

Malaysian Journal of Analytical Sciences (MJAS)

Published by Malaysian Analytical Sciences Society



MINI REVIEW: SENSOR AND BIOSENSOR FOR FOOD FRESHNESS DETECTION

(Ulasan Mini: Sensor dan Biosensor untuk Pengesanan Kesegaran Makanan)

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Received: 11 October 2020; Accepted: 30 December 2020; Published: xx February 2021

Abstract

The freshness of food is one of the important qualities that need to be considered by consumers while selecting the food. However, many people are having trouble to determine the level of freshness without the presence of devices or instruments. Therefore, the development of food freshness sensor has become one of the promising analytical techniques, which provides more benefits as compared to other instruments such as gas chromatography mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Food freshness sensors offer faster analysis and most importantly, it is non-destructive to food sample. In this review, we focus on the development and performance of various types of sensors for food freshness detection such as electrochemical sensors, optical sensors, potentiometry sensors and pH sensor. For each type of sensors, there are several main elements that will be emphasized, which include type of samples, reagents used, immobilization matrix and performances of the sensors. A section that discusses the future works, which have the potential for the application in the sensor and biosensor of freshness detection is also highlighted.

Keywords: sensor, biosensor, immobilization matrix and food freshness detection

Abstrak

Kesegaran makanan ialah salah satu kualiti penting yang perlu diambil kira oleh pengguna semasa pemilihan makanan. Walau bagaimanapun, pengguna akan mengalami kesukaran untuk menentukan tahap kesegaran makanan tanpa kehadiran peralatan atau instrumen. Oleh itu, pembangunan sensor kesegaran makanan telah menjadi salah satu teknik analisis yang menjanjikan lebih banyak faedah berbanding instrumen lain seperti kromatografi gas-spektrometri jisim (GC-MS), kromatografi cecair prestasi tinggi (HPLC) dan elektroforesis rerambut (CE). Sensor kesegaran makanan menawarkan analisis yang lebih cepat dan yang paling penting ia tidak merosakkan sampel makanan. Ulasan ini memfokuskan kepada pembangunan dan prestasi pelbagai jenis sensor untuk pengesanan kesegaran makanan seperti sensor elektrokimia, sensor optik dan sensor potensiometri. Bagi setiap jenis sensor, terdapat beberapa elemen penting yang akan ditekankan termasuk jenis sampel, reagen yang digunakan, matrik pemegunan dan prestasi sensor. Bahagian yang membincangkan kajian masa depan yang berpotensi untuk aplikasi sensor dan biosensor untuk penentuan kesegaran juga disorot.

Kata kunci: sensor, biosensor, matrik pemegunan dan penentuan kesegaran makanan

Introduction

Food spoilage has become a major problem in our society as it could lead to many health problems. It is a complex ecological phenomenon caused by physical, chemical and microbiological activities [1]. Physical food spoilage occurs when dried food absorbs excessive amounts of moisture or moist foods are excessively dehydrated. Chemical food spoilage happened when various components in the food react with foreign components resulting in the characteristic changes of food such as enzymatic browning, non-enzymatic browning, and oxidation [2]. Microbiological food spoilage is caused by the microorganisms like yeasts, molds and bacteria. These microorganisms will produce enzymes and produce by-products in the food [1]. Pathogens in food have caused severe diarrhoea and meningitis, meanwhile chemical contamination has led to serious food poisoning and cancer in the long-term period. Therefore, the most important quality that needs to be measured is the food freshness. The freshness of food can be detected physically and chemically where it can be identified physically through colours, textures and flavours of the food [3]. Fresh food is the easiest one to be contaminated by microorganisms as compared to dry and processed food. There are several microorganisms that cause food spoilage such as *Pseudomonas* spp, *Acinetobacter* spp and *Botrytis* [4]. There are also a few compounds found in fresh stock such as proteins, carbohydrates, and fat, which are easily degraded by microbial actions [5].

Consumers can determine the food freshness physically, but it could become a problem when the food is chemically or biologically contaminated. There will be some difficulties in identifying the freshness level without using specific instruments and methods like gas chromatography-mass spectrophotometry (GC-MS) and high-performance liquid chromatography (HPLC) [6]. Although all the detection methods are available and

very accurate in determining the food spoilage, it has several drawbacks which are time-consuming, expensive and inaccessible to consumers [7]. In addition, the use of these instruments required a trained personnel to handle. Therefore, by developing a handheld sensor that will be able to detect food freshness can aid the consumers to measure the freshness easily in a shorter time as compared to by using the high-end instruments. Many researchers have developed various sensors that can determine the qualities of food. Researchers have found that a real-time monitoring of food quality becomes very important to consumers and also to food manufacturers [8]. There are many sensors that have been developed for food freshness detection by using different sensing methods such as optical and electrochemical techniques. Besides that, there are several indicators or reagents that have been used for food freshness detection, namely bromothymol blue (BB), bromocresol green (BCG), curcumin, anthocyanin and enzymes-based reagents, such as xanthine oxidase (XO) [3, 9-11].

The growing research interest on food freshness detection has been shown by many articles related to food freshness since 2010. Based on Figure 1, it shows that the number of publications on food freshness sensors continue to increase from 2010 to 2020. This shows that over the year, many researchers are interested in food freshness sensors as the number of publications are gradually increased. Moreover, there are also several articles that have been reviewed pertaining to food freshness sensors including the fabrication of electrochemical sensors using various type of indicators [12-14] and biosensor development [15-16]. Interestingly, there are few researchers that reviewed the intelligent packaging used for food freshness detection [17-19]. In this review, we will focus on the development of optical and electrochemical sensors or biosensors for food freshness detection.

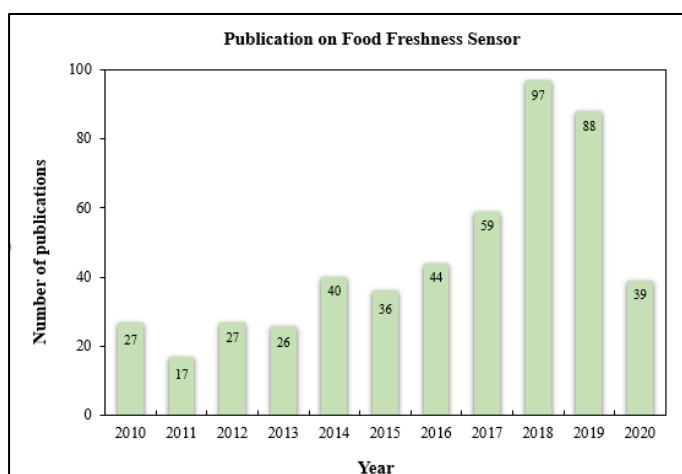


Figure 1. Number of publications on food freshness detection against year. Source taken from Scopus

Sensor and biosensor development for food freshness detection

Many sensors for food freshness detection have been developed by using natural, biological and chemical indicators. The chemical and biological reagents have been immobilized in various types of polymer matrix [20]. Freshness sensors can be defined as sensors that are able to indicate any changes such as pH, temperature, humidity and colour [21]. In addition, a freshness sensor is also sensitive to any changes occurred during degradation of the food, such as colour change, gas produced from food spoilage and bacterial count [20]. Here are two main types of freshness sensors that have been invented, which are direct sensor and indirect sensor. For the direct sensor, it senses the freshness of food directly by indicating the freshness level using ripeness, spoilage and microbial indicators. Meanwhile, the indirect sensors operate indirectly where they measure the freshness of the food by observing the variables such as temperature and storage time [22].

Optical sensor

Optical sensors are analytical devices that have attracted researcher's attention especially in analytical field. Optical sensors are considered a chemical or bio-based sensor that consist of chemical recognition phase (sensing receptors) coupled with optical transducers [23]. Generally, optical sensors are devices that have the capacity to detect and quantify various properties of

lights, such as frequency, wavelength, polarization and intensity [24]. Optical sensors have been widely used in the food industries for food quality measurements, such as pH, temperature and spoilage monitoring [25]. Besides the application in the food industries, optical sensors are also used in environmental monitoring, pharmaceuticals, clinical analysis and biotechnology [23].

In sensors development, the main important parts are receptors and transducers. A receptor is either chemical reagent or biomolecule that can react with analytes while transducer convert the chemical or biochemical signal produced from the analyte reaction and receptor [26]. For optical sensors, the receptors could be established by using various natural (biological) or synthetic (chemical) materials. The receptors are integrated on a sensor interface by many techniques, such as immobilization, molecular imprinting and self-assembled monolayers (SAMs) [23]. Moreover, most receptors are in a thin layer formed which are able to interact with the analyte molecules and selectively catalysed or participate in the chemical reaction. The receptors identify the changes that occur on the analytes like pH or temperature and produce the optical signals, such as colour changes. The colour changes will indicate the condition of tested samples. The optical transducers will translate the signal into readable signals that are suitable for the measurement purposes [27]. Optical

sensors give advantages such as high selectivity, inexpensive, non-destructive to analyte, can easily be miniaturized and possibility for multiple analyte detection.

There are several studies that have been done on the development of optical sensors for food freshness detection on different samples, such as milk, fruit, fish, and shrimp. Weston et al. have synthesized zinc oxide (ZnO) solution with conjugated polymers, polydiacetylene (PDAs) to detect freshness of full cream milk. The PDA/ZnO nanocomposite was prepared by using thin film hydration method and has been immobilized in an agarose to form a disc sensor. PDAs have unique colorimetric properties that make them an attractive building block for colorimetric sensors. In this study, the structure of the PDA/ZnO nanocomposite has been optimized to acquire the optimum pH value recorded from the spoiling milk. The colour of PDA/ZnO/agarose film have changed from blue to red with the increasing pH. Moreover, this sensor was able to differentiate between fresh, spoiling and spoiled milk based on the concentration of lactic acid produced by the milk. PDA/ZnO nanocomposite is a great indicator because it is sensitive towards acid where it will change colour due to disruption of steric forces within the nanocomposite. Lastly, PDA/ZnO also exhibited higher stability in a broad range of solvents than pure PDA. Therefore, it can be casted into various types of materials which is ideal to be imposed in food packaging systems [28].

Maftoonazad and Ramaswamy in their study have designed a pH biosensor that can detect fresh date fruit, *Rutab*. In this study, the sensor film was placed in the packaging where the changes in colour of the film was observed according to the pH changes of *Rutab*. Anthocyanin which is very suitable for colorimetric determination of pH has been extracted from the red cabbage and was immobilized inside polyvinyl alcohol (PVA). The use of anthocyanin red cabbage, which is naturally abundant as the indicator in developing the pH biosensor gives an advantage in terms of lower preparation cost. The pH biosensor developed was found to be reversible, stable and sensitive [10]. Hasanah et al. have successfully developed an optical

pH sensor to determine the fish freshness by using chromoionophore as the reagent. Chromoionophore was immobilized in pectin hydrogel membrane and the pH change on fish was detected based on the protonation and deprotonation of chromoionophore functional groups. Furthermore, pectin hydrogel membrane is suitable and can be used as sensor matrix due to the non-toxic property. In addition, pectin is a hydrophilic polymer, hence it has a higher permeability than hydrophobic synthetic polymer. The hydrophilicity properties can enhance the adsorption of chromoionophore into the polymer, thus allowing a rapid response towards pH change. The protonation in acidic condition (615 nm) and deprotonation in basic condition (535 nm) were analysed by using UV-Vis spectrophotometer. The changes in protonation and deprotonation processes produced an isosbestic point (**Error! Reference source not found.**) where it indicates the optimum absorbance of the optical pH sensor in both conditions. Based on the analytical performance tested, the sensor was able to give a fast response and produce desirable linearity and reproducibility [29].

Additionally, Lee and Shin have developed a freshness indicator to monitor the quality of skate (*Raja kenoeji*) during the storage time. Skate is fermented fish with unique smell that produces ammonia gas from the fermentation process. In this research, they indicate the freshness of skates by measuring the ammonia content produced during storage. The sensor was fabricated by using pH indicator, namely bromothymol blue-phenol red (BTB-PR) and immobilized in cellulose acetate solution. The sensor was coated with polyethylene terephthalate (PET) film to allow the gas to pass through. Moreover, the sensor was coated with polytetrafluoroethylene (PTFE) which acted as hydrophobic gas-permeable membrane to protect the indicator from humidity. From this study, they found out that the pH of the skates increased as temperature and storage time increased, thus changed the colour of the sensor film from yellow (fresh food) to purple (spoiled food). In this study the reagents used were free from leaching and the sensor showed a rapid response [30].

Although PANI is a conductive polymer and widely used for electrochemical sensor, Wang et al. have used PANI to develop a calorimetric or optical sensor to detect the freshness of tilapia fish by detecting the total volatile basic nitrogen (TVBN) released during the tilapia spoilage. In this study, hydrochloric acid was used to prepare doped-PANI. In the presence of spoiled tilapia, the indicator based on doped-PANI changed colour from green to peacock blue. The sensor developed was reusable for at least three times and offer some advantages such as easy to use and inexpensive for large production scale [31].

In another work, Rukchon et al. have developed a colourimetric sensor to indicate the spoilage of skinless chicken breast by using two types of pH-sensitive indicators, which were bromothymol blue and methyl red. The mixed reagents were then immobilized in polyethylene glycol. The films have been placed in the packaging of the chicken sample. The spoilage of the chicken was observed by the colour changes, which correlated to the amount of CO₂ produced by the spoiled chicken. Moreover, this sensor can also respond towards the temperature changes and microbial growth [11].

Kuswandi et al. reported an optical sensor for monitoring the shrimp spoilage based by using curcumin as reagent. The curcumin is a natural dye pigment that is isolated from herbs named *Curcuma longa*. Curcumin was immobilized in the bacterial cellulose membrane polymer matrix by absorption. The curcumin/bacterial cellulose membrane showed a colour change from yellow to orange with the increasing pH and then to reddish orange, which indicated the spoilage of the shrimps. Curcumin which is highly sensitive to acid-base reactions provides a simple method and this has enabled the mass production of low costs sensors [3].

Electrochemical sensor

Electrochemical sensors are one of the oldest chemical sensors that have been developed due to their advantages that can be miniaturized and simple [32, 14]. In general, electrochemical sensors are devices that provide information about composition in analyte by coupling a chemically selective layer (recognition elements/receptors) to an electrochemical transducer

[33]. Recent studies have shown that besides using chemical reagents as recognition elements, biological materials like protein and DNA also have become common because usually they are much more specific. Electrochemical sensors have been used in numerous applications due to its sensitivity, selectivity and ability to analyse samples with slight or no pre-treatment. One of the applications of electrochemical sensors in the forensic field where the sensors are developed for drug and poison detection [34]. In addition, the advancement of electrochemical measuring systems and bio-analytical chemistry has led to the development of electrochemical sensors in agricultural, environmental, medical (diagnosis of genetic disorders) and food analysis (genetically modified organism (GMO) content in food and freshness level) [32, 12].

Basically, electrochemical sensors are based on redox reactions where it involves the target analyte and three-electrode configuration which are working electrode, reference electrode and auxiliary electrode [35]. Electrochemical sensors can be divided into several types based on the signal transduction namely amperometric, impedimetric, potentiometric and chemometric [36]. Therefore, several studies have been reported on the development of electrochemical sensors for measuring the fish freshness. Thandavan et al. have reported the development of iron oxide (Fe₃O₄) nanoparticles-based electrochemical biosensor for quantitative determination of xanthine using amperometry technique. Xanthine is produced from degradation of nucleotides by dead organisms, such as fish. Therefore, the quantification of this molecule is suitable for determining fish freshness. In this study, they have used Xanthine Oxidase (XO) as reagent and it was covalently immobilized onto Fe₃O₄ nanoparticles which act as an interface between the reagent and electrode. The working electrode was fabricated by electropolymerization of XO/Fe₃O₄ on the gold (Au) electrode to promote higher electron transfer. Based on the study, it was found that the biosensor was able to determine the concentration of xanthine in the range of 0.4 to 2.4 nM. This biosensor showed better performance with higher sensitivity, mediator-free and less interference due to excellent electron transfer pathway by XO/Fe₃O₄/Au modified electrode [37].

Dervisevic et al. reported a study on developing amperometric enzyme-based biosensor to detect the fish freshness. In this study, they have developed a polymeric mediator by mixing two redox copolymers which are vinylferrocene (VFc) and glycidyl methacrylate (GMA) with multiwalled carbon nanotube (MWCNT). These polymeric mediators were used due to its excellent electron transfer properties. XO was immobilized in pencil graphite electrode (PGE) coated with GMA-co-VFc and MWCNT, (PGE/P(GMA-co-VFc)/MWCNT). Based on the result, the current response increased with the increasing of xanthine concentration produced by fish in the concentration range of 2-48 μM . The use of MWCNT in the development of this biosensors has offered some advantages such as higher sensitivity and selectivity towards xanthine analysis [38].

Dolmac et al. also reported the fabrication of amperometric biosensor for fish freshness. In their study, XO was immobilized in polypyrrole-polyvinylsulphonate (PPy-PVS) film by entrapment method to determine the level of hypoxanthine (Hx) in fish. In this study, XO/PPy-PVS was electropolymerized on the platinum (Pt) electrode and the electrode act as the working electrode. The level of Hx was electrochemically monitored based on the oxidation of uric acid liberated during enzyme activity on the electrode surface. Based on the finding, this biosensor was able to determine the concentration of Hx from 1.0×10^{-7} to 1.0×10^{-3} M. In general, this biosensor provides a simple and rapid detection with a low detection limit [39].

Bourigua et al. have prepared amperometric and impedimetric biosensors to detect trimethylamine (TMA), a volatile compound that is also one of the parameters to determine fish freshness. In this study, the reagent used was flavin-containing monooxygenase 3 (FMO3). FMO3 is a NADPH-dependant enzyme that catalyses the oxygenation of TMA to trimethylamine N-oxide (TMAO). The conducting polymer (CP), polypyrrole was substituted with ferrocenyl and used for enzyme immobilization. CPs can be considered a good polymer matrix for enzyme biosensor because it can preserve the catalytic activities of enzyme from any

alteration. Meanwhile, the ferrocenyl group was used as a redox probe due to its higher sensitivity towards electrochemical response. The copolymer film has been electrodeposited on Au working electrode by chronoamperometry method. This technique was used to control the thickness and reproducibility of the film. In this study, FMO3 was attached to the polymer-modified electrode by covalent immobilization where the amine group from lysine was bound to N-hydroxyphthalamide. This biosensor showed a linear response in the range of $0.4 \mu\text{g mL}^{-1}$ to $10 \mu\text{g mL}^{-1}$ and $0.4 \mu\text{g mL}^{-1}$ to $80 \mu\text{g mL}^{-1}$ for both amperometric and impedance methods. The charge transfer resistance for impedance was increased while the current response for amperometric has decreased with the increase of TMA concentration. The fabricated biosensor showed high selectivity and exhibited a broad linear range for the evaluation of fish freshness [40].

Potentiometry method is the measurement of potential difference between indicator and reference electrode [41]. Generally, potentiometric method can be considered as a simple method and are suitable for the development of electrochemical sensors to determine the food freshness [33]. Basically, potentiometric sensors consist of ion-selective membrane that measures the changes in membrane potential between two electrodes when in contact with the analyte solution. Ion selective electrodes (ISEs) are electrochemical ion sensor that convert activity of desired ion into electrical signal and coupled with reference electrodes for potentiometry application at zero current condition [42]. The ISEs membranes can be made either from solid, liquid or glass [43]. The most familiar ISEs are glass electrodes for pH measurements, which respond to H^+ concentration in analyte solution. Generally, ISE is a non-destructive method and it is considered inexpensive.

In a study conducted by Park et al., they have developed a flexible potentiometric pH sensor that can measure the pH for a spoiled milk and curved surface apple. The pH sensor has two-electrode configuration which were **polyaniline (PANI)** nanofiber array-based sensing electrode and Ag/AgCl reference electrode. The working (carbon) and reference electrodes were

produced by using screen printing technique on a flexible polyethylene terephthalate (PET) substrate (**Error! Reference source not found.**). PANI nanofiber array was deposited on the surface of carbon electrode through chemical polymerization method. The use of PANI as electrode provided higher stability to the pH sensor because it enabled redox reactions to happen. The performance of the pH sensors showed that the pH decreased from pH 7.1 to 6.2 after 48 hours which indicated the spoilage of the milk. This is because spoiled milk contains higher acidity due to amount of lactic acid produced. Meanwhile, for the pH determination of apple showed that the spoiled apple has lower acidity than the fresh apple. The advantage of this sensor is it demonstrated excellent selectivity and flexibility as compared to standard pH meter because it can measure the pH level of dent surface on fruits [44].

Kaneki et al. have developed a potentiometric gas sensor that were used to determine the freshness level of salmon and sardine by measuring the response from

volatile gases compound produced from decomposed fish. They have configured a three-sensor system that included oxidation-reduction (ORP) gas sensor, ammonia gas sensor and hydrogen sulphide gas sensor. In this study, the ORP-sensor was developed by themselves meanwhile the other two sensors were obtained commercially. ORP-sensor consists of reference electrode (Ag/AgCl), Au detection electrode and inner solutions (Ce^{3+} , Ce^{4+} and KCl). Based on the study, the gas sensor measured dimethylamine (DMA) and TMA that were produced from decomposed fish. In general, it was found that the potential change between electrodes increased as the storage time increased. This indicates that the emission of volatile compound increases with the deterioration of fish freshness. Potentiometric gas sensor was successfully developed, and it showed high sensitivity towards the product from fish degradation and showed no interference from the humidity [45]. Table 1 shows the summary of the different freshness sensor that have been fabricated.

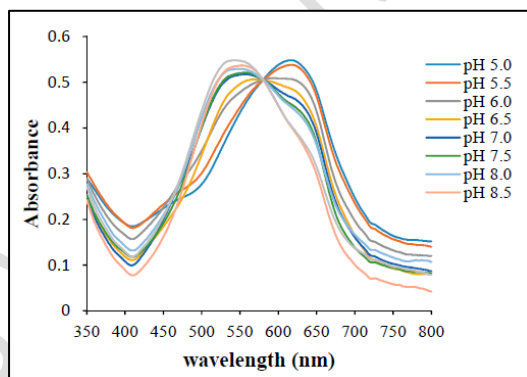


Figure 2. The response of optical pH sensor towards various pH [29]

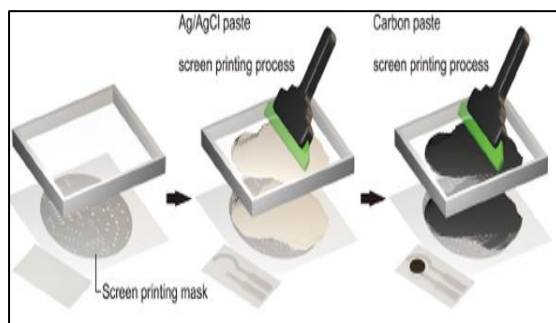


Figure 3. Schematic diagram on the fabrication of potentiometric pH sensor

Table 1. Summary on the various fabrication of freshness sensors

Reagent/ Indicator/Enzyme	Immobilization Matrix	Type of Sensor	Linear Range	Application	Reference
XO	PGE/P(GMA-co-VFc)/MWCNT	Electrochemical Sensor	2 - 48 μM	Monitoring fish freshness	[38]
XO	Fe_3O_4	Electrochemical Sensor	0.4 - 2.4 nM	Monitoring fish freshness	[37]
XO	Polypyrrole-polyvinylsulphonate (PPy-PVS)	Electrochemical Sensor	1.0×10^{-7} - 1.0×10^{-3} M	Monitoring fish freshness	[39]
FMO3	Polypyrrole-ferrocenyl	Electrochemical Sensor	0.4 $\mu\text{g mL}^{-1}$ - 10 $\mu\text{g mL}^{-1}$ (amperometric) 0.4 $\mu\text{g mL}^{-1}$ - 80 $\mu\text{g mL}^{-1}$ (impedance)	Monitoring fish freshness	[40]
PANI nanofiber	Carbon electrode	Potentiometric pH Sensor	pH 3.9 - 10.1	Monitoring pH of spoiled milk and apple	[44]
ORP, Ammonia and Hydrogen sulphide gas sensors	Gas permeable membrane Teflon	Potentiometric Gas Sensor	-	Monitoring salmon and sardine freshness	[45]
PDA/ZnO nanocomposite	Agarose gel	Optical Sensor	- Fresh (pH 6.8 - 6.0) - Spoiling (pH 6.0 - 4.5) - Spoiled (pH 4.5 - 4.0)	Monitoring milk freshness	[28]
Anthocyanin	PVA	Optical Sensor	pH 2 - 12	Monitoring freshness of date	[10]
Chromoionophore	Pectin hydrogel membrane	Optical Sensor	pH 5 - 9	Monitoring fish freshness	[29]
Bromothymol blue-phenol red (BTB-PR)	Cellulose acetate	Optical Sensor	5,000 and 10,000 mg/L of ammonia - early state of fish fermentation, showed 50,000 mg/L of ammonia - fish fermentation	Monitoring freshness of skate (<i>Raja kenoei</i>)	[30]

Table 1 (cont'd). Summary on the various fabrication of freshness sensors

Reagent/ Indicator/Enzyme	Immobilization Matrix	Type of Sensor	Linear Range	Application	Reference
Doped PANI	-	Optical Sensor	• Green-Fresh • Dark peacock blue - Spoilage	Monitoring milk freshness	[31]
Bromothymol blue and methyl red	Polyethylene glycol	Optical Sensor	-	Monitoring freshness of skinless chicken breast	[11]
Curcumin	Bacterial cellulose membrane	Optical Sensor	• Reddish orange - Spoilage	Monitoring freshness of shrimp	[3]

Future works

The development of sensor and biosensor for food freshness detection has attracted many researchers especially in the food sector. Despite of the achievement obtained so far, there are still some improvements needed. The used of computational chemistry to study the binding interaction between sensing agent and the analyte could be carried out to fabricate a more selective sensor. In addition, nanostructured material can be used for enzyme immobilization as it increases the electrochemically active surface area and enhances the efficiency of electron transfer. In addition, the development of e-tongue for food freshness detection has attracted researchers although there are some problems with the stability of the data obtained. Therefore, improvement in the e-tongue system need to be made and also for the development of smartphone-based sensor.

Conclusion

The development of sensors to determine the freshness of food has attracted many interests. In this review, we have discussed a brief overview on the food spoilage. The present review also highlights on the various type of sensors fabrication for food freshness detection. Based on the current trends, the development of electrochemical sensors involves the use of nanomaterials to achieve the low detection limit and

enhance the sensitivity of the sensors. Besides that, the use of highly selective reagent is one of the important factors in determining the performance of the sensor. Until now, there has been so much progress in the sensor research, but there is still room for improvement to fabricate sensors with a rapid response, higher sensitivity, renewable and more importantly can be miniaturized. **Despite of the achievement obtained so far, there are still some improvements that can be done.** The binding interaction between reagent and the analyte can be predicted using computer simulation to produce a more selective sensor. In addition, the use of mediator-less electrochemical sensor can provide a good sensor response and improve the selectivity. Therefore, more studies on the fabrication of mediator-less sensor or biosensor can be conducted.

Acknowledgement

The authors wish to thank Universiti Teknologi MARA Cawangan Negeri Sembilan for the services and facilities to carry out the work.

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