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REVIEW ARTICLE

Application of biosensors in food quality control

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ABSTRACT

Food producers gradually demand for effective quality control procedures to satisfy and regulate the requirements of consumer to enhance the production feasibility, automation, quality sorting and decreases time and cost of production. Also, there is the requirement for rapid and efficient techniques to identify the allergens and pathogens present in the food product which can be fulfilled by the biosensors. Biosensors have the ability to overcome all these disadvantages by offering quick, inexpensive as well as non-destructive procedures for quality control and pave way for the quick identification of allergens, pathogens, and pesticide residues present in food. In this review, the basic principle behind biosensors and the generations of biosensors have been highlighted. Various biosensor on the basis of technique used such as optical biosensor, potentiometric biosensor, amperometric biosensor, thermometric biosensor, electrochemical biosensor, piezoelectric biosensor, impedance biosensor, fluorescence label biosensors have been emphasized. In this peculiar review, the various applications of biosensors in food safety, process monitoring, as well as assessment of quality, including the detection of contaminants, pathogenic microorganisms, determination of antioxidants, heavy metals, quality evaluation of various fruits and vegetables have been discussed in detail.

Keywords: Nanosensor, transducer, food industry, food safety, quality control

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INTRODUCTION

Food can be defined as a material which is either in raw, processed, or formulated form that is utilized by humans orally or animals for their satisfaction, growth, as well as health purpose. Preservation of food is a process of keeping foods at an acceptable characteristic level for their maximum advantages. Generally, every step involved in handling, processing or production, storage as well as distribution influences the properties of food that could be undesirable or desirable. Hence, knowing the impact of every preservation technique and handling method on food system is crucial in food processing area that may result in the food safety (Rahman 2007). Safety monitoring as well as nutritional parameters of food is vital. The traditional analytical methods for safety and quality monitoring are tiresome, takes much time and needs well-trained operators, thus there is necessity to produce rapid, sensitive as well as reliable methods to monitor food safety and quality rapidly. Therefore, biosensor can be a suitable substitute to the traditional methods. Biosensor devices are the most relevant diagnostic methods for food, environmental and clinical examining as they are specific, rapid, easy to fabricate, and have field and economics applicability. Specificity is attained from these devices by biological binding reaction, obtained from a variety of interactions which involve antigen or antibody, enzymes, cofactor/ substrate, ligand /receptor, nucleic acid hybridization and chemical interactions in combination with large number of transducers.

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BIOSENSORS

A biosensor is described as combined receptor transducer system, having capability of giving discriminative semi quantitative or quantitative analytical description by means of a bio-recognition unit. It could be described as "analytical system integrating a bio-substance, a bio-derived substance or biomimic well linked with or within a physiochemical transducer, that could be thermometric, electrochemical, optical, magnetic or piezoelectric"(IUPAC 2000). Miniaturized nanosensors could be incorporated into the packaging material and integrated with wireless tracing techniques to produce tracking information regarding the food system as well as the supply chain. For foodborne pathogen identification, traditional methods mostly need around a week to produce an output, whereas nanosensors give result within just hours. Also, nanosensors are fabricated to various types of analytes revelant to food safety. These nanosensors are used to determine contaminants like metal ions, gases, vapors biomolecules, organic molecules and various food borne microbes in order to enhance the food quality (Zhang et al. 2019; Wang and Duncan 2017). Biosensor contains a sensor-component and a bio-element. Living cells, an enzyme, tissue, or an antibody acts as bio-elements, whereas, the sensing component can be electric potential, electric current etc. Various combinations of sensor elements and bio-elements form various kinds of biosensors to be applicable for large number of applications. The bio-receptor is basically a biomolecule having a capability of recognizing the target analyte whereas the transducer helps to convert that identification event into a particular quantifiable signal (Arora 2013).

The rareness of a biosensor is that the 2 compounds are combined in a single sensor. These combinations permit to detect the target analyte in absence of any reagents. For instance, the concentration of glucose in blood sample could be detected using a biosensor directly (specifically made for glucose determination) by directly putting the sensor in blood sample, which is in contrarily to the traditional methods where number of stages are involved and every stage needs a chemical agent to ascertain the specific sample. The conveniences as well as the swift measurement are considered the significant benefits associated with a biosensor. The “bio” and the “sensor” components are joined in any of the 4 potential ways such as: physical adsorption, membrane entrapment, entrapment in matrix, and covalent bonding. Undergoing membrane entrapment, a membrane for example, partial permeable are capable to split up the analyte and the bio-component from one another, and the sensor is combined with the biological substance. The physical adsorption process is based on association of ionic bond, hydrogen bonds, hydrophobic, Van der Waals to combine the bio-component surface of the sensor. The process of entrapment depends on making a porous encapsulation matrix throughout the bio- component thus aids to bind it to the sensor. In covalent bonding process the surface of the sensor acts as a reactive group to bund a bio-component. (Meshram et al. 2018).

GENERATIONS OF BIOSENSORS

Depending upon their integration level biosensors is divided into three generations.

First generation

In this generation, the biocatalyst is bound or trapped between the membranes which is then fixed to the transducer surface.

Second generation

The instant covalent or adsorptive binding of the bio-active compound to the surface transducer allows removal of the semi permeable membrane.

Third generation

The biocatalyst is bind to an electric equipment that help in transducing and amplifying the signal like the gate of a field effect transistor, is essential for an additional miniaturization of nano-biosensors (Thakur and and Ragavan 2013).

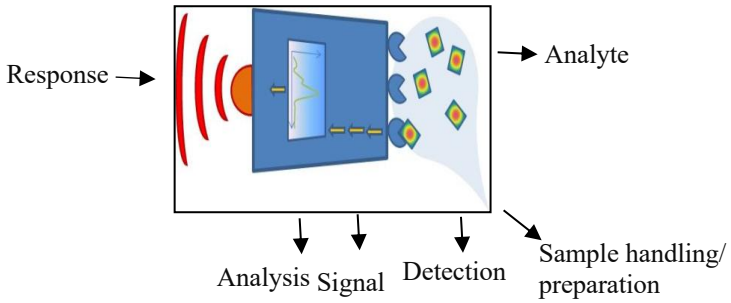

Fig.1. Diagrammatic illustration of Biosensor

CHARACTERISTICS OF A BIOSENSOR

The subsequent requirements/ characteristics are extremely important and needs to be considered for developing a novel and commercially feasible biosensor system.

- Selectivity: These devices must be extremely specific for the particular analyte and display less or no cross reactivity with moieties possessing same chemical composition.
- Sensitivity: These devices must have the ability to determine in the variety of interest for an analyte requiring lesser further steps like pre concentration or pre cleaning of samples.
- Linearity of response: These systems should cover-up the concentration above which the specific analyte is intended to be identified.
- SRR (signal response reproducibility): Whenever trial samples possessing similar amounts tend to be determined repeatedly, they must provide similar response.
- Recovery and quick response time: These devices must have quick responses that actual time monitoring of the particular analyte could be completed with efficacy. Also, the time of recovery must be little for reusability of these devices
- Operating life and stability: Majority of the bio-materials are less stable under various environmental and biochemical environments. The bio-materials used must be interfaced so as the activity could be maintained for a longer time thus making these devices marketable and practically beneficial in the field (Arugula and Simonian 2014).

WORKING PRINCIPLE

The basic principle behind this technique is the transformation of biologically produced recognition event (such as antibody, enzyme) into a particular measurable signal, through transducer followed by processor. The output is basically a display showing the concentration and the presence of the target analyte. The bio-receptor is a biological molecule having ability of detecting the target analyte (Meshram et al. 2018). A bioreceptor may a microorganism, tissue, cell, organelle, enzyme, antibody, biomimic and nucleic acid. The transducers simply transform the recognition event into a particular detectable signal.

Transduction can be electrochemical, optical, piezoelectric, thermometric, micromechanical and magnetic or combinations these methods.

Biosensor = Bioreceptor + Transducer

An essential role is played by analytical chemistry in food quality parameters as about each sector of public service and food sector depends on quality control. A biosensor used to control the quality of food system is basically a device, that responds to certain characteristics of food and transforms into a detectable signal, mostly an electric signal, which gives information regarding the quality factors to be determined or may have a direct relation with the food quality factors.
The important immobilization methods, mostly for enzymes, comprise physical adsorption, covalent binding, matrix entrapment (using printing inks, polymers, or gels), or photo-polymerization as well as electrochemical polymerization. Physical adsorption method depends on interactions between the transducer and the bio-material via van der Waals.

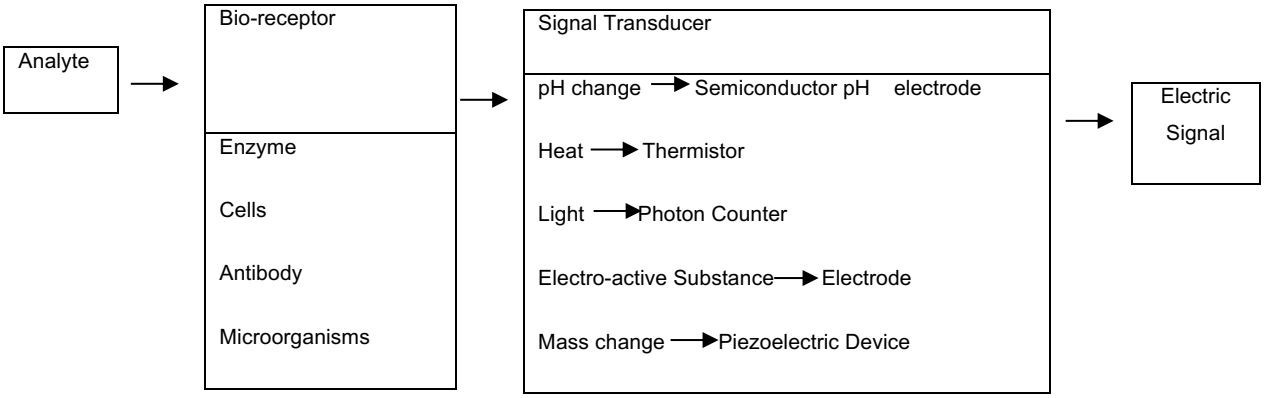

Fig. 2: Principle of biosensor

NANOBIOSENSORS

Nanosensors can be described as the chemical, biological, or sensory attributes which are primarily used form interpretation of functional, applicable information regarding the nanoparticles at microscopic level. Silicon nanowire (SiNW) was the first synthesized nanomaterial used for sensors was, it had been made from Silicon powder by thermal evaporation by iron and hot pressing at temperature of 1200°C at 100 Torr pressure using argon gas as inert gas. The sensors based on SiNW typically uses field-effect transistors (FETs) comprising of source, gate electrodes and drain (Zhang and Ning 2012). Various parameters such as carrier densities, diameter, mobility, as well as surface chemistry affects the efficiency of the biosensors. The elevated surface area: volume proportion, enhanced electrical attributes, as well as bio-compatibility tend to be vital specifications of SiNW that could be used for tagged or tag-free identification of the target analytes (cells, micro/ macro-biomolecules, pathogen, and ions (Wanekaya et al. 2006). An ideal biosensor depending on the addition of monomeric gramicidin-A ion channels (MGIC) into a tethered lipoprotein membrane is formed, which uses the disassociation and association prospects of gramicidin dimmers (Patra et al. 2019). Lipoprotein membrane attached with electrode of gold origin comprising of MGIC along with an immunoglobulin molecule is the main part of an ion channel switch. These biosensors are mainly utilized for cell typing and identification of viruses, protein molecules, pesticides, cells, micro-molecular drugs as well as

electrolytes (Krishnamurthy et al. 2010). These also have the potential for environmental detection, medical diagnostics, bio-hazard estimation. The novel nanomaterial for biosensors includes quantum dots (QDs), graphene, carbon nanotubes, and certain metal nanoparticles (Wang et al. 2003).

Carbon Nanotubes (CNTs)

The most important and commonly used material for fabricating sensor is nano-carbon. Particularly carbon nanotubes (CNTs), these nanotubes are either single walled or multiple walled depending on the number of rolled layers used in the design (Pérez-López and Merkoçi, 2011). These SWNTs and MWNTs have novel ideal electrical, mechanical, as well as electrochemical properties to be used in biosensors as transducers and electrodes. CNTs plays an essential part in improving the biocompatibility in a food matrix/ food system (Singh et al. 2013). The important functions include the addition of carboxyl or amino groups, or the incorporation of thin films or polymers, and ionic liquids. CNTs are also used in food matrix as sensing element to detect certain toxins like staphylococcal enterotoxin B (Inbaraj and Chen 2016). SWNTs are inconstant to environmental conditions surrounding them. Chemical resistors and chemically versatile FETs embedded with pristine CNTs are used to identify biological molecules (Kong et al. 2000). The FET based biosensor was 1st utilized to identify the ion concentration of the target analyte in 1970, and then to identify DNA hybridization, and concentration of nanomolar DNA (Fritz et al. 2002). Such nano-channel FET sensors are mostly used to identify viruses, micromolecules, nucleic acid, proteins, and elements in single or complex format (Patolsky et al. 2004; Wang et al. 2008; Hahm and Lieber 2004; Wang et al. 2005; Zheng et al. 2005).

Graphene materials

Graphene (GR) is basically a 2-D nano-sheet detected by Geim and Novoselov (Geim 2007). Graphene and its derivatives (such as graphene oxide (GO) and reduced graphene oxide (rGO)) are ideally used in the food sector for safety purpose because of their ideal physicochemical characteristics. Various noble metal nanoparticles are embedded with GR in various forms to make composites. AuNPs/GR nanocomposite are successfully used as the functional material, reflecting more favorable electrochemical properties and providing an auspicious platform for electrochemical sensors in safety of food system. For instance, Zhang et al. (2013) found a voltammetric sensor for caffeic acid detection based on AuNPs/GR, depicting electron transfer kinetics favorably as compared to the bare GCE. The hexagonal Ag nanoplates at nanoscale were formed and embedded with GR to form a vanillin based sensor with improved selectivity by incorporating appropriate proportion of polyvinyl pyrrolidone and tri-sodium citrate (Huang et al. 2014) The Ag/ GR nanoplates with larger surface area and improved conductivity could lessen its potential and rise peak current in electrochemical identification. Ag nanoparticles are ideally deposited electrically on the sulfonated functionalized GR, and the nanocomposite could be utilized to develop a sensor to determine metronidazole and chloramphenicol in aquatic foods. (Zhai et al. 2015). Additionally, Ni nanoparticles can also be used embellish ultrathin GR, possessing improved assimilation and catalytic activity the simultaneous identification of tartrazine and Sunset Yellow in foods (Gan et al. 2013). The graphene derivatives, such as GO, rGO, and graphene quantum dots (GQDs), are potentially used in biosensors (Vilian et al. 2014), to incorporate functional groups in chemical reduction and oxidation steps in the formulation phenomena.

Quantum Dots (QDs)

A quantum dot (QD) is ascribed as the captivity of quantum impact reflected by the particle (Xu et al. 2015). The captivity impact refers that the tangible framework and confirmation of a nanodot is lesser as compared to the exciton Bohr radius

(Shanehsaz et al. 2013). Owed towards quantum captivity attributes, optical-fluorescent characteristics are demonstrated. Tang et al. 2008) established the QD in fluorescent carbon dot (CDs) form and has been reported first, while as (Zhou et al. 2017) characterized and discovered it 16 years ago in 2004CDs is quite utilized as carbon nanomaterial, mainly if the dimensions are lower than 10 nm. The definite working of a CD's photoluminescence characteristic is not clearly known yet, and this is mostly because of the variation in structure and synthetic principles that has been formed (Pellegrino et al. 2005). The fluorescent property of CDs is used by altering the surface chemical groups of CD and their size during nanomaterial formation process. Literature also revealed that a sensor using CD-MnO2 based fluorescent recovery determining ascorbic acid can also be used in the identification of the target analyte in matrices like fruit juices and fresh fruits, vegetables (Liu et al. 2015). CDs are also utilized to detect microbial growth and various produced toxins produced by microbes, such as aflatoxin-B1 and Salmonella typhimurium (Lv et al. 2018). A CD-aptamer complex is used to quantitatively identify the Salmonella typhimurium in eggshells (Wang et al. 2016).

Metal nanoparticles

Ideal metal nanoparticles are extensively used to develop the sensing interface in nano-biosensors due to their ideal biocompatibility and catalytic activity. Among them, AuNPs is commonly used as the combination of AuNPs results in change in color visible to the eyes, depicting the presence of particular analyte (Bathinapatla et al. 2016). Even in absence of the carbon-based nanomaterials, AuNPs reflects excellent properties in nano-biosensors. For instance, the incorporation of AuNPs and CdTe QDs via chitosan microspheres for immobilization of AChE endorsed electron transfer and catalytically the electro-oxidation of thiocholine, hence amplifies the identification monocrotophos sensitivity (Du et al. 2008a). The other significant example consists of the recognition of malathion having an AChE-based biosensor (Du et al. 2008b). Incorporating planar gold electrode elevated using AuNPs electro-chemical deposit upon chitosan hydrogel accustomed to identify malathion was designed in line with the desorption or chemisorption phenomena of thiocholine. The Au-SiO2 nanocomposites incorporated with ferrocene, fullerene and thiolated chitosan composite nano-layer based immunosensor is used to detect E. coli O157:H7. To amply the signals, glucose oxidase incorporated with Pt nano-chains can also be used to trace tag to label signal antibodies (Li et al. 2013).

TYPES OF BIOSENSORS

Transducers

A transducer device can be activated by energy from one system which is then supplied in a new form, to another system. In biosensors, the energy obtained indirectly or directly by a bio-reaction is transformed to an electrical/ detectable signal. The 4 important kinds of transducers in biosensors include thermal, optical, piezoelectric, electrochemical tranducer. To select the particular type of transducer, the biological element plays an essential role. Enzymic reactions are simply observed, thermometrically or electrochemically, while as mass sensitive devices are less preferred for enzymic biosensors. On another note, piezoelectric sensors measures affinity reactions of antibody and antigen or DNA and does not require a label. While, other transducers often need certain type of tag for affinity detection (Leonard et al. 2003).

Electrochemical transducers

Electrochemical sensors work on the principle of electrochemistry (Villalonga et al. 2019). For nano-biosensing applications also, the similar principle is used, and when an electron is produced or used in the biological interaction phenomena. This alteration results in a number of detectable signals. Thus, an electrochemical nano-sensor, is based on chemical reactions

within the nano-substances or the nanomaterial surfaces are adhered to, that can be bio-molecules, chemicals, the bio-materials, and target analytes, to use or produce electrons or ions. These electron or ions variations could thus be detected as current, voltage, impedance (Lozano et al. 2019). Number of electrochemical methods is used in biosensors such as, potentiometry, amperometry, conductivity and impedance method.

Amperometric transducer

These types of biosensors rely on the persistence of a steady-state electrical current developed when the consistent potential is implemented applying potentiostat. The produced current could be associated to the electrochemical substances that are produced or consumed by the bio-material. The electro-chemical system regularly comprises of electrode like glassy carbon, platinum, gold, or graphite Ag or AgCl are used as a reference electrode, mostly formed from platinum or carbon.

Amperometric method is mostly used in biosensors for detection as it is simple and sensitive. The bio-material is mostly an enzyme and can be directly attached to the electrode. In an enzyme-based reaction, an electrochemically active material is consumed or formed using an enzyme and this is reduced or oxidized at that electrode. In an ideal situation, the analyte concentration is correspondent to the current. One example is glucose oxidase enzyme being used in glucose recognition biosensor. The enzymic response utilizes oxygen as well as gives H_2O_2, and is identified amperometrically. The majority of newer bio-sensors makes use of mediators in order to exchange electron support enzymes by way of an exchange of electron straight from the dynamic center to the electrode (Glaser 2000).

Potentiometric transducers

These types of biosensors can determine potential changes beneath zero current situations. The binding of antibody-antigen produces less alteration in the charge of the proteins that could be potentiometrically identified and as this charge is extremely minute, the process is less sensitive. Ion selective electrodes (ISEs) rely on potentiometric data and are mostly utilized in enzyme biosensors. The pH alteration because of its enzymatic activity for instance is monitored using pH sensitive ISE. The potential which is developed throughout the ion selective membrane is determined. Guilbault and Montalvo for urea in year 1969 developed the Ist potentiometric biosensor. Current potentiometric devices depend on FET systems (Mello and Kubota 2007).

Optical transducers

Optical biosensors differ from the electrochemical sensors, because these sensors assist in to determine the variance within the optical signal generated by the sensing unit inside the food system (Narsaiah et al. 2012). These electrodes could be employed in various different optical phenomenona like Surface Plasmon Resonance (SPR), fluorescence, absorbance, phosphorescence, bioluminescence polarization and chemiluminescence, (Roberts et al. 2018). Moreover, numerous of these methods could be used in a variety of modes like decay or time intensity. The increasing market for devices of fiber optics is extremely advantageous for researchers. The growth today is boosting because of the interest of telecommunication company in opto-electronics. The money spends by the firms in research on these optical fibers permit advancements rapidly. The other significant advancement is the accessibility of cheap, accurate and more stable light sources. Where, conventional devices were costly and usually larger light sources, the advancement of LEDs permits the assimilation of such lesser and usually

inexpensive systems into sensors. Large-scale manufacturing or optic fibers for the laser diodes as well as telephone wires, CD-ROM players has reduced the cost of these units substantially (Meshram et al. 2018).

Surface Plasmon Resonance (SPR)

It happens once illumination is reverberated at interface of any material from the inside having more/less index of refraction. Between these 2 layers, a thin layer having good conductivity like silver or gold is essential (Glaser 2000) an evanescent waves (EW) formed at the interface could associates with electrons existing within the conductive region. An absolute energy sources are vital to raise these types of surface plasmons. The innervation energy of plasmon is disappeared from reverberated illumination and ought to be identified making use of monochrome light that's reverberated at various angles. A minimization in reflectance at a specific angle (resonance angle) shows the plasmon excitation.

The energy and the evanescent waves needed to elevate the plasmons from the surface depends on index of refraction of medium having less index of refraction. The refractive index within the penetration depth of the electromagnetic wave is influenced by molecules attached to the interface layer surface. Thus, Deoxyribonucleic acid (DNA) binding or antibody antigen interactions could be detected label-free with SPR. SPR is associated with a surface plasma wave (SPW) and SPR is also charge density oscillation. This wave penetrates the less dense as well as the optical dense medium. The SPW is almost 10 times much powerful than the EW and have high sensitivity to the alterations in refractive index ortho dielectric layer ((Meshram et al. 2018). SPR is determined by the resonance angle and intensity and several other methods, moreover the angular technique is one of the most famous biosensor applications. The method permits the label- free identification of biological-affinity reactions, and washing steps are not required in in certain cases. BIAcore was the first the SPR based-biosensor and is regularly updated and improved. Companies like Texas Instruments and Quantech made SPR biosensors.

Piezoelectric transducers

For mass sensitive transducers piezoelectricity is used (Wang and Duncan 2017). Quartz is the substance which is typically used for piezoelectric biosensors. Once an oscillatory electrical field is put on through the quartz disc in between the electrodes, a wave is produced which transmits across the crystal. The frequency is based on the characteristics of crystal-like orientation, density, as well as chemical structure. The frequency also depends on the mass deposited on the surface of crystal (or in several cases on the surface of electrode that are deposited onto the crystal). This permits highly sensitive identification of mass alterations on the surface of crystal. AT-cut crystals are often utilized, because of their zero temp coefficient and also these types of crystals vibrates in their thickness shear setting, reducing obstructions and is triggered as a result some other oscillation processes. Surface acoustic wave (SAW) system is utilized in these biosensors. Large frequencies of 30-200 MHz provide the crystal an extremely high sensitivity, but these sensory chiefly rely on bulk acoustic wave (BAW) systems due to their practical complexities (Rocha-Gaso et al. 2009).

Thermometric transducers

These biosensors exploit the alteration of heat evolution or absorption that takes place in biochemical reactions. The fluctuation in heat causes the temperature variation in the reaction medium. The sensitive thermistors are utilized for monitoring the variation in temperature. Most of the bio-reactions are exothermic. Enzymatic reactions are related to the change in enthalpy of 20100kJ/mol. Inadequate enthalpy changes could at times be improved by the series of reactions with

greater changes in enthalpy. Measurements could be enhanced by high-protonation enthalpy buffers like TRIS or by co-immobilizing enzymes for signal amplification (Giese 2002).

Bio-recognition layer

Bio-recognition gives the biosensor with more appropriate analysis opportunity (Rotariu et al. 2016). In biosensor the sensing element is a very definite bio-receptor. These bio-receptors exhibit notable affinity towards a specific analyte, or a group/ family of analytes as compared to most chemical sensing substances. Various bio-elements are used in biosensor. For biosensors, the essential biological molecule can be enzymes with their high affinity towards the definite substrates. Bio-recognition between antigen and antibody are also essential to be used in immunological sensors. Various other biomolecules mostly used include DNA strands with their affinity towards other molecules or complementary strands. Lecitin was mostly used because of their affinity towards sugars. Other systems include entire cells, yeasts, bacteria, tissue slices, subcellular proportions and membrane receptors.

Based on the bio-element, biosensors possess catalytic activity or high affinity (Tothill 2001). In catalytic sensors, an enzymatic reaction involves the analyte, results in the change in concentration of an identifiable component. In affinity sensors, the receptor and analyte binding is examined, for example with DNA or antibodies.

Immobilization of Biological Components

For reliable operation, the biomaterial used in biosensor must be bound to the surface of transducer. The bio-component is immobilized in this process. In immobilization biomaterial is bought (nucleic acids, antibodies, enzymes, oligonucleotides) into insoluble form by adding it into an inert support or by physical binding to the surface of transducer. There are 5 ways of immobilization of bio-components (Eggins 2008).

Adsorption: It is one of the easiest processes which do not require any significant pretreatment of sensor compounds or specific-purpose chemicals. Activated carbon, alumina, silica gel, collodion, cellulose, hydroxylapatite, glass, clay, and various new materials adsorb enzymes with no impact on their native configuration. The chemical as well as physical adsorption can be used in such case. In case of physical adsorption, the biological material is placed on the surface either by ionic interactions, or van der Waals, Coulomb, or hydrogen bonding. In cellular structure immobilization, the adsorptive attachment of cells to the previously treated polymer surface is enough powerful in which the elimination of the surface cells of polymer leads to their disintegration. The output of adsorptive immobilization is mostly depending on the characteristics of the surface of transducer, the existence of polar groups, its charge, energetic uniformity, and its redox potential. Adsorption cannot afford large concentration of biomaterials. To raise the quantity of biomaterial adsorbed, the transducer is usually previously treated to form charged or polar groups increasing the adsorption of bio-components. This is carried out by many methods of oxidation as well as surface alteration using functionalizing reagents or polymers. For instance, the oxidation of carbon electrodes and gold raises the nucleic acid, protein, and their microbial adsorption capability. A much strong bond between the support and bio-component is delivered by chemisorption giving covalent bonds. The identification elements formed by adsorption are highly sensitive to temperature, pH, substrate concentration deviations and ionic strength. Adsorption is mostly utilized at the research stage, when the weak binding between the transducer and bio-component is adequate and the sensor is used for short-term operation (Korotkaya 2014).

Microencapsulation: It is most commonly used process of forming electrochemical sensors. A bio-component is kept close to the transducer to separate it from the remaining solution using a semipermeable membrane which permits analyte molecules and the catalytic reaction products to pass through it. In this method, an inverse or direct emulsion is firstly formed from a polymer solution in a solvent (of organic nature) and an aqueous solution of the biomaterial. The emulsion is then dried to attain a polymeric membrane which adds microcapsules of water comprising micro-molecular electrolyte ions and biomolecules. This method of immobilization leaves intact the hydrophilic nature of the biopolymer at every immobilization stage, in order to achieve significantly large residual enzyme activity. The immobilized enzyme is basically free but confined to a specific portion of the measurement cell. Various kinds of membranes can be utilized in microencapsulation. In addition to cellulose acetate (dialysis) membranes, that are not permeable to proteins and decreases the transfer of various micro-molecular components, membranes prepared by the protein collagen, polycarbonate (Nucleopore), and from polytetrafluoroethylene (Teflon) are used, the latter having permeability to only few gases (Eggins 2008).The Nafion polymer are utilized to form membranes possessing negative charge to be utilized for glucose sensors. This method of immobilization is mostly used in number of sensor models, ensuring the high performance of the enzyme, shielding it from degradation and contamination. Hence, microencapsulated enzymes are mostly resistant to change in ionic strength, temperature, pH, and the medium composition. However, certain species and molecules, like minute gas or electrolyte molecules can pass through these membranes.

Inclusion: Bio-component inclusion to make polymer matrix is a widespread process used in number of detection components. The polymer is deposited from a solvent (of organic nature) by dissolving the solution in water or from a micro-emulsion by dehydrating it onto the surface of sensor. The polymer is also attained by the process of gelation from polyacrylamide, agar, alginate solution or gelatin, or by condensation of various chloroanhydrides or organic esters. chloroanhydrides. The latter method is known as sol–gel immobilization (Korotkaya 2014).

A matrix comprising of a synthetic polymer is formed in existence of a bio-component. A crosslinking element is mostly incorporated to assemble separate polymer strands into 3-D network. The bioactive compounds are entrapped into the polymer bulk. One of the benefit of this process is its versatility. While the network inhibiting diffusion and impeding the permeation of analyte is one of its limitation. Additionally, if the molecules involved into the network are devoid of chemical binding, they are washed away. Nucleic acids and proteins are immobilized in polymers of p-aminophenol, thionine, o- and m-phenylenediamine, some phenoxazine and phenothiazine dyes, and thiophene derivatives, aniline and pyrrole. The inclusion of bio-components in poly-ionic complexes leads to the complexion in the layer-by layer deposition of polyelectrolytes from the solution. The native surroundings of the enzyme can be attained in synthetic lipid membranes, particularly, Langmuir– Blodgett films having same composition and characteristics as that of natural bio-membranes. These membranes function as a model in the examination of membrane methods, and in nucleic acid and protein immobilization (Koval'chuk 2003).Langmuir–Blodgett films possess less mechanical strength; due to which, they are attached to the inert polymer surface having hydrophobic nature(Teflon, polyvinyl chloride).

Cross-linking: In this process, the biological material is chemically bind to a gel or a solid support by bi-functional reagents, such as glutaraldehyde (Gol'dfarb 2005). Example of this method is the glutaraldehyde action, forming Schiff bases with hydroxyl, amino, and thiol groups of proteins or nucleic acids. Different kinds of polymers (cyclodextrins, gelatin, polyvinyl chloride, agar, polyacrylamide, and various other polymers and gels) were examined as matrices for a biological material, but they were not found to be perfect (Kellner et al. 2004). In encapsulation process, diffusion of substrate by the resulting substance can be quite slow. Bio-active components in these substances can degrade progressively. One more drawback of

the process is that the resulting substances possess low mechanical characteristics. But, this process could be used to improve the stability of adsorbed biomaterials.

Covalent bonding: It is one of the most universal process of immobilization. Following from its name, it can be described as the covalent bond formation between a support and a bio-component. Depending on the support material and the molecules to be bound, the selection of chemicals is done. Covalent bonding can be completed in three steps: the 1st step involves the purification or clarification of the support and functionalization of its surface with the important groups, the 2nd step involves the binding of biomaterial, and lastly, the third one involves the elimination of weakly bound molecules using solvent (pure). Evidently, the order of chemical reactions needs to be selected so that the bonds formed remains from the initial to the later stages. The sensors are produced using the following support materials: carbon, metals, (silver, gold, or platinum), polysaccharides (cellulose and their derivatives), glass, poly(methyl methacrylate), nylon and substances possessing imidazol groups or free –SH, $–NH_2$, or –COOH groups. Proteins having covalent binding via nucleophilic functional groups in their side amino acid chains mostly that does not affects their enzyme activity. Covalent bonds are mostly formed at optimal temperature, low physiologic pH values and ionic strengths. To shield the active site of an enzyme based reaction, the latter is conducted using substrate. The immobilization of oligonucleotides and DNA is done by cross-linking it to the chitosan to attain a series of amide bonds (Gol'dfarb 2005). Various processes of covalent binding of DNA to aminodextrins and silanized supports were given by Shlyapnikov (2010).A universal process includes the alteration of terminal nucleotide residues. The addition of thiol groups in the nucleotide residues gives ways to attain, through chemisorption on gold, even layers of oligonucleotides which are usually oriented orthogonally to the surface. The major benefit of this covalent binding is that it ensures strong biocomponent–support binding and inhibits the loss of bio-component and, on another note, it helps to make sensors with more service life (Eggins 2008). In certain cases, a bio-component is attached to the transducer by various processes. It is important to check the efficacy of binding by every process; particularly, it is essential to equate the activity of the same enzyme in the immobilized state and the enzyme activity in solution. The matrix (support) and binding process needs to be selected before fabricating the sensor.

APPLICATIONS IN THE FOOD INDUSTRY

The food sector requires various techniques of analysis to check the food safety, maintenance and quality, to enhance the product yield, to optimize the energy input, to monitor processing, and to increase the level of process automation. Identification of biological and chemical contaminations in food product is of major significance to assure the healthy nutrition for consumers. In the food industry biosensors used are mainly proposed to determine various contaminants, also to include some essential food components, like alcohols, sugars, amino acids, lactic acid, phenolic components, malic acid, acetic acid and vitamin C (Eggins 2008). Thus, it is essential to spend in enhancement of food quality biosensors, as these sensors are viable option to traditional analytical methods, like chromatography. Though, only few biosensors play a significant role to control the quality in various food industries. Substantial efforts are necessary to fabricate sufficiently reliable and inexpensive biosensors that will be able to work under real circumstances (Korotkaya 2014).

Important applications of biosensors in the food sector includes:

i. Safety of foods:

   a. Xenobiotics such as additives,fertilizers, pesticides drugs, and other contaminations like: PCB's, dioxins, PAH's, biotoxins and various heavy metals.
   b. Bacterial toxins such as marine toxins, mycotoxins.

c. Pathogens such as viruses, protozoa, bacteria.

ii. Food quality:

a. Food composition like amino acids, sugars, organic acids, alcohols, cholesterol

b. Shelf life: such as polyphenols and fatty acids (rancidity), biogenic amines (for freshness index), sugars and organic acids (maturation), aliina (onions and garlic).

c. Ensures food safety in various fresh poultry, meat or fishes.

d. Measures the concentration of organophosphate pesticides in dairy based products such as milk.

iii. Technological methods such as amino acids (fermentation), sugars (pasteurization and fermentation).

Biosensors in food sector are mostly used to carry out 2 basic purposes. 1st is enzyme based biosensor, i.e. mostly used in beverages and liquor industry to determine carbohydrates from alcohol, amines, amino acids, phenol amides, etc. The given table shows the food components and the enzymes sensed by various biosensors to identify the specific compounds.

Table1: Enzymes sensed by food biosensors

Food Components	Enzyme used
Fructose	Fructose -5-dehydrogenase
Glucose	glucose oxidase
Sucrose	Invertase, Glucose oxidase,
Ethanol	Alcohol Dehydrogenase
Glycerol	Glycerol Dehydrogenase
Lactose	Peroxidase, Galactose oxidase
Essential fatty acids	Lipoxygenase
Cholesterol	Cholesterol Oxidase

(Sarvesh and Kumar, 2013)

The 2nd type of biosensor used in food sector offers the identification of microbes. This includes:

Bioluminesent Biosensor: A genetically modified cell via incorporating reporter gene, and its expression is measured by regulatory protein or a receptor. When an analyte is associated with the cell, the protein receptor is bind and the expression of the reporter gene is activated which results in the formation of mRNa and consequently the reporter protein. The reporter protein measurement gives the analytical signal. Such as. Luciferase reporter phage where the gene encoding Luciferase embedded into the bacterial viruses genome.

Optical Biosensor: These sensors are mainly used to identify bacteria in food system directly. They depict the alterations in refractive index when cell is attached to receptor that are immobilized on the transducer (Tainaka et al. 2010). Such as optical biosensors are resonant mirror, elapsometric, and piezoelectric biosensor.

Electrical impedance biosensor: These sensors determines the electrical impedance of an interface in AC steady state with steady DC bias circumstances. These depends on impedance measure of inter digitized electrode structure or adherently growing cells. Cell growth, cell density on electrode alters the impedance of the biosensor.

Fluorescence labeled biosensor: These biosensors comprises of a receptor element to detect the particular ligand and a signal transduction element to change the ligand-binding event to detectable signals, like chemiluminescence, colorimetric, fluorescence, electrochemical, or magnetic responses. Particularly, fluorescence identification is commonly used technique in the biomolecular imaging because of its higher selectivity and sensitivity, expensive for use, and adequate temporal and spatial resolution (O'Connell et al. 2000).

Flow immune sensors: Most of the assays for microbial detection are based on Elisa using micro titration plates on completing Chromogenic reaction, the quantitative measurement is carried out by Elisa reader such as *E.coli* identification (Rustagi and Kumar 2013).

Microbial metabolism-based biosensors: Microbes have the potential to transduce their metabolic redox reaction to quantify electric signals by utilizing mediator and oxido-reductase reaction.

Table 2: Use of Biosensors for Identification of Toxins

Type of Biosensor	Matrix	Toxins
Electrochemical immunosensor	Cereals	Nivalenol, Deoxynivalenol
	Corn	Fumonisin
	Water	Alternariol, alternariol monomethyl ether
	Corn, Maize, babyfood cereal	Zearalenone
Electrochemical (amperometry)	Rice	Citrinin
	Corn, barley, grapes, milk	Aflatoxins (AFB1, T2, HT-2, AFM1)
	Lake water	Microcystin-LR
Electrochemical	Vegetable soup, Milk, Tomato juice	Ricin
	Soy milk, Watermelon juice, Pork apple juice,	Staphylococcal enterotoxin B
	Milk	Staphylococcal enterotoxin B
	Skim and full cream milk	Botulinum neurotoxin type A
Electrochemical (square wave voltammetry)	Razor clams, Mussels, cockles	Brevetoxin B
	Mussels	Okadaic acid

(Patra et al., 2019)

DETECTION OF MICROORGANISMS

Microbial contamination is one of the major contaminations that can be controlled, but the type of contamination which is publicly handled as food poisoning takes its toll on health and can result in mortality (Rajapaksha et al. 2019). Traditional methods to specify and determine microorganisms takes more time and are laborious. They depend on solid media's colony counts and also involve certain enhancement and various isolation phases on selective media. The validation of the detection of the specific microbe is attained using microscope, immunological and biochemical properties, leading to the identification times of few days that is the main drawback of traditional plating techniques. Though, enhanced analytical processes are

developed that primarily utilize the benefits provided by t-based or immunological techniques for the past years. Biosensors are becoming more significant to determine various microorganisms. Few specific antibodies are formed against surface antigens of number of microbes. An immunosensor in this way have the ability to discriminate between various microbes. On combining various transducers (such as optical fibers or piezoelectric materials) antibodies are significantly used to detect microorganisms. Main applications are concerned on confirming the absence of pathogenic organism like L. monocytogenes, *E. coli,* and *Salmonella* species as these pathogens cause foodborne health related outbreaks (Velusamy et al. 2010). Various strains of *E. coli* O157:H7 are mostly hazardous human pathogenic microbes and are worth to mention that they could lead to critical situations such as hemorrhagic colitis, bloody diarrhea, meningitis and renal failure (Kuhnert et al. 2000). Detection of Salmonella using SPR-based assays using antibodies as bio- recognition component is investigated by numerous researchers (Vaisocherová-Lísalová et al. 2016; Singh et al. 2015). SPR-based optical fiber sensors are also used to identify Salmonella (Romanov et al. 2011). An aptamer-based electrochemical biosensor to detect Salmonella has also been established (Ma et al. 2014). An improved SPR system has also been developed to identify E. coli within 20 min, it is cheap and label free (Tawil et al. 2012). A biosensors employing a ferrocene-antimicrobial peptide conjugate on the surface of gold based on impedance can also be used to identify E. coli (Li et al. 2014). Electrochemical biosensors can be used to identify Listeria cells rapidly (Tolba et al. 2012). Hybridization reactions by covalently immobilized DNA probe have developed a paper-based microfluidic system to identify L. monocytogenes possessing more reliability and sensitivity (Liu and Zhang 2015). These sensors have also been used to estimate oxygen stress in microbes like Campylobacter jejuni, Clostridium perfringens, and Listeria monocytogenes under certain conditions, causing improvement in safety of foods under vacuum packed and other atmospheric packed systems (Al-Qadiri et al. 2015). Recently, various biosensors are developed to help to control the overall quality food processing industries by identifying microbes in few minutes of sampling. If pathogenic microbes are present on- or near-line biosensors, the food processors could take decision speedily regarding various treatments, reducing the presence of contaminations in final food system (Velasco-Garcia and Mottram 2003).

QUALITY CONTROL OF MODIFIED ATMOSPHERE PACKAGES

Inadequate temperature abuse or package design while vegetables and fruits handling in modified atmosphere package to be subjected to less, injurious O_2 percentage results in quality loss of fermentation volatile compounds and ultimately breakdown of product (Velasco-Garcia and Mottram 2003). Extreme less O_2 in package also encourages expansion of various pathogenic microorganisms (such as *Clostridium botulinum*).The identification of ethanol gives subtle methods for less-O_2 injury detection. A commercially available ethanol biosensor comprising of chromagen and immobilized enzyme: peroxidase and alcohol oxidase are being tested. Alcohol oxidase increases oxidation rate of ethanol into acetaldehyde and Hydrogen peroxide in presence of oxygen and peroxidase increases the oxidation rate of the chromagen resulting in color change. The biosensor identifies ethanol to the level lesser than the human olfactory threshold (l0µl/l) ethanol in gaseous phase at 50° with the exposure of 15seconds. The inception of less O_2 injury was identified in slightly processed cabbage, broccoli, lettuce, and cauliflower modified atmosphere systems as detected by accretion of headspace ethanol (Smyth et al. 1999). The biosensor response was almost same to the one detected by gas chromatography technique, that is costly and needs technically expert operators. The biosensors are used to determine ethanol under controlled atmospheric storage of apple varieties, rot production in potato tubers and also helps to control quality loss related to the ethanol accumulation.

Fresh fruit and vegetables

In industry fresh produce demonstrates number of challenges encountered throughout the entire food industry in generally implementing archaic QC practices (Manikas 2002). For instance, the quality of low processed or /fresh-cut foods is firstly

evaluated by appearance; other essential quality parameters involve smell, taste, as well as texture. All of these 4 quality parameters could be evaluated either objectively or subjectively. Usually 10% of fruits intakes are discarded by fruit processors. Superior choice via enhanced quantitative QC at less-cost would certainly lead to the improved overall quality for intact as well as minimum processed fruit-based products. Enhanced quality control would possibly, in the short-term, cause greater denial and decrease the chances of concession for fruit processors. This concession is done when fruit could be utilized and is likely to sustain a cost penalty because of eater quality control costs. Enhanced QC leads to the ca. 25% saving on concessions for fruit processors (Cockerill, M. Orchard House Foods, United Kingdom, Personal communication, 2004; Terry et al. 2005). The biosensors immobilized with enzymes such as alcohol peroxidase and alcohol oxidase and a chromogen can be used to recognize injuries caused due to less oxygen in lettuce, broccoli cauliflower, and cabbage which are lightly processed and packed in modified atmosphere. These biosensors can also be used to determine ethanol during apples storage in a controlled atmosphere, the decay in potato tubers (Castillo et al. 2003), or in various other applications where accumulation of ethanol is related to the loss of quality. These biosensors are also used to detect various organic acids and sugars as maturity index in fruit and vegetables (Cañas and Macias 2004). Fruit sugars are the major soluble compounds in fresh foods which are essential for flavor. Composition of sugar as well as total titratable acidity could influence flavor and might control cellular pH, affecting the fruit pigments appearance in the tissue while processing. These parameters act as freshness/ ripeness indicators and can also be detected with the help of biosensors. A sensor with CD-MnO2 based fluorescent recovery detects vitamin C/ ascorbic acid, it can also be applied to determine various analytes in matrices like fruit juices, fresh fruits, vegetables (Liu et al. 2015).

Monitoring of wine quality

Wine, a complex mixture of various components, present simultaneously, at varying concentration. The chief components being ethanol, water, sugars, glycerol, certain ions and organic acids. Except glycerol and ethanol, amino acids and phenolic compounds, other aliphatic and aromatic alcohols, are present at very less concentrations. The 3 Pyrroloquinoline quinone (PQQ)-dehydrogenases such as alcohol dehydrogenase (ADH), glucose dehydrogenase (GDH), and glycerol dehydrogenase (GlDH) recently extracted and purified from Gluconobacter spp or Erwinia spp. are utilized to form biosensors to determine major components present in wine. The important enzyme substrate (glycerol for GlDH, glucose for GDH, and ethanol for ADH) is initially oxidized and the enzymes cofactor is decreased simultaneously. The enzyme in its active form is redeveloped through the association with the electrochemical mediator (Os modified redox polymer), that can be conserved in its oxidized form by applying potential at the electrode. There are Various compounds are responsible for unpleasant flavors and aromas in beverages and these could be detected with biosensors, such as 2,4,6-tricloroanisole in wine (Varelas et al. 2011), that is associated with wine bottle corks, whose presence may cause considerable losses to the wine industry.

Antioxidant and heavy metals detection

Antioxidants are the essential compounds which protect food parameters by inhibiting oxidation occurring during processing, distribution as well as end preparation of food product. Various antioxidants are incorporated into the foods to raise the food attributes. Amperometric biosensors have the potential to determine various antioxidants present in food products (Mello and Kubota 2007). The heavy metal ions can spread from industries to the environment and can lead to the major public health risk. These metals are non-biodegradable in nature and have higher density than iron, such as mercury (Hg), cadmium, and lead (Pb). The main sources of these metals are chemical fertilizers, emission from vehicles, or lead-acid batteries (Gammoudi

et al. 2010). In order to guard environment and human well-being, the essential measures need to be taken to eliminate these heavy metals from food system. Microbial biosensors are potentially used to identify heavy metal ions at cheaper rates with higher sensitivity. These biosensors use genetically modified microbes and enzymes like glucose oxidase, urease, alkaline phosphatase, cholinesterase, peroxidase and ascorbate oxidase (Tsai et al. 2003), incorporated to electrochemical and optical transduction systems. The microbial fluorescence-based biosensor devices (Amaro et al. 2014) utilizes reporter genes which reacts only when biochemical reactions take place between inducer molecules and cellular reporters. The association of microbial biosensors and a chemostat-like microfluidic platform is significantly used to identify molecular analyte on a chip (Kim et al. 2015). A combination of DNA optical biosensor combined and evanescent wave is potentially used to identify heavy metal ions rapidly (Long et al. 2013). Also, an on-chip label-free sensing devices are developed to identify heavy metals like cadmium-chelate conjugates with high throughput (Yan et al. 2016). The enzymatic biosensors could be used to recognize enzymes inhibition in water. Enzymes such as alkaline phosphatase and acetyl cholinesterase are inhibited due to heavy metals like carbamates and organophosphates

CONCLUSION

Quality control is a main thrust area in the food industry, the requirement for quick techniques to monitor the food quality is urgent. Advancement of proficient sensors speed up the quality monitoring process, and are cost effective also. Various scientific and technological obstacles needs to be addressed prior the nano-biosensor's benefits can be efficiently used in contaminant identification in foods. To overcome the challenges of biosensor technologies and their application in food matrix, a more detailed study regarding the interaction of food system and technological aspects needs to be analyzed. Developments of future sensors should focus on multiple-analyte identification combined with signal transmitters for remote sensing. These advancements will significantly accelerate various application bases for the food sector, and thus retains certain standard levels of animal, human, and environmental health for a speedily changing world.

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