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A REVIEW ON BIOSENSORS AND THEIR APPLICATIONS IN FOOD AND BEVERAGE INDUSTRY

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Abstract

The foremost challenge in food and beverage industry is the need to develop quick and cost effective tools in the detection of contaminants, toxins, non-Halal components and pathogens in the food. For this reason, biosensor is one of the best tool to detect and verify the existence of those components. They are modern analytical devices which consist of bio-receptor, transducer and signal processing to produce specific reaction when in contact with a specific component. Biosensors are recently gaining much interest and widely used in analysis of bio-materials for better understanding of their bio-compositions, structures and functions by converting biological responses into electrical signals. In this review, various biosensors reported in the literatures for the detection of pork in food, detection of alcohol in fermented beverages, monitoring of fructose level and detection of harmful contaminants in dairy products are summarized, highlighting their principles, advantages, and limitations together with their simplicity, sensitivity, and multiplexing capabilities.

Keywords: biosensor devices, bio-receptor, transducer, signal processing, food and beverage industry

INTRODUCTION

Biological sensor or biosensor is an analytical device having potential applications in various fields, such as medical diagnostics, fermentation, pharmaceutical, drugs, food and beverage industry. Food and beverage is indeed a complex industry, consisting a global collective of varied businesses that supply much of the food and is responsible for the food energy consumed by the world population. As the human life style changes, the demand for processed food increases leading to a multiplied production and huge development in the food and beverage industry. This large productions of food and beverage can cause serious contamination if safety measures and precautions are not properly applied and monitored. For example, repeated cases of food poisoning among primary school students in Malaysia were reported after consuming milk which was contaminated by pathogens. In the incidents, contaminated milk was distributed to students during School Milk Program, by the Malaysian Ministry of Education (Zailani & Yusoff, 2008). Thus, to curb such problems from repeating, a reliable device such as biosensor is of utmost important, especially when school children and the human essential needs such as food and beverages are in concern.

In helping Muslim consumers around the world, biosensor can be best applied in food and beverage industry for the detection of Haram and Syubhah ingredients, where Muslims are obligatory to the Islamic dietary law which rely on the *Halalan Toyyiban* concept. *Halalan Toyyiban* merely means allowed and permissible for consumption with relation to Syariah law as long as they are safe and not harmful. By definition, halal food are those that are free from any components that Muslims are prohibited from consuming (Riaz & Chaudry, 2004), as stated in the Quran:

"O mankind, eat from whatever is on Earth [that is] lawful and good and do not follow the footsteps of Satan. Indeed, he is to you a clear enemy"

(Al-Baqarah: 168)

The Quranic verse clearly mentioned that Muslims are prohibited from consuming Haram food, which include pork, animals that are not properly slaughtered, blood, carrion, intoxicants and alcoholic beverages as mentioned in the following verse:

"He has only forbidden to you dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah. But whoever is forced [by necessity], neither desiring [it] nor transgressing [its limit], there is no sin upon him. Indeed, Allah is Forgiving and Merciful".

(Al-Baqarah: 173)

Thus, development of a useful device, such as the biosensor would not just help Muslims around the world to obey the dietary law according to Syariah, but would also serve as a tool in helping other consumers to enjoy food and beverages that are safe from harmful contaminants, prions and pathogens.

Principle and Components of Biosensor

In general, biosensor uses living organisms or biological molecules, especially enzymes or antibodies to detect the presence of chemicals (analyte). According to the International Union of Pure & Applied Chemistry (IUPAC), biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds which are usually accompanied by electrical, thermal or optical signals (Monošík *et al.*, 2012). The major components of biosensor include bio-recognition/bio-receptor, transducer and signal processing (Figure 1). Thus, in the development of a biosensor, the bio-receptor is the key part which must be selective and sensitive enough to detect the presence of analyte, while a transducer is responsible to determine the effectiveness of the biosensor in detecting the analyte.

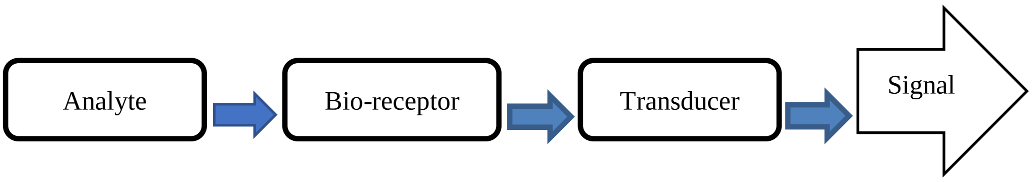


Figure 1. Basic Components of Biosensor

During signal processing, there is a conversion of the physical-chemical parameters occurring on the surface of the sensor due to interactions between the bio-receptor with analyte, which then produces quantitative electrical signal by the transducer. The electrical signal can be changed into analogue signals or digital signals which involve the process of amplification, filtration and correlation induced also by the transducer. Finally, the signal can be correlated to the concentration of analyte which is analysed qualitatively or quantitatively. Referring to the working principle of bio-recognition/bio-receptor, biosensor can be divided into different types as shown in Figure 2 (Kuswandi, 2010).

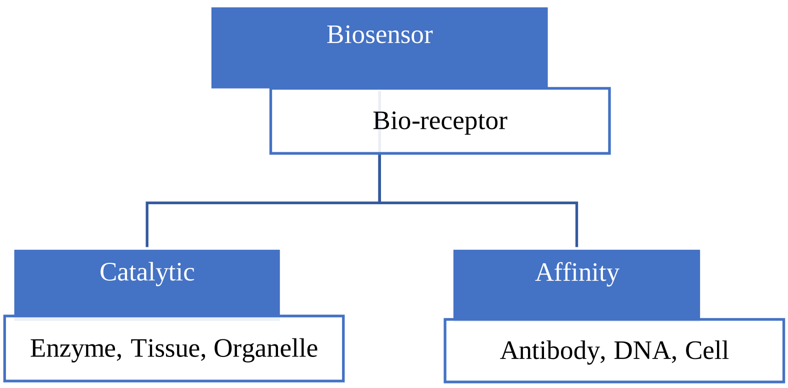


Figure 2. Types of Biosensor Based on the Working Principles of its Bio-receptor

Meanwhile, biosensors can also be classified according to the types of transducer used, and the types of signals and outputs, such as the electrochemical biosensors, optical biosensors, electronic biosensors, piezoelectric biosensors, gravimetric biosensors and pyro electric biosensors.

In line with the application of a biosensor, physical and/or chemical immobilization are the common techniques used to improve performance of biomolecules and the effectiveness of a bio-receptor in a biosensor system.

Immobilization Techniques of Bio-receptor/Biomolecule

In order to ensure proper bio-detection of analyte by bio-receptor/biomolecule, the biological components must be permanently fixed onto the surface of the sensor or transducer. Accurate technique of immobilization ensures high stability of biomolecule and appropriate sensitivity of a biosensor system. Among the various methods of immobilization, adsorption, encapsulation and entrapment are the examples of physical immobilization technique. While chemical immobilization technique includes the covalent bond and cross-linking methods (Kuswandi, 2010).

APPLICATIONS OF BIOSENSOR IN FOOD AND BEVERAGE INDUSTRY

Detection of Pork Adulteration

Pork, an adulterant according to Islam, is most widely used in food as additive, emulsifier, and etc. due to its low prices. It is therefore a serious issue to mix pork or its derivatives in food and beverage as it is prohibited by the Islamic laws. The prohibition of consuming pork in Islam is clearly stated in the following Quranic verse:

Say, *"I do not find within that which was revealed to me [anything] forbidden to one who would eat it unless it be a dead animal or blood spilled out or the flesh of swine - for indeed, it is impure - or it be [that slaughtered in] disobedience, dedicated to other than Allah. But whoever is forced [by necessity], neither desiring [it] nor transgressing [its limit], then indeed, your Lord is Forgiving and Merciful"*

(Al-An'am: 145)

The prohibition of pork is indeed in line with the scientific evidence which proved the existence of harmful bacteria which cannot be easily destroyed. Besides, an individual may suffer allergic effect due to consumption of pork (Che Man *et al*., 2007; Ballin *et al*., 2009). Besides that, the fat content in pork is also relatively higher than in any other poultry meat. This could lead to diabetes and cardiovascular diseases (Ali *et al*., 2011).

In a study by Ali and his co-workers (2011), pork adulteration was successfully detected in beef and chicken meatballs using 20 nm gold nanoparticles (GNPs) as colorimetric sensors. The 20 nm GNPs changed its colour from pinkish-red to grayish-purple, where the absorption peak at 525 nm is red-shifted by 30 - 50 nm in 3 mM phosphate buffer saline (PBS). Adsorption of single-stranded DNA protects the particles against salt-induced aggregation. Mixing and annealing of a 25-nucleotide (nt) single-stranded DNA (ssDNA) probe with denatured DNA of different meatballs differentiated well between perfectly matched and mismatched hybridization at a critical annealing temperature. The probes became available in non-pork DNA containing vials due to mismatches and interacted with GNPs to protect the non-pork DNA from salt-induced aggregation. However, all the pork containing vials, either in pure or mixed forms, consumed the probes totally by perfect hybridization and turned into grey, indicates aggregation. This was clearly reflected by a well-defined red-shift of the absorption peak and significantly increased absorbance in the 550 - 800 nm regimes.

In another study by Ali *et al*. (2012), a swine-specific hybrid nanobioprobe was developed to identify pork adulteration in Halal food industry. As this biosensor work based on the hybridization concept, identification of a targeted DNA required ssDNA probes between lengths of 15 - 30 pb. In this case, *Sus scrofa* (pig) mt-genome was used as swine-specific markers. On the other hand, a hybridized swine nanobioprobe was designed by a 3 nm gold nanoparticles and 18 nt fluorophore-labeled oligo-probe of swine-mitochondrial origin. Schematic description of quantification and operating principles of swine nanobiosensor probe is as shown in Figure 3.

The biosensor managed to identify the probe and quantify the existence of 1% pork DNA in comprehensively auto-claved pork-beef binary admixtures. Hence, this hybrid nanobioprobe could be extensively applied in analysis of pork adulteration in Halal food industry (Ali *et al*., 2012).

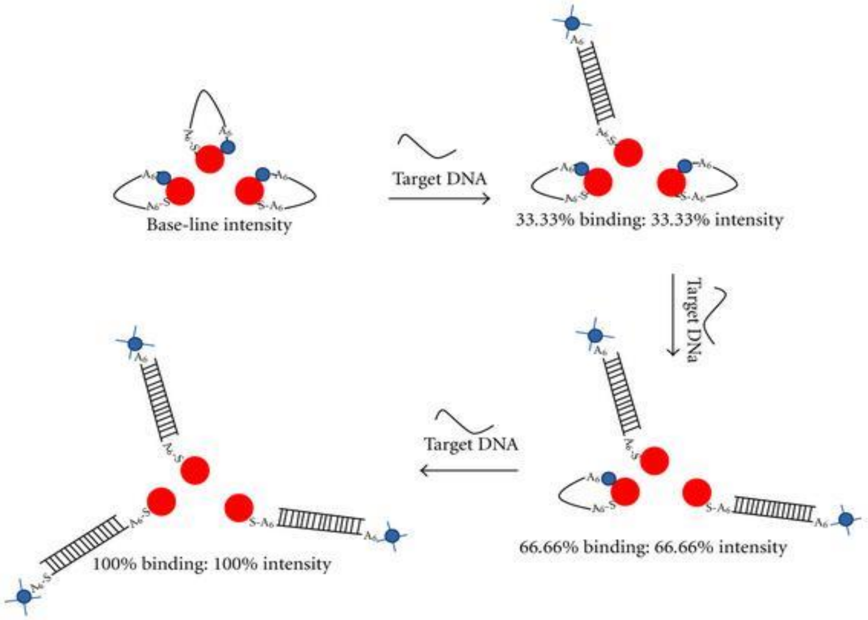


Figure 3. Working Principles of Swine Nanobiosensor Probe (Ali *et al*., 2012)

Detection of Alcohol in Fermented Beverages

Alcohol is a group of organic compounds with various functions in food and beverage industry. They are also sometimes found in food and beverages as a result of natural fermentation. According to the Muslim dietary laws, consuming food and beverages containing alcohol are forbidden due to the intoxicating effects of the compound to the human mental coherence. This was also mentioned in the Holy Quran, in the following verse:

"O you who believe! Do not go near prayer when you are intoxicated until you know (well) what you say"

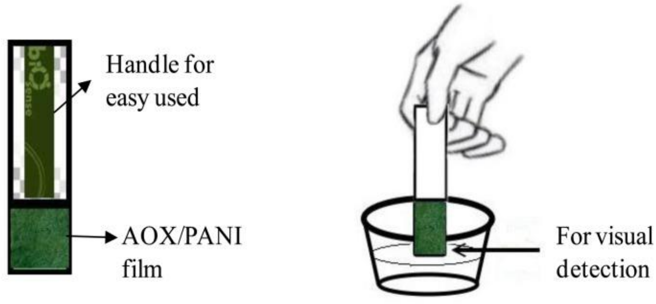
(An-Nisa': 43)

Ethanol is a type of alcohol commonly produced during fermentation or added for the aid of processing in food and beverage industry. Its consumption is often associated to health and social consequences via intoxication (drunkenness) and other biochemical effects in the human body (WHO, 2004).

According to Kuswandi and his co-workers (2001), biosensors are accurate, reliable, simple, low-cost, and rapid tools to detect the presence of ethanol in food and beverages. This can be done by using simple, visual ethanol biosensor which uses alcohol oxidase (AOX) immobilized onto polyaniline (PANI). In the detection, the colour change from green to blue could be clearly observed by naked eye when in the presence of alcohol (Kuswandi *et al*., 2014).

The developed biosensor was in the form of a dip stick that is easier and simpler to handle. Figure 4 depicts the biosensor dip stick made of

AOX/PANI film connected to a transparent plastic tape made of cellulose, which serves as a handle. By just dipping the biosensor stick into a small amount of sample, rapid colour change could be detected visually if the sample contain alcohol. Interestingly, the dip stick was designed to detect ethanol with a minimum concentration of 1%. This is in line with the Islamic dietary law, supported by scientific evidences that excessive consumption of alcohol with concentration higher that 1% can cause harm to the body system (Kuswandi *et al*., 2014).



(a) (b)

Figure 4. Biosensor Containing AOX Immobilized onto PANI in the form of a Dip Stick (a) Used by Dipping the Non-transparent End of the Stick into a Sample for Rapid Detection of Alcohol (b) (Kuswandi *et al*., 2014)

The simplicity of the biosensor lies in the use of molecular oxygen (O_2) for co-factor regeneration. The oxygen is needed by enzyme (AOX) to aid oxidation of ethanol into acetaldehyde and hydrogen peroxide. This chemical reaction is responsible for the color change from green to blue (Kuswandi *et al*., 2014). Since ethanol at concentrations higher than 1% gives bad effects to human health, this simple and rapid identification of ethanol will not only benefit Muslim consumers but also non-Muslim consumers who are conscious over their health.

Monitoring of Fructose Level

Fructose is an inexpensive sugar that is widely used as sweetener in fruit juices, adulterant in honey, carbonated beverage, sports drinks and food for diabetic patients. Its wide use in food and beverage industry is due to the sweetness of fructose, which is greater than the sucrose and glucose. However, high fructose consumption can lead to diabetes and heart diseases.

For the detection of fructose in food and beverages, fructose biosensor can be developed using the basic principle of oxidation of D-fructose to 5-keto-D-fructose by enzyme fructose dehydrogenase (FDH) in the presence of mediator, osmium redox polymer (Scheme 1)

$$\text{D-fructose} + 2Os^{3+} \longrightarrow \text{5-keto-D-fructose} + 2H^{+} + 2Os^{3+}$$

Scheme 1. Oxidation of D-fructose to 5-keto-D-fructose

For a more rapid and reliable monitoring device, a fructose biosensor can be developed based on the modification of a carbon nanotube paste (CNTP) electrode, as proposed by Antiochia *et al.* (2013). The fructose biosensor exhibited a detection limit of 1 μM, with huge linear range (0.1 to 5 μM), high sensitivity (1.95 μA cm^{-2} mM), good reproducibility (RSD = 2.1%) within a fast response time (4 s). Detection of fructose in food and beverages conducted using FDH biosensor showed results which were comparable to the detection done using enzymatic test kits. A high similarity was achieved between those two methods as tabulated in Table 1.

In another study, Bassi *et al.* (1998) developed a fructose biosensor based on the entrapment of the enzyme FDH underneath a thin non-conducting electro polymerized film of 1,3-phenylene-diamine-resorcinol. Two types of electrochemical mediators which were soluble hexacyanoferrate(III) (FeCN) and the water insoluble tetracyanoquinodimethane (TCNQ) were applied as redox mediators for the amperometric measurement of fructose. The use of TCNQ mediated biosensor was compared to chemical assay method, for monitoring of fructose in honey samples. Interestingly, the results obtained showed a good correlation between the use the biosensor and the chemical assay method.

Table 1. Comparison of Fructose Content in Real Samples Using FDH Biosensor and the Enzymatic Test Kit (Antiochia *et al*., 2013)

Sample	Fructose (%)[a]	Fructose (%)[b]
Orange juice	20.82 ± 0.15	23.15 ± 0.22
Apple juice	27.34 ± 0.20	29.12 ± 0.30
Pineapple juice	18.30 ± 0.34	20.14 ± 0.25
Peach juice	29.38 ± 0.52	31.68 ± 0.35
Cherry juice	27.68 ± 0.28	28.94 ± 0.34
Honey (thousand flowers)	36.22 ± 0.44	37.11 ± 0.24
Honey (rosemary)	39.05 ± 0.37	39.98 ± 0.22
Coco-cola	13.95 ± 0.18	15.14 ± 0.30

[a] Fructose level detected using fructose dehydrogenase biosensor
[b] Fructose level detected using enzymatic kit

Detection of Contaminants in Milk

Milk in general, is essential to human health and a major constituent of human diet. It is a main source of vitamins and minerals, especially calcium.

According to Kumar *et al*. (2013), consumer demand for milk and dairy products with stable quality, excellent taste and most importantly, safe for consumption, increased over the years. Monitoring the milk and dairy products before dispatching them into the global market is indeed not an easy work.

This is because, milk might be contaminated during various productions and processing procedures from cow to consumers (Angulo *et al*., 2009). According to Foster (1990), the disease spread through milk contaminants is common and the epidemiological impact of such disease is considerable. Milk are usually contaminated with microbial and non-microbial contaminants which lead to economic setbacks and health problems (Thakur *et al*., 2014).

To overcome this situation, a biosensor based on the use of bacterial spore had been produced. In order to detect the targeted analyte in milk samples within the detection limits as prescribed by the various regulatory authorities, the spore based system must be first developed. Using this method, the response to the presence of targeted analyte based on germination or inhibition of spores could be obtained within 3 - 4 hours (Thakur *et al*., 2013). This type of biosensor enables rapid and sensitive detection of analytes and is expected to facilitate the on-site detection of harmful contaminants in milk and dairy products.

In addition, Kumar *et al.* (2013) stated that spore germination is affected by the presence of microbial and non-microbial contaminants. Antibiotics and aflatoxin are examples of non-microbial contaminants which inhibit the spore germination and prevent the spore from releasing germination mediated enzymes.

Antibiotics, on the other hand, are a group of antimicrobials that are excreted into milk as residues (Thakur *et al*., 2014). Based on the research by Kumar and co-workers (2013), antimicrobial paves its way into the milk due to usage of unapproved antibiotics, extra label dosages and lack of proper treatment records. Once the residue of antibiotics enters the body, it will cause a number of problems such as allergic reactions, decreased antimicrobial susceptibility in bacteria of medical importance and potential spread of antibiotic resistance.

In the work by Kumar *et al.* (2013), the spores were seeded in sporulation medium and were incubated at 64ºC for 2 - 3 hours for germination and outgrowth in presence of specific germinant mixture containing dextrose. This was done when powder and skimmed milk powder were added in specific ratio with reconstituted milk as negative control and milk samples. The antibiotic residues were then detected when there is a change in colour of the sporulation medium from purple to yellow.

Meanwhile, *Enterococci* is an example of microbial contaminants which act on specific complex sugars and convert them into simple sugars by their specific marker enzymes action (Thakur *et al*., 2013; Prandini *et al*., 2009). These simple sugars act as germinants and lead to spore germination. The presence of microbial contaminants in milk will therefore increase the spore germination and this germination process can then be detected. This is known as germinant-germinogenic substrate principle (Kumar *et al*., 2013). This principle which was applied in the development of biosensor to detect contaminants in milk is a novel effort to produce safe and nutritive milk for consumers.

CONCLUSION

In conclusion, biosensors are the most applicable analytical devices to detect analytes due to its favourable characteristics which are sensitive towards targeted analyte, easy to handle, portable and rapid detection. Biosensor technology have and will continue to benefit the expanding food and beverage industry. The application of simple mechanisms and working principle on the targeted analytes to produce very specific signal is useful for consumers, especially in the Halal authentications. This enables Muslim consumers to select food and beverage that are Halal and permissible according to the Islamic dietary laws. Nevertheless, biosensor technology contributes toward improving health of global consumers, enabled through the consumption of safe, clean and nutritive food and beverages, as suggested in Islam.

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