custom-built instruments. SPR measures biomolecular interactions in real-time in a label-free fashion. The antibodies, other molecules or even cells can be immobilized to the sensor surface, while the molecules of interest are free in solution and passed over the surface. The interactions between different proteins, protein and DNA or small molecules can be studied. In addition, the detection of large molecular analytes like viruses is also possible. There is no need for modifying the molecules or cleaning the sample and the interaction is detected rapidly in real-time. SPR occurs in thin conducting films at an interface between media of different refractive index and results in a sharp dip in the intensity of reflected light at a specific angle, which is monitored by the detector. SPR depends on several factors, but mainly on the refractive index of the solution circulating over the sensor surface. If the molecule of interest (in the SPR terminology this molecule is termed analyte) binds to the detecting molecule attached to the gold layer (termed ligand), the refractive index is changed and so is the angle at which the SPR occurs. Most SPR refractometers measure the intensity of light at a fixed angle of the incident light.

The method offers several applications. The interaction between different proteins (de Mol, 2012), proteins and DNA (Walter *et al*. 2014) or proteins and lipid membranes (Beseničar *et al*. 2006; Hodnik and Anderluh, 2013) are most studied by using this technology. In the last decade the technology improved greatly offering the instruments and setups which can detect very low molecular mass analytes like steroid hormones, toxins, drugs and explosives residues (Mitchell, 2010). It is also possible to determine the specificity of the interaction by deriving equilibrium and kinetic rate constants. Furthermore, concentration measurements can be easily obtained. Some instruments offer very useful measurements of the active concentration of a molecule without conducting the calibration curve. The approach overcomes the limitations of measuring protein concentrations spectroscopically, which may overestimate the true concentration (Richalet-Sécordel *et al*. 1997). The surface plasmon-based sensors were shown many times to give quick outcomes at low cost in environmental and food safety analysis. One of the advantages is also that they offer high sensitivity of small analytes. The development of the instruments is in the direction of simple, user friendly and portable sensors. The latest feature is of great importance in the food quality control all the way from the food producer to the customer.

The routine application of biosensors in toxin detection has been long limited due to high cost of the instruments available on the market and low throughput. But in recent years many research groups developed custom-built instruments that allow cheaper and easier measurements (Waswa *et al*. 2007; Lan *et al*. 2008; Yakes *et al*. 2011). These instruments usually do not offer the most precise kinetics data, but they serve as fast and simple biosensors in food safety, medicine and environmental awareness. Recently, sensors based on localized surface plasmon resonance (LSPR) have been developed as miniaturized high-throughput devices (Manzano *et al*. 2016). The SPR signal amplification using gold nanoparticles has been found to be very attractive (Nie *et al*. 2014). LSPR occurs in metallic nanostructures, such as nanoparticles, nanorods or nanostars. Particle size, shape and the method of preparation (time, temperature, thickness of metal deposition layer, and so on) are important factors in preparing LSPR-based biosensor.

Direct detection of small molecules (<1000 Da) is not routine in SPR biosensors because the sensitivity of the detection is mass related. In many cases the toxins in the samples have low molecular weight so the mass change after the binding to the sensor surface is too small to cause

a change large enough in the refractive index and a notable SPR signal. As a result indirect sensing approaches have been developed, that is, sandwich and competitive/inhibition techniques. Sandwich technique is accomplished by using the signal enhancement molecule after the analyte is already bound to a sensor, but the change in mass is negligible (Stern *et al*. 2016). Usually antibodies against the toxin are used as enhancing molecules. In inhibition assays the analyte sample is mixed with the fixed concentration of the binding molecule, most commonly antibody, and injected over the sensor surface immobilized with the molecule of interest. The binding response is inversely related to the analyte concentration: the higher the concentration of the toxin in the solution, the lower the binding of the antibody to the surface. Calibration curves based on samples spiked with toxins' standards are compulsory (Campbell *et al*. 2007; Geschwindner *et al*. 2012; Yu *et al*. 2005). Some other methods (e.g. nanoparticle labels) can be also used to increase signal intensity (Hu *et al*. 2014).

SPR performs similarly or better in comparison to other analytical approaches used in food quality assurance due to the versatility and high sensitivity. The standard method accepted universally for testing different toxin potency in food matrices is the mouse bioassay (MBA). Mice are injected intraperitoneally with a sample suspected of containing toxin and monitored in the following days. Live animal testing has low sample throughput, analytical imprecisions, and it is expensive and requires a lot of effort. Enzyme-linked immunosorbent assay (ELISA) has become popular in recent years because it is inexpensive, sensitive, and selective. Various chromatographic techniques such as liquid chromatography (LC), ultra performance LC (UPLC), and high performance LC (HPLC), mass spectrometry (MS), polymerase chain reaction (PCR), and many other analytical approaches are also being used in toxin monitoring, but they have many drawbacks, for instance requiring expensive instrumentations, different reagents and trained personnel. For detection of diarrheic shellfish poisoning toxin in mussels, the MBA was for a long time the method of choice since ELISA did not always give quantitatively reliable results and HPLC method was limited to the determination of okadaic acid only (Draisci *et al*. 1994). Since then many new approaches were used, like direct competitive chemiluminescent-ELISA (Vdovenko *et al*. 2013), which made employing of MBA redundant in many cases. The detection capability of ELISA is quite comparable with SPR, but the second is faster and can be used on field, which cannot be said for the long-lasting ELISA procedure. Examples of different analytical approaches used for the detection of toxins and toxicants in food safety analysis are gathered in Table 7.1. For each toxin we report first the representative SPR example of toxin determination which is then followed by some additional approaches that were also successfully applied in toxin determination. Limits of detection (LOD) is the lowest quantity of a substance that can be reliably measured (Armbruster and Pry, 2008), and its value depends greatly on technique employed and substance that is measured. The development of a certain approach can increase the sensitivity and consequently decrease the value of LOD. In addition, sample preparation, costs and duration of the analysis also play very important roles when considering the most appropriate method in food safety analysis.

A great advantage of SPR is its versatility, which enables a detection of many different analytes. A biomolecule, termed 'ligand", is immobilized on the sensor surface (Malmqvist, 1999). There are many different types of molecules available, such as antibodies, aptamers, enzymes, lectins, lipids, living cells, organelles, peptides, proteins, etc. The ligand immobilization must be performed on a clean sensor surface, on which structural features and biological activity should be preserved. The most commonly used methods for the immobilization is based on physico-chemical interactions (chemisorption, covalent binding, electrostatic coupling, high affinity molecular linkers in multilayer systems, and so on) between ligand and surface. For a successful detection, the analyte in the sample and a ligand should possess a high affinity. It is desirable that the complex composed of ligand and analyte is possible to break, and such regeneration of

Table 7.1 Toxin determination in food safety by using different approaches.

Ara h1					
LOD	0.09 μg/ml	6.3 ng/ml	0.34 ng/ml	0.14 mg/ml	1.3×10^{-17} mol/l
Method	SPR	Amperometric sensor	ELISA	SERS	Electrochemical DNA biosensor
Reference	Pollet *et al.* 2011	Montiel *et al.* 2015	Peng *et al.* 2014	Gezer *et al.* 2016	Sun *et al.* 2015
Atrazine					
LOD	5.0×10^{-8} M	1.6 μg/kg	0.01 ng/ml	0.15 μg/kg	0.03 ng/ml
Method	SPR	LC-MS	GC-MS	UPLC–ESI-MS	ELISA
Reference	Tomasetti *et al.* 2015	Andrade *et al.* 2016	Williams *et al.* 2014	Dong *et al.* 2016	Barchanska *et al.* 2012
Casein					
LOD	10 ng/ml	0.1 mg/l	0.03 μg/ml	1.3 mg/kg	0.01 μg/g
Method	LSPR	ELISA	Colorimetric	LC–MS/MS	microHPLC–ESI-MS/MS
Reference	Minh Hiep *et al.* 2007	Deckwart *et al.* 2014	Li *et al.* 2014	Cristina *et al.* 2016	Monaci *et al.* 2014
Chloramphenicol					
LOD	ppb-level	1 μg/l	0.7 ng/l	0.8 μg/kg	33 nM
Method	SPRi	gel-based visual immunoassay	CL-ELISA	HPLC	Fluorescence
Reference	Rebe Raz *et al.* 2009	Yuan *et al.* 2012	Tao *et al.* 2014	Lu *et al.* 2016	Tan *et al.* 2015
E. coli					
LOD	3×10^3 CFU/ml	1.9×10^3 CFU/ml	5×10^5 CFU/ml	0.4 CFU/g	10 CFU/25 g
Method	SPR	LC-MRM–MS/MS	LC–ESI–SRM	ELISA	PCR
Reference	Wang *et al.* 2013	Banu *et al.* 2014	Martelet *et al.* 2014	Feng *et al.* 2013	Delbeke *et al.* 2015
L. monocytogenes					
LOD	10^3 CFU/ml	1.61 CFU/ml	10^3 CFU/g	1000 CFU/25 g	10^4 CFU/ml
Method	SPR	rt-PCR	ELISA	rt-PCR	ELISA
Reference	Ohk and Bhunia, 2013	Quero *et al.* 2014	Cavaiuolo *et al.* 2013	Gómez *et al.* 2013	Tu *et al.* 2016

Ochratoxin A					
LOD	0.042 ng/ml	0.04 µg/kg	0.1 µg/kg	0.85 ng/ml	0.05 µg/kg
Method	SPR	HPLC-FLD	LC–ESI-MS/MS	ELISA	MEF
Reference	Yuan *et al.* 2009	Brera *et al.* 2014	Fernandes *et al.* 2013	Novo *et al.* 2013	Todescato *et al.* 2014

Ovalbumin					
LOD	0.03 µg/ml	0.4 µg/ml	0.51 ng/ml	100 ng/l	0.8 µg/ml
Method	SPR	microHPLC–ESI-MS/MS	ELISA	LC-MS/MS	LC-ESI-MS/MS
Reference	Pilolli *et al.* 2015	Monaci *et al.* 2014	Peng *et al.* 2014	Tolin *et al.* 2012	Mattarozzi *et al.* 2014

PSP					
LOD	0.34 µg/g	0.01 µg/g	89 µg/kg	2.9 µg/kg	3 µg/kg
Method	SPR	ELISA	HPLC-FLD	HILIC/MS/MS	HILIC-ESI-MS^2
Reference	Cambell *et al.* 2009	Cambell *et al.* 2009	Sayfritz *et al.* 2008	Zhuo *et al.* 2013	Mattarozzi *et al.* 2016

TTX					
LOD	0.091 ng/ml	0.23 mg/kg	7.3 g/kg	0.005 µg	0.7 pmol
Method	SPR	ELISA	UPLC–MS/MS	LC-MS	LC/ESI-MS
Reference	Yakes *et al.* 2014	Reverté *et al.* 2015	Nzoughet *et al.* 2013	Tsai *et al.* 2006	Shoji *et al.* 2001

Salmonella					
LOD	10^6 CFU/ml	3×10^3 CFU/mL	5×10^{-3} ng/µl	10^3 CFU/ml	2×10^4 CFU/ml
Method	SPR	Colorimetric DNAzyme probe self-assembled gold nanoparticles	LAMP	MRS immunosensor	ELISA
Reference	Lan *et al.* 2008	Luo *et al.* 2014	Sayad *et al.* 2016	Wang *et al.* 2015	Wang *et al.* 2015

LC-electrospray tandem MS (LC–MS/MS); micro HPLC coupled to a dual cell linear ion trap MS (microHPLC–ESI-MS/MS); surface-enhanced Raman spectroscopy (SERS); LC coupled to MS (LC-MS); gas chromatography coupled to mass spectrometry (GC-MS); UPLC–electrospray ionization-MS (UPLC–ESI-MS); LC-multiple reaction monitoring tandem mass MS (LC-MRM–MS/MS); LC-triple-quadrupole tandem MS with electrospray ionization (LC–ESI–SRM); loop mediated isothermal amplification (LAMP); magnetic relaxation switch (MRS); HPLC method with fluorescence detection (HPLC-FLD); hydrophilic interaction liquid chromatography-ESI-MS^2 (HILIC-ESI-MS^2); UPLC coupled to tandem MS (UPLC–MS/MS); LC–electrospray ionization-MS (LC/ESI-MS); HPLC fluorescence detection (HPLC-FLD); LC–triple quadrupole tandem MS (LC–ESI-MS/MS); metal–enhanced fluorescence (MEF).

binding surface enables repetitive use of sensor surface (Muñoz and Ricklin, 2015; Rich and Myszka, 2000). SPR was routinely employed for highly sensitive detection of chemical and biological analytes. Even detection of small molecules like testosterone at picogram per millilitre was reported (Cao and Sim, 2007). Sensitive detection was even further improved recently by developing a novel water-compatible macroporous molecularly imprinted film, which lowered detection of hormone with a LOD down to 10^{-15} g/ml (Zhang *et al*. 2014). Other groups achieved similar detection of small molecules by combining the molecularly imprinted gold nanoparticles and SPR (Riskin *et al*. 2009).

Several review articles describe SPR employment in toxin detection in various samples (Soelberg *et al*. 2005; Hodnik and Anderluh, 2009) as well as the use of the technology in food safety control (Homola, 2004; Bergwerff and van Knapen, 2006; McWhirter and Wahlstrom, 2008; Situ *et al*. 2010). In this chapter we present the ability of SPR to detect the unwanted molecules in various food samples by showing some interesting examples (Table 7.2).

7.2 Detection of Toxins and Other Toxicants in Food Samples

7.2.1 Food Allergens

Allergy is an abnormal response of the immune's system to an ordinarily harmless substance called an 'allergen'. Allergens are typically naturally occurring proteins in foods or derivatives of them, and even though they do not present any threat to the majority of the population, the contaminated food could lead to serious health problems when consumed by allergic people. A hardly traceable amount of allergen can trigger unpredictable, even life-threatening reactions, varying with the dose and the sensitivity of affected individual. The allergic consumer should avoid allergen-containing food completely. Most of the food allergies are caused by eight major groups of allergenic foods, also known as Big-8, which includes plain and popular foodstuff such as milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (Wilson *et al*. 2005). Moreover, some of these foodstuffs are very often used in the food industry as additives. SPR was shown to be a suitable method for the detection of different allergens in food.

For the detection of casein, an allergen located in milk, Minh Hiep and colleagues (2007) developed and successfully applied a new type of LSPR-based immunosensor dependent on gold-capped nanoparticle substrate. Protein A was covalently immobilized to capture anti-casein antibodies through the Fc region for the analysis of raw milk samples. The LOD of their immunosensor was determined as 10 ng/ml, which is well below the lowest observed adverse effect level, determined to be 350 mg/ml of protein for children (Bellioni-Businco *et al*. 1999).

Two research groups made interesting studies in the detection of allergens in chocolate. Pollet *et al*. (2011) investigated peanut allergen Ara h1 in candy bars using a fiber optic SPR biosensor. In this study, a label-free assay, a secondary antibody sandwich assay and a nanobead enhanced assay were compared. The first was proved to be the most straightforward, but the enhancement with the magnetite nanoparticles as a secondary label (Figure 7.1) improved the LOD by two orders of magnitude, to as low as 0.09 μg/ml. Cookies and dark chocolate products from different manufacturers were analyzed by Rebe Raz *et al*. (2010) using imaging surface plasmon resonance (SPRi) in combination with antibody array for rapid and quantitative detection of different allergens present in different sorts of nuts (peanut, hazelnut, pistachio nut, cashew nut, almond, macadamia, Brazil nut, pine nut, and pecan), lupine, soy and egg. Antibodies against different allergens were spotted in duplicates randomly over the sensor chip surface while different chocolate and cookie samples where simply mixed 10 minutes with preheated allergen extraction buffer and centrifuged twice prior to injecting over antibodies. Detecting was done

Table 7.2 Some examples of allergen, toxin, toxicant and pathogen detection in food samples.

Toxicant	Type of detection	Source	Reference
Allergens			
Casein	Indirect	Raw milk	Minh Hiep *et al.* 2007
Ara h1	Indirect	Candy bars	Poulett *et al.* 2011
Nuts allergens	Indirect	Cookies and dark chocolate	Rebe Raz *et al.* 2010
Ovalbumin	Direct	Wine	Pilolli *et al.* 2015
Casein, β-lactoglobulin, ovalbumin and ovomucoid	Indirect	Pasta, bread, cereals	Gomma and Boye. 2015
Pathogen microorganisms			
E. coli O157:H7	Indirect	Apple juice, pasteurized milk, ground beef	Waswa *et al.* 2007
E. coli O157:H7	Indirect	Cucumber, ground beef	Wang *et al.* 2013
L. monocytogenes, E. coli O157:H7, *S. enterica*	Indirect	Beef, chicken, turkey meat	Ohk and Bhunia. 2013
S. typhimurium	Indirect	Chicken	Lan *et al.* 2008
Salmonella	Indirect	Milk	Mazumdar *et al.* 2007
E. coli O157:H7, *S. enteric* serovar Enteritidis strain	Indirect	Mineral water, raw milk, ground beef	Bouguelia *et al.* 2013
C. jejuni	Indirect	Broiler meat samples	Wei *et al.* 2013
B. bruxellensis	Indirect	Wine	Manzano *et al.* 2015
Feline calicivirus	Indirect	Oyster	Yakes *et al.* 2013
Toxicants			
Amoxicillin	Indirect	Chicken eggs	Yola *et al.* 2011
Atrazine	Indirect	Natural water	Farre *et al.* 2007
Atrazine, simazine, atrazine-desethyl, azinphos-ethyl	Direct	Bovine milk	Tomassetti *et al.* 2015
Neomycin, gentamicin, kanamycin, streptomycin, sulfamethazine, chloramphenicol, Enrofloxacin	Indirect	Milk	Rebe Raz *et al.* 2009
Bovine somatotropin	Indirect	Milk	Ozhikandathil *et al.* 2012
Ractopamin	Indirect	Liver, urine	Thompson *et al.* 2008
Toxins			
Ochratoxin A	Indirect	Cereals, apple and grape juice, wine	Yuan *et al.* 2009
PSP	Indirect	Mussels, cockles, clams, scallops, oysters	Campbell *et al.* 2009
PSP	Indirect	Shellfish	Yakes *et al.* 2012
TTX	Indirect	Puffer fish liver and muscle extract, human urine	Taylor *et al.* 2011

(*continued*)

Table 7.2 (*Continued*)

Toxicant	Type of detection	Source	Reference
TTX	Indirect	Sea snail	Campbell *et al.* 2013
TTX	Indirect	Puffer fish	Reverté *et al.* 2015
TTX	Direct	Puffer fish	Yakes *et al.* 2014

employing the IBIS SPRi instrument. The sensitivity of the assay was comparable to commercially available ELISA but was much faster and cheaper due to multiple measurements using a single chip.

The first application of SPR usage in winemaking was recently shown by Pilolli *et al.* (2015), with the development of a SPR-based biosensor, suitable for the fast detection of egg-related allergens that could be located in wine. During the winemaking process, the egg white powder is used as a substance that promotes wine clarification. The study was focused only on the ovalbumin (OVA) due to its high abundance. The polyclonal anti-OVA antibody was used as a bio-specific receptor for the allergen detection in OVA-spiked wine samples.

The field of antigenicity of allergens in foods subjected to gamma irradiation and thermal processes is not very well known. Some studies have reported that gamma irradiation used to preserve food and reduce the risk of foodborne illness affects the allergenicity of foods (Byun *et al.* 2002) since the exposure of proteins to radiation can lead to a variety of chemical and physical properties (Garrison, 1987). Therefore, a study done by Gomaa and Boye (2015) about recovery of milk and egg allergens provides us with some valuable information. Irradiated flours with the allergens casein, β-lactoglobulin, OVA and ovomucoid were used for the preparation of pasta, bread and cereal, which were also produced as controls by non-irradiated flours. The influence of the irradiation and food processing (i.e. boiling, baking or extrusion) on the allergenicity did not appear to show any significant decrease in antigenicity under the

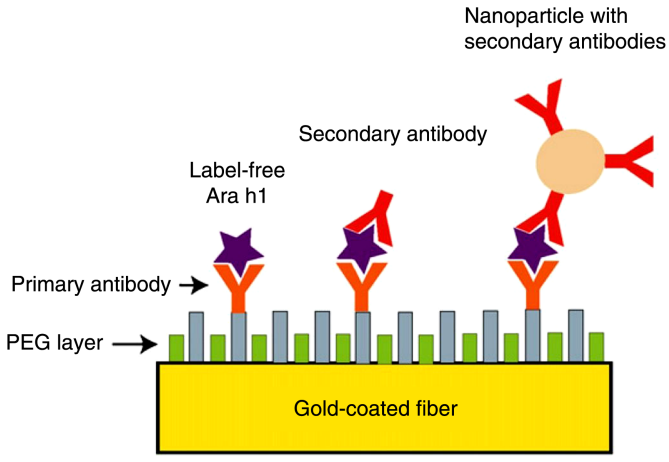

Figure 7.1 Enhanced SPR signal was achieved applying the nanoparticles with secondary antibodies in a sandwich assay. Source: Pollet (2011). Reproduced with permission of Elsevier.

experimental conditions used. On the other site, a big difference was observed in the difficulty of detection of allergens between thermally processed food matrices and flours.

7.2.2 Pathogen Microorganisms

Currently, foodborne pathogens are the most common cause of food poisoning, probably due to their regular presence in many popular sorts of food, such as meat, fish, dairy products, sea food, vegetables, candies, fruits, water and beverages. The foodborne pathogens family is very diverse and includes bacteria such as *Escherichia coli, Salmonella enterica, Listeria monocytogenes*, and some species which produce different toxins, such as tetrodotoxin or paralytic shell fish poisoning toxin, viruses like norovirus or hepatitis A virus, molds or fungi (*Brettanomyces bruxellensis*), and so on. Despite the fact that most of microorganisms are more or less harmless, some of them may lead to life-threatening conditions or even death. Therefore, rapid and reliable diagnostic methods, such as SPR, for the detection of foodborne pathogens in food are needed.

Waswa and colleagues used a SPR-based biosensor with specific antibodies against *E. coli* O157:H7 immobilized on the gold surface for detecting the target pathogen in apple juice, pasteurized milk and ground beef extracts, for the first time. The samples were spiked with *E. coli* O157:H7 at varying concentrations and injected over the sensor surface immobilized with antibodies. The biosensor assay was specific to *E. coli* O157:H7 as other organisms which were chosen due to their genetic similarity to the target pathogen (*E. coli* K12 and *Shigella boydii*) did not result in any binding. This detailed study was a good basis for establishment of further application of SPR in detecting foodborne pathogens in more complex and specific food matrices (Waswa *et al*. 2007).

Lectins, a plant or animal proteins or glycoproteins, are much smaller than antibodies. They can bind selectively and reversibly with carbohydrates located on the bacterial cell surface (Safina *et al*. 2008), and this led to their usage as possible ligands for specific identification of target bacteria. In comparison to antibodies or nucleic acids systems, which always require some background knowledge about the targets, lectins are also suitable choices when targets are unknown. Furthermore, the molecular size of lectins allows higher densities of carbohydrate-sensing elements leading to higher sensitivity and lower non-specific adsorption (Gamella *et al*. 2009). *E. coli* O157:H7 was successfully determined in the real food samples, including cucumber and ground beef since these are the foods most commonly contaminated with *E. coli* O157:H7. They used five different lectins from *Triticum vulgaris* (WGA), *Canavailia ensiformis* (Con A), *Ulex europaeus* (UEA), *Arachis hypogaea* (PNA), *Maackia amurensis* (MAL) to screen the optimal lectin that would bind *E. coli* O157:H7 effectively (Figure 7.2). A correlation between the SPR signal and the concentration of pathogen was found when a lectin from WGA as the binding molecule was used. Since different lectins used exhibited different affinities with *E. coli* O157:H7, *E. coli* DH 5a and *L. monocytogenes*, the selectivity of the approach was additionally proved (Wang *et al*. 2013).

An interesting approach for the detection of foodborne organisms from ready-to-eat meat samples was presented by Ohk and Bhunia (2013). Their goal was to develop and optimize a fiber optic sensor for simultaneous detection of the three most common foodborne bacterial pathogens *L. monocytogenes, E. coli* O157:H7 and *S. enterica* from food. In the sandwich assay the streptavidin-coated optical waveguides were immobilized with biotinylated pathogen-specific polyclonal antibodies and exposed to the bacterial suspensions or enriched food samples. After reacting with Alexa-Fluor 647-labeled monoclonal antibodies, pathogens were detected. Ready-to-eat beef, chicken and turkey meat were inoculated with each pathogen separately or a mixture of all three and tested with the biosensor, which was able to detect each pathogen,

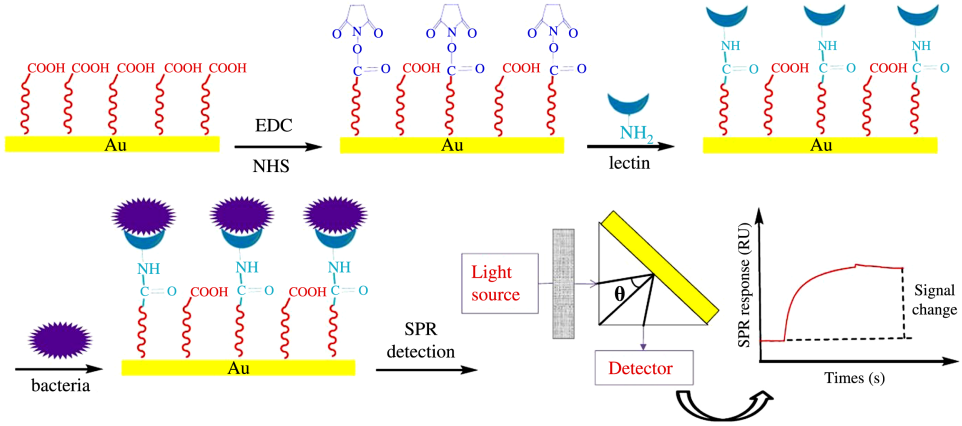

Figure 7.2 An approach for sensing *E. coli* O157:H7 detection utilizing lectins. Source: Wang (2013). Reproduced with permission of Elsevier.

individually or in a mixture, with very little cross-reactivity. Furthermore, the biosensor successfully detected each pathogen grown in a mixture from enriched meat samples under 24 hours. The pathogen presence was further verified by PCR and immunofluorescence assay.

Detection of *Salmonella typhimurium*, commonly associated with the processing of poultry in chicken carcasses was showed by Lan and colleagues (2008). They used an optical SPR biosensor immobilized by *S. typhimurium* antibodies on the surface of the sensor. Although the presence of this pathogen in chicken samples was confirmed successfully at 1×10^6 CFU/ml, the method sensitivity and detection limitations could be improved. Another example of detection of *S. typhimurium* was shown by Mazumdar *et al.* (2007). They developed a sandwich SPR-based assay to detect *Salmonella* in milk. A polyclonal antibody was used as a capture and detection antibody, which enabled detection down to the concentration of 1.25×10^5 cells/ml. A specificity of the assay to this pathogen was tested by cross-reactivity test in relation to *E. coli* since both bacteria belong to the same family and share many characteristics. The obtained results, especially the binding kinetics, showed that it is possible to clearly distinguish between them and this also confirmed the specificity of the assay.

The aim of Bouguelia *et al.* (2013) was a detection of bacteria in different sorts of liquid and solid foods using biochip micro-arraying and SPRi (Figure 7.3). The food samples were spiked with *E. coli* O157:H7 (mineral water and raw milk) and *S. enteric* serovar Enteritidis strain (ground beef). For the *Salmonella* detection, two monoclonal antibodies against *S. enterica* serovar Enteritidis and another commercial polyclonal anti-*Salmonella* spp. antibody were used. Anti-*E. coli* O157:H7 polyclonal antibody was used for the *E. coli* O157:H7 detection. As a negative control, two antibodies against *S. typhimurium* were used. Then, all antibodies were conjugated to NHS-pyrrole and immobilized by electrocopolymerization to the biochips. To decrease the non-specific binding of proteins to biochips, the surface was blocked with bovine serum albumin (BSA) and washed with buffer before running SPRi experiments. The method shown is suitable for the monitoring of different types of viable cells using the protein microarrays (Bouguelia *et al.* 2013). *Campylobacter jejuni* in broiler meat samples was also investigated (Wei *et al.* 2007). In this study, SPR was primarily employed for the determination of the effectiveness of *C. jejuni* detection in artificially contaminated chicken rinse. Different polyclonal rabbit antibodies against *C. jejuni* were used. Although the *C. jejuni* determination in meat samples was done successfully, the method was not optimal due to the high background when the food samples were analyzed.

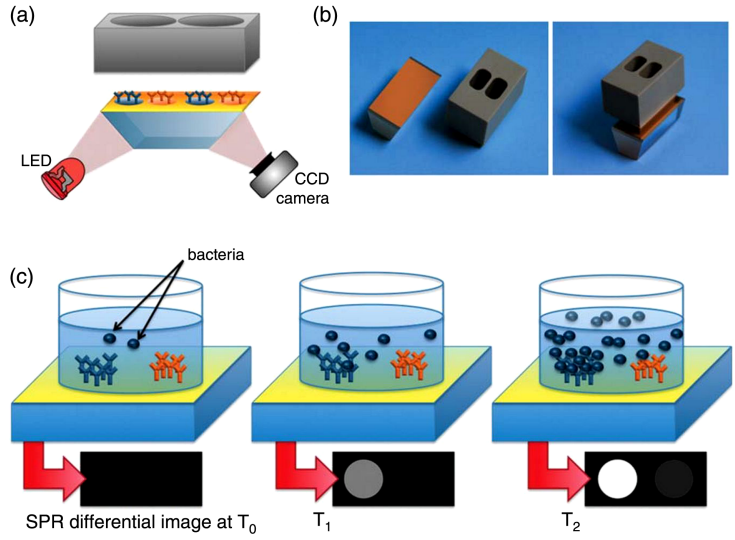

Figure 7.3 Detection of viable bacteria by SPRi. (a) Biochip microarrayed with antibodies. (b) A double culture chamber reactor device. (c) Development of the SPR response upon time. T0 – deposition of contaminated samples; T1 – bacteria start multiplying to yield SPR signal; T2 – high levels of bacterial concentrations are reached, the SPR response saturates on specific chamber and may increase on non-specific chamber. Adapted from Bouguelia *et al.* (2013). Reproduced with permission of Elsevier.

Despite the fact that *B. bruxellensis* does not present any risk to human life, its metabolic products can be a cause of unpleased aromas in wine. Therefore, an LSPR nanobiosensor presents an interesting approach for the determination of this yeast for the wine industry. For the LSPR analysis, a gold nanostructured surface on the sensor was immobilized by the Thiol-Brett DNA probe, where a thiol-modified DNA probe was used as a receptor for the target DNA molecules extracted from wine yeast (Manzano *et al.* 2016).

SPR can also be used for sensitive detection of viruses by employing monoclonal antibodies (Gutiérrez-Aguirre *et al.* 2014). In comparison to other foodborne pathogens, there are not many studies available regarding the detection of viral pathogens in food samples using SPR. As reported, Yakes and colleagues (2013) were the first to develop SPR sensor for viral pathogen detection in seafood. A sample of oyster was spiked with non-human pathogen feline calicivirus (FCV), which is, however, not expected as a natural contaminant of the seafood. FCV was primary chosen as a surrogate for human norovirus (NoV) due to the lack of a NoV reliable cell culture system and its genetic variability. Nevertheless, FCV and NoV are genetically related and have similar morphology. A biosensor with the shorter carboxymethylated dextran matrix was chosen because of the reduction of potentially steric hindrance between surface and large analytes. The FCV assay was performed by injecting intact virus over the antibody sensor chip followed by the injection of a secondary FCV antibody. The biosensor detected intact FCV particles with LOD of approximately 10^4 $TCID_{50}$ FCV/ml (50% tissue culture infective dose per millilitre) from purified cell culture lysates.

7.2.3 Toxicants

7.2.3.1 Antibiotics and Hormones

Most of the antibiotics produced in the developed countries are used in farm animal production to prevent diseases (Collignon and Voss, 2015). An uncontrolled antibiotic overuse is a cause of

the bacterial resistance to the antibiotics, which may lead to the serious health risk (Landers *et al*. 2012). Therefore, the precise determination of antibiotics in livestock production is mandatory.

Amoxicillin (AMOX) is a β-lactam antibiotic that belongs to the penicillin group. Its usage is mostly limited to veterinary medicine because it affects Gram-positive and Gram-negative bacteria. High concentrations of AMOX in meat or animal products could present a potential treat for humans. The determination of this antibiotic in chicken eggs was done by Yola *et al*. (2014). They modified the gold surface with allyl mercaptane, on top of which AMOX-imprinted poly(2-hydroxyethylmethacrylate-methacryloylamidoglutamic acid) nanofilm was generated. The assay was tested for the selectivity against AMOX in chicken egg in the presence of ampicillin (AMP) and cephalexin (CEP) as competitors, and the sensor was 11.8 and 14.0 times more selective for AMOX than AMP and CEP, respectively.

An extensive study of detection of different antibiotics in milk was done by Rebe Raz *et al*. (2009). The subject of the research was members from the four major antibiotics families: aminoglycosides (neomycin, gentamicin, kanamycin, and streptomycin), sulfonamides (sulfamethazine), fenicols (chloramphenicol) and fluoroquinolones (enrofloxacin). For the multiple antibiotic detection, a microarray biosensor, based on the SPRi platform was developed. For all analyses, a single sensor chip was spotted with different antibiotics while the unreacted groups in the hydrogel were blocked with ethanolamine. The milk samples where premixed with suitable antibodies and injected over the surface. The assay showed parts per billion-level sensitivity for the target compounds in buffer and in diluted milk. The sample preparation was rapid since it demanded only dilution in buffer and centrifugation.

The antibiotics are not the only contaminant that can be found in milk. Bovine somatotropin (bST) or bovine growth hormone has often been used in dairy farming to increase production. The concentration of natural growth hormone in milk is 1–10 ng/ml (McGrath *et al*. 2008); however, upon misuse the concentrations can be up to 10 times higher. A fast and low-cost method of detecting growth hormone in milk samples was described. Solid-phase extraction was used to extract growth hormone from milk. Glass substrates with gold nanoislands were functionalized with anti-bST. The portable setup was able to detect recombinant growth hormone in milk at concentrations as low as 5 ng/ml (Ozhikandathil *et al*. 2012).

Another animal growth promoter which is licensed for use in livestock in 20 countries worldwide, including the United States and Canada, but prohibited in at least 160 countries, including many European Union countries, is ractopamine (RCT). RCT is a β-agonist drug that increases protein synthesis and reduces fat, thereby increasing the profit per animal. European consumers consider the RCT residues unwanted in meat products. Due to the different requirements all over the world, the ability to detect residues of the drug at low concentrations is needed. The new sensor was developed by combining the chip with antibody against RCT and the inhibition assay. The detector was sensitive enough to detect low microgram per kilogram concentrations of the drug in urine and liver (Thompson *et al*. 2008).

7.2.3.2 Herbicides

Herbicides offer a selective method of managing certain weeds. Moreover, herbicides increase the amount of harvest and improve its quality, safety and shelf-life. Rapid and reliable methods for their detection in food are important for safety. Triazines are most heavily used selective herbicides for the control of grasses and broad-leaved weeds. Due to their high perseverance, they are often found in ground waters. One member from this class, atrazine, was banned in the European Union in 2003 (Bethsass and Colangelo, 2006) because of its ubiquity in drinking water since it was connected with cancer in many studies. The first portable SPR-based biosensor for atrazine identification in natural water samples was developed in 2007. The

developed sensor allowed the detection of atrazine in the field in 25 minutes in the inhibition format (Farré, 2007). Just recently, Tomassetti and colleagues (2015) focused on triazine pesticide detection, a pesticide that can be found in commercial bovine. These herbicides are mostly used as weed killers in corn, wheat, barley, legumes and several fruit crop productions. Milk samples were spiked with four different triazine compounds: atrazine, simazine, atrazine-desethyl and azinphos-ethyl. For the SPR detection of pesticides, a gold surface was modified by a self-assembled monolayer layer. The anti-atrazine antibodies were covalently bound via amine groups through classic immobilization procedure. The obtained SPR results were compared with those collected by two amperometric devices. The LOD and selectivity toward several triazine pesticides displayed great similarity among different biosensors used, but since SPR was the only one applied in direct format, it was quicker and easier to perform.

7.2.4 Toxins

7.2.4.1 Mycotoxins

Mycotoxins are small secondary metabolites of certain fungi with toxic effects on humans. The toxicity of mycotoxins varies greatly, but only a few of them are regularly found in food such as grains and seeds. Usually, five mycotoxins are found in food: deoxynivalenol/nivalenol, zearalenone, ochratoxin, fumonisins and aflatoxins. Their detection in food is very important because of their possible teratogenic and carcinogenic properties.

An example of detection of mycotoxins in food using SPR was done by Yuan *et al*. (2009). They developed an ultrasensitive SPR assay for small molecules, where an enhancement of signal on a mixed self-assembled monolayer surface was made by gold particles. The subject of their investigation was very toxic ochratoxin A (OTA) located in cereals, apple juice, grape juice, red wine, and white wine. Based on the immobilization of target OTA through its OVA conjugate with a polyethylene glycol (PEG) linker, a new OTA conjugate (OTA-PEG-OVA) exhibited remarkably boosted performance characteristics compared with the commercially available BSA-OTA conjugate without a PEG linker. Furthermore they applied large gold nanoparticles that lowered the LOD to 0.042 ng/ml.

7.2.4.2 Paralytic Shellfish Poisoning Toxins

Paralytic shellfish poisoning (PSP) in humans is a serious illness caused by ingestion of shellfish containing PSP toxins. Toxins accumulate in shellfish after contamination with dinoflagellate, diatoms or cyanobacteria that produce these harmful toxins. PSP toxins could be found in all bivalve molluscan shellfish including clams, mussels, oysters, geoducks and scallops. Heating or freezing does not destroy PSP toxins, and even a single toxic shellfish can be fatal to humans. Therefore, this popular seafood presents a huge potential threat and demands a rapid detection of PSP toxins.

SPR has been employed many times for the detection of PSP toxins in shellfish. Mussels, cockles, clams, scallops and oysters were analyzed by Campbell *et al*. (2009), where the saxitoxin–jeffamine–BSA protein conjugate was used to produce polyclonal antibody that was later used as a sensor to detect PSP toxins. Two simple, rapid extraction procedures were tested with the conclusion that the extraction in sodium acetate buffer is more rapid and more effective than using 90% ethanol. The toxin was detected in inhibition format. ELISA was performed on the same samples, and both methods were finally evaluated and compared with the classical mouse bioassay (AOAC official method 959.08) and HPLC method (AOAC official method 2005.06). AOAC, the Association of Official Analytical Chemists, develops analytical methods for a broad spectrum of safety control in foods and beverages. Yakes reported combining three antibodies to develop a sensor which would have a higher correlation of

response with PSP toxin potency (Yakes *et al*. 2012). Quite interesting was the first reported SPR biosensor interlaboratory study that was done in cooperation between seven independent laboratories from seven different countries (within the EU and North America) using a Biacore Q SPR instrument. The PSP toxin prototype kit containing a sensor chip with saxitoxin immobilized, and all the materials and instructions for the sample extraction and SPR assay were delivered to the laboratories. In each laboratory, 20 naturally contaminated and spiked shellfish samples were investigated. All participants completed the analysis successfully with the HorRat values (Horwitz and Albert, 1995) being <1 for all the samples tested, demonstrating that the method of performance, in terms of interlaboratory precision, can be considered to be suitable for PSP detection (van den Top *et al*. 2011). Moreover, this study proves that the method can be used as a routine tool for the detection of these and other toxins and pollutants in crude samples and can also replace some of the current existing tests that are no longer the most appropriate for the task.

7.2.4.3 Tetrodotoxins

Tetrodotoxin (TTX) is a low molecular weight neurotoxin and one of the most toxic molecules that can be found in nature. TTX selectively blocks voltage-sensitive Na^+-gated ion channels, and in the case of consuming TTX-contaminated food, serious life threats are inevitable. It has numerous known analogues of varying toxicities and can be found in xanthid crabs, marine gastropod, puffer fish, blowfish, balloon fish, toads, sunfish, porcupine fish, toadfish, globefish, and swellfish. TTX in the food is the most recognizable in connection with the special Japanese delicacy made of raw flesh of puffer fish (i.e. fugu).

In the case of TTX poisoning the potential food source of this toxin is usually not available for the analyses. Therefore, Taylor et al. (2011) showed a great example of how TTX poisoning could be determined also in clinical matrices. SPR sensor spotted with antibodies was successfully employed for the determination of TTX in 10 samples, comprising three from a poisoning occasion, two control samples, and five toxic pufferfish samples in puffer fish liver and muscle extract and human urine. A custom-built instrument and sensor chips were used in the study. The chip was manufactured with self-assembled monolayer consisting of amine- and hydroxyl-terminated alkane thiols, and TTX molecules were covalently linked to amine groups exposed on the surface. The calibration curve was made for the inhibition assay. The data corresponded well with LC electrospray-ionization multiple reactions monitoring MS analysis also reported in the study.

The sea snail *Charonia lampas* was responsible for the first TTX human poisoning in Europe. The Campbell group (2013) produced a monoclonal antibody raised to TTX–BSA protein conjugate, diluted it 1:300 and mixed it with equal volume of either the TTX standard or sample extracted from sea snail or puffer fish. Besides the sensitivity, the ability of the method to discriminate between the TTX and some other potentially interfering substances in the samples was also evaluated. The limit of detection was 10 times lower than the regulatory limit required for Japan, thus appropriate for general application. The protocol has been recently confirmed in a study where the authors developed a new ELISA configuration based on the immobilization of TTX through self-assembled dithiol monolayers which enabled an ordered and oriented molecules on the surface. Different toxin analogues were detected in puffer fish matrix with new ELISA and SPR. Both methods correlated well with LC-MS/MS analysis, whereas the MBA overestimated the concentration (Reverté *et al*. 2015). The first direct detection of TTX in puffer fish matrix using SPR biosensor was lately presented. This approach was focused on the characterization of antibody/toxin interaction yielding affinity constant in nanomolar range (7.96×10^{-9} M). This data can provide valuable information in future development of better antibodies. The direct assay offers much quicker toxin determination and was achievable by

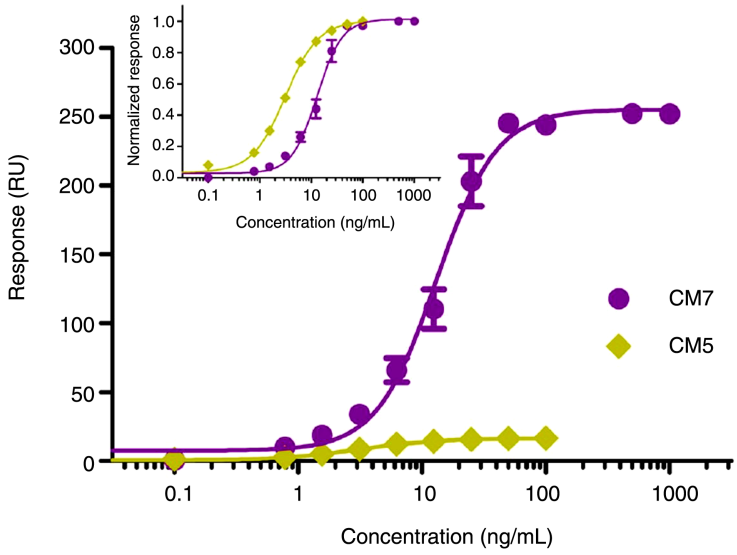

Figure 7.4 Direct TTX detection was achievable utilizing a sensor chip CM7 with higher antibody immobilization capacity. Source: Yakes (2014). Reproduced with permission of American Chemical Society.

utilizing a sensor chip with higher antibody immobilization capacity (Figure 7.4). Besides, in comparison to other indirect SPR detections, this approach enables faster analysis, more confident toxin detection and less biological reagents usage (Yakes *et al.* 2014).

7.3 Conclusion

Biosensors are powerful analytical tools for food safety monitoring. Those based on SPR are very efficient in providing data rapidly and in real time, they tend to be simple to use, and they can be portable and cost-effective. In this chapter we overviewed the current progress in toxin and other toxicant detection in food by using SPR. In most cases, food matrices do not contain high concentrations of targeted contaminants and are usually very complex, thus detection is difficult. The sensors were developed for a wide range of analytes, from small molecules like RCT up to bacteria and viruses. The detection can be done in various liquids, such as tapped water, wine, juice or milk, but with slight preparation also in food like eggs, cucumber, seafood or meat. The ongoing reports of using different detecting molecules and new materials prove that the sensitivity can be pushed into femtomolar area. New ligands, new methods for immobilizing receptors on sensors surfaces, and new methods for amplifying the response will allow successful sensing employing SPR, and this will have a positive impact on food quality.

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