Enzymes in Food Biotechnology
Production, Applications, and Future Prospects

Edited by Mohammed Kuddus
ENZYMES IN FOOD BIOTECHNOLOGY
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Dedicated to my beloved family
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   HITA RASTOGI, SUGANDHA BHATIA

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Biosensors for Food Quality and Safety Monitoring: Fundamentals and Applications

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40.1 INTRODUCTION

The food industry has grown rapidly over the last 50 years to meet the needs of the population as well as react to changing lifestyles. The consumption of ready-to-eat foods makes food as an important issue because it has socioeconomic and health impacts (Kurbanoglu et al., 2017). Food scientists and the food industry are responding by way of developing strategies and technologies for rapid, sensitive, reliable, and cost-effective analytical methods to determine the presence of foodborne pathogens and contaminants (Ibrišimović et al., 2015; Luong et al., 1997). Biosensors represent an important tool in food analysis. Their advantages over traditional methods such as chromatography, spectrophotometry, etc. are due to their economics, their ability so save time, their real-time monitoring, and, above all, their lack of need for highly trained personnel (Mello and Kubota, 2002). Basically, biosensors use biological components known as a bioreceptor and a transducer, which convert the bioreceptor response into an analytical signal. Biological binding reactions can be performed by various interactions such as enzyme/substrate, antigen/antibody, receptor/ligand, chemical interactions, and nucleic acid hybridization (Monosik et al., 2012; Thakur and Ragavan, 2013). However, recent advances in protein engineering present opportunities to improve the specificity of the target as well as the sensitivity by modifying the structure of proteins, ligand binding, etc. That will be dealt with in this chapter.
40.2 PRINCIPLES OF BIOSENSORS

The term “biosensor” is used to describe an analytical device based on a combination of bioreceptors such as antibodies, enzymes, tissues, organelles, and cells along with a variety of transducers, namely, electrochemical, thermal, optical, and piezoelectrical, depending on the specific purpose of the biosensor (Paddle, 1996). Biosensors can be classified either by the type of bioreceptor or the transducer (Gaudin, 2017).

40.2.1 Components of Biosensors

40.2.1.1 Bioreceptors

Bioreceptors interact with the substance of interest and the specificity of the interaction determines the efficiency of the biosensor. Bioreceptors are divided into three categories: (i) biocatalytic receptors, including enzymes, whole cells, cell organelles, or tissues; nevertheless, enzyme sensors are widely used due to their high substrate specificity, (ii) bioaffinity receptors such as chemoreceptors, antibodies (immunobioreceptors), or nucleic acids that form a stable and specific complex with their respective ligand, and (iii) hybrid receptors that employ DNA and RNA sequences that are complementary to one specific sequence present in the target microorganism (Mello and Kubota, 2002).

40.2.1.2 Transducers

Transducers convert the biological recognition of bioreceptors into a detectable signal that is measured as well as being proportional to the target concentration. The choice of transducers depends on the type of signals emitted by the bioreceptor. Electrochemical biosensors are based on monitoring electroactive species that are either produced or consumed by the bioreceptor. Transduction is carried out by an amperometric, potentiometric, or conductometric method, depending on the specific compound. The amperometric transducer measures the current produced due to the chemical reaction of an electroactive species to an applied potential, which is directly related to the concentration of the target. However, the selectivity of the amperometric devices is governed by the redox potential of the electroactive species present in the sample. Consequently, this is disadvantageous because the signal measured could be due to the noise from another chemical species. Potentiometric transducers are based on the generation of a proportional potential of the working electrode to the concentration of the active species with respect to a reference electrode. Meanwhile, conductometric biosensors depend on the conductivity changes that occur due to biochemical reactions (Mello and Kubota, 2002). Thermal transducers are regarded as a small microcalorimeter that detects changes in temperature. Because most whole microbial cell- or enzyme-catalyzed reactions are accompanied by heat generation, thermal transducers have broader applicability. A piezoelectric transducer consists of an oscillating quartz crystal immersed partially or completely in a liquid. Based on the changes in the physicochemical properties of the sample, such as viscosity, density, and conductivity, the variations in vibration frequency of the quartz crystal coupled with enzymes, antibodies, and antigens are measured and related to the analyte of interest (Luong et al., 1997). The optical transducers are based on light phenomena such as UV-Vis absorption, bio/chemiluminescence, fluorescence/phosphorescence, reflectance or scattering,
Quality is an important character that determines the growth and success of the food industry. Common biosensors employed and/or developed in monitoring the quality of different food products are discussed here.

40.3.1 Beverage Industry

40.3.1.1 Biosensors for Sugar-Sweetened Beverages

40.3.1.1.1 GLUCOSE

Glucose is an important component of beverages and is present in a considerable concentration (0.7%–6.5%) in sugar-sweetened beverages (SSBs), due to the use of high fructose corn syrup (HFCS), fruit juices, or sucrose (Ventura et al., 2011). Glucose is added in beverages to confer sweetness; however, it represents the major source of calories to the human diet, resulting in metabolic disorders such as obesity and diabetes mellitus (Kahn and Flier, 2000). Consequently, biosensors for glucose determination in food products and humans are the most studied and developed, and account for nearly 85% of the biosensor market (Wang, 2007). Enzyme-based biosensors are the most utilized for the monitoring of glucose. As biological recognition elements, glucose oxidase (GOx) and glucose-1-dehydrogenase (GDH) are widely used. Among these two enzymes, GOx is the most employed due to its specificity, turnover rate, and method of obtaining.

40.3.1.1.2 FRUCTOSE

D-Fructose is a low-cost monosaccharide widely distributed in food and beverages, either as a free sugar or in association with glucose as sucrose, a disaccharide. Nevertheless, its consumption has been related to metabolic diseases (Stanhope et al., 2013). In addition, this can cause health problems in some people due to the deficit of fructokinase (Steinmann and Santer, 2016), resulting in fructosuria. Therefore, fructose monitoring in the beverage
industry is essential both for consumer protection and for beverage quality. d-Fructose-5-dehydrogenase (FDH) is the widely employed enzyme for fructose measurement. However, there are some studies in which hexokinase coupled with fructose-6-phosphate kinase (F6PK) are utilized as the bioreceptor (Bhand et al., 2010).

### 40.3.1.1.3 ASPARTAME

Aspartame is a low-calorie artificial sweetener widely utilized in dietetic beverages because it is 200 times sweeter than sucrose. This substance is composed of aspartic acid, phenylalanine, and methanol, which also can produce ill effects on human health (Balgobind et al., 2016). The neurotoxic effects of phenylalanine and methanol have been well documented, specifically phenylalanine toxicity for people with phenylketonuria (Medinsky and Dorman, 1995; Van Spronsen et al., 2009). Therefore, the determination of aspartame in dietary beverages to monitor permissible levels or to determine its desirable presence in nonaspartame dietetic drinks is essential. The common basis employed by aspartame biosensors is based on the hydrolysis of aspartame and the detection of its metabolites.

### 40.3.1.1.4 ASCORBIC ACID

L-ascorbic acid is added to SSBs for its antioxidant and stabilizing properties, which are desirable characteristics in food products (Varvara et al., 2016). Furthermore, it is a good indicator of beverage freshness because of its easy oxidation (Favell, 1998). A daily intake of ascorbic acid (70–90 mg) is reported to be beneficial for health, such as the activation of an immune response. Nevertheless, a lower or a higher consumption of ascorbic acid can produce negative effects on human health (Hu et al., 2010). Vermeir et al. (2007) constructed a biosensor for ascorbic acid detection on fruit juice samples. Ascorbic acid was measured employing ascorbate oxidase, which in the presence of oxygen produced dehydroascorbate and water. Microplate differential calorimetry (MiDiCal) technology was applied as the transducer in which the enthalpy change for ascorbate oxidase activity was correlated to the ascorbic acid concentration. The biosensor showed a linear range from 2.4–350 mM with a detection limit of 0.8 mM. Later, Barberis et al. (2015) developed an amperometric biosensor for detection of ascorbic acid in fruit juices. This biosensor was designed to simultaneously detect ascorbic acid and the antioxidant capability of either ascorbic acid or phenolic compounds. The biosensor was composed of ascorbate oxidase in combination with fullerene C_{60} (FC_{60}), fullerene C_{70} (FC_{70}), single-walled carbon nanotubes (SWCN), and multiwalled carbon nanotubes (MWNT), with a limit of detection of 0.10, 0.13, 0.20, and 0.22 μM, respectively. However, the ascorbic acid selectivity index was almost zero in sensors with ascorbate oxidase with fullerene while it was 0.13 and 0.22 for the sensors coupled with SWCN and MWCN, respectively. Bioreceptors, transducers, and the characteristics of biosensors used in the beverage industry are presented in Table 40.1.

### 40.3.1.2 Biosensors for Wine Component Analysis

The wine-making industry is very much interested in monitoring substrates and metabolites during the process in order to avoid economic losses. Compounds such as ethanol, glucose, and lactate play an important role in the quality, taste, and flavor of wine. Therefore, they are employed as process control indicators (Goriushkina et al., 2009).
40.3 Biosensors for the Quality of Food Products

40.3.1.2.1 ETHANOL

The monitoring of ethanol in wines is crucial to controlling the desirable levels of alcohol in the final product as well as to avoid their inhibitory/toxic effect on yeast and thereby conserving the quality of wine. Enzyme-based biosensors employed to analyze alcohol utilize

### TABLE 40.1 Analysis of Compounds in the Beverage Industry Using Biosensors

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Bioreceptor</th>
<th>Transducer</th>
<th>Characteristics</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Glucose</td>
<td>Lactate oxidase and Glucose oxidase</td>
<td>Amperometric electrodes</td>
<td>DL = 5 × 10^{-6} M</td>
<td>Wine</td>
<td>Shkotova et al. (2016)</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glucose oxidase</td>
<td>Fiberoptic</td>
<td>LR = 0.05–5.0 mM</td>
<td>Beverages and human serum</td>
<td>Ho et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amperometric</td>
<td>DL = 0.01 mM</td>
<td>Blood samples</td>
<td>Rafighi et al. (2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LR = 1–100 µM</td>
<td>Fruit juices</td>
<td>Ayenimo and Adeloju (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 0.32 µM</td>
<td>Beverages</td>
<td>Gokoglan et al. (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LR = 0.5–24 mM</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 26.9 µM</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LR = 0.02–0.5 mM</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 7.035 × 10^{-3} mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>Fructose dehydrogenase</td>
<td>Amperometric</td>
<td>LR = 0.1–5 mM</td>
<td>Honey, fruit juices, soft and energy drinks</td>
<td>Antiochia et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 1 µM</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LR = 05–0.3 mM</td>
<td>Agave, cola, honey, maple syrup</td>
<td>Siepenkoetter and Salaj-Kosla (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 1.2 mM</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LR = 0.5–6.0 mM</td>
<td>Syrup</td>
<td>Bhand et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 0.12 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartame</td>
<td>Carboxyl esterase and alcohol oxidase</td>
<td>Amperometric</td>
<td>LR = 5 × 10^{-8} to 44 × 10^{-7} M</td>
<td>Soft drinks</td>
<td>Odaci et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 3.68 µM</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>DL for methanol = 0.1 µM</td>
<td>Soft drinks</td>
<td>Radulescu et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL for aspartame = 0.2 µM</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LR = 0.056–3.07 mM</td>
<td>Soft drinks</td>
<td>Xiao and Choi (2002)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Alcohol dehydrogenase</td>
<td>Amperometric</td>
<td>LR = 0.1 to 2.0 M</td>
<td>Wine</td>
<td>Samphao et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DL = 0.07 M</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol oxidase</td>
<td></td>
<td>DL = 0.52 mM</td>
<td>Beers</td>
<td>Cinti et al. (2017)</td>
</tr>
</tbody>
</table>

LR = linear range, DL = detection limit.
alcohol oxidase (AOX) and alcohol dehydrogenase (ADH). The detection of alcohol by AOX is based on its ability to convert low molecular weight alcohols to aldehydes and hydrogen peroxide in the presence of oxygen whereas ADH is more stable and selective to ethanol without the requirements of oxygen. For this reason, ADH is the most used biosensor, although it needs the addition of NAD$^+$ (Azevedo et al., 2005). Pyrroloquinoline quinone-dependent alcohol dehydrogenases (PQQ-ADH) also have been used in alcohol detection (Niculescu et al., 2002). These enzymes do not require oxygen and can directly transfer electrons between their active center and the electrode. PPQ-ADH was coupled with a redox polymer Os-complex-modified poly(vinylimidazole), with poly(ethylene glycol) diglycidyl ether as a cross-linker. This device demonstrated a sensitivity of 0.336 ± 0.025 A M$^{-1}$ cm$^{-2}$ for ethanol and a detection limit of 1 μM while functioning satisfactorily in a flow-injection system.

40.3.1.2.2 MALIC ACID AND LACTIC ACID

Malic acid is an important parameter of wine quality due to its high acidity. Its conversion to L-lactic acid decreases this acidity, contributing to wine freshness and providing stability. Hence both L-malic acid and L-lactic acid are considered for development of methods to test wine quality (Goriushkina et al., 2009).

Giménez-Gómez et al. (2016) developed an amperometric biosensor for L-lactate in wines. This biosensor is composed of lactate oxidase (LOX) and horseradish peroxidase (HRP), immobilized on a three-dimensional matrix of polypyrrole (PPy) with a thin-film gold electrode functioning as an electrochemical transducer. The role of HRP was to hydrolyze the hydrogen peroxide produced by LOX. The biosensor showed a linear range response of $1 \times 10^{-6} – 1 \times 10^{-4}$ M, with a detection limit of $5.2 \times 10^{-7}$ M and a sensitivity of $−13,500 ± 600 \mu$A M$^{-1}$ cm$^{-2}$.

Recently, Giménez-Gómez et al. (2017) constructed a biosensor for L-malic acid detection, employing malate dehydrogenase (MDH) and diaphorase (DP) enzymes as the selective receptor and NAD$^+$ to act as the redox mediator. Similar to the previous study, the PPy membrane was used as an immobilizing agent and a thin-film gold electrode as the transducer, which showed a sensitivity of $1365 ± 110 \mu$A M$^{-1}$ cm$^{-2}$, with a detection limit of $6.3 \times 10^{-8}$ M.

40.3.1.2.3 POLYPHENOLS

Polyphenols are compounds of great importance to wine character and quality because they produce several sensorial characteristics and have a high antioxidant capacity. These compounds are monitored to classify wines according to their taste and color and in some cases, they are employed as indicators of quality alteration. In general, enzyme-based biosensors are utilized, and the common ones used in phenolic amperometric quantification are tyrosinase (Abhijith et al., 2007), peroxidase (Yang et al., 2006), pyrroloquinoline quinine-dependent glucose dehydrogenase (Makower et al., 1996), and cellobiose dehydrogenase (Stoica et al., 2004).

Di Fusco et al. (2010) quantified polyphenol in wines utilizing laccase-based biosensors. Laccase activity was monitored amperometrically by its oxidative action on phenols producing water and an oxidized compound. This method is similar to the method of Folin–Ciocalteau in the determination of polyphenols.

Tyrosinase, which oxidizes monophenols, was employed initially (Adamski et al., 2010), and the authors later (Adamski et al., 2016) developed another one with laccase. In both systems, a glass-carbon electrode was used for the amperometric quantification of phenolic compounds. It was reported that the limit of detection of polyphenols by tyrosine and a
laccase biosensor was 10.3 and 27.4 mg/L, respectively. Unfortunately, interferences by other wine components were observed with both biosensor systems.

### 40.3.1.2.4 GLYCEROL

Glycerol in wines is formed by yeasts for alternative regeneration of NAD$^+$ via fermentation (Wang et al., 2001). Production of glycerol is a 1:10 ratio of the ethanol formed (Mataix and Luque De Castro, 2000). It has been described that glycerol contributes considerably to the taste properties and smoothness of wine, and its sudden diminution can be related to the presence of undesirable microorganisms (Oliveira et al., 2006). Due to the established ratio of glycerol:ethanol, glycerol detection aids in the determination of a possible adulteration of wine or a change in wine quality (Mataix and Luque De Castro, 2000; Šehović et al., 2004).

Monošík et al. (2012) developed a multienzymatic biosensor for glycerol determination in wines, which consisted of five enzymes: glycerol kinase (GK), creatine kinase (CK), creatinase, sarcosine oxidase, and peroxidase. Though these enzymes were immobilized in chitosan, the authors used a gold planar electrode (GPE) for the first biosensor system and nanocomposite-containing MWNTs for the second biosensor system. Gold and nanocomposite biosensors showed a linear range of 5–640 μM and 5–566 μM with detection limits of 1.96 and 2.24 μM and sensitivities of 0.8 and 0.81 nA μM$^{-1}$, respectively; both had a response time of 70 s.

### 40.3.2 Dairy Industry

#### 40.3.2.1 Lactose

Lactose is a good indicator of dairy product quality as well as for detection of mastitis in cows, which decreases the lactose content in milk (Conzuelo et al., 2010). Measurement of this carbohydrate is also crucial in functional foods such as lactose-free milk directed to people with lactose intolerance. A biosensor for lactose detection in milk using GOx and β-galactosidase (β-gal) was tested by Jasti et al. (2014). Lactose concentration was determined by the hydrolytic activity of β-gal on lactose, which produced galactose and glucose. The glucose produced was further oxidized by Gox-producing hydrogen peroxide and was detected by employing a colorimetric method. Both enzymes were immobilized on BSA-coated allyl glycidyl ether (AGE)-ethylene glycol dimetacrylate (EGDM) copolymer. The addition of glutaraldehyde after immobilization provided a high thermal stability to the enzymes, and this aided in lower limits of lactose detection (0.17 mg mL$^{-1}$). Tasca et al. (2013) constructed a new amperometric biosensor for lactose, which was composed of cellulose dehydrogenase (CDH) as the bioreceptor and SWCNT facilitating the direct transfer of electrons. CDH allows this transference because of its two domain structures. At the FAD-containing dehydrogenase domain (DH$\text{CDH}$), lactose is oxidized and electrons are subsequently transferred to the heme b-containing cytochrome domain (CYT$\text{CDH}$). This biosensor demonstrated a detection limit for lactose of 0.5 μM with a linear range of 1–150 μM, high sensitivity (476.8 nA μM$^{-1}$ cm$^{-2}$), and a fast response time of 4 s.

### 40.3.3 Meat Industry

#### 40.3.3.1 Nitrates

Nitrate and nitrite are compounds of special interest in the meat industry because they are used as preservatives against pathogens and as antioxidants at the meat curing process
(Jadán et al., 2017). Nonetheless, these compounds can provoke serious health issues as nitrate in meat is reduced to nitrite, a precursor of the carcinogen nitrosamines (Tannenbaum and Correa, 1985). Hence, the determination of nitrate and nitrite in meat is essential in quality control to find whether their levels are within the permitted levels in the finished product.

Dinckaya et al. (2010) developed a voltametric biosensor for nitrate detection in meat samples using nitrate reductase that reduces nitrate to nitrite. The biosensor recorded a linear range of $5.0 \times 10^{-9} - 90 \times 10^{-9}$ M and a detection limit of $2.2 \times 10^{-9}$ M. Later, Jadán et al. (2017) also utilized a nitrate reductase immobilized on an oxygen electrode and an amperometric detector to evaluate the decline in oxygen concentration to hydrogen peroxide and water, which is proportional to the nitrate amount. The linear range for nitrate was $10–70 \mu$M and the results of this sensor without oxygen interference are comparable with that of the HPLC method.

### 40.3.3.2 Amines

The most common biogenic amines presented in meat are tyramine, cadaverine, putrescine, and histamine (Ruiz-Capillas and Jiménez-Colmenero, 2005), causing toxicological effects in humans such as migraine, headaches, nausea, vomiting, and respiratory and blood pressure disorders (Ladero et al., 2010). Biosensors in the food industry use amine oxidase, xanthine oxidase, or hypoxanthine oxidase as bioreceptors along with either electrochemical or optical transducers. Recently, Omanovic-Miklicanin and Valzacchi (2017) developed two new chemiluminescence biosensors for putrescine in meat. This sensor is based on the enzymatic reaction of putrescine oxidase or diamine oxidase on putrescine in the presence of $O_2$, producing 4-aminobutyraldehyde, $H_2O_2$, and ammonia. Consequently, the hydrogen peroxide produced is the equivalent of putrescine levels and was quantified by measuring the chemiluminescence of the reaction between hydrogen peroxide and luminol. Putrescine oxidase and diamine oxidase biosensors showed a linear range of $1–2$ mg/L with a detection limit of 0.8 and 1.3 mg/L, respectively. Previously, Pospiskova et al. (2013) also utilized a diamine oxidase bioreceptor in fiberoptic biosensors for the detection of biogenic amines. The biosensor was coupled with a ruthenium complex, and the consumption of oxygen due to amine oxidation was determined by measuring the suppression in the fluorescence lifetime of the ruthenium complex. The results obtained were favorable for putrescine and cadaverine detection with a linear range concentration of $0.075–0.1$ mM L$^{-1}$ and a detection limit of 25 and 30 $\mu$mol L$^{-1}$, correspondingly.

### 40.3.4 Bakery Industry

#### 40.3.4.1 Acrylamide

Acrylamide is an aliphatic amide produced during baking at high temperatures due to the reaction between the reducing sugars and the asparagine present in bread. It is a neurotoxicant and a possible carcinogen; regrettably, the mechanism of acrylamide synthesis is still not fully understood. Hence, the improvement in manufacturing practices to avoid acrylamide production is still not achievable and thereby, acrylamide detection is crucial (Stobiecka et al., 2007). Silva et al. (2009) developed a biosensor for acrylamide with whole cells of Pseudomonas aeruginosa with amidase activity, which hydrolyzes acrylamide-producing acrylic acid and ammonia. It was detected potentiometrically with a linear response in the range of $0.1–4.0 \times 10^{-3}$ M of acrylamide and a detection limit of $4.48 \times 10^{-5}$ M.
40.4 BIOSENSORS FOR FOOD SECURITY

The Centers for Disease Control and Prevention (CDC) have estimated that one in six Americans gets sick each year due to the consumption of contaminated foods and/or beverages (Cdc.gov., 2017). Also, more than 9.4 million episodes of foodborne illness and 1351 foodborne bacterial contaminations associated with death occur every year in the United States (Scallan et al., 2011). Food safety is a serious issue that the food industry must face due to the potential harmful agents present in processed food, such as pathogens, pesticides, antimicrobial compounds, allergens, antibiotics, etc. (Luong et al., 1997). Biosensors represent a promising tool not only to ensure food safety at the industry scale during processing and packing, but also be for consumers to verify the quality of the food they consume.

40.4.1 Pathogens

Pathogenic microorganisms are a nuisance not only to human health but also to the health of the food industry. The CDC’s Food Net in 2015 reported 20,098 confirmed infections across the United States, including 4598 hospitalizations and 77 deaths related to pathogen infections per every 100,000 persons. The most common foodborne pathogens were reported to be Salmonella, Campylobacter, Shigella, Cryptosporidium, Escherichia coli, Vibrio, Yersinia, Listeria, and Cyclospora. Conventional methods for the detection and identification of foodborne pathogens are mostly based on microbiological and biochemical identification but are greatly restricted by a long assay time that, in some cases, may take up to several days to yield results due to enrichment requirements. Biosensors for foodborne pathogens should comply with a high sensitivity (ability to detect lower population densities) as a desirable feature because some pathogens are harmful at low densities (Adley and Ryan, 2015). In recent years, biosensors have become an alternative tool over conventional methods due to their ability to perform rapid response analyses, high-throughput capacity, good selectivity, low cost, speed of operation, portability, and the ability to measure samples with minimal sample preparation (Moran et al., 2016). Different bioreceptors and transducers employed for the detection of pathogenic microorganisms present in different samples are presented in Table 40.2.

40.4.2 Pesticides

Although environmental concerns are growing, pesticide use has been estimated at 2 million tons per year (Verma and Bhardwaj, 2015). Pesticides are known for their persistence as well as their accumulation in soil, water, agricultural products, and eventually in biological systems affecting human health (Arjmand et al., 2017). This highlights the importance of detection of pesticides in foods. Organophosphate compounds (OP), inhibitors of the acetylcholinesterase (AChE), are by far the largest class of urban and rural pesticides. Amperometric AChE biosensors are generally used to detect these compounds. They are reported to have a linear range from 1 nM to 5 μM with a detection limit of 0.7 nM for paraoxon and from 5 nM to 1 μM for dimethoate (Lang et al., 2016). Further, they developed a strategy to minimize the irreversible inhibition of cholinesterase by submerging the biosensor in a cholinesterase reactivator solution and by this way, restored more than 95% of the original sensibility of the biosensor. Previously,
### TABLE 40.2 Detection of the Main Foodborne Pathogens in Food Industry Using Biosensors

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Bioreceptor</th>
<th>Transducer</th>
<th>Characteristics</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>Immunosensor</td>
<td>Optical</td>
<td>DL = $1 \times 10^3$ CFU mL$^{-1}$</td>
<td>Turkey breast and chicken drumsticks</td>
<td>Abdelhaseib et al. (2016)</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Aptamer</td>
<td>Impedimetric</td>
<td>LR = $1 \times 10^4$ to $1 \times 10^8$ CFU mL$^{-1}$ DL = 6 CFU mL$^{-1}$</td>
<td>Apple juice</td>
<td>Bagheryan et al. (2016)</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>Immunosensor</td>
<td>Optical</td>
<td>LR = $1.6 \times 10^3$ to $1.3 \times 10^4$ DL = $9.7 \times 10^2$ CFU mL$^{-1}$</td>
<td>Turkey</td>
<td>Sapsford et al. (2004)</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>Immunosensor</td>
<td>Electrochemical</td>
<td>LR = $1 \times 10^3$ to $1 \times 10^7$ CFU mL$^{-1}$ DL = $2.1 \times 10^4$ CFU mL$^{-1}$</td>
<td>Chicken carcass</td>
<td>Che et al. (2001)</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>Immunosensor</td>
<td>Optical</td>
<td>LR = $7.8 \times 10^5$ to $1.3 \times 10^7$ CFU mL$^{-1}$ DL = $7.8 \times 10^2$ CFU mL$^{-1}$</td>
<td>Milk</td>
<td>Sapsford et al. (2004)</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Immunosensor</td>
<td>Optical</td>
<td>DL = Up to 10 oocysts mL$^{-1}$</td>
<td>Drinking water</td>
<td>Thiruppathiraja et al. (2011)</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Immunosensor</td>
<td>Piezoelectric</td>
<td>LR = $1 \times 10^2$ to $1 \times 10^4$ oocysts mL$^{-1}$</td>
<td>Drinking water.</td>
<td>Campbell and Mutharasan (2008)</td>
</tr>
<tr>
<td><em>STEC non-O 157</em></td>
<td>Immunosensor</td>
<td>Potentiometric</td>
<td>LR = $1 \times 10^{-1}$ to $1 \times 10^7$ cells mL$^{-1}$</td>
<td>Lettuce and sliced carrots</td>
<td>Ercole et al. (2003)</td>
</tr>
<tr>
<td><em>STEC O 157</em></td>
<td>Immunosensor</td>
<td>Optical</td>
<td>LR = $4$ to $4.0 \times 10^6$ CFU mL$^{-1}$</td>
<td>Milk, orange juice</td>
<td>Hu et al. (2016)</td>
</tr>
<tr>
<td><em>STEC O 157</em></td>
<td>Immunosensor</td>
<td>Colorimetric</td>
<td>LR = $1.8 \times 10^5$ to $1.8 \times 10^6$ CFU mL$^{-1}$</td>
<td>Water</td>
<td>Park et al. (2008)</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>Immunosensor</td>
<td>Optical</td>
<td>LR = $1 \times 10^3$ to $1 \times 10^7$ CFU mL$^{-1}$</td>
<td>Food products</td>
<td>Sungkanak et al. (2010)</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>Electrochemical</td>
<td>Amperometric</td>
<td>DL = $2.17 \times 10^2$ CFU mL$^{-1}$</td>
<td>Blueberrys</td>
<td>Davis et al. (2013)</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>Immunosensor</td>
<td>Impedance</td>
<td>LR = $3.0 \times 10^3$ to $3.0 \times 10^4$ CFU mL$^{-1}$</td>
<td>Lettuce</td>
<td>Chen et al. (2015)</td>
</tr>
</tbody>
</table>

LR = linear range, DL = detection limit, STEC = Shiga toxin producing *E. coli.*
Nunes et al. (1998) also developed an amperometric biosensor based on cholinesterase (ChE) for the detection of carbamate residues and its limit of detection for aldicarb, carbaryl and carbofuran, methomyl and propoxur in potato, carrot and sweet pepper samples was in the range of $5 \times 10^{-5}$ to $50 \text{ mg kg}^{-1}$. A portable AChE screen-printed electrode biosensor was used to determine the total concentration of organophosphorus and carbamate pesticides in water and food samples with a lower limit of detection of $2 \mu \text{g L}^{-1}$ (Hildebrandt et al., 2008). Iprovalicarb, a fungicide derived from carbamates, has been detected in onion or potatoes by using antibody-based biosensors with a sensitivity of $0.0128–5000 \text{ ng mL}^{-1}$ (Cho and Kyung, 2008). Biosensors have also been developed to determine the presence of herbicides in contaminated ecosystems, agricultural products, and foods. Sulcotrione, an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD), is a group of herbicides widely used to protect corn crops against grassweeds. This herbicide has been detected in water samples by an amperometric biosensor based on the inhibition of HPPD with a lower limit of detection of $1.4 \times 10^{-10} \text{ M}$. This detection limit is in good agreement with European Union legislation, which sets a maximum concentration of $0.1 \text{ mg L}^{-1}$ of drinking water for pesticides, equivalent to $3.10^{-10} \text{ M}$ for sulcotrione (Rocaboy-Faquet et al., 2016).

### 40.4.3 Antibiotics

Antibiotic resistance is growing at alarming rates and is a great threat to human health (Su et al., 2014). Trace amounts of antibiotic residue present in food products could trigger adverse side effects on human health such as allergic reactions in the case of hypersensitive individuals (Conzuelo et al., 2013). As a new screening method, biosensors offer a big opportunity for a rapid and cheap detection of antibiotic residue in food products (Bargańska et al., 2011). A summary of bioreceptors, transducers, detection limits, and types of samples is presented in Table 40.3.

### 40.5 PROTEIN ENGINEERING FOR BIOSENSORS

The most common recognition element of a biosensor is a protein basing its action on enzyme activities, protein interactions, and posttranslational modifications (Wang et al., 2009). However, proteins in their native form are not always able to cover the limits of detection and the level of stability required for a sensor. For this reason, researches have looked for a mechanism to modify these characteristics by manipulating their structure and obtaining a more stable, sensitive, selective, and specific biomediator that can be employed as a biosensor. Protein engineering plays an important role in food industries because, by its application, it is possible to maintain or even enhance the quality of the final products. With recent advances in molecular biology, protein engineering offers the possibility to enhance biomolecules for catalysis, recognition, structural integrity, signaling, locomotion, and defense. Progress in technology gives the advantage of computational predictions to modify or design a protein to acquire or redefine a specific structure, specific function, or novel applications (Arnold, 2001), which later can be translated experimentally. Additionally, computational tools lend more control on biomolecular recognition (Looger et al., 2003). Despite its potential character, three main challenges are to be resolved before developing applications (Damborsky and Brezovsky, 2014; Fowler and Fields, 2014; Tiwari et al., 2012): (i) structure modeling and prediction, (ii) protein stability at the desired conditions, and (iii) protein-protein and DNA-protein interactions.
### TABLE 40.3 Detection on Antibiotics Residues in the Food Industry Using Biosensors

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Bioreceptor</th>
<th>Transducer</th>
<th>Characteristics</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
</table>
| Penicillin G   | Immunosensor| Electrochemical | LR = $3.34 \times 10^{-3}$ to $3.34 \times 10^3$ ng L$^{-1}$  
DL = $2.7 \times 10^{-4}$ ng L$^{-1}$ | Milk        | Li et al. (2015)                  |
| Tetracycline   | Aptamer     | Optical    | LR = 0.20–2.0 μg mL$^{-1}$  
DL = 0.039 μg mL$^{-1}$               | Raw milk    | Luo et al. (2015)                |
| Chloramphenicol| Aptamer     | Optical    | LR = 0.01–1 ng mL$^{-1}$  
DL = 0.01 ng mL$^{-1}$               | Milk        | Wu et al. (2015)                 |
| Thiamphenicol  | Immunosensor| Optical    | DL = 25 μg kg$^{-1}$                  | Kidney      | Thompson et al. (2017) |
| Kanamycin      | Aptamer     | Electrochemical | LR = 1.2–75 ng mL$^{-1}$  
DL = 0.11 ng mL$^{-1}$               | Milk        | Sharma et al. (2017)             |
| Sulfonamide    | Immunosensor| Electrochemical | LR = 0.12–8.41 ng mL$^{-1}$  
DL = 1 ng mL$^{-1}$               | Milk        | Conzuelo et al. (2012)           |
| Streptomycin   | Enzyme      | Electrochemical | LR = 0.05–20 ng mL$^{-1}$  
DL = 10 pg mL$^{-1}$               | Honey/Milk  | Liu et al. (2013)                |
| Nitroimidazole | Immunosensor| Optical    | DL = 1 μg kg$^{-1}$                  | Kidney      | Thompson et al. (2009) |

LR = Linear range, DL = detection limit.
40.5.1 Protein Design

Four methods are considered for protein engineering of biosensors: rational protein design, directed evolution, semirational design (a combination of the two mentioned before), and de novo design. Of the four methods, rational protein engineering and directed evolution (Fig. 40.2) are considered to be efficient in application (Eriksen et al., 2014).

FIG. 40.2 Schematic diagram of rational protein design and directed evolution.
40.5.1.1 Rational Protein Design

Rational design needs prior information on a three-dimensional structure as well as the biophysical data and functions of the protein. It can be achieved either by single-point mutation, exchange of elements of secondary structure, exchange of whole domains, or by fusion of enzymes, creating hybrid enzymes (Nixon and Firestine, 2000; Nixon et al., 1998). Holland et al. (2012) improved the catalytic activity and stability of GOx by site-directed mutations in Aspergillus niger. They did this by combining the genetic elements responsible for the stability of A. niger and modifying its regions of catalytic activity with reference to Penicillium amagasakiense, which has a fourfold greater catalytic rate and sixfold superior substrate affinity. They reported four obtained mutants that demonstrated significantly higher catalytic activity and moderate improvement in stability than the parental A. niger. Other studies have reported the use of rational design to establish a new ligand to proteins. The specificity of biosensors based on proteins depends on the complex formed between the protein and the ligand (Bishop et al., 2000). Looger et al. (2003) redesigned computationally the ligand-binding site specificity of five members of the periplasmic binding protein (PBP) superfamily in E. coli, which binds to sugars or amino acids. The binding sites of the proteins were engineered to recognize trinitrotoluene (TNT), L-lactate, or serotonin and the engineered proteins showed higher detection of these compounds than sugars or amino acids. Nonetheless, Schreier et al. (2009) observed that there were false positive results when they experimentally validated this design, which highlights the importance of experimental studies to validate structure modification, ligand binding, etc. With respect to antibiotic biosensors, Tsang et al. (2015) developed a β-Lactamase-based fluorescent biosensor using site-directed mutagenesis, which offered a sensitive and rapid detection of cephalosporin. They employed the specific interaction between β-Lactamase and its cephalosporin substrates/inhibitors and replaced Tyr150 by serine, which impaired the catalytic activity of the protein and improved the fluorescence stability.

40.5.1.2 Directed Evolution

In directed evolution, experimental simulations using randomly distributed mutagenesis that represent natural genetic and/or protein evolution have been done to study the molecular diversity of the sequence of interest. By this method it is possible to improve protein structure, catalytic efficiency, binding, etc. Over time and through many cycles of mutagenesis and amplification of selected library members, the beneficial acquired characteristics will accumulate, enhancing the traits of the modified organism (Jäckel et al., 2008; Packer and Liu, 2015). Earlier, Sun et al. (2001) reported a 1.7-fold increase in substrate affinity (43 ± 2 against 57 ± 3 mM of the wild-type) and thermal stability of galactose oxidase in E. coli by using the directed evolution method. This method is extensively applied in the food industry, not only to obtain a higher yield and activity but also to replace the ligand for another compound of interest. Wu et al. (2017) used directed evolution in the effector-binding pocket of LacI to design a lactulose biosensor. They obtained a LacI mutant (LacI-L5) specifically induced by lactulose as an altered effector and observed the expression of a green fluorescent protein (GFP) at a concentration of 10 mM of lactulose, while the wild-type, LacI, showed no response.
40.6 CONCLUSION

It is evident that protein engineering is being implemented in increasing the range of targets and sensitivity by biosensors. With further advances in molecular biology and system biology, future studies will focus on the development of new biosensors to detect a wide array of harmful agents in foods with increased sensitivity. Therefore, this will aid in monitoring food quality, food safety during processing until consumption, and, most importantly, on issues related to global human health.

References


Enzymes in Food Biotechnology: Production, Applications, and Future Prospects, presents a comprehensive review of enzyme research and the potential impact of enzymes on the food sector. This valuable reference brings together novel sources and technologies regarding enzymes in food production, food processing, food preservation, food engineering, and food biotechnology useful for researchers, professionals, and students. Food applications include process of immobilization, thermal and operational stability, increased product specificity and specific activity, enzyme engineering, implementation of high-throughput techniques, screening to relatively unexplored environments, and development of more efficient enzymes.

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• Includes recent cutting-edge research on the pharmaceutical uses of enzymes in the food industry

About the Editor

Prof. Mohammed Kuddus has completed his doctorate degree in Enzyme Biotechnology from SHUATS, Allahabad, India. After completing his PhD, he served at Integral University, Lucknow, India. At present, he is working as a professor and chairman of the Department of Biochemistry at University of Hail, Saudi Arabia. Prof. Kuddus’ main research area includes enzyme technology, protein biochemistry, and microbial biotechnology. He has more than 15 years of research and teaching experience, and has published more than 55 research articles in reputed international journals along with 15 book chapters. He has presented more than 36 abstracts at various national/international conferences/symposia. He also worked as an organizing committee member for 7 international conferences/symposia in the related field. He has been serving as an editor/editorial board member and reviewer for more than 10 and 30 international peer-reviewed journals, respectively. He has also been awarded Young Scientist Projects from the Department of Science and Technology, India, and International Foundation for Science, Sweden.