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A Review on Utilization of Biosensors for Detection of Adulteration in Fish

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ABSTRACT

The consumption of fish has lately seen an upward trend in recent days considering the wide range of health benefits of fish and sea foods and with it, the issues of prevalent adulteration of fresh fish with unapproved chemicals and additives have came into light both through electronic and print media. Several instances of adulteration of fish and fishery products with unsafe chemicals like formaldehyde, ammonia and additives like sodium benzoate has been highlighted in a number of reports and research papers. Presence of these adulterants in freshly marketed fish seriously compromises health of consumers and daily ingestion of considerable amounts of these adulterants can generally cause severe abdominal pain, coma, renal injury, vomiting and possible death. This has aroused concerns regarding the safety and quality of fish as well as the necessity of a reliable and faster technology which can easily identify the contaminants and toxicants in food materials. As per regulatory guidelines of food quality some conventional methods are used but these conventional techniques possess various limitations. Biosensor is an analytical device which can replace these conventional techniques and offers a quick on-site monitoring with accuracy and provides an index of quality of the product in real time. This study aims to review the recent advancement in biosensors and their contributions in determination of adulteration and freshness concerning fish and seafood safety.

KEYWORDS: *Adulteration of fish, Formaldehyde, Ammonia, sodium benzoate, Biosensor.*

INTRODUCTION

Fish is a healthy food and serves as a most important source of animal proteins and great source of ω-3 PUFAs and micronutrients for people worldwide **(Mohanty et al. [1])** mainly in the underdeveloped and developing countries. High consumer demand for fish and fish products has resulted in enhanced fish trade in various countries. To cater the need of consumers, fish is exported from the place of production to various distances. As fish is perishable, the flesh of fish can spoil quickly and it should be eaten on the day of capture, unless cured **(Marzuki et al. [2])**. In order to extend the storage life as well as to improve the sensory attributes i.e. appearance, fishermen and fish vendors tend to carelessly use hazardous chemicals like formaldehyde and ammonia and additives such as sodium benzoate in various fish and sea foods. Formaldehyde has been identified "as carcinogenic to human" by IARC (International Agency for Research on Cancer) **(Bianchi [3])** .As consumption of these adulterants is hazardous to human body, it is important to investigate the presence of adulterants as well as the quality of fish. Various methods have been adopted for detection of adulterants like liquid chromatography, ultraviolet detection, mass spectrometry and fluorescence techniques, gravimetric methods etc. Colorimetric detection methods, such as Eegriwe's method and Deniges' method, are being familiar since the beginning of the 20th century **(Wang et al. [4])**. Unfortunately, these methods, reagents, and reaction products are often hazardous to human health. All of these conventional methods require similarly harmful reagents and suffer from a number of interventions, concluding in false positives. Additionally, these conventional techniques possess various limitations such as requirement of large sample volumes, time consuming, expensive and these techniques required highly trained personnel **(Duffy [5])**. Hence to get over these limitations recent efforts have turned towards the development of biosensor that enables direct, reliable, and reproducible measurements. Biosensors is a kind of device which recognises bio molecules such as enzymes, antibodies, nucleic acid, aptamers ,integrated with physiochemical transducer to identify the specific analyte **(Perumal [6])**. This review provides an overview on the recent developments and implementation of biosensors used for determination of adulteration as well as freshness of fish, in so as to know better the risks of fish consumption, to regulate the risks of consumption and to cater additional information in food safety.

MAIN ATTRIBUTES OF BIOSENSOR

Biosensors are defined as an analytical tool composed of biological components that are being used to detect the presence of a target ligand in order to generate a signal (**Turner [7])**. If we compare these devices with any other presently existing diagnostic device, these sensors are far advanced in terms of selectivity as well as sensitivity. An ideal biosensor must be fast, cost-effective, and stable for sensitive and selective towards target molecules. Among the different types of biosensors, electrochemical biosensors have gained special attention in the scientific community **(Kalcher [8])**.

Electrochemical biosensor consists of an electrode transducer which descry the electrons induce by the reaction with bio receptor and determine a measurable analysis of the contaminants **(Thevenot [9])**. Key to the operative fulfilment of biosensors is their biological recognition elements which provide a superior stage of specificity and binding affinity with the desired molecule. Such binding is termed coupling or specific binding. If interaction occurs, it creates an electrical signal which is recorded and amplified **(Sharma et al. [10])**.

Bio receptor is introduced in biosensor to cooperate with the actual analyte of interest to grant a control measured for the transducer. The key prerequisite of the bio receptor is: high selectivity for the analyte among a lattice of other substance or natural segments. Bioreceptor includes biological components such as tissue, organelles, microorganisms, enzymes, antibodies, cell receptors, nucleic acids, etc) which acknowledges the target analyte. The bio receptor may be a bio molecule which discriminates the target analysed, however the transducer changes over the acceptance of the occasion driven for a perceptible sign. On the opposite hand there's a large variation within the varieties of bio molecules used. The speciality of a biosensor is that: the two segments are synchronised into one single sensor.

COMMON FISH ADULTERANTS

FORMALIN

Formaldehyde is a colourless, strong signature smelling gas, used as preservatives in various purposes, like plywood, particleboard, glue, fabrics, paper coating, food, antiseptics, medicines and cosmetics etc **(Bolt [11]) and (Groah et al.[12])**, some insulation materials and in laboratory preparation of other chemical compounds. It is easily broken down in air and water, and so is water soluble. Formalin consists of 60-63% of water and 37-40% of formaldehyde (by weight), with most of the formaldehyde presents as low polymers **(Kiernan [13])**. It is widely used as industrial disinfectant and preservatives in biological and medical laboratories and funeral homes.

Formaldehyde also produces during some metabolic process. Since the samples in the Biological labs are preserved for decades with negligible loss of physical structure and biological lustre, it is clear and easily understandable that adulteration of zoological food materials has its influence. Fishes undergo adulteration with Formalin to keep them shiny and apparently fresh for a longer time **(Sayeeda et al. [14]),** neglecting the probabilities of nasal sinuses, leukaemia and cancer for the consumers of the same **(Hauptmann et al. [15])**.

AMMONIA

Ammonia is a colorless highly irritating gas with a sharp suffocating odour. It contains three atoms of hydrogen and one atom of nitrogen. Ammonia is strongly soluble in water and is frequently used in water solution to produce ammonium hydroxide. Ammonia exits in two forms: un-ionized ammonia (NH_3^0) and ionized ammonia (NH_4^+). The aquatic toxicity is primarily attributed to the unionized form (NH_3^0) whereas the ionized species (NH_4^+) is considerably non toxic or less toxic in nature **(Emerson [16])**. According to Indian and International regulations, the fresh fish and sea foods are mainly preserved in ice. But in practice, some substances other than ice are used to extend the keeping quality is a fraudulent practice. Ammonia is cheaply used during ice manufacturing to cut down the cost of ice or reduce the cost of ice. Although ammonia is not proved to be carcinogenic, but exposure to higher concentration of ammonia may cause severe health hazards. Ammonia is also severely toxic to fish as it affects the central nervous system which causes "acute ammonia intoxication" - that includes seizure and even death **(Randall [17])**.

SODIUM BENZOATE

Sodium benzoate is widely used as preservatives due to its good stability and high water solubility **(Ren et al. [18])**. Sodium hydroxide reacts with benzoic acid to produce sodium benzoate. It is the sodium salt of Benzoic acid, soluble in water, and so is easily usable in the field of fishery.

Fishes are doped with several toxic compounds, whereby Ammonia and Sodium benzoate are leading, which slow down the melting process, and so preserving the fresh appearance for a dramatically longer duration. However according to FDA it is considered "Generally Regarded as Safe" and can be used in foods in concentrations above 0.1% **(Lennerz et al [19]**). The notable point is the compound is able to cause numerous devastations to the consumers including Parkinson's disease, genetic disorders and cellular level of destruction **(Bruna [20])**. A survey cum inspection conducted by the FSSAI named Operation Sagar Rani exposed the malpractice.

DETECTION OF FISH ADULTERATION USING BIOSENSOR

FORMALIN

Fish and seafood are very much perishable in nature, the flesh of fish can spoil quickly and it should be eaten on the day of capture, unless cured **(Marzuki et al. [21])**. Though it can be kept in dry ice for maximum 8 to 14 days. For keeping the fish fresh, fish vendor uses formaldehyde as it is a well known preservative though it contains carcinogenic effects on human being. The electrochemical biosensor for detection of formalin provides lower cost, faster response and it is user-friendly. For the detection of formalin here electrochemical biosensor is used which consists of the following parts; gold nanoparticles (AuNPs), chitosan (nanocomposite membrane), ionic liquid [EMIM][Otf] and glassy carbon electrode for determination of formaldehyde. Here as Bio- recognition receptor we use formaldehyde dehydrogenase, which is very selective towards substrate, formaldehyde .Electro-catalytic properties of the electrode could be amplified through immobilization, or it enhances the rate of chemical reaction without being consumed in the process and better results can be obtained.

Formaldehyde biosensor (FDH) was primarily immobilized onto the surface of chitosan. This FDH hydrolyzes the formaldehyde and produces NADH and Formic acid **(Bohari [22]).**

$$HCHO + NAD^+ + H_2O \rightarrow HCOOH + NADH + H^+$$

Nanoparticles are also immobilized on the chitosan along with FDH to provide larger surface area for electrochemical reaction rate **(Siddiquee et al. [23])**. FDH is found to be very selective towards formaldehyde whereas for the higher stability of the reaction cofactor NAD^+ is used. Chitosan (CHIT) film is used in the sensor due to its zero toxicity and low cost**(Kumar [24]) and (Krajewska [25])** and easily controlled by manipulation of pH **(Rinaudo [26]).**This electrochemical biosensor can investigate formaldehyde content of fish in real time.

DETECTION OF FRESHNESS USING BIOSENSOR IN FISH QUALITY ASSESMENT

DETECTION OF HYPOXANTHIN ON ATP DEGRADATION

Hypoxanthine can be considered as one of the major freshness indicator of fish which yields due to metabolic degradation of ATP. The level of hypoxanthine in fish muscle either alone, or in conjunction with the levels of other ATP metabolites, is used as an indicator of quality

(Roberts et al. [27]) and(Yano et al. [28]). Hypoxanthine accumulates mostly in the animal muscle; therefore, a close correlation between the nucleotide catabolism and the foodstuff loss of quality can be established **(Agüi et al. [29])**. Certain enzymatic biosensors followed by colorimetric or electrochemical detection have been designed to measure the level of hypoxanthine **(Lawal [30])** .Here xanthine oxidase (XOD) is being used for bio recognition of hypoxanthine or xanthine **(Lin et al. [31]) and (Nakatani et al. [32]).** Xanthine oxidase (XOD) was immobilized in the sensor by cross-linking with bovine serum albumin (BSA) and glutaraldehyde on different types of electrodes: electrodeposited gold-over-gold disks and carbon-paste electrodes . In case of XOD immobilization highest sensitivity is obtained on a carbon paste electrode modified with gold nanoparticles (AuNPs). The sensor can be operated within the potential range between 0.00 to 0.6 V and it possess the capacity of working at 0.00 V which allows the exclusion of interfering compounds such as ascorbic acid. Detection of hypoxanthine by XOD consists of the following steps: firstly hypoxanthine oxidizes to xanthine, then to uric acid **(Fatima [33]).**

$$\text{Hypoxanthine} + O_2 \rightarrow \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + O_2 \rightarrow \text{Uric acid} + H_2O_2$$

The detection of xanthine using colorimetric sensor involves the oxidation of 3,30,5,50-tetramethylbenzidine dye (TMB) .The oxidation is enhanced for using copper nanoclusters in the presence of H_2O_2 which produces due to the oxidation of xanthine **(Yan et al. [34]).** The sensor displays a detection range of 3.8×10^{-7} M., and a linear range from 5.0×10^{-7} to 1.0×10^{-4} M., a multicolour sensor has been developed for hypoxanthine detection by using gold nanorods (GNRs). Hypoxanthine is oxidized by XOD and produces H_2O_2 which undergoes a Fenton reaction and generates hydroxyl radicals in the presence of Fe^{2+}.In the presence of hydroxyl radicals, a vivid color change is observed. Depending on the concentration of hypoxanthine in the range of 0 to 1.13 mM. Various colors were generated, such as purple, gray, pink, reddish brown, green, yellow and blue. The sensor is capable to semi quantitatively estimate the levels of hypoxanthine in fish extract by the naked eye.

VOLATILE GAS

Moist fresh fish has almost no fishy odour. The fishy odour develops with time after harvest. Generally, a kind of microorganism is found in gills and skins popularly known as specific spoilage organisms (SSO), the number increases gradually over time and spreads to different tissues when the fish die. During storage of non-fermented foods, a group of biogenic amines are produced known as Total volatile bases (TVB) **(Horsfall [35])**. The combined total amount of ammonia (NH_3) [produced by the deamination of amino-acids and nucleotide catabolises], dimethylamine (DMA) [produced by autolytic enzymes during frozen storage] and trimethylamine (TMA) [produced by spoilage bacteria] in fish is called as the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for freshness of fish **(Wu and Bechtel [36])**. TVB-N concentration of freshly caught fish varies between 5 to 20 mg per 100 g **(Muhammet and Sevim [37])**. Though the classic technique to measure the volatile gas is the use of gas chromatography **(Olafsdottir et al. [38])** but the quantification of this total volatile basic nitrogen (TVB-N) can be done by using an organic semiconductor gas sensor with a porous top metal electrode sensor with a nanostructure surface in order to get enhanced gas adsorption **(Chang et al. [39]).** The sensor possesses the capacity to detect up to 100 ppb whereas the accepted value for ammonia is 200-300 ppb. Another emerging technique of monitoring fish quality is the use of "electronic nose" **(Kuske [40])**. The e-nose is a scientific tool equipped with an array of gas sensors and an artificial intelligence algorithm system capable of discriminating such volatiles .The semiconductor of metal oxide (MOS-FET) gas sensor array is the most prevalent e-nose gas sensor. Small size, low power consumption, short response time, wide operating temperature and high efficiency are some of the advantages presented by this sensor type. The e-nose requires no sample transformation from solid to liquid state and is better suited for sensing meat and fish volatile compounds as observations can be made both in the liquid and solid states.

CONCLUSION

It is difficult to make the humans understand that how much risk they are in due to some dishonest person and for their over greediness, they are making the regular food poisonous and unhealthy. So, by the use of biosensor we can save the lives of human beings. The toxicity is very crucial and needs to be detected and evaluated repeatedly in order to ensure healthy lives. However for assuring connectivity of sensing devices and developing wireless, independently operated sensors are used to privilege rapid monitoring of large number of samples in order to provide real time status during shipping and storage for longer time. Moreover, the electrochemical biosensor is proven to be the most economic, reliable and high sensible, this is an effective and cheap way to regulate the formaldehyde content in fish. A novel electrochemical biosensor is always developed based on FDH/AuNPs/[EMIM][Otf]/CHIT/GCE using methylene blue as redox indicator. This developed kit contains many advantages over conventional methods like reliability, high sensitivity and stability.

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