

Smart Sensing of Aquatic Nutraceuticals: Emerging Biosensor Technologies for Quality and Safety Assurance

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Abstract

Nutraceuticals derived from both fresh and marine water environments have recently been under considerable scrutiny because of the diverse benefits that can be gained from them nutritionally and health wise. Such compounds are naturally occurring in plants like *Nelumbo nucifera*, *Lemna minor*, macro-algae from the seas, fish and crustacean waste products and contain a number of bioactive substances such as polyphenols, proteins, omega-3 fatty acids, pigments and polysaccharides which can be used as antioxidants, anti-inflammatory, antimicrobial and cardioprotective agents. Nevertheless, the maintenance of quality and safety of these nutraceuticals is an important challenge owing to the risks of contamination with pathogenic microorganisms, marine toxins, heavy metals, and spoilage. In this regard, biosensors are becoming increasingly popular as a rapid, sensitive, and reliable method of quality monitoring of nutraceuticals. This review illustrates the emerging role of biosensors in the better monitoring of aquatic nutraceuticals. It describes basic principles, components, and classification of biosensors and emphasizes on electrochemical sensors due to their sensitivity, portability, low cost, and ability to use on-site analysis. Moreover, some of the recent advances in the field such as biosensors for pathogen detection, heavy metal detection, spoilage metabolite detection, marine toxin detection, and bioactive compound detection are discussed. Additionally, the recent advances associated with the application of nanotechnology, artificial intelligence, wearable biosensors, and smart packaging in aquatic nutraceuticals quality monitoring are considered.

Keywords: Aquatic nutraceuticals; Biosensors; Electrochemical biosensors; Food quality monitoring; Marine bioactive compounds

INTRODUCTION

Biosensor is defined as a device, which transforms biological response into electrical signal. Normally, biosensor should be highly selective and not dependent on any physical parameters like pH and temperature and also be reusable. The term "biosensor" was introduced by Cammann and its definition was proposed by IUPAC. [1]

First true biosensor was invented by Leland C. Clark, Jr in 1956. It was made for measuring oxygen content. He is known as the father of biosensors and his invention of the oxygen electrode bear his name: 'Clark electrode'. [3]

Nutraceutical is a term derived from "nutrition" and "pharmaceutics." The term is applied to products that are isolated from herbal products, dietary supplements (nutrients), specific diets, and processed foods such as cereals, soups, and beverages that other than nutrition are also used as medicine. [3]

Seafood consumption demand has seen a marked increase that has contributed to the growth of seafood production. Marine biotoxins like tetrodotoxin, saxitoxin, okadaic acid, domoic acid, brevetoxin-1, and azaspiracid-1 contaminates the seafood. [4] Further, seafood serves as an excellent host for pathogenic bacteria that have the potential of causing infection in the consumers. Some common harmful microorganisms found in seafoods are *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Escherichia coli*, etc. [5] Traditional methods of testing seafood quality require expensive laboratory equipment. Traditional methods have little use in developing countries. [6]

The biosensors are functionally integrated and provide, through a biological recognition element, a (bio)transducer that recognizes the presence of a specific analyte in the reaction. [7] The signal formed as a result of the transduction process (through heat absorption, light absorption, redox reaction, change in the mass of reactants or products, etc.) lead to changes in the electricity/electronics output that is measured by converting them into a measurable signal via the operation of the transducers. Finally, the detector enhances the transduced signal. [8]

In case of fish usage, electrochemical biosensors have been developed to detect the existence of some compounds formed during the degradation process. These key compounds are divided into two types. The first type includes molecules based on purines such as xanthine which are build when ATP breaks down, also with its related substance like uric acid and hypoxanthine. The second

type includes gases like trimethylamine and dimethylacetamide. Spoilage bacteria in fresh meat convert these gases into TMAO (trimethylamine oxide) and at same time they break down urea and amino acids to produce ammonia. [9]

NUTRACEUTICALS

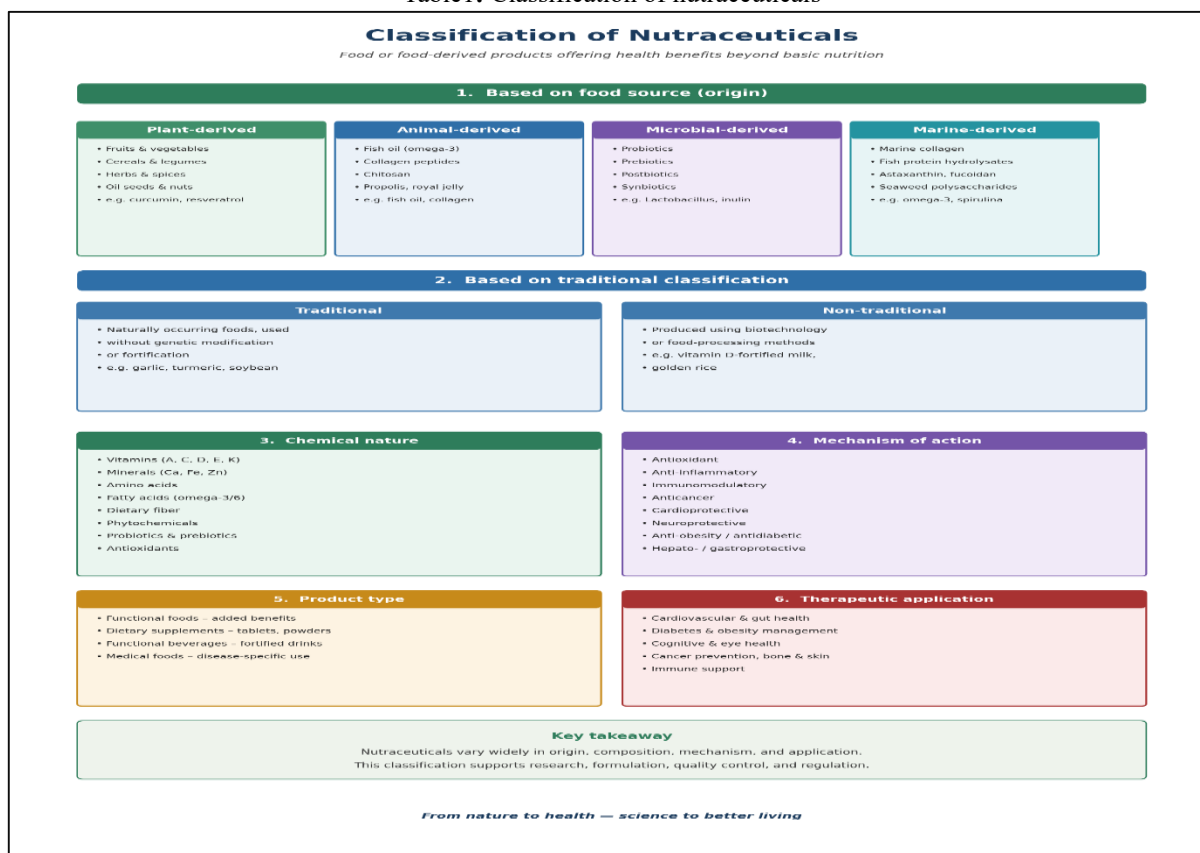
In 1989 the term nutraceutical was coined by Dr. Stephan De Felice. Nutraceutical refers to a product which cannot be considered as a conventional nutrient, but exerts positive physiological impact on the human body. Food and drug laws are not easily applied to nutraceuticals. [10]

Under dietary supplement, health and education act (DSHEA) dietary supplements are products intended to supplement the diet that contains the following dietary ingredients: a vitamin, a mineral, a herb or other botanical, an amino acid, a dietary substance is taken by man to enrich the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract, or combinations of these ingredients. [11]

Classification of nutraceuticals

Nutraceuticals may be broadly classified under a number of groups according to their sources, chemical structure, and mode of action. Table 1 below demonstrates the classification of nutraceuticals. [12]

Table1: Classification of nutraceuticals



AQUATIC NUTRACEUTICALS: AN OVERVIEW

One of the major components of earth is water. Approximately more than 70% of the earth is surrounded by water in the form of lakes, rivers, seas, oceans etc. [13] The aquatic plants serve as a great source for compounds having recognized biological significance. [14] Numerous biologically active metabolites have been isolated from aquatic plants which are being used as colouring agent, flavouring agent, nutraceuticals and in discovery of new drugs and molecules. [15] Terrestrial, semi-aquatic and aquatic plants are a rich source of phytochemicals with pronounced anti-oxidant and anti-inflammatory actions. These phytochemicals possess different or multiple mechanisms through which they act as anti-inflammatory and anti-oxidant such as inhibition of COX pathway, down regulation of pro-inflammatory cytokines, reducing the translocation of NF-KB to the nucleus, reducing oxidative stress via free radicle scavenging action etc. [16]

Freshwater derived nutraceuticals

The larger family of such aquatic plants includes the *Nelumbo nucifera*, while some studies have also outlined that other less discussed aquatic plants can also contribute to the above approaches, such as representatives from *Araceae* family (e.g., *Lemna* spp). [17]

Nelumbo nucifera: The lotus is a water plant that is widespread in Asia, America and Australia fresh waterbodies. Lotus (*Nelumbo nucifera Gaertn.*) is one of the species of the genus *Nelumbo* in the family *Nelumbonaceae*.^[18] *Nucifera Gaertn.* is cultivated in many countries, including China, India, Russia and Australia. The other species, *N. lutea Willd.* mainly grow in the eastern and southern parts of North America.^[19] Chinese lotus is usually divided into three types: rhizomatous lotus, lotus seed and lotus flower.^[20] Lotus seed is the one which has more carpid and seeds than any other, and the nutritional value of seeds is always better compared to that of rhizomatous lotus.^[21] As far as Chinese and Indian medicine is concerned; these four edible parts have medicinal qualities.^[20] Pharmacological properties such as antioxidant activity, anti-inflammatory, whitening, and anti-aging have made *Nelumbo nucifera* popular.^[22]^[23]^[24] The below figure 1 shows the health benefits of *nelumbo nucifera* along with phytochemical constituents responsible for it.

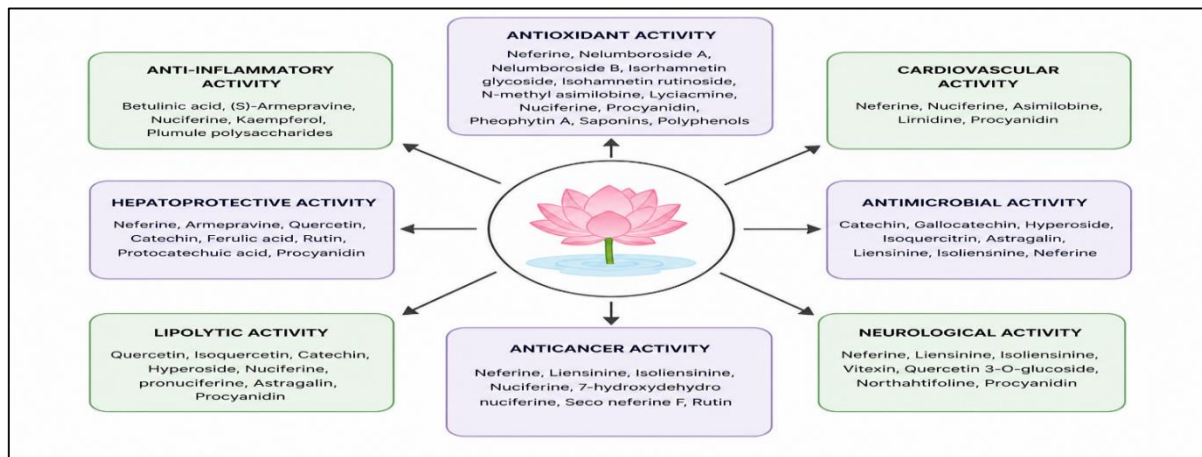


Figure 1: Health benefits of *Nelumbo nucifera*

Lemna minor: *Lemna* spp. is an aquatic plant also known as duckweed. The term 'duckweed' comes about due to the global spread of this plant in conjunction with birds like ducks, as well as its fast growth rate.^[17] The duckweeds are small floating water plants that occur in thick patches in waters that are stagnant or flowing slowly but have plenty of nutrients.^[25]^[26] Several bioactive have been detected in *Lemna*, with health benefits also being reported for the antioxidant, anti-inflammatory and antithrombotic health promoting effects of its bioactive, extracts and relative functional products against several manifestations of thrombo-inflammation, oxidative stress, etc. Figure 2 indicates the health advantages of *Lemna minor* plant

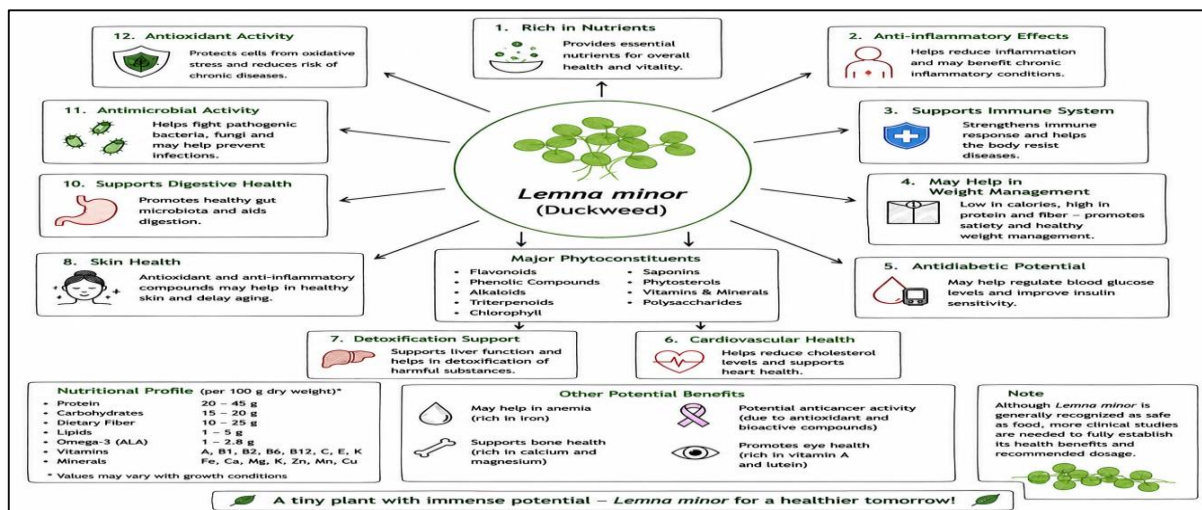


Figure 2: Health benefits of *Lemna minor*

Marine derived nutraceuticals

The natural compounds derived from marine resources which are considered as having a positive impact on health and a possible application in the fields of food and medicine include proteins and peptides, polysaccharides, ω -3 PUFA, enzymes, polyphenols, pigments, and vitamins.^[27] Chitosan ((1-4)-2-amino-2-deoxy- β -D-glucan) is the most common and natural cationic polysaccharide found in exoskeletons of crustaceans, molluscs, insects, and fungi. Seafood is used for the making of chitosan such as crab and shrimp prawn shell. Shell material is an example of such a product that provides chitosan.^[28] The Figure 3 depicts the health benefits of chitosan.

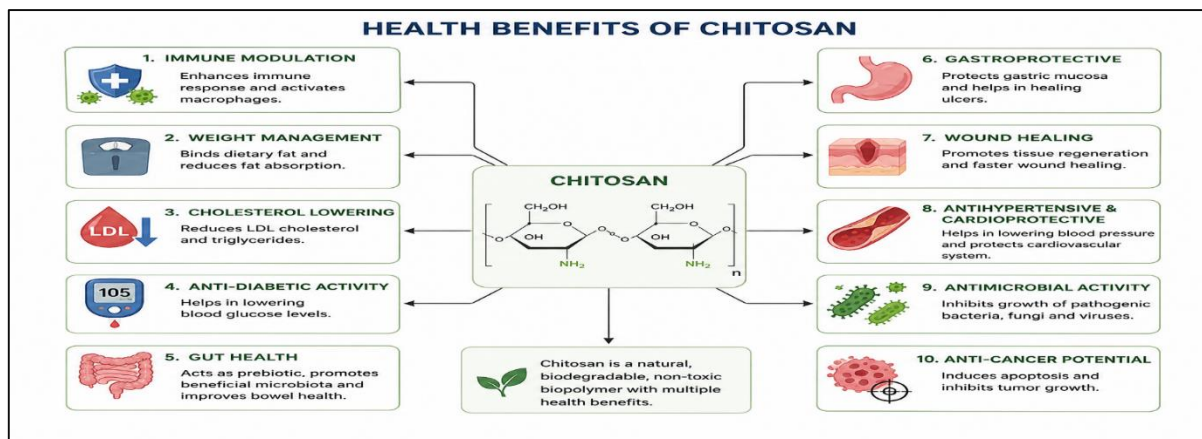


Figure 3: Health benefits of chitosan

Beneficial molecules from marine macroalgae: Among the biological species, marine algae are classified as one of the primitive producers of whole aquatic biomass making them a source of natural bioactive molecules. [29] The natural algal pigments are often bioactive, particularly from Chlorophyceae, Rhodophyceae, and Phaeophyceae (or green, red, and brown algae, respectively). The categories of naturally occurring pigments derived from marine algae comprises carotenoids, chlorophylls and phycobiliproteins [30] [31]. There are also health benefits associated with the natural pigments derived from algae which have been presented on Figure 4.

HEALTH BENEFITS OF NATURAL ALGAL PIGMENTS with Phytoconstituents Responsible			
ALGAL PIGMENT	MAJOR ALGAL SOURCES	PHYTOCONSTITUENTS (Responsible Compounds)	KEY HEALTH BENEFITS
CHLOROPHYLLS (Chlorophyll a, b)	<i>Chlorella vulgaris</i> <i>Spirulina platensis</i> <i>Ulva</i> <i>Dunaliella salina</i>	- Chlorophyll a - Chlorophyll b - Pheophytin - Pyropheophytin	- Detoxification (binds heavy metals and carcinogens) - Antioxidant & anti-inflammatory - Supports wound healing - May help in cancer prevention
CAROTENOIDS (β-Carotene, Astaxanthin, Lutein, Zeaxanthin)	<i>Haematococcus pluvialis</i> <i>Dunaliella salina</i> <i>Chlorella zofingiensis</i> <i>Isochrysis galbana</i>	- β-Carotene - Astaxanthin - Lutein - Zeaxanthin - Canthaxanthin	- Powerful antioxidant - Eye health (macular protection) - Skin protection (anti-photoaging) - Cardioprotective - Immune modulation - Cancer prevention
PHYCOERYTHRIN (PE)	<i>Porphyridium cruentum</i> <i>Gracilaria verrucosa</i> <i>Palmaria palmata</i>	- Phycocyanin - Phycocyanobilin	- Anti-inflammatory - Neuroprotective - Anticancer potential - Improves liver function
PHYCOCYANIN (PC)	<i>Spirulina platensis</i> <i>Arthrospira maxima</i> <i>Oscillatoria spp.</i>	- Phycocyanin - Phycocyanobilin	- Antioxidant & anti-inflammatory - Immunomodulatory - Hepatoprotective - Anti-diabetic potential
FUCOXANTHIN	<i>Phaeodactylum tricornutum</i> <i>Sargassum spp.</i> <i>Undaria pinnatifida</i>	- Fucoxanthin - Fucoxanthinol - Amarouciaxanthin A	- Anti-obesity - Anti-diabetic - Anticancer - Cardioprotective
VIOLAXANTHIN	<i>Dunaliella viridis</i> <i>Chlorella zofingiensis</i>	- Violaxanthin - Antheraxanthin	- Eye health (protects retina) - Antioxidant - Immune support

Figure 4: Health benefits of NPM

BIOSENSORS

A biosensor consists of biological components such as enzymes, antibodies or living cells along with a transducer in order to generate and convert the biological reactions to electronic or optical signals. Biosensors are made to offer sensitive and precise detection or measurement of a wide range of target analytes, including pathogens, chemicals, ions, and biomolecules. Interaction of the biological recognition element (bioreceptor) with the analyte forms the basis of a biosensor. A biological reaction or a signal is generated once the analyte gets bound to the bioreceptor and then transducer converts this signal into measurable output.

Components of typical biosensor

Bioreceptor: The biological element attaches on the target substance selectively. The bioreceptor may be an enzyme, an antibody, a nucleic acid (DNA/RNA), cells, etc.

Transducer: It is the element of the biosensor that translates the bio-chemical reaction by the bioreceptor into an output signal.

Signal Processing Unit: It is the part of biosensor that is mainly responsible for processing and amplifying the signal. [32] Figure 5 indicates biosensor's detection mechanism.

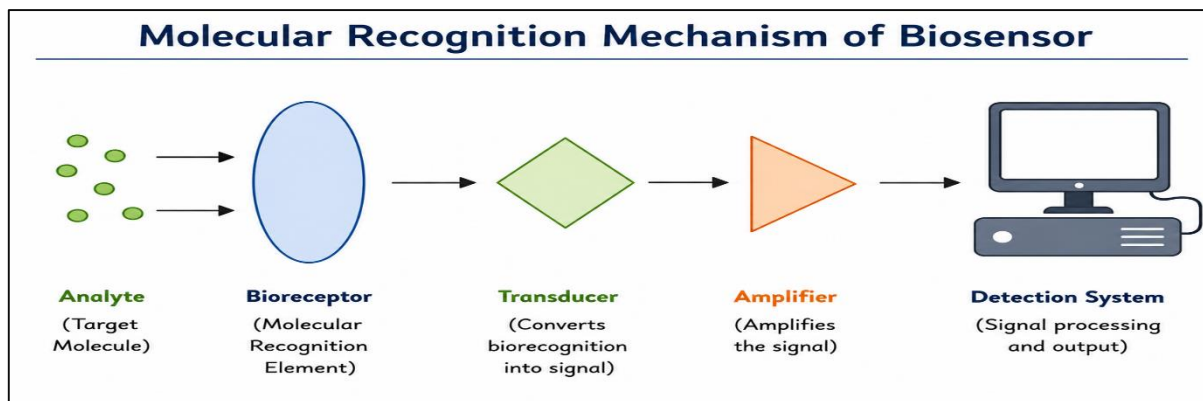


Figure 5: Molecular recognition by biosensor

Classification of biosensor

Based on biological receptors or transducer component, biosensors can be classified as below. A flowchart Figure 6 is illustrating the different types of biosensors based on the biological receptors and transducer elements. ^[33]

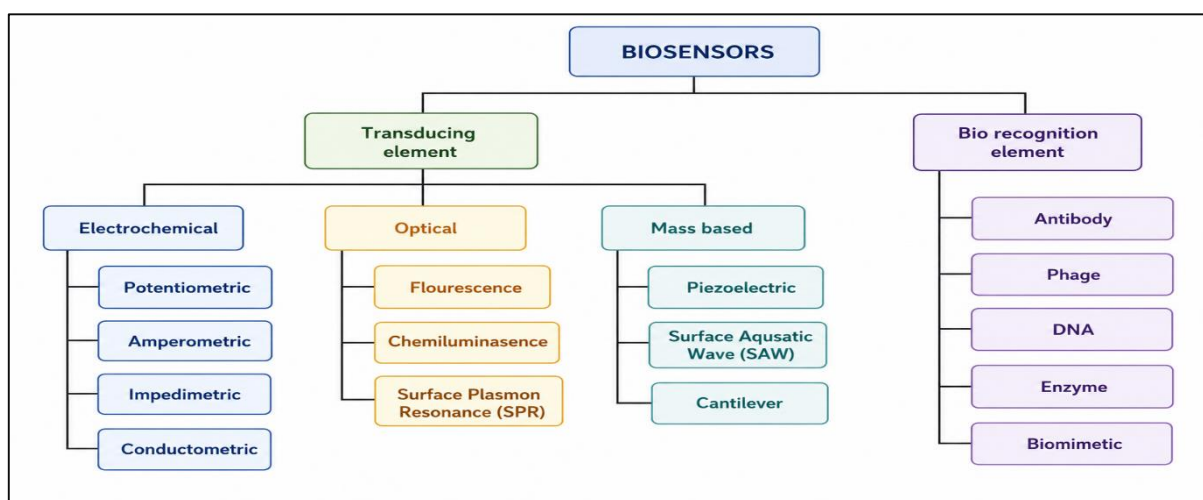


Figure 6: Types of biosensors

Biosensors used in Aquatic Nutraceuticals

Electrochemical biosensors have achieved lots of attentions over other biosensor owing to their different benefits. There are many advantages that come with electrochemical biosensors. One of them is their enhanced sensitivity as a result of the signal amplification effect in electrochemistry. ^[34] The reasons for popularity of electrochemical biosensors can be enumerated as follows: sensitivity, low cost, and simple production. They are able to help prevent spoiled foods from entering the food chain by detecting them early enough. Electrochemical biosensors can detect microbial metabolic products, volatile organic compounds, biological toxins, pathogenic organisms, chemical markers, and others, which makes them applicable for food quality control. ^[35]

Environmental pollution with heavy metals is also one of the problems that pose a threat to the quality of the fish and marine products. Metals like cadmium, lead, zinc and other heavy metals can accumulate in fish tissues, that exceeds safe consumption limits. They might cause toxicity and carcinogenesis, indicating the importance of monitoring. ^[36] Electrochemical sensor consisting carbon nanotubes are used to detect heavy metals in fish. The sensors took advantage of high conductivity and sensitivity. ^[37] Thandavan's biosensor incorporated iron oxide nanoparticles which had the capability to detect nanomolar amounts of Xa. The biosensor made it possible to detect early spoilage of fish. ^[38] Table 2 displays different kinds of biosensor and their respective application.

Table 2: Types of biosensors, target analyte and application of biosensors in nutraceuticals

Biosensor Type	Target Analyte	Application in Aquatic Nutraceuticals
Amperometric	Hypoxanthine, glucose	Fish freshness assessment and spoilage monitoring
Potentiometric	Heavy metal ions, pH	Detection of contaminants in algae, seaweed, and fish-derived products
Conductometric	Microbial metabolites, ammonia	Monitoring microbial spoilage during processing
Impedimetric	Pathogens, marine toxins	Safety evaluation of seafood and marine nutraceuticals
Voltammetric	Carotenoids, phenolics, algal pigments	Quantification of bioactive compounds in marine algae
Enzyme-based	Cholesterol, lactate	Quality assessment of fish oils and marine protein products
Aptamer/Immunoelectrochemical	Marine toxins, pathogens	Authentication and rapid detection of contaminants

DISCUSSION

The importance of biosensors in conjunction with aquatic nutraceuticals is becoming ever more important as far as the efficiency and safety of the products are concerned. Aquatic nutraceuticals derived from fresh water plants like *Nelumbo nucifera* and *Lemna minor*, as well as from sea sources like algae, fish, crustaceans, and chitosan have various types of bioactive components that serve as antioxidants, anti-inflammatory, antimicrobial, cardioprotective among others. There is a possible threat of contamination via microorganisms, metal ions, toxins from the sea, and biochemical degradation reactions.

Out of the several sensors available, there is one sensor which gains importance on account of several factors such as its sensitivity, rapid response time, portability, low-cost, and ability to analyse immediately whenever required. Electrochemical biosensors for pathogen, metal, spoilage product, sea toxin, and biological substance detection make it possible to monitor the process throughout the production, processing, storage, and distribution of food.

Recent advances in the areas of nanotechnology, artificial intelligence, biosensors wearables, and smart packaging have greatly improved biosensors' capabilities through increased analytical precision and automation, among others. These developments help in achieving prediction-based quality control and help in food safety regulation and increasing consumer trust. However, issues like standardization of sensors, stability, commercialization and validation pose problems in the adoption of these sensors at an industrial level. Future research should concentrate on development of affordable, multiplex biosensors which will be able to detect many contaminants and biomarkers. Such technologies help in implementing a system of prediction-based quality control and in regulating food safety and enhancing consumer confidence. This would help expedite the development of quality control systems that can be sustained for the nutraceuticals derived from aquatic sources. [23] [35]

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