DETERMINATION OF BIOGENIC AMINES IN FOOD USING BIOSENSOR ELECTRODE: A REVIEW

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Abstract

Recently, biogenic amines (BA) have become an interest to many researchers along with the development of modern technologies as well as analytical methods to determine the compounds. BA is one of the most important components in food as an indicator of freshness especially histamine due to its capability to cause health issues and food intoxication. In many sensing applications, the development of biosensor electrodes has been widely used towards this discovery. The latest advances are in the utilization of carbon nanomaterials in biosensor electrodes and enzymatic methods for electro-analytical sensing devices. The studies in quantifying the amounts of BA have been widely investigated as its level content can determine the quality and safety of food presence today. Hence, this paper will be focusing on the determination of BA using modified carbon nanomaterials biosensors electrodes.

Keywords: *Biosensor, food, biogenic amines, modified electrode*.

INTRODUCTION

Biogenic amines (BA) are chemical compounds that possess smaller molecular weights and are categorized under organic bases. They also can be found in the forms of aliphatic, aromatic and also heterocyclic structures. The presence and degradation of BA are caused by the cellular metabolism process carried out by microorganisms, plants and animals (Doeun et al., 2017). Various foods, beverages and fermented foods consumed by humans today contain BA such as fish, meat, eggs, cheeses, milk, fermented vegetables, fruits, nuts, chocolates, beers and wines (Benkerroum, 2016).

However, high levels of BAs can be harmful and cause toxic to human health. Therefore, food safety and quality can be examined from the level of BA contained in that food. The factors that influence high content of BA are the composition of the food itself and the growth of bacteria during the food is being processed and stored. The maximum level of BA that can harm human health varies, depending on regulations enforced by each country. For instance, in European Union (EC), the limit of food safety set for histamine is between 100-200 mg/kg. On the other hand, in the United States, foods that contained 500 mg/kg of histamine are said to be spoiled (Vanegas et al., 2018).

Many analytical methods that can be used for determination of BA in food such as capillary electrophoresis and chromatographic methods (Önal, 2007). However, among drawbacks of these methods are complicated, time-consuming and expensive. As an alternative analytical device, biosensors have been widely used which provides good sensitivity, low cost, easy to handle and shows high rapid response time (Alonso-Lomillo et al., 2010). Besides, it has been widely used in many applications such as disease detection, environmental monitoring, water quality management, biomedical research as well as in food quality control (Bhalla et al., 2016). In addition, biosensors with modified electrodes with suitable nanomaterials such as graphene or chitosan allow the biosensor to be more sensitive and selective in detection of the analytes.

BIOGENIC AMINES

Biogenic Amines in Food

The association between BA and food is leaning towards the determination of the amines' level in certain types of food to prevent food intolerance. There has been a fair amount of research done to investigate the relation between BA and food. Out of all BA that have been found and experimented, histamine shows the most significant availability in almost every food especially the ones fermented and protein-rich. According to Saaid, Saad, Hashim, et al., BA that are found to be in food and beverages have undergone decarboxylation, in the microbial state, of the corresponding amino acids available (Saaid et al., 2009). BA are also found not to reduce notably when exposed to high temperature treatment. Therefore, the existence of BA in food will cause deadly contamination if it exceeds certain amount stated in regulation for safety.

According to Spano and other fellow researchers, BA have been used conventionally to indicate and determine microbial activities in food products. As the amount of BA such as histamine is found high, the product will immediately be deemed as defected due to its deterioration of quality (Spano et al., 2010). The variety of BA are highly depending on its precursor which involves amino acid.

Every each of the amines has their own amino acid precursor, as an example, the amino acid precursor for histamine is histidine. Other than amino acid precursors, the BA also possess their properties based on the respective chemical structure categories which are aliphatic, aromatic or heterocyclic. The function of the composition of certain biochemical and also the presence of microorganism depicts the total amount of BA in any food product, for example, cheese and sausage need high concentration of lactic acid bacteria for their fermentation which can cause the occurrence of dense content of histamine, cadaverine, tyramine or putrescine stated by Vanegas et al. (Vanegas et al., 2018).

As a consequence of having drawbacks in consuming BAs, few sets of regulations have been made to ensure that the food will not intoxicate the consumers. The regulations must be followed to lessen the chance of food diseases. As said by Leuschner et al. in their research, the poisoning of histamine, vastly called as 'scombroid' food poisoning in fish products due to astonishing concentration of histamine has affected those who have inhibited natural mechanism or have flawed genetic. The name 'scombroid' comes from the specific species of fish; *Scombridae* such as tuna, mackerel, bonito and also *Scomberesocidae* such as saury, queenfish. Although the species represent the name of the illness, there are also reported cases coming from the intolerance of *Clupeidae* (herring, shad, sardine) species around the world (Leuschner et al., 2013).

Food intoxication cases that have been reported are particularly similar in the term of symptoms such as those who were affected often showed allergy-like symptoms upon consuming the BAs. Nausea, vomiting, diarrhea, headaches, acute rash and hypotension or hypertension are often depicted by the patients and documented by a group of researchers, however, in some severe cases where a patient digested BAs in extreme concentration, it can lead to death. In addition, there are BAs (spermidine and spermine) that are precursors for *N*-nitroso carcinogenic compounds Cao et al. and Erdag et al. reported specifically on the effects of BAs such as histamine, tyramine, putrescine, agmatine and polyamine. Erdag, Merhan and Yildiz et al. stated that due to the presence of aminoxidase enzyme inhibitors, the BA will avoid detoxification and cause problems related to health (Saaid et al., 2009).

Moreover, according to them, histamine also has an important role in controlling blood pressure as well as serving functions for the nervous system by binding with cardiovascular system and cell membrane receptors. Although it might seem to be given as an advantage, this may assist in neurotransmitter disarray which eventually leads to gastrointestinal problems and edema on account of blood vessel expansion. Erdag, Merhan and Yildiz also stated that polyamine might cause low dosage of colon cancer. Tabanelli in her research has connected between BA and health problems. She stated that tyramine can cause food poisoning and attributable to its abundance in cheese, a 'cheese reaction' food poisoning is found. This reaction is responsible for nausea, vomiting, migraine that is induced by diet, elevation of cardiac output and increased blood glucose (Tabanelli, 2020).

BIOGENIC AMINES AND BIOSENSOR

I. Biosensor

Biosensors can possess many advantages and benefits in the concentration determination of the target analyte. According to C. Cristea et al., (2014), biosensors are devices that are sensitive and selective in analyzing the chemical compounds associated with associating a bio-component to a transducer. Besides, in constructing a biosensor, the enzyme immobilization method plays an important role in choosing the adequate enzyme for determination of the target analyte. Apart from that, this new technology device offers fast time analysis, relevant limit of detection and only simple acquisition needed so that more accurate assessments in the food industry can be done. In addition, the performance of biosensors can be evaluated experimentally based on a few analytical considerations such as sensitivity, selectivity, reproducibility, linearity and the limit of detection (LOD) (Bhalla et al., 2016). Firstly is selectivity. This analytical characteristic is meant by the ability of bio-receptor to react with a specific analyte in samples that also containing other contaminants or interferences (Bhalla et al., 2016). For a clearer visual, the antigen-antibody interaction in living organisms. From this example, the antibody becomes the bio-receptors that immobilized on the transducer surface and a solution which usually being used is buffer solution that possess the antigen and will then be exposed to transducer where the antibody will react with the specific antigen.

Apart from that is sensitivity. This refers also to the detection limit. In other words, this characteristic means the minimum quantity of analyte that the biosensor able to detect. Moreover, this factor is very important in medical and environmental monitor field. This is due to the traces that are normally found in analyte samples from those two fields are in very low concentration such as ng/mL and fg/mL. For example in medical field, if there is the presence of 4 ng/mL of phosphate-specific antigen (PSA) component, the person will be diagnosed to suffer in prostate cancer (Apetrei & Apetrei, 2016). In addition, the analytical characteristic is reproducibility which refers to the capability of the biosensor to generate similar responses for repeated procedures in the same experiment. Under this characteristic, the precision and accuracy of the transducer in the biosensor device are two main important things. Precision refers to the ability of the device to generate an alike value of results every time the samples are tested. On the other hand, accuracy is ability of the device to show close results with the true values (Bhalla et al., 2016). Table 1 summarizes the characteristics of each prepared biosensor. The DAO/Pt-NP/Graphene/Chi/SPE sensor possesses the highest sensitivity compared to other biosensors.

Table 1 summarizes the characteristics of each prepared biosensor. The DAO/Pt-NP/Graphene/Chi/SPE sensor possesses the highest sensitivity compared to other biosensors. On the other hand, DAO-photoHEMA/SPE showed the lowest sensitivity towards the detection of histamine in prawn samples. The polymer-nanoceria hybrid stated by Apetrei et al., (2016) showed as the most sensitive ones towards histamine. However, the limit of detection (LOD) is quite low. From Table 1, the best sensor conformation is the DAO/Pt-NP/Graphene/Chi/SPE as it possesses the highest sensitivity and the lowest LOD. Different types of sensors conformation will give different performance towards the biosensor device. In addition, the standard analytical instruments used in previous studies such as TLC and GC were more accurate within these ranges however they are much expensive, longer time analysis, sample pre-treatments and skilled operators needed (Apetrei & Apetrei, 2016).

Veseli et al., (2016) had conducted research to determine histamine in fish sauce samples using heterogenous carbon electrodes modified with rhenium (IV) oxide. Under flow injection analysis (FIA) mode, the operating potential used to analyze the fish sauce samples was at -150 mV. In addition, other possible sample constituents such as aliphatic amines were also been studied regarding their interferences on the signals produced at the end of the experiment. However, the signals formed did not affect the slope of the calibration curve and thus the diluted fish sauce samples can be directly used without any procedures such as filtration or centrifugation. Apart from that, the samples were kept in an ice bath as the histamine properties were sensitive to high temperature. At the earlier procedure, the fish sauce samples were diluted with 0.1 M phosphate buffer solution under pH 7.5 so that linear range of concentration of the method can be obtained (Veseli et al., 2016).

Table 1 Enzymatic Biosensors Performance Characteristics For Determination of Biogenic Amines (Vanegas et al., 2018)

Sensor Conformation	**Food Sample (BA)**	**Sensitivity (μa/Mm)**	**LOD (μm)**	**References**
DAO/Pt-NP/Graphene/Chi/ SPE)	fish (His)	63.1	0.02	(Apetrei & Apetrei, 2016)
DAO-HRP/ polysulfone/CNT/ferrocene/SPE)	fish (His)	19	0.17	(Pérez et al., 2013)
DAO-photoHEMA/SPE	prawn (His)	0.62	5.8	(Keow et al., 2007)
DAO/CeO_2-PANI/GCE	prawn (His)	51.47	48.7	(Gumpu et al., 2014)
DAO/Nafion/MnO_2/SPE	chicken (His/Tyr)	5.95	3	(Telsnig et al., 2013)
DAO-HRP/SPE	fish (His)	17.66	0.18	(Alonso-Lomillo et al., 2010)
LSG-nCu-CNC/DAO (analytical grade materials)	fish (His)	58.7	7.7	(Vanegas et al., 2018)

LSG-Cu-MFC/DAO (locally sourced materials)	fish (His)	23.3	11.6	(Vanegas et al., 2018)
HMD/TTF/SPCE	octopus (His/Put)	10.2	8.1	(Henao-Escobar et al., 2016)

(DAO: diamine oxidase; SPE: screen-printed electrode; LSG-Cu-MFC: laser scribed graphene-Cu-microfibrilated cellulose).

II. Enzymatic method

The basic concept that is applied by enzymatic method is the interaction between biological elements with certain analyte used and then generation of physicochemical changes in several forms like electron transfer, pH and mass changes and redox process that involves the absorption and releasing of particular ions or gases (Phetsang et al., 2019). One of the devices used in this method is biosensor. Biosensor can be defined as a device to measure biological and chemical reactions by generating signals that are proportional to the concentration of the analyte being analyzed. Typical biosensors used in quantification of chemical compounds especially BA consist of several components such as analyte, bio-receptor, transducer, electronics and the display. First and foremost is the analyte. Analyte is any substance of interest that needs to be detected. In electrochemical biosensor, analyte is important as a medium for the ion to mobilize and allow the electrical flow. The next component is bio-receptor. It consists of a molecule that can recognize the analyte of interest specifically.

Among the examples of bio-receptors are enzymes, cells, aptamer, deoxyribonucleic acid (DNA) and antibodies. When this bio-receptor and analyte interact, signals will be generated in the computer system. The signals generated are in the form of heat, light, pH, mass and charge changes. The signal generation process is called as bio-recognition. Besides, another important component in biosensor is transducer. It is one of the elements that can convert one form of energy into another form. Its function is to convert the bio-recognition formed into signals that can be measured such as current. This energy conversion process is known as signalization (Bhalla et al., 2016). The function of electronic part in biosensor is to process the transduced signal and generate it for display. Besides, in this part, the amplification and conversion of signals from analogue into digital form will occur. The last component in biosensor is the display. It consists of the systems that can generate numbers or curves understandable by the users. The output signals can be in number, graph or images depend on the requirements of the users. Apart from that, biosensor can be categorized into a few types which are some of them are electrochemical biosensor, optical, thermal and piezoelectric biosensors.

FACTORS AFFECTING ELECTROCHEMICAL BIOSENSOR PERFORMANCE

Through the performances of biosensors, the efficiency of the surface of the electrode or transducers is a key in the immobilization of chemical samples. This contributes greatly to the sensitivity of the sensors being formulated. The transducers are responsible in analyzing the current generated by the oxidation and reduction of the electroactives materials. The working electrode must provide excellent signal-to-noise qualities and measurable responses (Azri et al., 2017). In addition, consideration must also be given to electrical properties, mechanical characteristics, possible windows, surface reproducibility, cost, accessibility, and toxic effects. The transducers, the screen-printed carbon electrodes themselves, have been capable in providing an appropriate and efficient operating site for biochemical analysis. However, numerous attempts were made to enhance productivity as well as sensor sensitivity, such as development with nanomaterials (Antuña-Jiménez et al., 2020). Nanostructures, nanotubes, and carbon nanotube offer excellent potential in interfacing of biorecognition activities with electrical signal transduction (Jadav et al., 2018). It is expected that the incorporation of these material properties would improve the current conduct of the analysis (Gerbreders et al., 2016).

Modification of Electrode

The main uses of screen-printed electrode (SPEs) are environmentalism control including water performance tests, diagnosis of toxic elements and pollution of harmful gases (Sarkar et al., 2013). It is also used in biochemistry and electrochemical performance. There are three main components in SPEs which consisting the three electrodes: auxiliary (AE), working (WE) and reference (RE) electrodes. A reference electrode (RE) is made from silver printing ink, meanwhile auxiliary (AE) and working (WE) can be modified by various conductive printing ink such as carbon, gold, and other elements. The principal electrode goes to the working electrode (WE) where the electrochemical responses are accomplished, meanwhile the RE and AE were utilized as crucial elements to complete the electronic circuit (Rountree et al., 2017). Researchers present that the whole process of modifying the SPE is by formulating a conductive ink and the process of screen-printing(Gerbreders et al., 2016). The function is to identify the electrochemical effecting on SPE. The crucial parts in characterization of biosensors are reaction rate, detection limit (selectivity) and protection from fouling effect. According to Jirasirichote et al., 2017, the screen-printed carbon electrode was exhibiting several significant benefits over common basic electrodes (Jirasirichote et al., 2017).

One of the additional materials which is widely used is carbon nanotube (CNTs) that has a special properties which are lightweight properties, electrical and thermal conductivity (Yang et al., 2013). They are pure carbon polymers that can be produced and manipulated by identified and super rich carbon chemistry (Antuña-Jiménez et al., 2020). Most of all the usage of carbon-based has been produced by screen printing with graphite, carbon black and activated carbon. Based on their result from previous research by Bergamini et al., 2007, the use of chemical carbon nanomaterial modified electrodes with the development of simple electrochemical sensors has been showing larger processes in electroanalytical chemistry for the past few years. One of the carbon nanomaterials, carbon black (CB), showed several advantages including cost-effective, suitable to attain stability and homogenous dispersion and mass-producible followed by currently existing processes for electrochemical performances (Bergamini et al., 2007).

Another significant benefit in using carbon nanomaterial for electrodes modification is its potentiality of scan rates and large catalytically available surface: the film, mechanically or chemically discharged on the sensor surface, causes the measurement of particles with elevated oxidation potential. Graphen has the potential benefits of low cost and high performance compared to CNTs (graphene does not contain metallic contaminants as CNTs do) (Puy-Llovera et al., 2017). However, some of the researchers present the graphene has more advantages in electrochemical performance because of its higher electron mobility in electrochemical (Lv et al., 2019). The innovation of nanotechnology and microelectronics has stimulated the growth of electrochemical biosensing process which provides a new technique for quick detection and classification in biomarkers of metabolic diseases (Lázaro et al., 2014). Carbon ink is a fitted platform to be customized with a range of materials, such as nanotechnology, natural macromolecules, or polymers, due to its resistance to a large variety of solvents, minimal background currents and large potential windows of application (Manuscript, 2020). However, the (Nano) modified surface responds to a particular electro catalytic behavior, based entirely on the design of the modification. Various modified electrodes are usually characterized by electron scanning microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) (Manuscript, 2020). Hence, the electrochemical performances were depending on the unique characteristics of the modified materials. From the results obtained by the researchers, the comparison with the screen-printed electrode without CNT were comparable with SPE-CNT. In addition, the result of cyclic voltammetry of non-functionalized and nano functionalized SPE was significant to the surfaces of both WE, which was enclosed by electron microscopy scanning (SEM) (Azri et al., 2017).

CONCLUSION

In this review paper, the relation of how BAs in food can cause food intoxication is correlated with the amount of certain amines, such as histamine, that can be detected. In this context, histamine poisoning can lead to serious symptoms in which rapid detection is needed, hence the advancement of biosensors comes into light. The performance of biosensors can be enhanced using modifying or supporting materials such as nanomaterials that can promote high sensitivity and selectivity towards the analytical testing. Nanomaterials were often used as carrier signals for indirect, but still strongly precise and efficient signifies in detecting chemical compounds. The review paper links few important points of BA in food and its determination method of electrochemical biosensing. Further with the growth of new biosensor technology, scientists were expected to speedily utilize them for the advancement of even better sensor technology and tools, as well as to discover a new advanced method in determining various biomolecules.

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