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Review: A Modest Approach Of Electrochemical Sensor To Determine Biogenic Amines In Food And Beverages

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Abstract: *Biogenic amines are chemical compounds that can be found in protein food. The analysis of biogenic amines in food samples is very important because the toxicity released by them is very adverse if consumed by human. Several methods have been applied and developed in order to detect biogenic amines such as liquid chromatography (LC) and gas chromatography (GC). Nevertheless, both of them are time consuming and using many chemical compounds. Thus, electrochemical sensors become the best solution in order to solve the issues from chromatography methods. Electrochemical sensors are very simple, easy to use and cheap. This paper reviews about various techniques of electrochemical sensor such as chemical sensors, biosensors and optical sensors that have been used for biogenic amines detection.*

1. INTRODUCTION

The more human population increases so the demanding of food increases. Furthermore, it will cause an impact of food security and become a challenging situation to guarantee the safety of food before distributed to market. Fish, meat, cheese and other variety of food that containing protein have been studied containing biogenic amines. These samples contaminated by chemical, biological and physical process, such as storage conditions of food, inappropriate transportation and processing conditions leading to microbial growth, such as the biogenic amines production in food and beverages. Foodstuffs contain protein have high possibility to produce biogenic amines. This is because the protein compound undergoing a degradation process causing amino acids convert to biogenic amines. The biogenic amines concentration formed strongly rely on the food and beverages characteristic and the microorganisms present. The main biogenic amines generally found in food samples are histamine, tyramine, putrescine and cadaverine (Onal et al. 2013). Consuming food that containing biogenic amines are not suggested because it can harm human body, that is the reason the review paper written in order to review about biogenic amines and their issues and the techniques used by researchers to detect biogenic amines in various samples.

2. BIOGENIC AMINES

Biogenic amines (BA) are nitrogenous organic compounds that can be found in protein foods. They have various structure such as aliphatic, aromatic and heterocyclic. The formation of biogenic amines in foods owing to amino acids decarboxylation where some specific bacteria react specific enzymes and amino acids and convert amino acids to biogenic amines (Alizadeh et al. 2017). The alpha-carboxyl group relocated from amino acid compound causing biogenic amines production such as histidine generates histamine, lysine generates cadaverine, tyrosine generates tyramine

and so on (Lazaro & Conte-Junior 2013) **Figure 1** shows the formation of biogenic amines where food protein triggered by proteinase become peptides, derivative of peptides such as histidine, tyrosine and lysine and three of them undergo decarboxylation process then convert to biogenic amines such as histamine, tyramine and cadaverine.

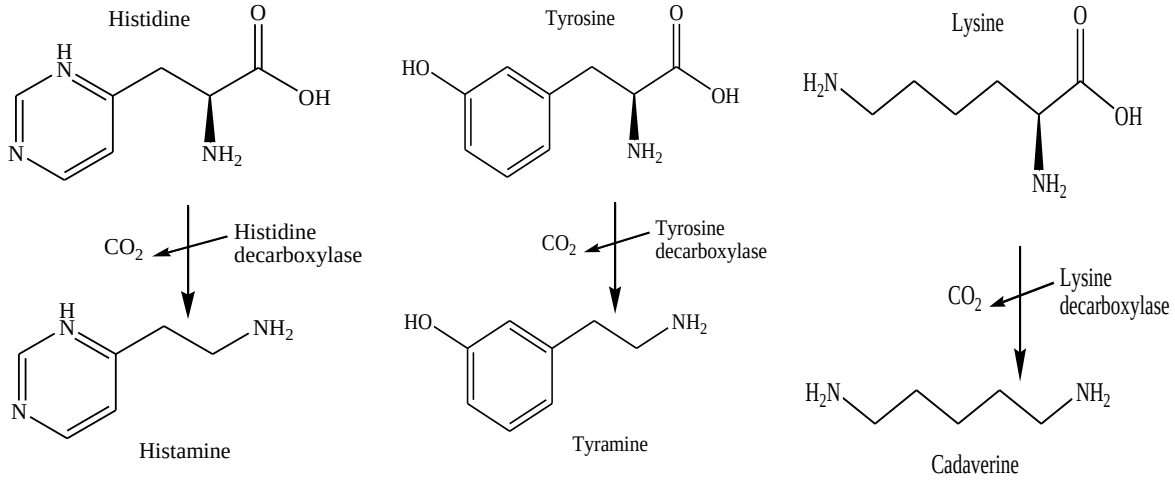

Figure 1: The biogenic amines formation by decarboxylation of the amino-acid through microbial or enzymatic processes (Lazaro & Conte-Junior 2013 with some mofications).

Consumption of high concentration of biogenic amines is not safe because it will lead human body become toxicity or called allergy issues. For an instance, the toxicity causing by histamine consumption currently popular with Scombroid poisoning. Histamine poisoning usually connected to fish samples despite some food and beverages can cause histamine poisoning. Nevertheless, based on some studies almost 80% cases of food poison in Europe in 2011 owing to fish consumption. Food allergy symptoms caused by histamine consumption such as hives, itching and diarrhea to life-threatening anaphylaxis (EFSA 2013; Jones et al. 2014; Wood 2016; Akdis & Akdis 2015; Yu et al. 2016). Therefore, due to these issues presented above , monitoring and measuring of biogenic amines particularly histamine is an imperative task for the food industry and food safety, especially us as researchers to controlling and finding the best method to detect biogenic amines in food and beverages before distributed to market.

3. ELECTROCHEMICAL SENSORS

Several approaches have been used in order to detect biogenic amines in various samples and the famous methods used are high performance liquid chromatography (HPLC) and gas chromatography (GC) (Cohen et al. 2015; Munir et al. 2016; Onal et al. 2013; Almeida et al. 2012). However, many conflicts occur during the application of these methods such as very expensive, require many chemical materials, time – consuming, only people who have background in analytical chemistry can use it, moreover, both of the chromatography methods need to be derivatized with a specific derivatizing reagent in order to increase the sensitivity of HPLC and GC. Derivatization step is needed owing to biogenic amines characteristics have low volatility and lack of chromophores so the step is a must in order to modify their characteristics (Papageorgiou et al. 2018). Because of those issues, several studies have been done in order to find a solution and electrochemical sensors are the best choice in order to detect biogenic amines. They show many advantages such as fast, simple, cheap, no need to be derivatized. This is imperative to ensure the foods are safe during production, distribution and consumption (Turner 2013). They are also friendly because do not use much organic solvents. The other advantages of sensors also could be non-destructive, adaptable to small sample volumes, the instruments are tiny and no need skill on chemistry analyst to operate this sensor (El-Nour et al. 2017; Roales et al. 2015).

Electrochemical sensors are analytical methods using dissimilar approaches as chromatography methods. Chromatography methods used specific detectors such as UV, fluorescence detector and

mass spectrometry detector whereas electrochemical sensors use chemical and biological in order to work as a receptor so the receptor can be used to react with analyte and give signal or data. Owing to the receptor divided into two parts, chemically and biologically so electrochemical sensors divided into two such as biosensor and chemical sensor. The objective of electrochemical sensor is to produce an electronic signals from a single analyte or group of analytes that can be interpreted easily. Assorted types of electrochemical sensors using chemical receptors or having chemistry modification (Stojanovic et al. 2016; Degefu et al. 2014; Geto et al. 2014) or using biology receptors such as immobilized amine oxidases and dehydrogenases (Apetrei & Apetrei 2016; Perez et al. 2013; Telsnig et al. 2012). These review paper elaborated the use of electrochemical sensors for biogenic amines determination in various food samples until now.

3.1. Biosensors and chemical sensors for biogenic amines detection

Electrochemical sensors have two parts that can work as a receptor, chemically and biologically. The modification of receptor chemically and biologically in order to acquire and convert the signal into an electrical signal where that can be performed by using divergent approaches such as amperometry, impedimetric and potentiometry methods (Justino et al. 2015). Amperometry method is a method that can measure the current generated by analyte, while potentiometry method is a method to measure the potential charge and in order to analyse the alteration of a medium between electrodes called conductometry method. Beside these methods, some studies reported the use of impedimetric method can be used which it can measure impedance which is resistance and reactance from analyte analysed (Faribod et al. 2011). Electrodes also play a crucial role for electrochemical sensors performance because reaction between analyte and receptor occur onto the electrode surface until the result of their reaction will be read by instrument. Electrochemical sensors usually need three electrodes such as a *reference* electrode, a *counter* or auxiliary electrode and a *working* electrode, also known as the sensing or redox electrode. The reference electrode, commonly made from Ag/AgCl because it has ability to maintain a distance from the reaction site in order to achieve a stable measurement. The working electrode serves as the transduction element in the biochemical reaction, while the counter electrode establishes a connection to the electrolytic solution so that a current can be applied to the working electrode (Ben et al. 2006). **Table 1** summarizes the electrochemical techniques used to determine biogenic amines in foods by biosensors and chemical sensor, in the last decade.

The biogenic amines determination using biosensors is a general method applied by some researchers compared to chromatography methods. They have several solutions to handle chromatography problems such as short time analysis, simplicity and can used easily by everyone and everywhere. In order to acquire reliable biosensor for biogenic amines determination, several receptors can be used in biosensor nevertheless enzymes become the popular receptor compared to others. Enzymatic reactions catalysed by amine-selective enzymes such as monoamine and diamine oxidase, putrescine oxidase and methylamine dehydrogenase applied by some studies to do the reaction with biogenic amines (Kiviranda & Rinken 2011).

Antibody based immunoassays known as the enzyme-linked immunosorbent assay (ELISA) had been proven to be rapid, sensitive and low-cost screening tools for chemical analysis. Several ELISAs have been developed and reported for histamine detection in food samples or even blood samples. Although ELISA method offering fast during the analysis but it lacks selectivity and sensitivity. Jiang et al. (2015) reported the use of ELISA method was tedious and time-consuming in order to prepare this method. Thus, in order to find a solution some researchers showed that the use of diamine oxidase for biogenic amines determination is better than ELISA. Several biosensors also have been studied for biogenic amines determination using diamine oxidase (Perez et al. 2013; Dai et al. 2014; Jiang et al. 2015; Veseli et al. 2016). Furthermore, diamine oxidase is cheaper than ELISA but enzymes also lack of stability. Immunosensors have superior characteristics compared to ELISA and diamine oxidase due to the high sensitivity, satisfactory specificity and higher stability (Dong et al. 2017; Apetrei & Apetrei 2016).

Table 1 – Various electrochemical methods based on biological and chemical sensor to detect biogenic amine in food samples.

Food Sample	Analyte	Technique	Ref.
Marine fish	His	Amperometric biosensor using diamine oxidase and peroxidase as molecular recognition element	Trevisani et al. (2017)
Food products		Voltammetric sensor based on molecularly imprinted polymer (MIP)	Akhoundian et al. (2017)
Chicken meat	Put, Cad, Tyr	Amperometric biosensor using pea seedling amine oxidase (PSAO) as molecular recognition element	Telsnig et al. (2012)
Beef, chicken, turkey and fish meat	Put	Chemiluminescene biosensor using putrescine oxidase and diamine oxidase as molecular recognition element	Micklicanin & Valzacchi (2017)
Fish	His	Amperometric biosensor using diamine oxidase as molecular recognition element	Apetrei & Apetrei (2016)
		Amperometric biosensor using diamine oxidase and horseradish peroxidase as molecular recognition element	Perez et al. (2013)
		Chemical sensor using square wave stripping voltammetric (SWSV)	Yilmaz & Inan (2015)
		Chemical sensor using differential pulse voltammetric (DPV)	Geto et al. (2014)
	His, Agm, Spd, Put, Cad	Amperometric biosensor using diamine oxidase as molecular recognition element and coupled to liquid chromatography	Muresan et al. (2008)
	His	Amperometric immunosensor	Dong et al. (2017)
		Surface plasmon resonance (SRP) based on a molecularly imprinted polymer (MIP) film as a biosensor	Jiang et al. (2015)
	His, Tyr, Phm	Quartz crystal microbalance (QCM) based on a molecularly imprinted polymer (MIP)	Dai et al. (2014)
Salted anchovy	His, Put, Cad, Tyr, Spm	Amperometric biosensor using monoamine oxidase, diamine oxidase and horseradish peroxidase as molecular recognition element	Alonso-Lomillo et al. (2010)
Sardine	His, Put, Spd, Spm, Tyr, Trp	HPLC with pulsed amperometric chemical sensor	Carralero et al. (2005)
Fish sauce	His	Amperometric biosensor using rhenium dioxide as molecular recognition element	Veseli et al. (2016)
Fish and pork meat	His, Put, Cad, Spd, Phm, Trp, Tyr	Amperometric biosensor using monoamine oxidase, putrescine oxidase and diamine oxidase as molecular recognition element	Boka et al. (2011)
Fermented food products	His	Amperometric biosensor using coenzyme pyrroloquinoline quinone (PQQ)	Young et al. (2013)
Tiger prawn		Amperometric biosensor using diamine oxidase as molecular recognition element	Keow et al. (2007)

Cheese and anchovy	His, Put, Cad, Spm, Spd	Amperometric biosensor using diamine oxidase as molecular recognition element	Carelli et al. (2007)
Aqueous medium	His	Chemical sensor using cyclic voltammetry (CV)	Castro et al. (2010)
Alcoholic beverages		Chemical sensor using differential pulse voltammetric (DPV)	Stojanovic et al. (2016)
Wine		Chemical sensor using square wave voltammetry (SWV)	Degefu et al. (2014)
Beer	Put	Amperometric biosensor using putrescine oxidase as molecular recognition element	Boka et al. (2012)
Cheese	His	Chemical sensor using chronopotentiometry with different electrodes	Svarc-Gajic & Stojanovic (2010 & 2011)
Biogenic amine: agmatine (Agm), amylamine (Am), butylamine (But), cadaverine (Cad), diethylamine (Det), dimethylamine (Dmet), dopamine (Dop), ethylamine (Et), ethanolamine (Eth), heptylamine (Hep), 1,6-hexamethylenediamine (Hex), histamine (His), isoamylamine (Iam), isobutyamine (Ibut), methylamine (Met), nitrosamine (Ntr), 2-phenylethylamine (Phm/2-PE), piperidine (Pip), propylamine (Prop), putrescine (Put), spermine (Spr), spermidine (Spd), tryptamine (Trm/Tryp), tyramine (Tyr).			

Amperometry method become the common method used by biosensor studies due to the enzymes can easily react with the analyte in order to obtain the current or known as a cyclic voltammetry (CV). Cyclic voltammetry (CV) measures the current that during the reaction between bio-receptor and analyte on the electrode surface. CV is performed by cycling the potential of a working electrode, and measuring the resulting current. A cyclic voltammogram is obtained by measuring the current at the working electrode during the potential scans. **Figure 2** shows a cyclic voltammogram resulting from a single electron reduction and oxidation. In **Figure 2**, the reduction process occurs from (a) the initial potential to (d) the switching potential. In this region the potential is scanned negatively to cause a reduction. The resulting current is called cathodic current (i_{pc}). The corresponding peak potential occurs at (c), and is called the cathodic peak potential (E_{pc}). The Epc is reached when all of the substrate at the surface of the electrode has been reduced. After the switching potential has been reached (d), the potential scans positively from (d) to (g). This results in anodic current (I_{pa}) and oxidation to occur. The peak potential at (f) is called the anodic peak potential (E_{pa}), and is reached when all of the substrate at the surface of the electrode has been oxidized.

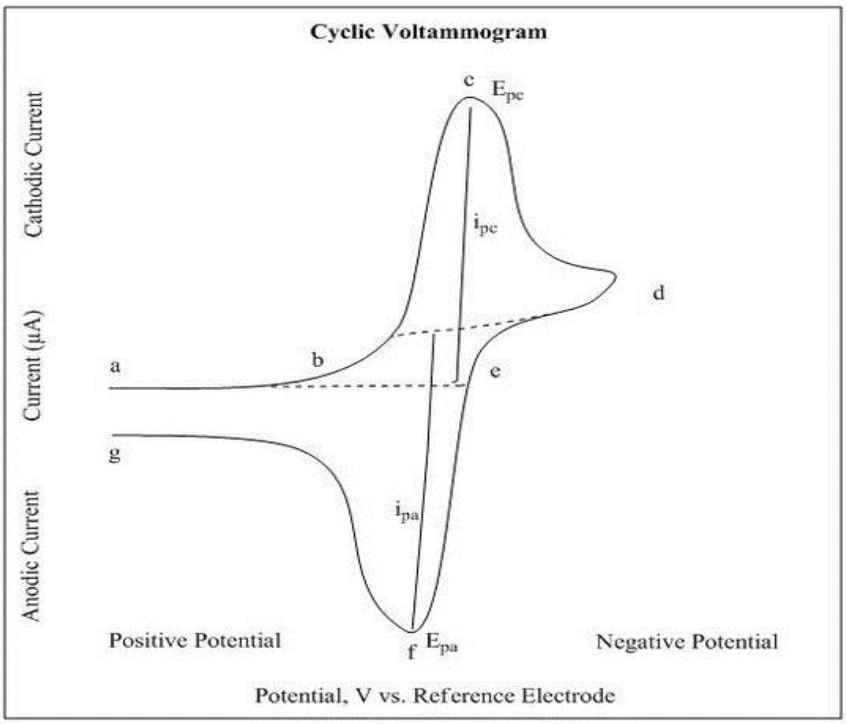

***Figure 2**: Voltammogram of a single electron oxidation-reduction*

However, there are some disadvantages of enzymes and one of the major problems is stability of the enzyme itself. They also cannot be reused owing to the difficulty to separate enzyme from the reaction media (Romaskevic et al. 2006). A solution made to tackle this problem such as using an enzyme immobilization, where it is a method of keeping the enzymes molecules confined or localized in a certain defined region of space with a retention of their catalytic activity. A study reported the use of enzyme immobilization in biosensor to detect histamine in prawn. The histamine biosensor operated at a lower potential where to achieve it, the process underwent the electrochemical oxidization of the product imidazole acetaldehyde, which was produced from the enzymic reaction of diamine oxidase on histamine, where the biosensor utilized a photocuring technique for the immobilization of the diamine oxidase enzyme where it was directly entrapped in a photocured membrane and deposited onto a carbon paste screen-printed electrode (SPE) (Keow et al. 2007). The use of diamine oxidase (DOx) also reported by Perez et al. (2013) where the enzyme combined with horseradish peroxide (HRP) for the determination of histamine in fish samples where these enzymes need to be immobilized in order to modify the stability of enzymes. They co-immobilized into a polysulfone/carbon nanotubes/ferrocene membrane by means of phase inversion technique onto screen-printed electrodes. These enzymes used in order to increase the possibility to detect H_2O_2 at lower applied potentials where it was a method to produce an amperometric biosensor. The electrochemical measurements have been carried out in phosphate buffer solution at pH 8.0 in batch mode and low applied potential (-50 mV vs. Ag/AgCl, KCl 0.1 M) in order to reduce the interferences.

Nanomaterials are also applied to modify the sensitivity of biogenic amines biosensor by amplifying their conductivity, catalytic activity and biocompatibility signals (Ma et al. 2013; Song et al. 2016). Prussian blue (PB) shows good electrocatalytic activity and generally used for the immobilization of enzymes to produce an enzymatic biosensor. A study by Dong et al. (2017) developing a sensitive and selective electrochemical immunosensor was constructed to analyse histamine by assembling a PB-CS-AuNP nanocomposite film on a screen-printed carbon electrode (SPCE) to capture histamine-antibody (HA-Ab) and histamine-antigen (HA-Ag). Its study acquired the satisfactory sensitivity and selectivity to detect histamine in fish samples by the catalytic reaction between the signal tag (HRP) and H_2O_2 using hydrquinone (HQ) as an electron mediator. The PB-CS-AuNP nanocomposite film was electrodeposited on the SPCE. Afterward, the blocking solution was coated on the electrode surface to block the possible remaining active sites to avoid nonspecific

binding. Lastly, the immunosensor was thoroughly washed with 0.01M phosphate buffer solution (PBS) and stored at 4^{o}C until used.

Jiang et al. (2015) also studied the use of surface plasmon resonance (SPR) based on molecularly imprinted polymers (MIPs) as a biosensor due to its selectivity and sensitivity in order to detect histamine. MIPs showed a satisfactory alternative during histamine detection. MIPs are synthetic receptors with imprinted nanocavities, which exhibit similar specificity and selectivity to the desired target molecules as their natural antibodies or enzymes. They are also suitable for detection of small molecules. Not only that, MIPs have several advantages such as it can be synthesised at a relatively low cost, they are robust and can withstand extreme temperature and pH and they also prepared using the noncovalent approach present regeneration potential (Akhoundian et al. 2017). It shows that MIP has good ability to recognize histamine from food samples.

Several techniques have been applied to immobilize enzymes on a solid support. They are based on chemical and physical methods. Both physical and chemical immobilization methods give advantages and disadvantages. During the chemical methods, the activity of enzyme was loss where the immobilization process can perturb the enzyme's native structure, nevertheless covalent bonding give a firm and stable enzyme attachment and in some cases can reduce the enzyme deactivation rates. The physical immobilization methods showed less perturbation but the enzyme cannot bind firmly. For some studies, immobilization enzymes also offer an instability, expensive test kits and tend to overestimate histamine (Akhoundian et al. 2017). Furthermore, biological recognition elements such as antibodies or enzymes are expensive, difficult to prepare and not always available for the desired target. Furthermore, these elements are unstable in organic solvents, high temperature or changing pH. Thus, a synthetic recognition element is strongly needed so it can overcome the temperature and pH issues.

Chemical sensors become another alternative in order to avoid the use of antibodies, enzyme and DNA. These methods use receptor to capture biogenic amines where the electrodes have modified chemically. The electrodes also must be both conductive and chemically stable. Platinum, gold, carbon and silicon compounds are commonly used by researchers as a electrode to detect biogenic amines. Furthermore, conducting polymer modified electrodes have attracted much attention due to their good stability, reproducibility, homogeneity in electrodeposition, strong adherence to electrode surfaces and their available active sites. Studies on carbon nanatubes (CNT) have been applied as a novel materials in electrochemical sensor applications owing to their unique properties including high chemical and thermal stability, high elasticity and high tensile strength make them suitable to be applied as electrode modifiers. The use of composite modified electrodes involving conducting polymers and carbon nanotubes for chemical sensor have also been reported to increase the catalytic role of both materials (Geto et al. 2014). CNT belong to a group of rather new nano-sized materials and they having ability to functionalize the electrode surface. CNT divided into two types such as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The structure of CNTs provides them unique electrical, chemical and physical properties (Stojanovic et al. 2016).

This study showed the importance of CNT as a conducting polymer in order to modify the electrode behaviour. The SWCNT-CPE showed higher current response accompanied with better defined peak shapes in comparison to the unmodified CPE where the response of histamine on SWCNT-CPE was 15-fold higher than a plain CPE. After effect of pH and scan were optimized and validated their study continued for optimization of experimental parameters of differential pulse voltammetry (DPV). Determination of histamine using SWCNT-modified carbon paste electrode (CPE) was applied by Stojanovic et al. (2016) where the electrochemical behaviour of histamine in beverages on SWCNT-CPE was investigated and a voltammetric method was elaborated by using differential pulse mode. The method was success and shown in **Figure 3.**

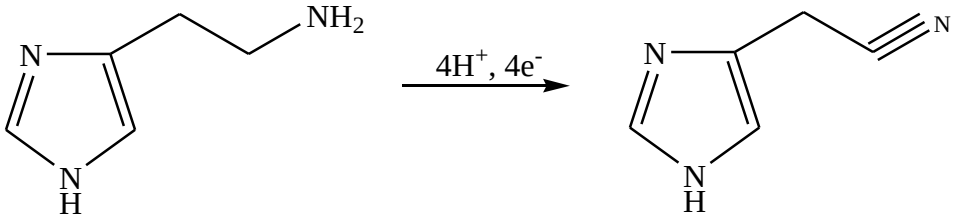

Figure 3: Schematic illustration of proposed reaction for the oxidation of histamine on SWCNT-CPE.

The SWCNT-CPE showed satisfactory result but it is undeniable that the method is expensive and the complexity of the modification electrode will be faced. Geto et al. (2014) reported the use of MWCNT modified glassy carbon electrode (GCE) to detect histamine in fish muscle. However, a chemical method using a cheap and material from biopolymer as electrode modifier should be developed. Several biopolymers reported having ability as a conducting polymer such as lignin, polyurethane and chitosan (Nezakati et al. 2018; Degefu et al. 2014).

The use of lignin modified glassy carbon electrode (GCE) as a chemical sensor in human urine and wine studied by Degefu et al. (2014) where cyclic voltammetry was used to investigate the electrochemical behaviour of histamine at the surface of lignin modified GCE and unmodified GCE. This study showed that GCE modified by lignin gave more sensitivity to analyse histamine due to the properties of lignin as a conducting polymer. Furthermore, this study also used square wave voltammetry (SWV) for the quantitative analyses of histamine due to the method was more sensitive than cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

3.2. Optical sensors for biogenic amines detection

Optical sensors for amines detection based on UV-Visible and fluorescence spectroscopic methods have been developing well owing to they do not consume the analyte and less diffusion-limited. Optical sensors for amines are usually applied in food technology and biotechnological processes. Two approaches used for the optical determination of amines. First is by embedding a pH indicator dye into a hydrophobic matrix and measuring the basicity of the amine through its deprotonation of the indicator dye whereas the second approach uses a more selective method of interaction between the indicator and the amine by employing trifluoroacetyl or aldehyde (Schaude et al. 2017). **Table 2** below shows the comparison data for the optical determination of the biogenic amines in various samples.

Table 2. Biogenic amines determination using optical sensors

Indicator	Analyte	Remark	Ref.
CR-528 & CR-555	Spermine, spermidine & ethanolamine	Absorbance-based assay & ethanol solution	Mastnak et al. 2018
Tyrosine-protected gold nanoparticles (Tyr-Au-NPs)	Spermine & spermidine	Absorbance-based assay, fluorescence-based assay; PBS buffer at pH 6.0	Rawat et al. 2016
Aggregates from [9,9-bis(6'-methylimidazoliumbromide)hexyl)-fluorene-co-4,7-(2,1,3-benzothiadiazole)](PFBT-MI) and surfactant	Spermine	Fluorescence-based assay; aqueous solution	Akhtar et al. 2016
Cu(II) complex of Schiff-base receptor organic nanoaggregates	Spermine	Absorbance-based assay, DMF/water (1/99, v/v) solvent system	Chopra et al. 2015

Indicator	Analyte	Remark	Ref.
Chameleon dye (Py-1) embedded in a polymeric (sensor microtiterplate)	Histamine	Fluorescence-based assay; methanol solution	Khairy et al. 2016
(Trifluoroacetyl)azobenzene dye added in carbon nanotube-Nafion® composites	Ethylamine, Cadaverine & putrescine	Ethanol/deionized water solution	Lin et al. 2015
ZnTriad poryphyrin thin films	Butylamine, propylamine& heylamine	Optical fiber spectrophotometer to record UV-Vis spectrum	Roales et al. 2015
4-(Dioctylamino)-4'(trifluoroacetyl)azobenzene (ETH4001)	Isopentylamine, propylamine & putrescine	solution	Nedeljko et al. 2017

Optical sensors have been applied to determine biogenic amines in various food samples such as histamine, tyramine, ethylamine, putrescine, agmatine, isopentylamine, methylamine and propylamine by using specific indicator dyes that exhibit different spectral characteristic when exposed to these analytes (Kumpf et al. 2015; Rawat et al. 2016; Nedeljko et al. 2017; Mastnak et al. 2018). Akhtar et al. (2016) reported the biogenic amines determination in liquid samples where this study synthesized the water-soluble cationic conjugated polymer using [9,9 – bis (6'-methylimidazoliumbromide) hexyl) – fluorene – co - 4,7 - (2,1,3-benzothiadiazole)] (PFBT-MI) and combined it with a surfactant in order to acquire aggregates, which enabled the spermine detection. Whereas Rawat et al. (2016) used tyrosine-protected gold nanoparticles (Tyr-Au-NPs) as a dual probe for colorimetric and fluorescene turn-on assays of spermine and spermidine in biological samples. Determination of biogenic amines in meat and cheese also reported by Khairy et al. (2016) using optical sensor based on fluorescence sensor microtiterplate against GC-MS. The data obtained from this study was match with data obtained from GC-MS and that can be concluded the sensor microtiterplate can be used for cheap pre-screening of the biogenic amines content in food samples.

4. CONCLUSION

The presence of biogenic amines in foods and beverages that containing protein are inevitable. Nevertheless, it is imperative to monitor foods and beverages that containing biogenic amines beyond FDA regulation in order to protect the health of consumers. The development of analytical methodologies for laboratory examining of biogenic amines content is strongly necessary. Without a question where the use of chromatography methods are not the best choices owing to some factors. Therefore, sensor methods such as electrochemical and optical sensors are promising technique where not merely they are cheap, fast and easy to use but also they have good sensitivity, selectivity, accuracy and precision. The advantages and limitations of each technique has been reviewed.

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