UNIT 8 APPLICATIONS OF BIOSENSORS IN FOOD SAFETY

Structure

8.0 OBJECTIVES

After studying this chapter, we will be able to:

- Understand biosensors and nanobiosensorsin food safety
- Identify its applications in detecting food contaminants
- Understand regulatory requirements for biosensors

8.1 INTRODUCTION

The rapidly changing food and climate landscape around the globe has resulted in newer challenges across the food chain. Global warming has resulted a shift in climate, agriculture and resultantly the microbial ecology, more resistant variants of pathogens. Consequently, more potent drugs and chemicals, pesticides, insecticides are employed to deal with these more resistant strains of microbes, pests and insects.

The entire food chain from primary processing, secondary processing and tertiary processing resultantly, gets exposed to several chemical and biological contaminants. These contaminants jeopardize the safety of the food and may result in risks and subsequently hazards generating in the food. In order to ensure safety of the food during processing and storage, for the consumers; food processors need to conduct timely analysis for varied parameters as per the food regulators requirements. However, many of the contaminants require tedious procedures and sophisticated instruments. A key solution to this is by the use and application of biosensors.

A biosensor is a type of analytical instrument, in which the measurement (quantitative, semi-quantitative) of a given analyte is done based on biochemical principles. In other words, the sensing element of the biosensor is of a biological origin.Food safety remains a constant and dynamic challenge to the food processing sector with newer strains of pathogenic microorganisms, their toxins, chemical contaminants such as microplastics, antibiotics, pesticide and veterinary drug residues from the natural and/or processing and handling environment. Conventional analytical methods require expensive, elaborate procedures and sophisticatedequipments, along with awell trained staff to handle the instruments, resulting in high cost and lengthy procedures. Biosensorsprovide rapid solutions for diagnosis/analysis for food, clinical, environmental etc. monitoring aspects, as they offer a reliable and accurate detection mechanism for these safety concerns.

In recent times, biosensors aregaining popularity due toease of usage, reliability and efficiency and are a great boon to food processing. In this chapter, we will be learning about the various applications of biosensors for detection of chemical and biological contaminants in food.

8.2 BIOSENSORS

The first biosensor was designed by Leland Charles Clark Jr. in the year 1956, for measuring oxygen concentration in blood. Thereafter, several advancements in the field of biosensing have taken place through different approaches, such as material science, physics, chemistry with applications in medicine through biomedical devices, environmental monitoring, and food safety monitoring etc. Biosensorsare a type of analytical instrument, in which the measurement (quantitative, semi-quantitative) of a given analyte is done based on biochemical principles. In other words, the sensing element of the biosensor is of a biological origin. A typical biosensor consists of an analyte, a bioreceptor, transducer, electronics and a display system.

Several biosensors have high specificity, high sensitivity, high selectivity towards the target analyte while excluding the interfering compounds. The typical food safety hazards associated with the food chain at primary processing, secondary processing and tertiary processing such as pathogens, pesticide residues, heavy metals, agrochemical residues, antibiotic residues, hormones, secondary metabolites, chemicals from food packaging, are the main target analytes for developing biosensors to ensure food safety and to strengthen the food traceability implementation.

Similarly, several applications relate to biosensing for assessing marker compounds to determine freshness of raw/processed foods, volatile organic compounds, allergens in foods, shelf-life determination and towards ensuring food authenticity.

8.3 GENERATION OF BIOSENSORS

There are three generations of biosensors which include: first generation, here the reaction product diffuses to the transducer resulting in an electrical response, then the second generation of biosensors, involving the specific 'mediators' in between the reaction and transducer to facilitate an improved response. Finally, the third generation of biosensors wherein, the reaction results in a response and no mediators or products are involved directly.

A biosensor typically consists of an analyte, a bioreceptor, a signal transducer, a method of conversion of signal from analog to digital and a display or monitor.

The transducer receives electrical signal which is often low, and this is affected by random high frequency signals that superimpose the low signal. The purpose of signal processing is to nullify or subtract this interference in amplification of the resultant signal difference and filtering out, electronically the unwanted signal or noise.

The relatively slow nature of the biosensor response considerably eases the problem of electrical noise filtration. The analogue signal produced at this stage may be output directly but is usually converted to a digital signal and passed to a microprocessor stage where the data is processed, converted to concentration units and output to a display device or data store.

Check Your Progress Exercise 1

Note: a) Use the space below for your answers.

b) Compare your answers with those given at the end of the unit.

1. State the three generations of a biosensor.

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2. Name the components of a typical biosensor?

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8.4 APPLICATIONS OF BIOSENSORIN IN DETECTION OF FOOD CONTAMINANTS

Food safety and quality is imperative to the food processing industry as it directly affects the health of the consumer. Additionally, regulatory requirements and the increasing incidences of contamination due to the rapidly changing environment and increased usage of chemicals at various stages of processing have resulted in increased demand for rapid detection methods with high sensitivity and specificity. Biosensing is one of the most effective approaches for detecting various chemical and biological contaminants in food matrices.

Based on their principle of working, biosensors are divided into different categories such as surface plasmon resonance, electrochemical, optical, impedance, thermometric, amperometric, fluorescence, piezoelectric, mechanical, biological, acoustic etc. Depending on the analyte of interest and suitability for the analyte, methodology for detection of the contaminant is selected. Biosensors find several applications in the field of food safety such as monitoring and control of food processes, assessment of quality and safety of raw material, on line during processing and end products during storage, transportation and retail, in terms of biological contaminants such as pathogens and chemical contaminants such as heavy metals, pesticide residues, antibiotics. Application of biosensors in detection of chemical and biological contaminants and food nutrients has been discussed in detail in the following paragraphs.

8.4.1 Chemical Contaminants

Food gets exposed to various chemical contaminants during processing from the agricultural fields until it reaches the consumers. Chemical contaminants in food essentially include heavy metals, pesticide residues, veterinary drug residues, toxins, food additives during processing, chemicals migrating from food packaging materials, and toxins etc.

- Heavy metals: Heavy metals such as mercury, cadmium, zinc, lead are bio-accumulative and find their way through the environment into food. These heavy metals are well known for their toxicity, can cause serious organ damage and even cancer due to their carcinogenic properties. Owing to their high toxicity, heavy metals have been subject to strict regulations globally. The standard method of detection of heavy metals, used currently involves usage of an expensive and sophisticated instrument – Inductively coupled plasma-mass spectrometry (ICPMS). Well trained and skilled operators along with controlled environments are essential requirements for operation of this sophisticated instrument. Therefore, it is essential to overcome these challenges and use biosensing technology to device rapid methods for detection of heavy metals which are portable, cost effective and, facilitate better enforcement of regulations thereby preventing occurrence of any contaminants and adverse health effects to consumers.

  Several researches have been developed to detect heavy metals using biosensing for example, a conductometric sensor has been developed to detect the presence of heavy metals by using alkaline phosphatase enzyme as a biorecognition element while the heavy metals act as enzyme inhibitors. In the presence of heavy metals, the change in pH and conductivity is sensed by the conductometric tranducer, with high sensitivity of 10 ppb (Limit of detection).

  Similarly, DNA based biosensor has been developed for identification of mercury. The formation of a thymine-mercury-thymine complex was the underlying principle for the detection method. This thymine-mercury-thymine complex is produced when the mercury ions attached to the two thymines resulting in an elevation in peak of faradic current, which is detected by square wave voltammetry.

Nadeje et al. (2014) developed a bienzymatic biosensor basedmethod by immobilization of *Arthrospira platensis* spp. on a gold transducer. The method was based on the key principle that presence of pesticides and heavy metals reduced enzymatic activity of esterase and phosphatase in the sample.

- Pesticide residues in food:Insects and pests cause infestation in the agricultural produce and therefore to deal with them, pesticides and insecticides are commonly employed both at farm and storage levels. However, these pesticide and insecticide residues are very harmful even for the human body if present beyond the permissible levels. In order to ensure that the food is safe for consumption and lower than the permissible limits, high sensitivity, high cost and sophisticated instruments such as gas chromatography mass spectrometry, high performance liquid chromatography are employed. However, several researches now facilitate use of biosensors, for example a biosensor with high specificity for analysis of pesticides in food was developed by Zhang et al. (2015). The principle of the biosensor was by inhibition of acetylcholine esterase (AChE), which hydrolyzes acetyl thio choline to thiocholine. This resulted in a peak due to oxidation which was visualized on the detection system. Based on the same principle of AChE inhibition,Schulze et al. (2002), developed an amperometric biosensor for direct detection of insecticides for testing in weaning foods for infants.

  Researchers have developed enzyme based biosensor methods for carbamate pesticides. The enzyme Trametes versicolor laccase was immobilized on graphene doped carbon paste electrode which was modified with Prussian blue films. Inhibition of laccase and 4-aminophenol was the key underlying principle of this method. Similarly, organophosphorus pesticides have been detected using a boron-doped electrode, which was designed with platinum carbon aerogels composites.

  Similarly, Flampouri et al., (2010) developed a cellular biosensor based on measuring the changes in the cell membrane potential of immobilized cells for detection of organophosphates and dithiocarbamates in tomatoes. Smartphone based biosensors have been developed for organophosphorus pesticide residue detection by using octahedral silver oxide particles enhanced with aptamer sequence. The enhanced aptamer sequence facilitated in chemical changes which resulted in color change in the solution. This color change was further quantified by the RGB values using a smartphone.

- Biotoxins: Toxins produced by bacteria, molds, algal toxins, marine toxins, all play a havoc and pose a threat to food safety. These toxins have often been studied for their mutagenic, carcinogenic and teratogenic effects and can even be fatal to the human body. Aflatoxin B1 was detected successfully by using a competitive inhibition immunoassay based surface plasmon resonance sensor developed by Daly et al. (2000). Electrochemical aptamer based biosensors were developed by Fetter et al. (2015) for detecting protein toxins – botulinum and ricin. Similarly, square wave voltammetry based electrochemical biosensors were developed by Tang et al., (2012) for detection. Of Brevetoxin B in mussels, razor clams and shellfish and by Eissa and Zourob (2012) for mussels.
- Chemical contaminants from packaging materials: Packaging materials are an essential aspect of food processing and aid in maintaining integrity and quality of the products during storage and transportation. However, due to their light weight, durability and ease of usage plastics are becoming the dominant and preferred option for food packaging however, some of these microplastics leach into the food and can cause harm to the consumer. One such frequently used material is Bisphenol A which acts as an endocrine disrupting chemical. An electrochemical aptamer basedbiosensor was developed byLiu et al. (2016) for detection of Bisphenol A in food packaging systems.

8.4.2 Biological Contaminants

Food poisoning and food borne illnesses are a major concern to food safety. Food borne illnesses have a significant impact on the health care systems and result in hospitalizations, deaths, lost man hours and a major economic loss. Hence, biological contaminants are a major hazard to food safety and thus, rapid detection methods using biosensors become imperative to ensure safety of the consumer.

Several optical biosensors have been employed to monitor and visualize the growth and multiplication of bacteria. As the deterioration of food progresses and multiplication of pathogens increases, the optical biosensors aid in distinguishing the microorganisms in food. As the volume of the pathogenic cells increase in the natural product, the optical biosensors through changes in the refractive index and/or colorimetric changes due to production of enzymes, detect through the receptors on the transducer surface. The principal techniques employed in optical biosensors are chemiluminescence, fluorescence, colorimetric and surface plasmon resonance.

Immunoassay based optical biosensors have been employed for detection of *Salmonella typhimurium*, *Staphyloccoccus aureus*, *Salmonella enteritidis* and *Campylobacter jejuni*. Here different coloured nanobead coupled antibody with immunomobilized lactoferrin on a cotton swab helped in determining the concentration and form of microbes by resulting in a change in color and strength of the cotton swab.

Yersinia enterocolitica has been detected in Kimchi solutions using electrochemical biosensors. Here, a single walled carbon nanotube immobilized successfully, with a polyclonal antibody for *Y. enterocolitica* was used for detection. The electrochemical sensor had a low detection limit of 4 log CFU/ml. Similarly, *Salmonella typhimurium* was detected using a potentiometric paper based biosensor. This biosensor contained a filter paper coated with a combination of ethylenedioxythiophene and polystyrene sulfonate, further antibodies were attached covalently onto the filter paper. The biosensor recorded a linear range of 4.07 log CFU/mL and, with 0.698 log CFU/ml as the detection limit. The biosensor successfully conducted the analysis and obtained results in less than 5 minutes.

8.4.3 Food Nutrients

8.5 RAFT (RAPID ANALYTICAL FOOD TESTING) KIT

In order to facilitate rapid testing of food to ensure food safety, Food Safety and Standards authority of India has introduced a policy to validate and approve rapid food testing kits. This will help reduce screening time of food products, and enhance surveillance activities. RAFT kits will provide results in 'real time'.

8.6 NANOBIOSENSORS

Biosensors have increasingly become popular across varied disciplines such as environmemtal monitoring, food pathogen detection and monitoring, contaminant testing and other aspects of food safety and quality as given below.

- Detection of microorganism and ensuring food safety
- Food quality of packaged food product in modified and controlled atmosphere
- Freshness of meat and fish products
- Evaluation of wine quality and alcohol percentage in alcoholic beverages
- Freshness of fruits and vegetable
- Measurement of growth hormones and urea in milk
- Detection and estimation of agrochemical residues such as pesticides, antibiotics, hormones

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Check Your Progress Exercise 2

Note: a) Use the space below for your answers.

b) Compare your answers with those given at the end of the unit.

1. State true or false:

i) Whole cell based biosensors are used in environmental monitoring.

ii) Bioreceptor in case of enzyme based biosensors is an Enzyme.

iii) Electrochemical biosensors are also sometimes referred to as optodes.

iv) Change in temperature is measured using calorimetric biosensors.

2. Biosensors are classified into different types based on ________ or __________.

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3. Discuss the features of a biosensor.

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8.7 FSSAI AND OTHER REGULATIONS FOR BIOSENSORS

8.8 LET US SUM UP

Biosensors are devices that measure the response produced in the biological reaction and convert them into an electrical signal, which is proportional to the concentration of the analyte. Biosensors essentially consist of an analyte, bioreceptor, signal transducer, electronics and a display system. Essential features of a biosensor include selectivity, sensitivity, linearity of response, reproducibility of a signal response, quick response and recovery time, and good stability and operating life. Due to the awareness among consumers and rising incidences of outbreaks due to food poisoning and food contamination, biosensors have become crucial elements of a food safety implementation mechanism. Food safety is integral to food processing and with increasing thrust on food processing, tools to ensure food safety are becoming a key concern. Biosensors offer a simple, rapid and reliable analysis of a range of food safety micro-organisms and contaminants with both quantitative and qualitative estimation. Due to their ease of usage and high sensitivity and specificity; biosensors are finding way in the entire food chain i.e. primary, secondary and tertiary processing with an aim to ensure food safety.

8.9 KEY WORDS

- Biosensors: Devices that measure the response produced in the biological reaction and convert them into an electrical signal, which is proportional to the concentration of the analyte.
- Analyte: Substance of interest that is being subject to chemical analysis.
- Bioreceptor: The biological recognition element that is responsible for recognizing the analyte.
- Sensitivity: The biosensors should be able to detect the analyte over the entire range. That means even minute quantities and high quantities should be detected.
- Selectivity: To avoid cross reactivity between analytes and also to be specific the biosensor needs to be highly selective in nature. The measured response should be for the analyte of interest and not other analytes.
- Reproducibility: The analysis results are consistent even when the sample is analysed multiple times.

8.10 TERMINAL QUESTIONS

1) What are biosensors?
2) What is the principle of biosensing? How do biosensors work?
3) Discuss the different types of biosensors.
4) Briefly specify the various applications of biosensors.

8.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) The three generations of biosensors include: first generation, here the reaction product diffuses to the transducer resulting in an electrical response, then the second generation

of biosensors, involving the specific 'mediators' in between the reaction and transducer to facilitate an improved response. Finally, the third generation of biosensors wherein, the reaction results in a response and no mediators or products are involved directly.

2) Components of a biosensor:
   - Analyte
   - Bioreceptor
   - Signal Transducer
   - Electronics
   - Display

Check Your Progress Exercise 2

1) (i) True

   (ii) True

   (iii) False

   (iv) True

2) Bioreceptors or transducers

3) Features of a biosensor are:
   - Selectivity
   - Sensitivity
   - Linearity of response
   - Reproducibility of signal response
   - Quick response and recovery time
   - Stability and operating life

8.12 ANSWERS TO TERMINAL QUESTIONS

1) Biosensors are devices that measure the response produced in the biological reaction and convert them into an electrical signal, which is proportional to the concentration of the analyte.

2) Biosensors are devices that measure the response produced in the biological reaction and convert them into an electrical signal, which is proportional to the concentration of the analyte. A biosensor typically consists of an analyte, a bioreceptor, a signal transducer, a method of conversion of signal from analog to digital and a display or monitor.

With respect to the functioning of a biosensor; the analyte of interest is sensed by the sensor and a signal is generated. Subsequently, the signal is processed and converted into a form which is compatible with human understanding. Mostly in biosensors, a specific type of biochemical reaction is employed and based on the progress/outcomes of reaction, a signal is generated and proceed to human understandable form.

3) Types of Biosensors:
- Enzyme Based
- Whole cell Based
- Affinity Biosensors
- Electrochemical
- Optical
- Calorimetric
- Acoustic
- Piezoelectric
- Surface Plasmon Resonance

4) Applications of biosensors
- Detection of microorganism and ensuring food safety
- Food quality of packaged food product in modified and controlled atmosphere
- Freshness of meat and fish products
- Evaluation of wine quality and alcohol percentage in alcoholic beverages
- Freshness of fruits and vegetable
- Measurement of growth hormones and urea in milk

8.13 REFERENCES/SUGGESTED READINGS

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