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Review

Biosensors as freshness indicator for packed animal and marine products: A review

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Abstract

There is growing interest in food quality and safety, thus creating the demand for the development of highly sensitive devices to detect freshness and quality of perishable food. The development of on-package dual sensor and smart packaging systems is gaining momentum at the end of the supply chain management, regarding the quality of packed marine products. The colour change provides a clear indication of the quality to the consumers. Simultaneously, the manufacturers can track the quality of the packed marine products, at any point in time, to take an appropriate call depending on the standard. The on-package double sensors were built dependent on two pH pointers, and applied to screen the freshness of marine products. Methyl red (MR) and bromocresol purple (BCP) are commonly used pH indicators to detect the freshness of marine products. Once the marine products start spoiling, the MR changes from red to yellow, while the BCP changes from yellow to purple, based on the pH. Therefore, the label can be used as a simple and practical freshness indicator to continuously monitor and detect the quality of packaged products by data capturing, and also collecting it by cloud computing and the internet of thinking (IoT).

Introduction

In recent years, more importance has been given to food safety in developing countries such as India. On the other hand, many products (mainly animal products) have been recalled from the market due to safety concerns in developed countries. For example, in the United States, issues related to finfish, poultry, and beef (Iwamoto *et al.*, 2010) have caused a significant impact to consumers' health. The main bacteria that cause spoilage in seafood are *Vibrio*, *Salmonella*, *Shigella*, and *Listeria*, which in turn could be controlled to a certain extent during harvesting fish, shrimp, or shellfish. Also, further spoilage could be prevented by introducing proper handling and processing. Nowadays, great attention is being paid to food safety in the seafood processing sector. Novel approaches are to be adopted to attain complete food safety for seafood.

In seafood, the quality deteriorates relatively faster since microbial activities are very high after harvesting, which in turn alters the sensory attributes and chemical compositions of the marine products. Sensory parameters are determined as subjective and objective outcomes. In the subjective analysis, the inspector makes assessment by using a 5- or 9-point Hedonic scale rating between "likes extremely" to "dislikes extremely". In the objective sensory analysis, the inspector makes assessment by using specific quality factors *e.g.*, degree of saltiness, firmness, *etc*. (Olafsdottir *et al.*, 1997). If the sensory methods are correctly done, then we can consider it as an accurate tool that can provide unique information. However, the result is subject to change as per the perception of the inspector (York and Sereda, 1994).

The present work review aimed to control food-related problems and ensure food safety needs

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by the application of new techniques capable of producing reliable data, and eventually establish regulatory organisation criteria. The achievement of new development and creative food packaging to guarantee food safety, quality, and traceability demands the use of biosensors linked with computer data capturing and monitoring systems. Consumer prefers to have a traceability mechanism in the pack which could be achieved by smart packaging. Here, the label provides all information including the origin, manufacturer details, nutritional information, *etc*. through radio-frequency identification (RFID) tags. Also, the label provides all the details to trace the current status of the product, previous storage conditions, harvesting conditions, *etc*.

Chemical methods are used in resolving issues by providing accurate results without much deviation. Total volatile basic nitrogen (TVB-N) is a commonly practised chemical method in measuring seafood quality. It is a simple technique to perform and generally reflects the spoilage at later stages, which could not be controlled. Other methods are based on trimethylamine (TMA) and related compound analyses. The total volatile bases developed during the stages of unfrozen fish consist primarily of ammonia and tri-methylene. But the accuracy of the results depends on other factors such as the time of the year, location of catch, stage of spoilage, type of processing, storage, and method of analysis (ElShehawy *et al.*, 2016).

Due to these existing strategies, we need an accurate, low-cost, and effective method for evaluating fish or seafood quality. The present review also aimed to describe a non-invasive technique or tool, and an alternative method for developing an on-package dual sensor on smart packaging, by which consumers could directly detect the freshness of the seafood. Many such inventions related to detecting seafood freshness have been explored earlier. Edible sensors are also available for the evaluation of fish freshness. The edible sensor film has a high sensitivity for gaseous amines. But the technique might have a negative impact due to its coating strength that could impair the detection of quality in the supply chain.

Dual sensor on package material is suggested to check the quality of the seafood in the current pandemic situation. The colour change in the dual-sensor image of methyl red (MR) and bromocresol purple (BCP) (Kuswandi and Nurfawaidi, 2017) in the packaging material could be monitored to detect fish freshness. The sensors are highly sensitive to pH, and therefore, could be used to continuously detect the freshness of fish.

Smart packaging system

Presently, shoppers are on the lookout for creative and useful brilliant bundling frameworks in grocery stores. The regular packaging system only provides normal quality parameters to assess the freshness, and detect spoilage in seafood. Also, they only provide basic information like use-by-date, best before date, *etc*. But in the case of highly perishable products like seafood, the basic parameters alone are not enough. Customer satisfaction is also poor here because of the lack of effectiveness in detecting spoilage based on these factors.

An effective solution for these issues could be the use of a smart packaging system with a sensor. The existent smart packaging system for seafood is based on the food quality indicator and time-temperature indicator. The internet of things, or IoT, is a network of interacting smart gadgets, which interacts and performs tasks in novel ways, for businesses and everyday living. Therefore, the authors have attempted to develop a smart packaging system with a sensor that indicates the spoilage on the basis of colour. Further, the authors also envisioned to link the packaging system with IoT and cloud computing, which in turn would pave the way for extensive research and benefit the start-up ventures in this regard (Schaefer and Cheung, 2018) (Figure 1).

Food freshness / quality detection

Few changes can happen in packaged food due to processing or microbial growth over time. For example, changes in gas production or microbial contamination give information about the level of spoilage condition of food. Sensors that can check such parameters could provide an overall result of food quality. Models that incorporate "on-package" pH indicators show a colour difference when food spoils as a result of pH changes associated with the release of unstable amines produced during meat or fish decay (Kuswandi *et al*., 2011). In this regard, many start-up companies have attempted to create economically accessible freshness indicators for various kinds of food including fish, meat, poultry, oat grains, organic products, and vegetables.

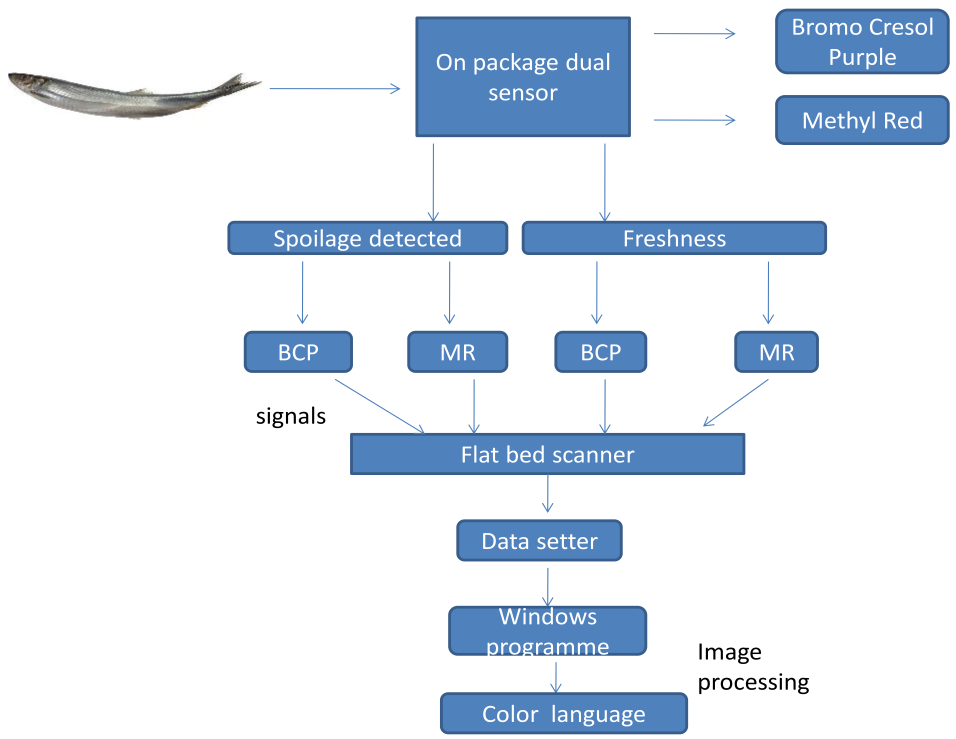

Figure 1. Flow chart of the whole process of fish packaging and sensor fixing.

Fish, meat, and poultry

The indicators that can measure the time when meat, fish, or poultry experience spoilage would indicate lipid breakdown, protein breakdown, and adenosine triphosphate (ATP) breakdown. The rate of decomposition is reliant upon numerous variables like temperature and other ecological components. The methodology that provides the result of quantitative markers of spoilage would provide an idea about the stage and nature of food. In fish, one of the standard markers is hypoxanthine, which is produced by the metabolic breakdown of ATP. Trimethylamineoxide (TMAO) is essential for the Non Protein Nitrogen (NPN) part, and evaluates the overall quality for marine (Hebard *et al*., 1982) and some freshwater fish (Gram *et al*., 1989; Anthoni *et al*., 1990). The spoilage of fresh fish is positively impacted by the presence of TMAO, especially under conditions where oxygen is unavailable. Anaerobic microorganisms can use TMAO as the terminal electron acceptor in an anaerobic respiration, thus retarding the off-flavour development (Gram *et al*., 1987; 1990; Dalgaard *et al*., 1993). Factors including meat constituents, temperature, pH, oxygen or carbon dioxide, and competing microbiota are significant in keeping up meat quality over time (Koutsoumanis *et al*., 2006; Lambert *et al*., 1991).

Types of biosensors

Electrochemical biosensors

In electrochemical biosensors, estimations of signs for natural examples are, for the most part, electrochemical in nature, while a bioelectrochemical component fills in as the transduction part. As a rule in electrochemical biosensors, natural responses either produce an adjustment in signal for conductance or impedance, quantifiable current, or charge amassed, which could be estimated by conductometric, potentiometric, or amperometric strategies (Mishra *et al*., 2018). Anodes assume a significant function in electrochemical biosensors. The location limit of the electrochemical biosensor can be influenced by the terminal surface measurement, its material, and modifications. Three types of anodes are utilised in the electrochemical biosensor, *i.e.*, reference cathode, working anode, and auxiliary cathode (Koyun *et al*., 2012). Electrochemical biosensors are distinguished into two kinds, contingent upon the sort of bioreceptor and transducer utilised.

Conductometric biosensors

Conductometric biosensors connect a conductance and a biorecognition procedure. When an electrolyte separates into its ionic components in a fluid, the fluid turns into a conducting medium (Rogers and Mascini, 1998). Various compound reactions, such as natural film receptors, may be detected by molecule conductometric or impedimetric devices using interdigitated microelectrodes. Since the affectability of the assessment is blocked by the equivalent conductance of the model course of action, ordinarily, a differential assessment is performed between a sensor with impetus and an indistinct one without protein. Analytes like urea, charged species, and oligonucleotides are recognised using this procedure.

Calorimetric biosensors

Calorimetric biosensors measure the change in temperature of the system, which contains the analyte following compound development, and interpret it throughout the activity. Since the majority of protein-catalysed responses are exothermic, the signal made by the response is utilised to pick the analyte. Calorimetric biosensors are widely used for the identification of pesticides and other enzymatic responses (Thakur and Ragavan, 2012).

Potentiometric biosensors

Potentiometric estimations are resolved dependent on the likely distinction between a marker and a reference cathode. The transducer might be a particle-specific terminal, which is an electrochemical sensor dependent on specific films as acknowledgement components. The most well-known potentiometric devices are pH anodes; ions (F^-, I^-, CN^-, Na^+, K^+, Ca^{2+}, NH^{4+}) or gas (CO_2, NH_3) particular terminals are also available (Alarcon-Angeles *et al*., 2018). The possible contrasts between these markers and reference anodes are relative to the logarithm of the particle movement or gas target, as depicted by the Nernst-Donnan condition (Grygolowicz-Pawlak *et al.,* 2013).

Optical biosensors

Optical biosensors are called "optodes" taking into account their similitude with anodes. The optodes operate by choosing the difference in light absorption between the reactants, and after-effects of a reaction, or assessing the light yield by a radiant cycle. Optical biodetectors arrange ocular strategy along a natural part for perceiving engineered or common kinds. Various ocular biodetectors were made subject to exterior plasmon resonation, spectrometry, and temporary signals, *etc*. The steering of phosphorescent signs from NADH to quantum dots (QDs) had been a matter of broad exploration for FRET-related applications. Cadmium Telluride (CdTe) QD also goes through paraphrasing cooperation with NADH because of expansive ghostly retention and different excitonic states coming about due to quantum restriction impacts. Thus, non-radiative essentialness move can happen from NADH to CdTe QD, thus improving the QDs phosphorescence. Centrality controlling the investigation of NADH-QD is used for the territory of formaldehyde-like representation analyte in the range 1,000 - 0.01 ng/mL, using a standard framework. It was possible to observe formaldehyde in the range of 1,000 - 0.01 ng/mL with a limit of detection (LOD) of 0.01 ng/mL. This technique would be utilised to accompany the action of NAD^+ subordinate proteins and zone of dehydrogenesis (Chen and Wang, 2020).

Microbial biosensors

For the most part, potentiometric microbial biosensors comprise of a particle-specific anode or a gas-detecting terminal, covered with an immobilised microbial layer. Due to microbial digestion, the take-up or arrival of analyte creates an adjustment in potential that comes out due to particle aggregation or consumption. Biosensors constitute significant explanatory instruments, and the instrumentation and packs are now turning out to be accessible for use. The significant necessities for biosensors are that they are practically identical to, or better than the traditional detection method, in terms of execution. Likewise, biosensors are relied upon to satisfy other standards.

Future prospects of biosensors as freshness indicators for packed marine products

Indicators are devices that detect change in features, such as colour, to provide information about the presence or absence of a chemical, or the degree of interaction between two compounds. Indicators differ from sensors, in that they lack receptor and transducer components, and instead convey information *via* direct visual changes (Kuswandi *et al.*, 2011). Time, temperature, gas, and freshness indicators are three well-known groups of indicators (Mohebi and Marquez, 2015). Because of microbial development or synthetic changes inside a food product, freshness indicators can provide immediate product quality data. The most successful technology for encapsulating responsive materials is encapsulation, which is simple to implement in industrial production. Polymers were used as support materials for oxygen, nitrogen, carbon dioxide, moisture, and pH sensors. The evaluation of the nutritional quality and the avoidance of poisoning mishaps are both important considerations while following the metabolic process of packaged food. This process can be observed by measuring the amount of gas released or the change in pH, as well as sensory characteristics such as colour, smell, and texture. The colour shift of the pH sensor is the simplest and most cost-effective method for food quality control (Ibrahim *et al.*, 2021).

Intelligent packaging using pH-sensitive polymers has been also used to show the freshness of packaged meat items. Raspberry anthocyanins were used as a pH-sensitive colour indicator in a recent study (Roy and Rhim, 2021). The indicator was first put into it to enhance the stability of low acyl gellan. The inner surface of a double-layer indicator film was made with indicator integrated gellan, while the outside layer was made with chitosan; the final film system was assembled layer by layer. The colour shift of anthocyanins in relation to pH was first examined. During refrigerated storage, the film method was utilised to pack and monitor the deterioration of pork patties and fish balls. The colour parameters of the double-layer indicator films (which reflected the changes in the quality of the meat products) were used as the input to the BP-ANN model, and the TVB-N content was used as the output (Sun *et al.*, 2022).

Versatile and adaptable biodetecting methods are needed in the examination for different checks utilising an individual device. One can observe the developing interest in lab-on-chip coupling with biosensor frameworks. The development of biodetectors with huge impact and accuracy needs to be simple eventually, so that they can be used universally with ease, even in the absence of the assistance of trained/skilled personnel. Therefore, research involving biosensor exploration ought to be empowered with adequate funding that enables progress in the field.

The study of sensor-based packaging also has a considerable scope when connected with the IoT. Then, the signals identified by the device can be transmitted, and easily read with the assistance of cloud computing. Such a product traceability is used in the supply chain and logistics system for many products. The potential applications of IoT are countless, and can optimise the process with a direct impact on the quality and safety of seafood. The most significant challenges regarding IoT could be converted into opportunities, especially in the developing countries.

Any methodology, when related to IoT, could give additional data exceeding the basic data provided by the routine food inspection. In addition, the system described is straightforward, and the customers can easily access it from their homes. Further, if the device is connected to IoT, it is possible to develop an interrelated device that can link actuators and sensing devices, and provide a common operating picture by sharing information over various platforms. The signals or data sensed by such devices can be transported wirelessly to a computer system which provides an interface, where the user can see the progression of product quality over time (Popa *et al*., 2019).

Conclusion

In the food industry, there is an all-time need to meet the consumers' demand to provide quality products. The demand in turn creates a need to develop new devices for survival in the market. The proposed package dual sensor is a promising area to be explored. It is an interdisciplinary approach to obtain appropriate result and to satisfy the quality-conscious consumer's advancement in the packaging system. The attempt involves the development of biosensors interlinked with IoT of different varieties. Therefore, the on-package dual-sensor label based on the pH indicators, namely BCP and MR, has been developed for seafood freshness quality monitoring. The colour change of the BCP and MR sensors will be from yellow to purple and red to yellow, respectively, as the pH increases. The use of BCP and MR in the smart packaging system as labels for the seafood freshness quality monitoring is a promising area of research.

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