Biosensors in food processing

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Abstract Optical based sensing systems that measure luminescence, fluorescence, reflectance and absorbance, etc., are some of the areas of applications of optical immunosensors. Immunological methods rely on specific binding of an antibody (monoclonal, polyclonal or engineered) to an antigen. Detection of specific microorganisms and microbial toxins requires immobilization of specific antibodies onto a given transducer that can produce signal upon attachment of typical microbe/microbial toxins. Inherent features of immunosensors such as specificity, sensitivity, speed, ease and on-site analysis can be made use for various applications. Safety of food and environment has been a major concern of food technologists and health scientists in recent years. There exists a strong need for rapid and sensitive detection of different components of foods and beverages along with the food borne and water borne pathogens, toxins and pesticide residues with high specificity. Biosensors present attractive, efficient alternative techniques by providing quick and reliable performances. There is a very good potential for application of biosensors for monitoring food quality and safety in food and bioprocessing industries in India.

Keywords Biosensors · Food processing · CFTRI · Nanosensor · Aptamer · Immunosensor · Food safety

Introduction

Foods are materials, raw, processed, or formulated, that are consumed orally by humans or animals for growth, health,

satisfaction, pleasure and satisfying social needs. Food preservation is an action or a method of maintaining foods at a desired level of properties or nature for their maximum benefits. In general, each step of handling, processing, storage and distribution affects the characteristics of food, which may be desirable or undesirable. Thus, understanding the effects of each preservation method and handling procedure on foods is critical in food processing which lead to the safe food (Rahman 2007). Monitoring of safety and nutritional quality of food are very essential. The conventional analytical techniques for quality and safety analyses are very tedious, time consuming and require trained personal, therefore there is a need to develop quick, sensitive and reliable techniques for quick monitoring of food quality and safety. In this connection biosensor is an appropriate alternative to the conventional techniques.

Biosensor devices are emerging as one of the foremost relevant diagnostic techniques for food, clinical and environmental monitoring due to their rapidity, specificity, ease of mass fabrication, economics and field applicability. They obtain their specificity from biological binding reaction, which is derived from a range of interactions that include antigen/antibody, enzyme/substrate/cofactor, receptor/ligand, chemical interactions and nucleic acid hybridization in combination with a range of transducers. Present review describes several applications of biosensors for food processing and safety.

Biosensor definition

A biosensor can be defined as a quantitative or semi-quantitative analytical instrumental technique containing a sensing element of biological origin, which is either integrated within or is in intimate contact with a physico-chemical transducer (Turner et al. 1987). A chemical sensor is a device that transforms chemical information, ranging

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from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. Chemical sensors usually contain two basic components connected in series: a chemical (molecular) recognition system (receptor) and a physicochemical transducer. Similarly, biosensors are chemical sensors in which the recognition system utilizes a biochemical mechanism interfacing the optoelectronic system (Cammann 1977; Turner et al. 1987). A device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals (Nic et al. 2006).

Prerequisites for a biosensor

While developing a biosensor system following requirements are very essential and considered for successful and commercially viable project.

- a. **Selectivity:** The biosensor device should be highly selective for the target analyte and show minimum or no cross reactivity with moieties having similar chemical structure.
- b. **Sensitivity:** The biosensor device should be able to measure in the range of interest for a given target analyte with minimum additional steps such as pre cleaning and pre concentration of the samples.
- c. **Linearity of response:** The linear response range of the system should cover the concentration range over which the target analyte is to be measured.
- d. **Reproducibility of signal response:** When samples having same concentrations are analyzed several times, they should give same response.
- e. **Quick response time and recovery time:** The biosensor device response should be quick enough so that real time monitoring of the target analyte can be done efficiently. The recovery time should be small for reusability of the biosensor system.
- f. **Stability and operating life:** As such most of the biological compounds are unstable in different biochemical

and environmental conditions. The biological element used should be interfaced such that the activity is retained for a long time so as to make the device marketable and practically useful in the field.

Working principle

General working principle of a biosensor is described below. The key component of a biosensor is the transducer (Fig. 1) which makes use of a physical change accompanying the reaction. This may be

- Heat output (or absorbed) by the reaction (Calorimetric biosensors)
- Changes in electrical or electronic output (Electrochemical biosensors)
- Redox reaction (Amperometric biosensors)
- Light output or light absorbance difference between the reactants and products (Optical biosensors) or
- Based on mass of the reactants or products (Piezo-electric biosensors).

The electrical signal from the transducer is often weak with heavy noise. To increase the signal to noise ratio a 'reference' baseline signal derived from a similar transducer without any bio catalytic membrane from the sample signal should be used. The difference between the signals is very weak and amplified as a readable output. The above process removes the unwanted noise from the signal. The analogue signal produced by amplifier is usually converted in to a digital signal and passed to a microprocessor. The data is processed, converted in to concentration units and output to a display device or data store (Chaplin 2004).

Historical developments

First generation enzyme sensors

Leland Charles Clark Jr is considered as the pioneer of the biosensor research. In 1956 he published his first paper on

Fig. 1 Schematic representation of biosensor components

the electrode to measure oxygen concentration in blood (Clark 1956). In 1962 while addressing at New York Academy of Sciences symposium in which he described “how to create electrochemical sensors (pH, polarographic, potentiometric or conductometric) more smart” by adding “enzyme transducers as membrane enclosed sandwiches”. The model was exemplified by an experiment in which glucose oxidase was entrapped at a Clark oxygen electrode using dialysis membrane. The decrease in measured oxygen concentration was proportional to glucose concentration. (Clark and Lyons 1962).

In 1967, Updike and Hicks further extended the work of Clark and demonstrated the first functional enzyme electrode based on glucose oxidase immobilized onto an oxygen sensor. Glucose concentration was measured in biological solutions and tissues in vitro (Updike and Hicks 1967). In the year 1970, Guilbault and Montalvo described the first potentiometric enzyme electrode. It was a urea-sensor based on enzyme urease immobilized at an ammonium (NH_4^+) selective liquid membrane electrode (Guilbault and Montalvo 1970). In the year 1973, Guilbault and Lubrano described glucose and a lactate enzyme sensor based on hydrogen peroxide detection at a platinum electrode (Guilbault and Lubrano 1973). In 1974, Klaus Mosbach developed a heat-sensitive enzyme sensor termed ‘thermistor’ (Mosbach and Danielsson 1974).

Clark’s ideas turned out to be commercial reality in 1975 with the fruitful re-launch (first launch 1973) of the Yellow Springs Instrument Company’s glucose analyzer based on the amperometric detection of hydrogen peroxide. The biosensor took a further fresh evolutionary route in 1975, when Divis put forward that bacteria could be harnessed as the biological element in *microbial electrodes* for the measurement of alcohol (Divis 1975). Lubbers and Optiz coined the term *optode* in 1975 to describe a fiber-optic sensor with immobilized indicator to measure carbon dioxide or oxygen (Lubbers and Optiz. 1975). They extended the concept to make an optical biosensor for alcohol by immobilizing alcohol oxidase on the end of a fiber-optic oxygen sensor.

Second generation enzyme sensors

In 1976, Clemens et al. integrated an electrochemical glucose biosensor in a bedside artificial pancreas and this was later marketed by Miles as the Bio-stator. Although the Bio-stator was commercially unavailable, VIA Medical introduced a novel semi-continuous catheter-based blood glucose analyzer. Later in 1976, La Roche (Switzerland) introduced the Lactate Analyzer LA 640 in which the soluble mediator, hexacyanoferrate, was used to shuttle electrons from lactate dehydrogenase to an electrode (Geyssant et al. 1985).

Third generation enzyme sensors

Third generation enzyme sensors bear a resemblance to second generation enzyme sensors based on the use of electron mediators. However, they have advanced into the implementation of co-immobilised enzymes and mediators onto the same electrode instead of freely diffusing mediators in the electrolyte. Direct interaction between the enzymes redox centre and the electrode was possible so either mediator or enzyme was not required. Thus, recurrent measurements were possible which abates the sensor design costs (Cass et al. 1984).

In the year 1983, Liedberg monitored affinity reactions in real time using surface Plasmon resonance (SPR) technique (Liedberg et al. 1983). In 1984, Turner and his colleagues published a paper on the use of ferrocene and its derivatives as immobilised mediators for use with oxidoreductases in the fabrication of low-cost enzyme electrodes. This formed the basis for the screen-printed enzyme electrodes launched by MediSense, Cambridge, USA in 1987 with a pen-sized meter for home blood-glucose monitoring. The electronics were redesigned into popular credit-card and computer-mouse style formats and MediSense’s sales showed exponential growth reaching US \$175 million by 1996. The global biosensor market will reach \$12 billion by the year 2015 (Anon 2012b) and offers immense potential for advancement and expansion.

Types of biosensors

Based on the working principle of biosensors they are classified into different types (Fig. 2). Some of the significant ones are explained below and tabulated (Table 1).

Electrochemical biosensors

An electrochemical biosensor uses an electrochemical transducer where electrochemical signals are generated during biochemical reactions and are monitored using suitable potentiometric, amperometric or conductometric systems of analyses. It is considered as a chemically modified electrode since electronic conducting, semiconducting or ionic conducting material is coated with a biochemical film (Durst et al. 1997; Kutner et al. 1998). The different types of biosensors in electrochemical category are explained below.

Conductometric biosensors

Many enzyme reactions, such as those of urease and many biological membrane receptors may be monitored by ion conductometric or impedimetric devices, using

Fig. 2 Schematic representation of biosensors with various combinations of physical and biological elements. Source: Adopted from Thakur (2012)

interdigitated microelectrodes (Cullen et al. 1990). Because the sensitivity of the measurement is hindered by the parallel conductance of the sample solution, usually a differential measurement is performed between a sensor with enzyme and an identical one without enzyme. Analytes like urea, charged species and oligonucleotides are detected using this principle.

Calorimetric biosensors

Calorimetric biosensors measure the change in temperature of the solution containing the analyte following enzyme action and interpret it in terms of the analyte concentration in the solution. Since most of the enzyme catalyzed reactions are exothermic, the heat generated by the reaction

Table 1 Information of biosensor, biosensor mechanism and its analytes

Type of biosensor	Mechanism	Analyte	Reference
Polarographic oxygen electrode	Electrode based	Oxygen in Blood	Clark 1956
Electrochemical	Enzyme electrode (Glucose oxidase)	Glucose	Updike and Hicks 1967
Potentiometric	Enzyme electrode (Urease)	Urea	Guilbault and Montalvo 1970
Amperometric	Electrode with immobilised glucose oxidase	Blood glucose	Guilbault and Lubrano 1973
Optical	Fluorescence	pCO ₂ /pO ₂ in fluids and gases	Lubbers and Optiz 1975
Amperometric	Dual enzyme electrode system	Organophosphorous pesticides	Gouda et al. 1997
Immuno-chemiluminescence	Charge coupled device	Methyl parathion	Chouhan et al. 2006
Optical	FRET (Forster resonance energy transfer)	Formaldehyde	Akshath et al. 2012
Immuno-chemiluminescence	Dipstick	Vitamin B ₁₂	Selvakumar and Thakur 2012a
Microbial	Whole cell immobilization	Caffeine	Babu et al. 2007
Optical microbial biosensor	Bioluminescence	Heavy metals and pesticides	Ranjan et al. 2012
Aptasensors	Aptamer	Vitamin B ₁₂	Selvakumar and Thakur 2012b

is used to determine the analyte. Calorimetric biosensors are extensively used for the detection of pesticides and other enzymatic reactions (Fig. 3).

Potentiometric biosensors

Potentiometric measurements are determined based on potential difference between an indicator and a reference electrode (Fig. 4). The transducer may be an ion-selective electrode, which is an electrochemical sensor based on thin films or selective membranes as recognition elements (Buck and Lindner 1994). The most common potentiometric devices are pH electrodes; several other ion (F^- , I^- , CN^- , Na^+ , K^+ , Ca^{2+} , NH_4^+) or gas (CO_2 , NH_3) selective electrodes are available. The potential differences between these indicator and reference electrodes are proportional to the logarithm of the ion activity or gas concentration as described by the Nernst-Donnan equation.

Amperometric biosensors

Amperometric biosensor systems are commonly used devices and are available in the market today. The first ever commercial biosensor designed by Leyland and Clark for monitoring glucose was an amperometric electrode. Amperometry is based on the measurement of the current resulting from the electrochemical oxidation or reduction of an electroactive species. It is usually performed by maintaining a constant potential at Pt, Au or C based working electrode or on array of electrodes with respect to the reference electrode, which may also function as the auxiliary electrode, if currents are low (10^{-9} to 10^{-6} A). The resulting current is directly interrelated to the bulk concentration of the electroactive species or its production or consumption rate within

the adjacent biocatalytic layer. As biocatalytic reaction rates are frequently preferred to be first order dependent on the bulk analyte concentration, such steady-state currents are usually proportional to the bulk analyte concentration (Thevenot et al. 2001). Analytes like sugars, alcohols, phenols, oligonucleotides and O_2 can be determined using amperometric biosensors. There are reports on both single as well as multienzyme based systems for OP pesticide detection (Gouda et al. 1997). Single enzyme based biosensors use either acetyl cholinesterase (AChE) or butyryl cholinesterase (BuChE) as the biological component and thiocholine production is monitored amperometrically (Rekha et al. 2000b) or acid production is monitored potentiometrically. Multienzyme based biosensor systems use cholinesterase in conjunction with choline oxidase and measure hydrogen peroxide production (Rekha et al. 2000a) or oxygen consumption (Gouda et al. 2001).

Optical biosensors

Optical biosensors are also known as “optodes” because of their resemblance with electrodes (Fig. 5). These include determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process. Optical biosensors integrate optical technique with a biological element to identify chemical or biological species. Many optical biosensors were developed based on surface Plasmon resonance, spectroscopy and evanescent waves etc. Recently our group at our institute has published several research papers to monitor pesticides, vitamins, carcinogens and toxins based on chemiluminescence and fluorescence (Gouda et al. 1997; Chouhan et al. 2006; Akshath et al. 2012; Selvakumar and Thakur 2012a).

Fig. 3 Diagrammatic representation of calorimetric biosensor

Fig. 4 Diagrammatic representation of potentiometric biosensor

The routing of fluorescent signals from NADH to quantum dots (QDs) has been a subject of extensive research for FRET based applications. CdTe QD may undergo dipolar interaction with NADH as a result of broad spectral absorption due to multiple excitonic states resulting from quantum confinement effects. Thus, non-radiative energy transfer can take place from NADH to CdTe QD enhancing QDs fluorescence. Energy routing assay of NADH-QD was applied for detection of formaldehyde as a model analyte in the range 1,000 ng/mL–0.01 ng/mL by proposed technique. It was possible to detect formaldehyde in the range 1,000 ng/mL–0.01 ng/mL with a limit of detection (LOD) at 0.01 ng/mL. This method can be used to follow activity of NAD⁺ dependent enzymes and detection of dehydrogenases (Fig. 6) in general (Akshath et al. 2012).

One more biosensing method based on immuno-chemiluminescence and image analysis using charge coupled device (CCD) for the qualitative detection of methyl parathion (MP) with high sensitivity (up to 10 ppt) was developed. MP antibodies raised in poultry were used as a biological sensing element for the recognition of MP present

in the sample. The immuno-reactor column was prepared by packing in a glass capillary column (150 µl capacity) MP antibodies immobilized on Sepharose CL-4B through periodate oxidation method. Chemiluminescence principle was used for the detection of the pesticide. Light images generated during the chemiluminescence reaction were captured by a CCD camera and further processed for image intensity, which was correlated with pesticide concentrations. The results obtained by image analysis method showed very good correlation with that of competitive ELISA for methyl parathion detection (Chouhan et al. 2006).

Microbial biosensors

A microbial biosensor is an analytical device that combines microorganisms with a transducer to facilitate rapid, accurate and sensitive detection of target. Conventional microbial biosensors used the respiratory and metabolic functions of the microorganisms to detect a substance that is either a substrate or an inhibitor of the processes. Microbial biosensors are more advantageous than enzymes biosensors since the construction of enzyme sensors are complex and costly. Some of the main types of microbial biosensors are amperometric, potentiometric, and conductometric sensors. Amperometric microbial biosensor operates at fixed potential with respect to a reference electrode and involves the detection of the current generated by the oxidation or reduction of species at the surface of the electrode. Amperometric microbial sensors are extensively used for detection of biological oxygen demand in industrial waste water and also used for detection of ethanol, total sugars, organophosphates, cyanide, phenols and phenolic compounds.

Generally potentiometric microbial biosensors consist of an ion-selective electrode or a gas-sensing electrode coated with an immobilized microbe layer. Due to microbial metabolism, the uptake or release of analyte generates a change in potential resulting from ion accumulation or depletion. Potentiometric transducers measure the potential difference between a working electrode and a reference electrode, and the signal is correlated to the concentration of analyte. The signals are expressed in logarithmic relationship between the potential generated and analyte concentration, so wide detection range is possible. Potentiometric microbial sensors are used for the detection of organophosphates, penicillin, tryptophan, urea, trichloroethylene, ethanol and sucrose. Whole cell biosensor immobilization of *Pseudomonas alcaligenes* MTCC 5264 having the capability to degrade caffeine were used for the development of caffeine biosensor. The biosensor system was able to detect caffeine in solution over a concentration range of 0.1 to 1 mg/mL. Caffeine biosensor acts as a rapid analysis system for caffeine in solutions (Babu et al. 2007).

Fig. 5 Diagrammatic representation of optical biosensor

Fig. 6 NAD based “turn off” and NADH based “turn on” of QD fluorescence. Source: Akshath et al. (2012)

Most of the microbe-catalyzed reactions involve a change in concentration of ionic species. Due to the change in concentration there is a net change in the conductivity of the solution. Even though the detection of solution conductance is non-specific, conductance measurements are extremely sensitive. Single-use conductivity and microbial sensor were fabricated to investigate the effect of both species and concentration/osmolarity of anions on the metabolic activity of *E. coli*. This hybrid sensing system combines immuno-chemical and biological sensing and comparative data could be obtained (Lei et al. 2006).

Optical microbial biosensor

Optical biosensors are based on optical properties such as UV–Vis absorption, bio- and chemi-luminescence, reflectance and fluorescence. Bioluminescence is related with the emission of light by living microorganisms and it plays a vital role in real-time process monitoring. The bacterial luminescence *lux* gene has been widely applied as a reporter. Bioluminescence based biosensors are used for the detection of metal ions, heavy metals, phosphorus, naphthalene, genotoxins and chlorophenols. It has huge potential in monitoring the sanitation and pollution levels in industries.

Similarly *gfp* gene coding for the green fluorescent protein (GFP) has also been widely applied as reporter. GFP is very stable and not known to be produced by microorganism indigenous to terrestrial habits. GFP-based microbial biosensor has been shown to be useful in assessing heterogeneity of iron bioavailability on plant. It is also applied for assessing compounds like arsenite, galactosides, toluene and related compounds, N-acyl homoserine lactones and biological oxygen demand. Bioluminescence microbial sensor based on *P. leigonathi*, a marine luminescent bacterium, was used for the monitoring of environmental toxicants especially heavy metals and pesticides. The microbes are immobilized into

biophotonic beads for the monitoring purpose and the detection range was 2 ppm (Ranjan et al. 2012).

Affinity biosensors

Affinity based biosensors are analytical devices that use an antibody, sequence of DNA or protein interfaced to a signal transducer to measure a binding event. Different types of affinity biosensors are designed based on the transducer types. Binding of an analyte to a bioaffinity based sensors are stoichiometric in nature. Affinity biosensors are designed for monitoring a wide range of compounds including drugs, hormones, pesticides, explosives, insulin, serum proteins, toxins, ethidium and poly aromatic hydrocarbons (Rogers and Mulchandani 1998). Recent reports indicate that efficient nano-biosensor could be designed using nanoparticles interfacing with biomolecules such as enzymes, antibodies and oligonucleotides. While interfacing the nanoparticles with biomolecules give very characteristic photonic properties which could be used for development of sensitive biosensor (Fig. 7) (Vinayaka and Thakur 2011).

Luminescent quantum dots (QDs) possess unique photo physical properties, which are advantageous in the development of new generation robust fluorescent probes based on Forster resonance energy transfer (FRET) phenomena. Bio-conjugation of these QDs with biomolecules create hybrid materials having unique photo physical properties along with biological activity. Colloidal CdTe QDs capped with 3-mercaptopropionic acid were conjugated to different proteins by the carbodiimide protocol using N-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride and a coupling reagent like N-hydroxysuccinimide. The photoabsorption of these QD-protein conjugates demonstrated an effective coupling of electronic orbitals of constituents. A proportionate quenching in BSA fluorescence followed by an enhancement of QD fluorescence point toward

Fig. 7 Schematic representation of photo-absorption and resonance energy transfer in protein conjugated CdTe quantum dots. Source: Vinayaka and Thakur (2011)

nonradiative dipolar interactions. Bioconjugation has significantly influenced the photoabsorption spectrum of QD conjugates suggesting the formation of a possible protein shell on the surface of QD (Vinayaka and Thakur 2011).

Surface plasmon resonance (SPR) biosensors

SPR is a physical optics phenomenon which is used for development of non-labelled/marker based biosensor system. SPR biosensor is an analytical device, which works on optical phenomenon in which **plasmon** waves are excited at the metal dielectric interface. The surface **plasmon** waves are extremely sensitive to the refractive index changes at the sensor surface and are proportional to the sample mass. SPR is a surface sensitive technique in which small change at the surface due to binding or dissociation of biomolecules brings variation in SPR signal. Antibodies are the commonly used biomolecules for the capture of the analyte from the sample. For a consistent assay of bimolecular interaction in a SPR biosensor, one species should be immobilized. Covalent attachment of biomolecules at the sensor surface is the most frequently used method for reliable assays condition (Syed et al. 2011). SPR biosensors offer unique

opportunity of rapid, label free, real time and cost effective detection and identification of biomolecules.

A simple method for the immobilization of antibodies on gold substrates has been developed for surface plasmon resonance (SPR) applications (Schmid et al. 2006). Gold surface on a glass slide was modified with Protein A with cross linker dithiobissuccinimide propionate (DSP) to get uniform, stable and sterically accessible antibody coating. The deposition of the antibodies bound to Protein A was examined as a function of the protein concentration, buffer pH, buffer type and reaction time. The modified gold surface was stable for several weeks and the reproducibility was satisfactory. Thus this technique can be applied to construct immunosensors or biochips.

Acoustic sensors

Acoustic sensors have microbalances that determine an increase in mass due to formation of an immunocomplex. Acoustic sensors are also known as electroacoustic devices. Acoustic biosensors use piezoelectric materials for the acoustic transduction, coupling mechanical deformation to electric potentials and vice versa. Different types of acoustic waves can be generated, which are detected by either bulk

acoustic wave (BAW) sensors or surface acoustic wave (SAW) sensors. Although SAW devices are basically more sensitive than BAW devices, the latter are frequently used in food analyses. BAW sensors contain quartz wafers in the form of 10 to 16 mm disks, squares, or rectangles, which are ~0.15 mm thick and sandwiched between two gold electrodes. These crystals can be used as chemical sensors by covering the electrode surfaces with a selective coating that binds analyte. Binding of the antigen is detected by a decrease in the resonant frequency. This allows real-time monitoring of the affinity reaction (Thakur 2012).

Aptamers

Aptamers are single-stranded oligonucleotide sequences with the capability to recognize various target molecules ranging from small ions to large proteins with high affinity and specificity. Aptamers can be made up of DNA, RNA or peptides. Even modified nucleic acids are also employed for biosensor development in special cases. Aptamers mimic properties of antibodies in a variety of diagnostic formats. They undergo change in their structure after binding with the target. Aptamers are selected *in vitro* by a process known as systematic evolution of ligands by exponential enrichment (SELEX) (Tuerk and Gold 1990). Aptamers may have advantages over antibodies in designing biosensor devices. Aptamer offer chemical stability over a wide range of buffer conditions, resist harsh treatments without losing its bio-activity and the thermal denaturation is reversible for aptamer. Aptamer has a wide range of molecular and

therapeutic targets, including amino acids, any class of proteins (enzymes, membrane, proteins, viral proteins, cytokines and growth factors and immunoglobulins), drugs, metal ions, other small bio-/organic/inorganic small molecules, and even whole cells. Aptamers are small in size, cost effective, and offer remarkable flexibility and convenience in designing their special structure. Moreover, combinatorial chemical synthesis offers a wide variety of methods for aptamer sequence modifications such as the terminal tagging chemical groups. Biosensors based on aptamers as bio-recognition elements (Fig. 8) have been coined Aptasensors (Selvakumar and Thakur 2012b).

Molecularly imprinted polymers

Molecular imprinting is a technique that is based on the preparation of polymeric sorbents with selectivity predetermined for a particular chemical, or group, or structural analogs. Functional and cross-linking monomers such as methacrylics and styrenes, are used and allowed to interact with a ligand and polymerization is induced. During this process, the molecule of interest is entrapped within the polymer either by a noncovalent, self-assembling approach or by a reversible, covalent approach. After the polymerization, the template molecule is allowed to wash out. The imprint of the template is maintained in the rigid polymer and possesses a steric size, shape and chemical arrangement of complementary functionality. The molecularly imprinted polymer can bind the analyte with specificity similar to that of the antigen-antibody interaction (Ansell et al. 1996).

Fig. 8 Scheme of the vitamin B₁₂ RNA Aptamer and colorimetric detection of vitamin B₁₂ Source: Selvakumar and Thakur (2012b)

Immunosensors

Immunosensors are affinity ligand-based biosensor devices in which the immunochemical reaction is coupled to a transducer. They are solid state devices. The fundamental basis of all immunosensors is the specificity of the molecular recognition of antigens by antibodies to form a stable complex which is similar to the immunoassay methodology. Immunosensors can be classified based on the detection principle applied. The main developments are electrochemical, optical and microgravimetric immunosensors (Luppa et al. 2001). In contrast

to immunoassay, modern transducer technology enables the label-free detection and quantification of the immune complex. Immunosensors based on Chemiluminescence for detection of vitamin B₁₂ (Fig. 9) was designed and developed by Selvakumar and Thakur (2012a).

World scenario including work done in India

Government of India under the Ministry of Food Processing Industries (MOFPI) has formulated a Vision 2015 action plan which includes trebling the size of the food processing

Fig. 9 Schematic representation of (a) immunochemiluminescence based dipstick technique for detection of vitamin B₁₂, (b) Enzymatic dephosphorylation of dioxetane by alkaline phosphatase. Source: Selvakumar and Thakur (2012a)

industry, raising the level of processing of perishables foods from 6 % to 20 %, increasing value addition from 20 % to 35 % and enhancing India's share in global food trade from 1.5 % to 3 % (MOFPI 2012). The food processing industry, accounting for 32 % of the total food market, is one of the largest industries in India, and is ranked fifth in terms of production, consumption, export and expected growth.

Quality assessment and safety of food during the manufacturing process are the essential requirements in the food industry. The quality assessments should be carried out during the food processing at appropriate points to get an immediate quality status of the product being manufactured. The biological, chemical and/or physical status of the food may be the result of environmental contamination or failures during food handling, processing, packing and distribution (Thakur et al. 2010). Food contaminants can be classified into pre-processing contaminants and post-processing contaminants. Earlier to processing the raw materials carry many contaminants along with them as a result of agri-process. They are classified as pre-processing contaminants and they may be brought under safe levels as per regulatory measures during various processing operations. However, during the processing operations certain compounds are formed which may be injurious to health thereby making the food unsafe for human consumption. These compounds are of major concern since they are formed during the food processing operations.

During food processing operations it not only increases the shelf life of the product but may introduce new compounds into the food item which is being processed. Mostly the compounds with ill effects on human health are of major concern of food safety. Therefore a strong need exists to monitor those compounds with care to prevent them from entering into the food chain, even though there are many conventional analytical methods which are sensitive enough to monitor the compounds. But they lack on-site applicability, laborious process, needs skilled labour, time consuming and expensive. Detect, correct and prevent these failures are the basis for the development of quality management systems to ensure food safety.

Some of the important compounds that are noted globally in processed foods at increased amounts so as to cause illness in humans are described below.

Acrylamide

Acrylamide is formed when certain carbohydrate-rich foods are fried, baked or roasted at temperatures above 120 °C and is of concern as a possible carcinogen. Acrylamide was accidentally discovered in foods in April 2002 by Swedish National Food Agency and Stockholm University. They reported that acrylamide was formed in high concentrations

of 30–2,300 µg/kg in starchy foods, such as potato chips, French fries and bread that had been fried or baked and not in boiled foods (Tareke et al. 2002). In fried or baked goods, acrylamide may be produced by the reaction between asparagine and reducing sugars (fructose, glucose, etc.) or reactive carbonyls at temperatures above 120 °C (248 °F) (Mottram et al. 2002) (Fig. 10).

Acrylamide causes cancer in rats when administered orally in high-dose, increases tumors in the nervous system, oral cavity, peritoneum, thyroid gland, mammary gland and uterus. Acrylamide also shows neurotoxicity in vertebrates, mutagenicity in somatic and germ cells, and carcinogenicity in experimental animals. This finding has focused the world's attention on public health problem (Friedman 2003). Recent food processing methods such as vacuum frying lowers the acrylamide formation (Granda et al. 2004). Acrylamide content in the fried foods is increased when silicone is used as a foam inhibitor in food industries (Gertz and Klostermann 2002).

Biosensors for acrylamide

Due to the toxic effects of the acrylamide different types of biosensors have been designed for the detection of the compound at very low concentrations with high selectivity and specificity (Agata et al. 2007; Silva et al. 2009). Some of the important ones are Ion selective electrode using whole cells of *Pseudomonas aeruginosa* containing amidase activity. This biosensor exhibited a linear response in the range of $0.1\text{--}4.0 \times 10^{-3}$ M of acrylamide, a detection limit of 4.48×10^{-5} M acrylamide, a response time of 55 s, a sensitivity of 58.99 mV mM^{-1} of acrylamide (Silva et al. 2009). An alternative to ammonium ion selective electrodes few devices consist of electric circuits with a sensitive zone where a mixture of 50 μL of whole cell suspension and 10 μL of glutaraldehyde 2.5 % (v/v) is deposited. This mixture is incubated overnight at 4 $^{\circ}\text{C}$ allowing an adequate immobilization of *Pseudomonas aeruginosa* cells. The transduction process is based on conductivity or resistivity changes in the sensitive zone due to the increase of NH_4^+ amount. On the other hand, pH changes are also detectable, since the concentration of hydroxide ion (OH^-) increases as the reaction proceeds.

Fig. 10 Formation of acrylamide from asparagine by Strecker reaction. Source: Granvogl et al. (2006)

Another biosensor was developed based on carbon-paste electrode modified with hemoglobin (Hb). Interaction between Hb and acrylamide was observed through decreasing of the peak current of Hb-Fe(III) reduction. Validation of the method done by extracting acrylamide from potato chips, showed that the electrodes were suitable for the direct determination of acrylamide in food samples (Agata et al. 2007). Another method was described by Agnieszka and his colleagues using glassy carbon electrodes coated with single-walled carbon nanotubes and Hb for voltammetric detection of acrylamide in water solutions (Agnieszka et al. 2008).

Benzene in soft drinks

Presence of benzene in soft drinks is of concern for human health due to the carcinogenic nature of the benzene. In 1993, benzene formation from benzoic acid in the presence of vitamin C was reported by Gardner and his coworkers (Gardner and Lawrence 1993). The benzene results from decarboxylation of the preservative benzoic acid in the presence of ascorbic acid (vitamin C), E300 or erythorbic acid (E315), especially under heat and light. Benzoic acid is commonly added as a food preservative in the form of its salts sodium benzoate (E211), potassium benzoate (E212), or calcium benzoate (E213) (Gardner and Lawrence 1993). Citric acid is not thought to induce significant benzene production in combination with benzoic acid, but some evidence suggests that in the presence of ascorbic or erythorbic acid and benzoic acid, citric acid may accelerate the production of benzene (Fig. 11). Other factors that affect the formation of benzene are heat and light. Storing soft drinks in warm condition speeds up the formation of benzene.

Microbial biosensor based on flow injection analysis was developed for the detection of benzene. In the sensor cells *Pseudomonas putida* were immobilized between two cellulose acetate membranes and covered on a Clark dissolved oxygen electrode. The *P. putida* aerobically degrades benzene and the working of the biosensor is simple and at the same time it has good reproduction of results (Rasinger et al. 2005).

Fig. 11 Benzene formation in soft drinks due to benzoic acid and ascorbic acid. Source: Adopted and edited from Gardner and Lawrence (1993)

Nitrosamine

In processed foods, nitrosamines are generally formed from nitrites and secondary amines, which are found in the form of proteins (Hinuma et al. 1990). Nitrosamines formation occurs in case of strongly acidic conditions during digestion in stomach (Scanlan 1983; Hinuma et al. 1990). High temperature processing such as frying, can promote the formation of nitrosamines. The presence of nitrosamines may be identified by the Liebermann's reaction (Mulliken 1916). Nitrosamines can cause cancers in a wide variety of animal species, a feature that suggests that they may also be carcinogenic in humans (Table 2).

A biosensing method for determination of nitrate in food and water samples by using nitrate reductase (EC 1.9.6.1) was developed based on the detection of oxidation peak current of redox mediator, methyl viologen, related to nitrate concentration. This method is selective and sensitive to determine the nitrate levels of water samples and processed meat samples. Detection limit of the method was $5.0\text{--}90.0 \times 10^{-9}$ M for nitrate concentration with a 10 s response time (Dinckaya et al. 2010).

Polycyclic aromatic hydrocarbon (PAHs)

The PAHs are a class of organic compounds consisting of condensed aromatic rings. Contamination of food with polycyclic compounds can come from the atmosphere having industrial gases, by direct drying of cereals with combustion gases, by smoking or roasting of food (grilled/barbecued meat, vegetables, oils, grains/cereals, smoked fish, sausage, ham, coffee roasting) (Anon 2012a). PAHs accumulate in high-fat containing tissues. Benzo[a]pyrene (Bap) is one of the major PAH and is a potent carcinogen in animal experiments and is embryotoxic and teratogenic in mice. The

Table 2 Nitrosamines in food. Source: Belitz et al (2009)

Food product	Compound	Content µg/kg
Frankfurter (hot dog)	NDMA	0–84
Fish (raw)	NDMA	0–4
Fish, smoked and pickled with nitrites or nitrates	NDMA	4–26
Fish, fried	NDMA	1–9
Cheese (Danish, blue, gouda, goat milk cheese)	NDMA	1–4
Salami	NDMA	10–80
Bacon (hog's hind leg smoked meat)	NDMA	1–60
Pepper-coated ham, raw and roasted	NPIP	4–67
	NPYR	1–78

NDMA N-Nitrosodimethylamine, NPIP N-nitrosopiperidine, NPYR N-nitrosopyrrolidine

concentration of PAH in the above said foods ranges from as low as 0.001 ng/g of the food sample (Kazerouni et al. 2001). Bap usually serves as an indicator compound of PAH (Belitz et al. 2009).

Contamination of Bap detection is a challenging task because of its low molecular weight and poor solubility in water. Optical and piezoelectric immunosensors for the determination of Bap by using Polarisation-Modulation Infra-red Reflection-Absorption Spectroscopy (PM-IRRAS) or Quartz Crystal Microbalance with dissipation measurements (QCM-D) as transduction techniques have been developed. Immunosensors design is based on a direct or indirect competitive format. Bap was detected by PM-IRRAS with both immunosensor formats with sensitivity range of 5 μ M (Boujday et al. 2010). An electrochemical immunosensor, based on a dual amplification strategy by employing a biocompatible layer as sensor platform was designed for detection of Bap (Lin et al. 2012). There are different types of biosensing methods for the detection of polycyclic aromatic hydrocarbons at sensitive levels.

Freshness of fish

Generally enzyme based biosensors are used to evaluate the freshness of fish. Different types of metabolites are determined to estimate the freshness of fish like hypoxanthine (Watanabe et al. 1983; Shen et al. 1996), inosine (Watanabe et al. 1984), octopine and amines (Shin et al. 1998), histidine, cadaverine, putrescine (Male et al. 1996), polyamines (Chemniti et al. 1992) and sulphates (Mulchandani et al. 1991). Biosensors for other quality parameters like micro-organism content, pesticides and heavy metals detection are explained in detail by Venugopal (2002).

Free radicals and antioxidants

Antioxidants are one of the main ingredients that protect food attributes by preventing oxidation that occurs during processing, distribution and end preparation of food. Different types of antioxidants are added to food products to increase the attributes of food product. Amperometric biosensors are generally preferred for the determination of antioxidants in various food products (Mello and Kubota 2007).

Work done at our institute

1. Biosensors for pesticide monitoring in food and environmental samples

A highly sensitive immuno-sensor system (Fig. 12) was developed for the detection of ethyl and methyl

Fig. 12 Immuno-Chemiluminescence based biosensor system-CFTRI, Mysore

parathions, 2,4-Dichlorophenoxyacetic acid and atrazine at picograms concentration (ppt) based on the immuno-chemiluminescence principle. Antibodies against pesticides were raised in chicken (IgY) and rabbit (IgG). An economical IgY was produced for highly sensitive detection system based on immuno-chemiluminescence biosensor (Chouhan et al. 2006). The high sensitivities of pesticides detection achieved in the project show promise of excellent applications of our immunosensors for field application.

A variety of pesticides and herbicides have been extensively used in agricultural practices to increase productivity, leading to pesticide residues in soil, water and food (Chouhan et al. 2006). These contaminants create serious health hazards to human population. Following biosensor systems were developed for the detection of pesticides.

i. Acetyl choline esterase (AChE) inhibition based biosensor

AChE based biosensor system was developed for monitoring of organophosphorus (OP) pesticide. Electrode was polarised at +410 mV and signals were correlated with OP pesticide concentrations. While biosensors based on AChE inhibition have been known for monitoring of OP pesticides, in food and water samples. However, strong inhibition of the enzyme is a major drawback in practical application of the biosensor. This can be at least partially overcome by reactivation of the enzyme for repeated use. In our laboratory, study on enzyme reactivation by oximes was explored. Two oximes viz., 1,1-trimethylene bis 4 – formylpyridinium bromide dioxime (TMB-4) and pyridine 2-aldoxime methiodide (2-PAM) were compared for the reactivation of the immobilized AChE. TMB-4 was found to be a more efficient reactivator under repeated use retaining more than 60 % of initial activity (Gulla et al. 2002).

ii. Detection of OP pesticides using ascorbic acid oxidase

A laboratory biosensor has been constructed for paraoxon with a sensitivity of 0.5 ppm. This sensitivity is not quite adequate for practical applications and efforts are in progress to improve the biosensor performance. Considerable research has been carried out on the development of single and multi-enzyme based amperometric biosensors for OP pesticides detection. It is known that organophosphates exhibit their pesticide power through a strong inhibition of acetylcholine esterase (AChE) activity. This inhibition principle has been used to develop a biosensor for detection of OP pesticides (Rekha et al. 2000a).

iii. Acid phosphatase inhibition-based detection

An amperometry-based biosensor has also been developed to analyze the OP pesticide using the dual enzyme system. The system consists of potato layer (rich in acid phosphatase) and immobilized glucose oxidase (GOD) membrane which is operated with a Clark type dissolved O_2 electrode. It detects paraoxon at a level of 1 $\mu\text{g/mL}$. A notable advantage of this biosensor is that the inhibition of acid phosphatase by the pesticide is reversible and thereby eliminates the serious problem of enzyme inactivation (Gouda et al. 2001).

2. Construction of a prototype biosensor instrument for glucose and sucrose analysis

A prototype biosensor instrument (Fig. 13) for the analysis of glucose and sucrose has been fabricated, and subjected to various test conditions in the laboratory. It is a kind of electrochemical sensor where the amplified and processed signal is proportional to concentration of glucose in the sample. Three enzymes (invertase, mutarotase and Glucose oxidase (GOD)) are employed for

the analyte detection. Due to the action of invertase, sucrose is converted into α -D-glucose and fructose and further into α -D-glucose, which is converted into β -D-Glucose. GOD reacts with glucose and forms gluconic acid and H_2O_2 . In order to commercialize the instrument collaboration was established with an instrument manufacturing company. The prototype biosensor system was successfully field tested in various industries like sugar mills and confectionery industries (Thakur and Karanth. 2003).

3. Construction of a lactate monooxygenase (LMO) enzyme electrode

A batch type L-lactate biosensor was developed in our institute. This biosensor system is a basically a electrochemical sensor and the working principle is based on electrochemical changes due to biochemical reactions catalyzed by the LMO enzyme with Clark's dissolved oxygen electrode as a sensing element. The output current from the electrode is amplified and converted into a detectable signal in terms of voltage, which is proportional to the L-lactate content.

Detection was in the concentration range of 50–800 mg/dl and the technology has been transferred to industry (M/s Solid State Electronics, Pune). It features an enhanced operating life of 60 days for enzymes sensing element. This biosensor can be used to detect L-lactate in fruit pulp, fermented samples and dairy products (Thakur and Karanth. 2003).

4. On-line monitoring of fermentation process using biosensor

A biosensor with Flow Injection Analysis (FIA) system (Fig. 14) useful for continuous monitoring and control of food and fermentation processes was developed. On-line data acquisition and real time control of food and fermentation processes is a difficult task and limits the use of a batch type of biosensor. Through FIA

Fig. 13 Industrial biosensor system for glucose and sucrose analysis developed at CFTRI, Mysore

Fig. 14 Flow injection analysis system

system it was possible to detect glucose and L-lactate subsequently.

5. Biosensor for ascorbic acid analysis

Work has been carried out on the development of a tissue based biosensor for L-ascorbic acid analysis in food and pharmaceutical samples. An immobilized Ascorbic acid oxidase enzyme was used for the detection of ascorbic acid oxidase obtained from cucumber peels. We found that ascorbic acid oxidase was suitable enzyme for the development of several biosensor systems for detection of pesticides, vitamin C and copper ions.

6. Detection of copper ions by biosensor

An ascorbic acid oxidase based system was used for the detection of Cu^{++} ions. This enzyme contains Cu^{++} in its active site. Based on its folding and unfolding activity, a biosensor was constructed. It was able to detect Cu^{++} ions in water sample.

7. Tea biosensor

India is exporting a large quantity of black tea all over the world. Tea polyphenols play a crucial role in determining quality of black and green tea. Major quality attributes such as colour and astringency are directly linked with polyphenol contents. Therefore, it is necessary to know quantity of polyphenols in tea. Also, tea polyphenols are gaining importance due to their strong antioxidant properties for nutrition and health. In this context in our lab, we have successfully developed an enzyme based amperometric biosensor (Fig. 15) for the determination of total polyphenol content in tea infusions. Both lab and industry trials were satisfactory for tea polyphenols detection and tea biosensor technology (Sujith Kumar et al. 2011).

8. Detection of pesticides and toxins using Q-dots

Efforts are on for the detection of pesticides and

microorganisms and microbial toxins using nanoparticles such as quantum dots (CdSe, CdTe, and CdS) and gold (Au) nanoparticles. Studies have been conducted on conjugation of atrazine with CdTe quantum dots for highly sensitive detection based on fluorescence. Antibodies were raised against pesticides/toxins and these nanoparticles were successfully used for detection of pesticides. Novel phenomenon called Fluorescence Resonance Energy Transfer (FRET) between QD-Nanoparticles and Protein molecules was proved (Thakur et al. 2010).

9. DNA nano probes

Quantum dots are being used for the detection of food pathogens such as *Staphylococcus aureus* and *E. coli*. The simple process of hybridization between complementary strands of targeted ssDNA is being used for detection. For this purpose the gene for the SEB/Ent B toxins are being used as the target sequence. Biotinylated complementary probes will be conjugated with streptavidin coated quantum dots. These DNA probes on binding with the target sequence will show a 20-fold increase in fluorescence compared to conventional dyes and hence very low number of target sequence in sample solution can be traced.

Future R & D needs

Multifunctional and versatile biosensing systems are required for analysis of multiple analytes using a single device. At the same time, reduction in physical size of the device and multi-array analysis are preferred without compromising the specificity and sensitivity of the device. There is a growing demand of lab-on-chip interfacing with biosensor systems. Novel biosensing materials aimed at high sensitivity, selectivity, stable and low material synthesis cost will boost the market for biosensors and also their applications in different areas. Handling of biosensors should be made simple so that even a common man can use it without the help of qualified persons. Biosensor research should be encouraged with the allotment of funds and good infrastructure for research groups in order to advance in the biosensing field.

Summary

The current trend is biosensor application in the food processing field. Basic working principle of biosensor followed by the history of biosensor development with classification of various generations of biosensors are discussed in this article. Major contaminants in food processing are identified along with their pathway of production in food chain. The ill

Fig. 15 Tea biosensor designed in CFTRI, Mysore

effects of contaminants on human health are noted down with reference to animal tests. Biosensing systems for the contaminants are explained briefly. The article also highlights the various biosensing methods and devices developed at CFTRI, Mysore.

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