Review article

Journal of Human, Health and Halal Metrics; 2020, 1(2): 94-103
https://doi.org/10.30502/jhhhm.2021.233755.1024

Biosensors as a rapid method for detection of non-halal ingredients in food products

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Submission: 20 September 2020 ***Revision***: 21 April 2021 ***Acceptance***: 2 May 2021

Abstract

Background and objective: Market of halal products has been growing in last decades due to the increasing demand for halal foods by the consumers. Indeed, insertion of halal sign on labeling of food products is a requirement for export of the products to some countries. There are several approaches to identify non-halal ingredients in the matrices such as polymerase chain reaction, Fourier transform infrared spectroscopy, gas chromatography mass spectroscopy, and high performance liquid chromatography, but their complexity and/or high cost of the experiments has led to more attention to biological-based approaches. In this review, we studied biosensors for authentication of halal food products.

Results and conclusion: More than two billion Muslims live around the world that are halal food consumers. Biosensors are biological-based detectors used in food control purposes to identify minor elements. They have advantages such as fast detection of the target agent, portability, cost-effectiveness, and high sensitivity. Biosensors act by converting the chemical reactions to measurable signals. For example, some biosensors contain enzymes able to react with ethanol and diagnose it in the formula followed by a detectable response. Therefore, presence of ethanol which is now allowed in foods by Islam can be monitored by the biosensors within the least time. Moreover, tracing non-permitted meats such as pork meat in the food mixtures, which is commonly determined by gene sequencing techniques such as polymerase chain reaction, can also be detected by biosensors. In conclusion, well-designed biosensors are in-place diagnostic tools which can be considered as alternative to the other time-consuming and expensive tracing methods.

Keywords: Alcohol, Biosensors, Food control, Non-halal meats

1. Introduction

Nowadays, concept of halal in food products is of special importance for Muslims. Along with development of various foods produced by different resources, methods of preparation, and occasionally adulteration in raw materials and/or final products, adherence to halal status is essential [1]. Other than Islamic countries, it is globally accepted that halal mark on the food labels quarantine quality and safety of the products and they are preferred by the consumers even by non-Muslim [2].

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Analytical approaches should be efficient in determination of trace ingredients in foods. Alcohol is produced during fermentation process and its complete removal from the products is impossible. In Islamic countries, a maximum level of ethanol is allowed in Halal foods that is up to 0.5% by volume [3]. Conventional methods for quantification of volatile compounds such as gas chromatography-mass spectroscopy (GC-MS) and high performance liquid chromatography (HPLC) are qualified enough for determination of alcohol in food products. In this regard, HPLC as a specific and sensitive analytical method can determine the ethanol content in foods to find out whether it is in accordance to the permitted level [4–6]. In comparison, no pork meat should be used in food preparation. Fourier transform infrared (FT-IR) spectroscopy is another analytical approach for evaluation of foods. In some studies, sharper peaks were observed for porcine meat compared to bovine meat in FT-IR spectra [7,8]. High sensitivity of HPLC coupled with tandem mass spectrometry was also reported in discrimination of beef and horse/pork meats [9].

Despite high capability of the mentioned analytical methods, they are expensive and needs complicated preparation. In recent years, biosensors have been developed as alternative analytical method to determine the inclusion of alcohol, non-halal meats, and other non-permitted microbial metabolites in a mixture within a short time. These biological systems are selective (by their specific reactions) and high sensitive in identification of the target ingredients that is important in quality control of the products [4,10].

With regard to the importance of rapid and sensitive approaches development in food control, at this article, we reviewed the use and efficiency of biosensors in detection of non-permitted ingredients including non-halal meats and ethanol in food products.

2. Halal concept and its importance in trading in the world

Halal covers a wide range of fields including foods, pharmaceuticals, cosmetics, healthcare services, and travel and tourism. The word of Halal in food industry refers to the foods accepted by the holy Quran and Islam. Indeed, it means the cleanliness of the food from not-allowed ingredients for Muslims [3,11].

Halal food products was produced in Malaysia for the first time. In recent years, many efforts have been made by the Islamic countries for globalization of halal concept, so that it has been welcomed by non-Muslim consumers as well [3]. By 2025, the Muslims population will grow up to 30% of the world's population which will be important for international trade. Commercialization of halal product should be high enough in the next years to respond the global need because the halal products are popular among most of people in the world based on its slogan of high quality and safety [12].

Due to the importance of halal concept in food, FAO/WHO Joint Committee provided an official definition about halal food for the first time in 1997 which focused on production, processing, transportation, and storage of products. Halal products are free of the ingredients forbidden by Islam such as pork and alcohol. On the other hand, their method of preparation and production should be in accordance to the Islamic law. For example, the animals that their meat is halal in nature must be slaughtered based on the Islamic manner [13].

In 2005, global market of halal with regard to food and non-food commodities included $ 2,000 billion a year, of which $ 547 billion was related to halal foods equals 27.35% of the total financial turnover, and it was assumed that halal food market will worth $ 739.59 billion by 2025 [11, 14]. Malaysia is known as the prominent country in halal food in the world. Brazil is one of the main exporters of meat and agricultural crops worldwide. Interestingly, significant amount of the Brazilian exported food commodities are halal. For example, approximately 30% of the exported Brazilian deboned meat, accounted as 1.1 million tonnes, are halal. In addition, among the countries of the Persian Gulf Cooperation Council, Iran and Egypt are the major buyers of the Brazilian meat products [11,13,15,16].

France is one of the prominent exporters of poultry. It is reported that nearly 75% of the exported French frozen meat are halal. They are mainly exported to the Islamic countries such as Saudi Arabia, Kuwait, the United Arab Emirates, and Yemen. In addition, The United States is the third exporter of meat in the world, which exports more than 80% of its products to the Islamic countries. The US exports have halal certificate and it is labeled on the packages. New Zealand is the fourth exporter of beef worldwide, by exporting 40% of its deboned meat (which is certified as halal) to more than 12 Islamic countries [14-17].

Iran as a large Islamic country in the region has a great potential to successfully enter in halal food market. For this, investigation of the current and future situation of the market, the competitors, and strengthening the internal resources are necessary to catch a big share in halal food market [17]. There are various compounds in the food industry which need to be investigated their inclusion in the foods taken by Muslim consumers. The most common tracing approaches are included to genetic-based methods such as PCR, chromatography techniques, and spectrophotometry [13]. However, development of more feasible techniques in term of cost-effective and fast outputs has been requested all the time. Therefore, use of the other techniques able to trace non-permitted ingredients in halal foods within a desirable time at low cost is interested especially in Muslim societies.

3. Biosensors: new diagnostic technology

Biosensors are a group of sensors that are designed to react with specific substances. Product of the reaction provides input for a microprocessor to analyze it. In recent years, biosensors have been improved in several fields. According to the International Union of Pure and Applied Chemistry (IUPAC), biosensor is a tool that uses specific biochemical reactions to detect the chemical elements of a substance, usually electrically, optically, or thermally, through isolated enzymes, tissues, or cells [18,19]. The concept of biosensor was first developed by Dr. Leland C. Clark in early 1960s to measure glucose concentration in diabetic patients by glucose oxidase enzyme [19]. Biosensors include two separate parts: the first is biological part including biological compounds such as antibodies, enzymes, receptors, or microbial cells. The second is the processor which converts the biological signal to optical, audio, electrochemical and symmetric signals (Figure 1).

3.1. Alcohol as target of biosensors

Microbial metabolites and fermentation products are of interested components in food industry and also other technologies. However, they are restricted occasionally due to the presence of alcohol produced during fermentation process that is a critical point in Islam. Some enzymes are able to use ethanol as energy source and convert it to other compounds, that is a basis for alcohol detection by biosensors. The enzymes can act within the living cells without the need of their extraction. For example, species of *acetobacter* are used in fabrication of biosensors and can detect alcohol in range of 0.01 to 0.3 mg/l [4,10]. There are several enzymes able to detect alcohol including Nicotinamide Adenine Dinucleotide-dependent Alcohol Dehydrogenase (NAD-ADH), Pyrroloquinoline Quinone-dependent Alcohol Dehydrogenase (PQQ-ADH), and Alcohol Oxidase (AOx) [20].

ADH is a tetramer and is mostly active in the presence of ethanol. Its activity decreases by change in size of the alcohol molecule.

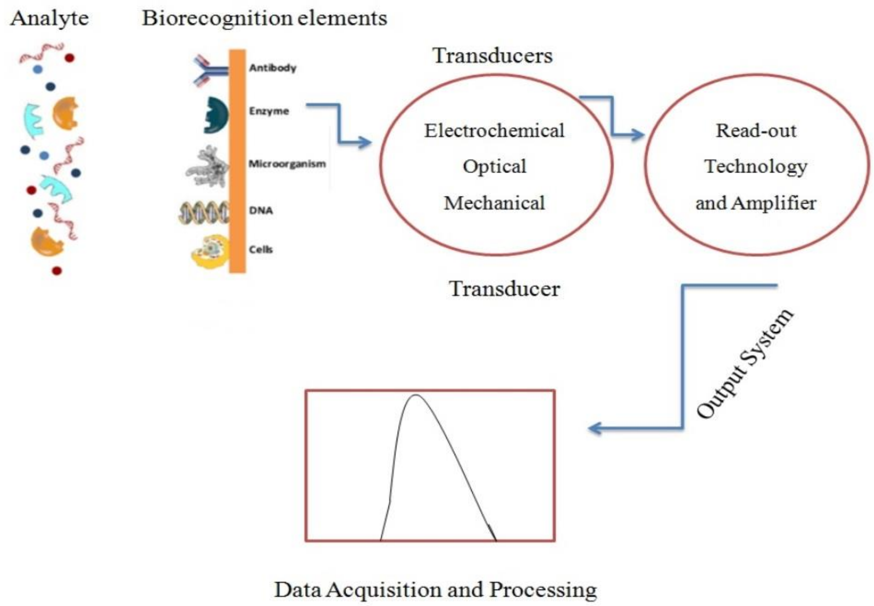

Figure 1- Schematic of a biosensor components

Yeast ADH is widely used in production of alcoholic biosensors. AOX (a complex peroxisomal, oligomeric flavoprotein) is an octamer and contains Flavin Adenine Dinucleotide (FAD) in its subunits as a cofactor. Irreversible catalysis of alcohol to the other carbonic compounds and no need of further addition of cofactor to the reaction medium to complete the reaction have made AOX as an interested ingredient in biological systems [4].

Majority of the enzyme-based biosensors for detection of ethanol operate by NAD^+ (as cofactor of ADH) or oxygen consumption and H_2O_2 production (in case of AOX-based biosensors). In fact, AOx-based biosensors are bounded to the cofactor originally, compared to ADH-based biosensors which need further addition of the cofactor to the sample or its stabilization on the biosensor's surface. On the other hand, AOX-based biosensors are simpler than ADH-based biosensors because they use oxygen molecules to restore the cofactor to its active form. The enzyme requires molecular oxygen to oxidize ethanol and change it to acetaldehyde and hydrogen peroxide. The problem with AOX is its inability to choose ethanol specifically, so that it reacts with short chains primary alcohols and converts them to aldehyde. For example, it converts methanol to formaldehyde that are more toxic than ethanol and acetaldehyde, respectively (Figure 2) [4,10,21].

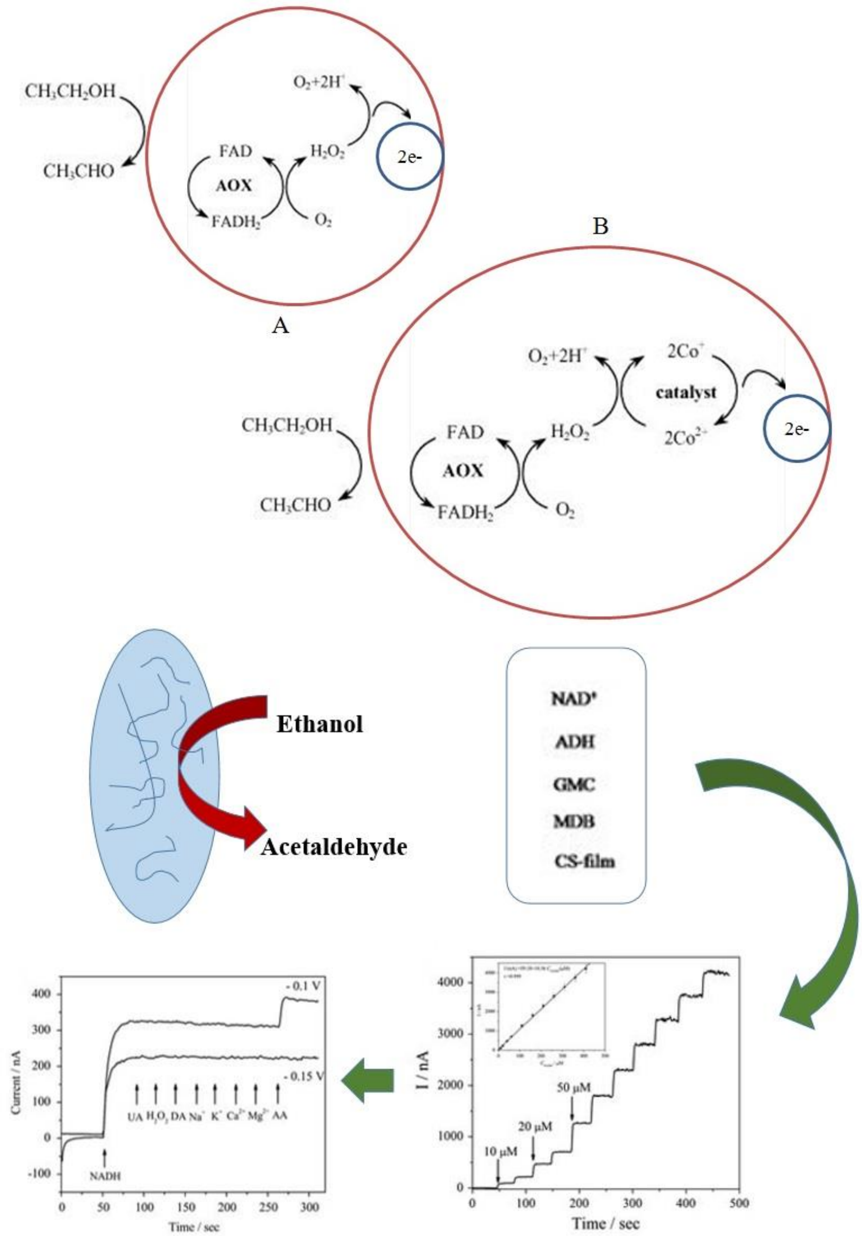

Figure 2- Schematic of ethanol biosensors based on alcohol oxidase (up) including direct and indirect H_2O_2 detection (A and B, respectively), and alcohol dehydrogenase (down); Reproduced with permission from Salimi et al. [10]

3.2. Non-halal meats and their derivatives as target of biosensors

Scientists have studied pork meat and found several germs in its tissue which cause several diseases such as dysentery and paralysis of respiratory muscles. In Europe, about 80% of edible gelatins are produced from pigs' skin, but vegetable-derived, halal, and kosher gelatins produced from seaweeds, fish bones, and non-pig sources are also available [15,16,22].

Addition of pork and its products including lard, blood plasma, collagen, and gelatin to foods is a major concern for Muslims and Jewish. Therefore, it is necessary to discriminate non-halal meats and their by-products in foods in order to confirm their halal status. Development of such diagnostic tools helps better competition in international trade for the countries [23,24]. Determination of animal species in food mixtures are mainly done by DNA or protein analysis [10]. Protein-based approaches are efficient in identification of non-heated matrices, but they are not effective in analysis of thermally-processed foods because of protein denaturation after heating [25]. In addition, in highly processed products such as gelatin, protein-based analyzes may obscure the increasing denaturation of protein markers (is used to estimate the size of proteins in analytic methods) due to extreme temperature and pH treatment during production [12].

High sensitive biosensor for discrimination of pork from beef in ready-to-eat kebabs has been developed [26,27]. Gold nanoparticle (GNP) has been proven as a potential sensor for differentiation of pork from beef and poultry in the products. The developed method by GNP is based on color changes that are visually detectable by the operator (Figure 3). The special characteristics of AuNPs have made it an excellent sensor in biological systems [28,29]. They are easily synthesized by several physicochemical and biological methods [30,31], are stable to oxidation [32], and have great sensitivity owing to their small size [33]. Initially, AuNPs show red color in the solution. Their further aggregation leads to their high absorption [34] and changes the color to blue [35,36].

DNA-based biosensors are valuable tools in determination of halal status of a complex system. In such systems, a fragment of DNA or oligonucleotide is used as sensor. This tool has been developed based on a highly specific hybridization of DNA molecules in the system. On the other hand, a specific reaction between target DNA in the sample and a DNA capture probe immobilized on the electrode surface is conducted [37–39].

Ali et al. introduced a biosensor by using a pig DNA marker (containing 18 nucleotides) to target pig's mitochondrial gene by real-time PCR.

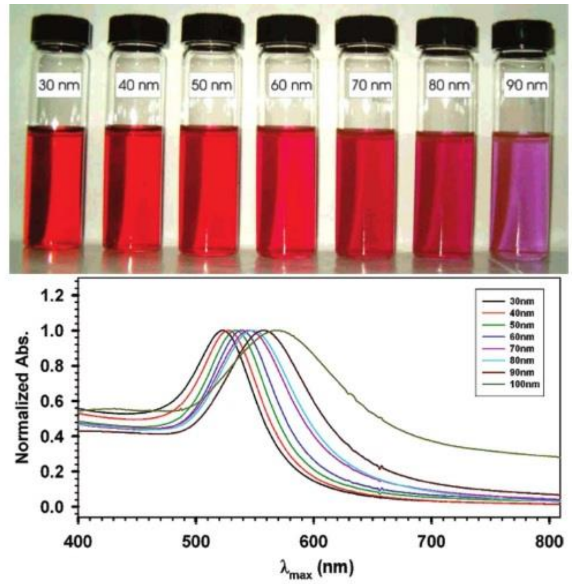

Figure 3- Color of gold nanoparticles at various sizes; Reproduced with permission of Subara et al. [34]

In the absence of the target sequence, the marker protects GNPs from accumulation and a red color is observed in the medium. While the target sequence presents, accumulation of GNPs occurs and a blue color appears. This method was developed to identify pig genes in a pig-cow mixture and was able to detect 1% impurity [26]. In another study, Malaysian researchers distinguished pork from poultry meats by using GNPs with naked eye. Similar to Ali et al., their method was based on accumulation of GNPs and further colorimetric changes [40].

Other scientists developed nano-biosensor for discrimination of swine impurity in formulation of meatball by using pig's cytochrome gene as target. Three oligonucleotide probes formed an arc-like structure by interaction of their thiol group at one end and fluorine group at the other end. The fluorophore group was extinguished by the nanoparticles in the environment in the absence of the target gene. In comparison, in the presence of the target gene, it bound to the fluorophore and changed the structure to rod-like shape. The last hybrid was fluorescent and its emission rate was determined by amount of hybridization. Such system could detect up to 1%

impurity in raw and cooked meatballs. In addition, it was able in detection of 20% impurity visually (without the need for PCR) [41]. Figure 4 shows mechanism of the introduced nano-biosensor. GNPs and fluorophores are presented by red and blue balls, respectively. Moreover, detection of the target sequences and the nucleotide mismatches are shown in Figure 5. Tracing the pork genes within different meat mixtures at 545 nm by Ali et al. in 2014 is also presented in Figure 6 [41].

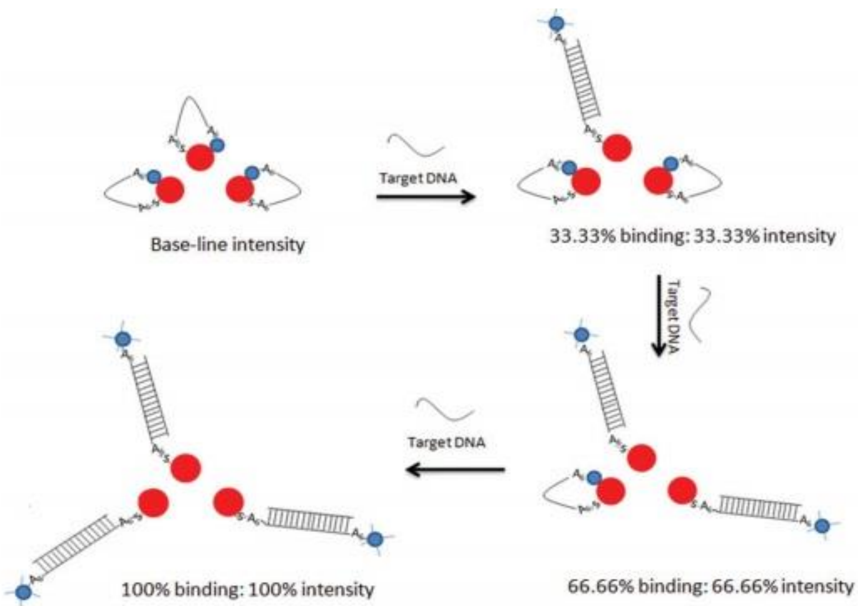

Figure 4- Schematic of a nano-biosensor developed by using swine gene sequence, gold nanoparticles (red balls), and fluorophore (blue balls); Reproduced with permission of Ali et al. [41]

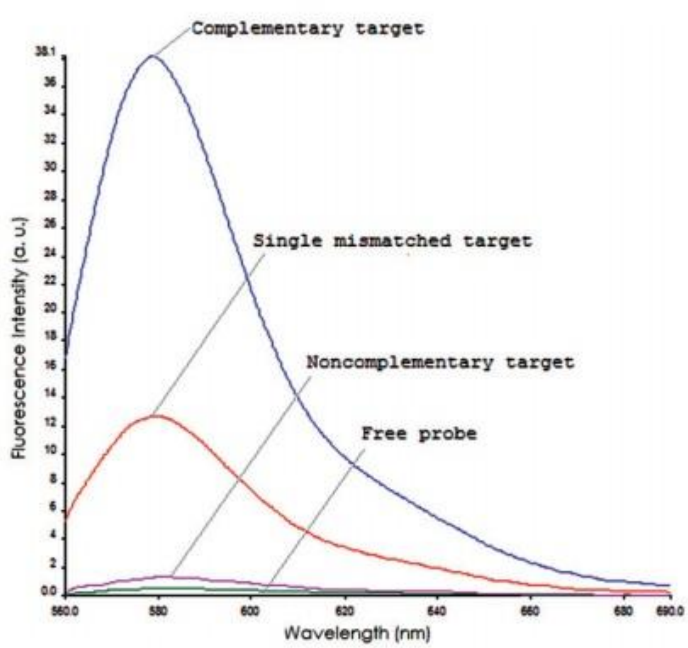

Figure 5- Emission spectra of a nano-biosensor developed by using swine gene sequence, gold nanoparticles, and fluorophore at 545 nm; Reproduced with permission of Ali et al. [41]

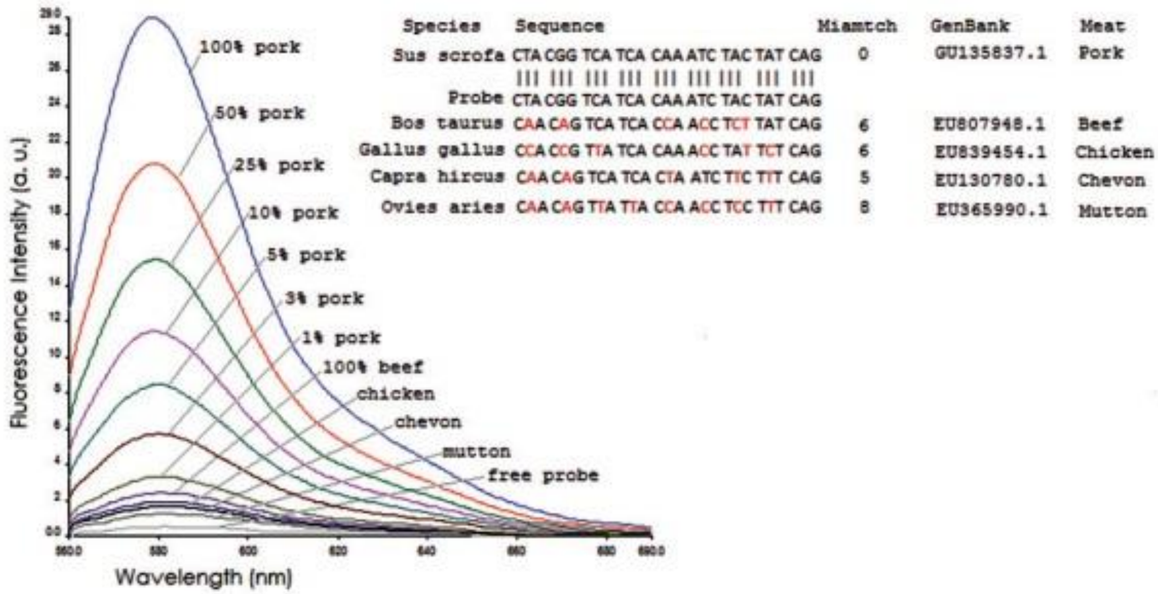

Figure 6- Emission spectra of a nano-biosensor developed by using swine gene sequence, gold nano-particles, and fluorophore at 545 nm. Each spectrum is related to a product containing a different meat mixture than the others. The mismatched bases are shown in red and grey; Reproduced with permission of Ali et al. [41]

4. Conclusion

Halal foods are popular worldwide even among Christian and Jewish. Therefore, there is a need for development of tracing tools to diagnose non-halal ingredients in products. Several analytical approaches are developed in this regard but they are complicated and time-consuming to some extent. Biosensors have been introduced as alternative to the other complicated approaches. They are small, portable, and high-sensitive enough which can measure a molecule, specifically, at very low concentrations. Two common biosensors in detection of trace amounts of alcohol and non-halal meat in food mixtures have been introduced. Alcohol can be detected by enzyme-based biosensors and meats are traced by gene-based biological systems. In some cases, the results can be observed visually with no need for any detector.

5. Conflict of interest

The authors declare no conflict of interest.

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