Chapter 2

Review of Literature

2.1 Fish and its Composition

Fish are abundant in natural water bodies and have been a valuable food source since ancient times, with around 250 different edible species. They are classified into two main categories based on anatomical differences. Finfish, possessing vertebrae and fin appendages that aid in balance and movement through water, have scales covering their skin and are coated with mucous. Shellfish, lacking a skeleton but covered by a hard shell, are another category. There are more varieties of finfish available for consumption compared to shellfish, and their types vary by location, found in both saltwater and freshwater. Common freshwater fish include sardine, mullet, catfish, common carp, rohu, perch, and tilapia, etc. Fish are also classified based on their fat content, categorized as lean (less than 2 % fat), medium (2-5 % fat), and fat (more than 5 % fat) fish. Examples of fatty fish include salmon, sardine, mackerel, and tuna. Fish with higher fat content typically have more pigmented flesh compared to low-fat varieties, which are generally white-fleshed (Manay et al., 2008).

The composition and nutritional properties of fish vary widely depending on several factors, including species, diet, degree of maturity, and environmental conditions such as water temperature, salinity, breeding season, geographical location, and whether the fish is farmed or caught wild. Most fish are typically composed of 14-20% protein, 0.2-20 % fat, 18-35 % total solids, and 1.0-1.8 % ash (**Table 2.1**) (Potter et al., 2012; Taşbozan et al., 2017). Fish is recognized as an excellent source of protein, offering highly digestible proteins that are comparable in amino acid content to those found in red meat. The primary constituents of fish oils are glycerides of fatty acids, comprising about 95 % of the oil content. The polyunsaturated fatty acids (PUFA) found in fish oils, especially EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid), are known to be abundant in fish and are mainly obtained through fish consumption (Manay et al., 2008). The number and position of double bonds in the molecular structure of fatty acids determine both their physical and functional characteristics (Taşbozan et al., 2017). While some fatty acids are synthesized by the human body, others that contain essential n-3 and n-6 polyunsaturated fats cannot be produced by the body. In human nutrition, the ideal n-6/n-3 fatty acid ratio is 5:1 and for best nutrition and disease prevention, the World Health Organization (WHO) recommends a n-3/n-6 fatty acid ratio of 1:1 or higher, highlighting the significance of increasing fish consumption or consuming n-3 fatty acids rich foods (Simopoulos, 1991). In addition to protein and fatty acids, fish is

also a good source of essential minerals such as copper, sulphur, and phosphorus. Fish oils are notably rich in vitamins A and D; however, vitamin reserves can vary significantly among different fish species, highlighting the importance of dietary diversity for obtaining essential nutrients.

The internal anatomy of fish reveals a well-balanced structure that enables them to survive and navigate in underwater environments. A representative diagram is illustrated in **Fig. 2.1**. The spine is the central structural framework of their body, consisting of hollow vertebrae that protect the delicate spinal cord and connect it to the skull and tail. The brain processes sensory inputs and controls automatic functions like respiration. The lateral line, a sensory organ, detects vibrations in the water and helps fish navigate and detect movement. The swim bladder is a gas-filed organ that regulates air volume to help fish maintain neutral buoyancy and conserve energy. Gills are essential for underwater respiration because they efficiently extract oxygen from the water and expel carbon dioxide (Olson, 2002). Pyloric caeca, located at the stomach-intestine junction, secretes digestive enzymes and aids in nutrient uptake. The vent or anus eliminates metabolic byproducts. Fish have reproductive organs called gonads (ovary or testis), which are visible during spawning in females and produce milt to fertilize eggs in males. Muscles made up of muscle tissue, are required for locomotion and are frequently consumed by humans due to their nutritional value.

The flesh of healthy fish is considered bacteriologically sterile, but various types of bacteria naturally live on the digestive tract, gills, and skin of living fish. Spoilage typically occurs shortly after the death of the fish, as bacteria begin to attack and break down the tissue components (Manay et al., 2008).

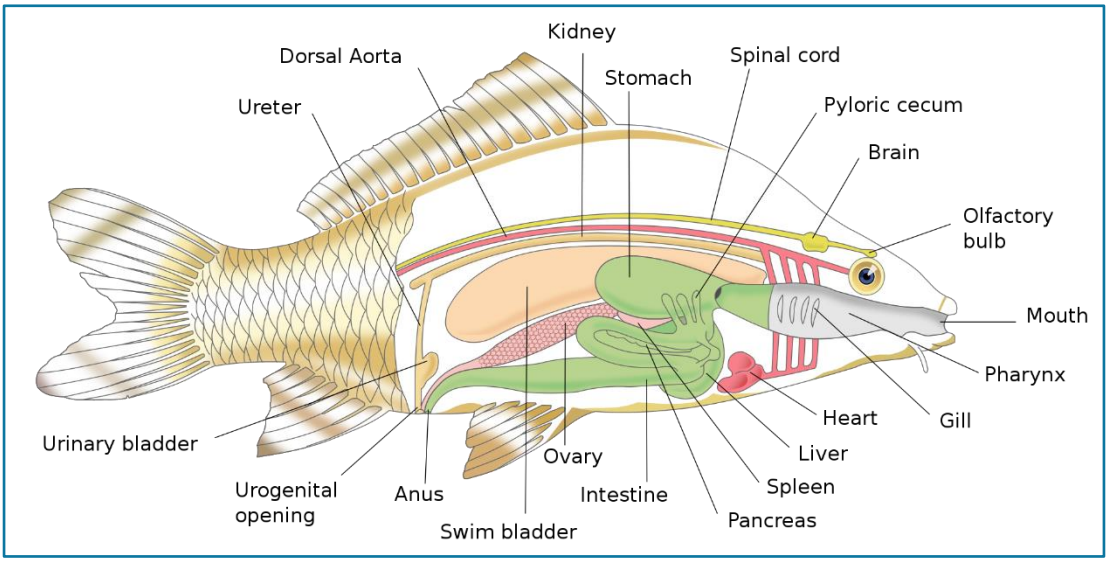


Figure 2.1 Representation diagram of the internal anatomy of fish. (Source: *Wikipedia* [https://en.wikipedia.org/wiki/Fish_anatomy]).

Table 2.1. Typical composition of fish (%) (Source: Potter and Hotchkiss, Food Science, Fifth edition.)

Fish	Protein	Fat	Ash	Carbohydrate	Water
Nonfatty	16.40	0.50	1.30	-	81.80
Fatty	20.00	10.00	1.40	-	68.60
Crustaceans	14.60	1.70	1.80	2.60	79.30
Dried	60.00	21.00	15.00	-	4.00

2.2. Causes of fish spoilage

Physiological and spoilage changes in fish result from a combination of biological, chemical, and physical factors. These factors can individually or collectively contribute to the deterioration of fish quality. Physiologically, fish undergo rigor mortis, a post-mortem muscle stiffening due to the depletion of adenosine triphosphate (ATP), leading to texture changes. Additionally, autolytic enzymes in fish tissues contribute to flavor and texture alterations as they break down proteins and lipids. Spoilage occurs primarily

due to microbial growth, particularly by psychrophilic bacteria that thrive in cold environments. The odor developed due to spoilage has been amalgamated to amines (e.g., dimethylamine (DMA), trimethylamine (TMA), putrescine, cadaverine, histamine, ammonia), sulphur compounds, acids, short-chain carbonyls, unsaturated aldehydes, N-cyclic compounds, etc., that may be used for monitoring fish freshness. Microbial degradation of fish leads to an increase in TVB-N, primarily composed of TMA, DMA, and ammonia as well as TBA levels (indicates the degree of secondary lipid oxidation), which are used widely to evaluate fish freshness. Their presence is a significant indicator of spoilage and is responsible for the development of unpleasant off-odors (Tilami et al., 2018; Hao et al., 2021). Xanthine and hypoxanthine are two important chemical compounds of high importance as these indicate the freshness of meat, fish, etc. Generally, these compounds are produced due to the degradation of adenosine triphosphate (ATP) due to respiration and biosynthesis of ATP after death. Xanthine oxidase (XO) is an important enzyme that is used for sensing for the detection of the presence of xanthine and hypoxanthine (Dervisevic et al., 2019; Mustafa et al., 2020). Alongside the formation of volatile amines, changes in physical characteristics, such as alterations in color, textures, and muscle softness, are observed in the fish tissue undergoing spoilage. From the moment fish is harvested at its source to its arrival at the market and eventual consumption, its value diminishes due to several transformations. These changes encompass bacterial putrefaction, lipid oxidation leading to rancidity, and the degradation of ATP within the fish. The mechanism of fish spoilage is presented in **Fig. 2.2.** In the fish industry, sensory evaluation, including assessments of color, odor, and appearance, plays a crucial part in determining freshness. Additionally, parameters like pH, TVB-N value, and microbial counts are used to assess the freshness of fish during storage. The acceptable thresholds for these indicators have been extensively researched under varying temperatures and environmental conditions to comprehend their response to different conditions. Maintaining TVB-N levels below 35 mg N/100 g serves as an acceptable limit, although this limit might vary among different species. Moreover, the European Commission (EU law 95/149/EC, 1995) has set specific TVB-N limits for various species: 25 mg/100 g for *Sebastes* spp. (*Helicolenusdactylopterus*, *Sebastichthys capensis*), 30 mg/100 g for Pleuronectidae family species (excluding halibut: *Hippoglossus spp*.), and 35 mg/100 g for *Salmosalar* (species within the *Merlucciidae* and *Gadidae* family). Hence, the formation of TBV-N in stored fish is

liable to factors such as fish composition, species, storage temperature and duration, and environmental conditions (Prabhakar et al., 2020). Understanding these factors is crucial for prolonging the shelf life of various fish varieties. The establishment of limits for fish freshness considers two key points: when spoilage begins and when the product becomes unfit for human consumption. This division is crucial because even if fish is rejected in the market for being unfit, poor individuals often still consume it as a nutritious option until spoilage is evident. While the value of the fish decreases when it becomes unfit but not yet spoiled, there are consistent buyers for whom it remains a viable option. This presents a dual scenario where fish harvesters face a loss in the value of their sale, but many impoverished people benefit from accessing this food source before spoilage. The recommended limits set by researchers have played a vital role in determining shelf life based on human convenience, and ongoing efforts will leverage these limits to further extend shelf life in the future. Recent technological advancements aim to extend the shelf life of fish and reduce its losses. Nowadays, total volatile basic nitrogen is widely considered as fish spoilage index due to its consequent concentration increase with microorganism growth.

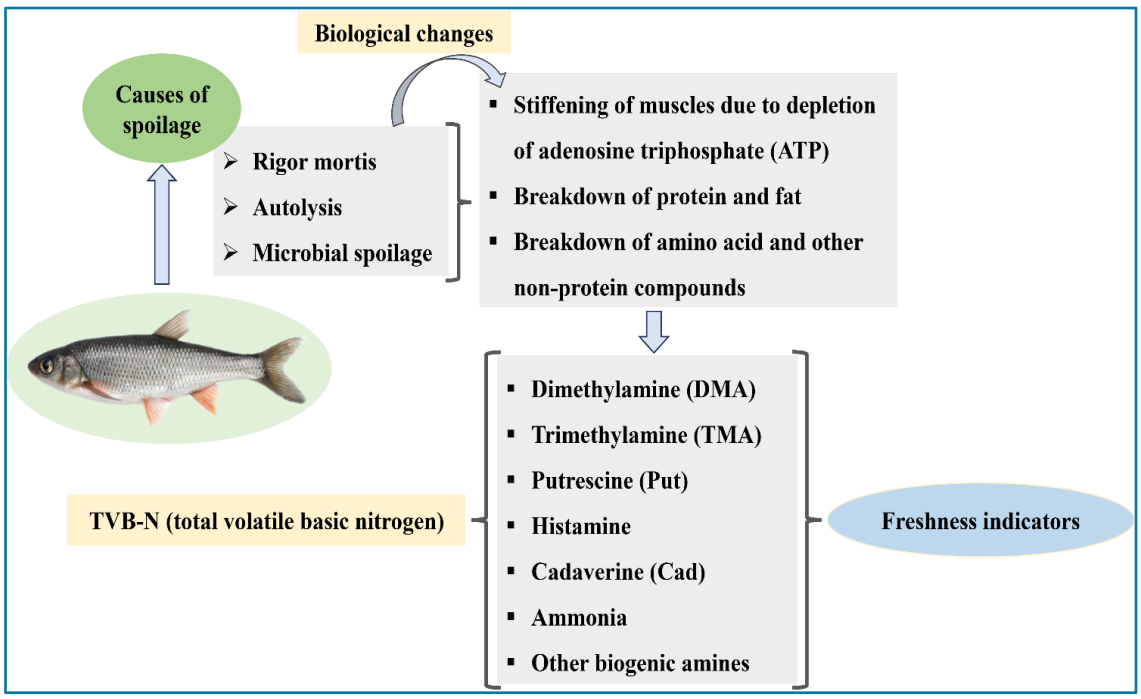


Figure 2.2. Mechanism of fish spoilage: Biological changes in fish lead to the release of total volatile basic nitrogen (TVB-N), which serves as an indicator of fish freshness.

2.3. Method of detection of fish spoilage

2.3.1. *Conventional techniques*

Fish being highly vulnerable and perishable, the quality assessment of fish as a part of quality assurance has become the main concern in the fish industry globally. Ensuring quality involves sensory evaluation and instrumental/biochemical techniques to evaluate fish freshness. Sensory methods rely on human senses to assess characteristics like odor, texture, and appearance, while instrumental methods include chemical and biochemical analyses.

2.3.1.1. *Sensory evaluation*

The sensory method is one of the conventional techniques for evaluating the freshness and quality of fish. Sensory tests, focusing on attributes such as skin, eyes, gills, and texture, are widely used in the fish supply chain to assess the quality of raw whole fish. These tests scientifically quantify and interpret variations in characteristics like odor, taste, texture, and appearance using human senses. Common grading methods for assessing fish freshness include the European Union (EU) scheme, the Quality Index Method (QIM), and the Torry Scoring System (FAO).

2.3.1.1.1. *European Union scheme*

The EU scheme is widely used in European countries, and it relies on general parameters, not fully accounting for species differences. The "Multilingual Guide to EU Freshness Grades for Fishery Products" proposes updates with specialized schemes for whitefish, dogfish, herring, and mackerel. The scheme categorizes freshness into three grades: E, A, and B. Grade E represents the highest quality, while fish below grade B are deemed unfit for human consumption (FAO). The criteria are detailed in **Table 2.2.**

Table 2.2. Criteria of EU scheme (adapted from Green, 2010)

Parts of fish inspected	CRITERIA			
	FRESHNESS CATEGORY			Not Admitted
	E	A	B	
Skin	Bright, iridescent color, shining, no discoloration	Dull color, loss of lustre and shine	Lustreless, dull, skin creased	Pigmentation very dull
Skin mucus	Transparent, Aqueous	Slightly cloudy	Milky	Opaque mucus, grey, Yellowish,
Eyes	Convex, blue black, transparent cornea, bright pupil	Dark pupil slightly sunken and convex,	Opaque pupil, opalescent cornea, opaque pupil, flat	Milky cornea, grey pupil, concave in the centre
Gills	No mucus, silvery	Transparent, less colored,	Brownish, thick opaque	Milky, yellowish,
Peritoneum on gutted fish	Bright, smooth, difficult to detach from flesh	Easy detachable from flesh, slightly dull	Apart from flesh, speckled	Not sticking
Gills smell and abdominal activity	Fresh seaweedy smell	Smell neutral	Slightly sour, fermented, rancid smell	Rotten sour
Flesh	Firm and elastic, smooth surface	Less elastic	Slightly soft, less elastic	Soft, scales easily detached from skin, surface rather wrinkled

2.3.1.1.2. *Quality Index Method*

For effective quality management, a sensory system must clearly and simply reflect different quality levels. One such system is the Quality Index Method (QIM), which has unique characteristics. QIM is based on observable changes in raw fish,

focusing on attributes such as skin, eyes, gills, and odor. It uses a scoring system from 0 to 3 demerit (index) points (**Table 2.3.**). The QIM involves summarizing scores for various characteristics to produce an overall sensory score, called the Quality Index. The QIM scientific aim is to evaluate the Quality Index of fish during storage, making it useful for production management. Each parameter's score description is listed in the QIM scheme, and assessors evaluate all parameters (Hyldig et al., 2004; FAO). This makes QIM effective for teaching inexperienced evaluators, training panellists, and monitoring their performance. The total demerit score, which predicts the remaining shelf life, is less influenced by minor differences in any single criterion. Lower scores indicate fresher fish.

Table 2.3. Consumer QIM for assessment of raw whole non-specific fish species (adapted from Hyldig et al., 2004)

Parameter	Instruction	Description	Point
Texture	Thumb press and forefinger at the back of the fish	Firm	0
		Firm/soft	1
		Soft	2
Odor	Belly	Sea, Seaweed	0
		Neutral	1
		Off odor	2
Appearance	Brightness of skin	Bright	0
		Reduced brightness	1
		Dull	2
Sum of demerit point			

2.3.1.1.3. *The Torry Scoring System*

The Torry Freshness Score is an objective sensory scoring system that was developed in the UK and used to assess fish freshness by evaluating the gill odor of raw and cooked fish (without ingredients). This species-specific method correlates the presence of spoilage causing microorganisms with the freshness score where lower

scores indicate higher microorganism density. While traditionally based on human sensory perception, electronic tools have been developed to aid in validation. The scoring system ranges from 10 to 0, with 10 indicating newly caught fish, 7 being neutral, 6 as the borderline, and 3 or below considered spoiled. Fish are typically rejected when the score drops to 6 or lower, which corresponds to around 11 days on ice and marks the onset of off-flavors and odors (Gopakumar, 2002). The Torry-scores method is widely used to assess the freshness of cooked fish, with score sheets describing the cooked odors and flavors of iced fish. Quality degradation starts with a loss of fresh fish flavor (sweet, seaweedy) and progresses to a neutral odor/flavor. The appearance of spoilage-related sensory attributes like sour, pungent, and TMA (trimethylamine) odors and flavors indicates the shelf life (Howgate, 2009).

2.3.1.2. *Instrumental or biochemical methods*

2.3.1.2.1. *Total Volatile Basic Nitrogen (TVB-N)*

Total Volatile Basic Nitrogen (TVBN) measures the level of volatile bases generated from nitrogen derivatives in fish, primarily indicating protein decomposition. This includes ammonia and various amines resulting from the bacterial breakdown of amino acids in fish muscle (Huss 1988). Formalin-bound nitrogen (FBN) comprises ammonia and primary amines, while trimethylamine (TMA) represents the unbound fraction. TVB-N serves as a key indicator of fish freshness, with levels typically below 35-40 mg/100 g of fish muscle considered acceptable (EC. 1995). Higher TVBN values correlate with increased spoilage. To measure TVB-N, a Trichloroacetic Acid (TCA) extract sample is treated with saturated sodium carbonate to liberate, which is then captured in sulfuric acid. The excess acid is titrated with sodium hydroxide, yielding the TVB-N value (Jeyakumari et al., 2018).

2.3.1.2.2. *Trimethyl Amine (TMA)*

TMA is considered a freshness indicator for sea fish derived from Trimethylamine Oxide (TMAO), which is required for osmoregulation in these fish. TMAO, an odorless non-protein nitrogen compound, varies in content depending on season age, and size of the fish (Peleg, 2016; Prabhakar et al., 2019). During spoilage, enzymes convert TMAO to TMA, with amine concentrations in fish tissues varying with time and temperature, corresponding to fish deterioration. TMA concentration, which

indicates decay, is commonly used to assess fish quality, with TMA-N levels of 10-15 mg/100 g muscle deemed acceptable for round, whole chilled fish. However, this index does not apply to freshwater fish or heat-treated fish products. TMA, a non-protein nitrogenous volatile compound, is primarily determined by the concentration of its precursor, TMAO in fish muscle.

2.3.1.2.3. *Thiobarbituric Acid (TBA)*

The TBA index is a widely used indicator of advanced lipid oxidation, specifically measuring malondialdehyde produced during fat oxidation. The method is based on the reaction of TBA with oxidized lipids, which produces a red complex that can be quantified spectrophotometrically. Malondialdehyde, a product of oxidative rancidity, is thought to be involved in this reaction with TBA. Thus, the TBA value is calculated as milligrams of malondialdehyde/kg of the sample (Jeyakumari et al., 2018).

2.3.1.2.4. *Peroxide Value (PV)*

Fish lipids that are highly rich in unsaturated fatty acids are susceptible to oxidation, primarily resulting in lipid hydroperoxides. Chemical methods, utilizing their oxidation potential, are commonly employed to detect these compounds, either by oxidizing iodide to iodine or iron (II) to iron (III). Hydroperoxide concentration is determined using titrimetric or spectrophotometric methods, providing the peroxide value (PV) in milliequivalents (mEq) peroxide/1 kg of extracted fat. The widely used iodometric titration method measures iodine generated from potassium iodide (KI) by the fat's peroxide content. PV acts as a reliable indicator of fat quality, with fresh oil ideally having a PV of 1 mg oxygen/kg, which can increase to 10 mg/kg during storage. The formation of peroxides during fat oxidation leads to oxidative rancidity, underscoring the importance of PV as a measure of peroxides in the oil. Determining PV typically involves volumetric methods, where potassium iodide reacts with peroxide oxygen in acidic conditions, followed by titration of liberated iodine with sodium thiosulfate solution (Jeyakumari et al., 2018).

2.3.1.2.5. *Free Fatty Acid (FFA)*

Lipid degradation is a major concern in the fish industry, and it falls in two ways: oxidation, which causes unpleasant odors and flavors, and hydrolysis, which releases

FFA and causes hydrolytic rancidity. Fish muscle contains lipase, which aids in the hydrolysis of short-chain triglycerides. Free fatty acids, primarily derived from phospholipids, rise during spoilage, possibly because of bacterial activity, enzyme action, or non-enzymatic catalysis (Salonen et al., 1995). To assess fat spoilage, a common chloroform extract is used to measure the FFA and the PV. The FFA in the extract is diluted with alcohol and neutralized by titration with sodium hydroxide. The resulting FFA content is expressed as % Oleic acid present in the extracted fat (Jeyakumari et al., 2018).

2.3.1.3. *Biological method*

The Total Plate Count (TPC) is one of the methods that measures the total microbial flora present in fish or fish products, providing a microbiological perspective on their quality. It quantifies the number of bacteria/g of fish. TPC is determined by culturing bacteria present in a fish sample with appropriate bacteriological media to obtain the greatest number of bacteria. Aseptically minced fish samples undergo serial decimal dilutions and are pour-plated onto agar media, such as nutrient agar for fresh fish and tryptone glucose beef extract agar for processed fish products. Incubation at 37 °C for 24 hours allows bacterial colonies to develop, which are then counted to calculate TPC by multiplying with the result by the suitable dilution factor. Fish freshness cannot be determined solely by TPC because even low TPC fish can contain pathogens that could be harmful if consumed. Thus, to determine whether pathogenic or dangerous bacteria are present, qualitative analysis is carried out. To find bacteria like *Salmonella*, *Shigella*, and *E. coli* among other coliform bacteria in fish samples, a variety of ordinary or selected media are used (Lyhs, 2009).

2.3.2. *Innovative techniques*

2.3.2.1. *Polyaniline-based sensor*

Polyaniline (PANI) is a notable conducting polymer categorized within the semi-flexible rod polymer family. Renowned for its electrical conductivity and mechanical resilience, it stands as one of the extensively researched conducting polymers. Initially discovered in the 19th century, polyaniline gained substantial interest from the 1980s onwards (Heeger 2001). Originally identified as black aniline, polyaniline (PANI) exhibits various forms depending on its oxidation state. Moreover, PANI is known for

its simplicity, environmental stability, and its ability to doping by protonic acids (Park et al., 2016; Bhadra et al., 2020). Its structure is established through the coupling of aniline monomer units via 1, 4-coupling and may exist in multiple oxidation states, with its characterization often involving the determination of FTIR benzenoid to quinonoid ratios. This polymer can exist in three distinct oxidation states: leucoemeraldine, emeraldine, and pernigraniline. The leucoemeraldine state is clear and colorless, representing the fully reduced form, while the emeraldine state manifests as green (in the emeraldine salt) or blue (in the emeraldine base), exhibiting neutrality and heightened electrical conductivity upon doping with acid. Conversely, the pernigraniline state appears as blue or violet, representing the fully oxidized form (Zhou et al., 2009). These oxidation states find applications in sensors and electrochromic devices owing to the associated color transformations. Furthermore, polyaniline's electrical conductivity undergoes a substantial enhancement following treatment with acids, rendering it invaluable across diverse domains such as sensor technology, optoelectronics, and photonic devices (Boeva et al., 2014, Beygisangchin et al., 2021).

Polyaniline and other conducting polymers have been used by researchers for the analysis of ammonia and other gas sensors. A chip-based solution-cast PANI sensor was described by Kukla et al. (1996) and measured a wide range of ammonia concentrations up to 2000 ppm. The sensor showed high electrical and chemical stability. Lee et al. (2005) demonstrated a resistive ammonia sensor with a sensitive PANI film that had a sensitivity of about 40 % at 50 ppm ammonia. In another study, Huang et al. (2012) developed an ammonia gas sensor which was based on PANI nanoparticles and reduced graphene oxide (RGO) with a detection limit of 50 ppm NH_3. Tripathi et al. (2013) developed a PANI gas sensor, which detects ammonia gas concentrations ranging from 100 to 1500 ppm. With the detection limit of 0.6 mol/L, Yang et al. (2014) developed an amperometric nanosensor to detect sulphite in samples using PANI-coated copper hexacyanoferrate modified GCE (PANI/CuHCF/GCE), which showed higher electrocatalytic activity and suitable stability toward sulfite oxidation. An enhanced electrochemical nano biosensor was developed by Chawla et al. (2012) to identify phenolic compounds in fruit juices. With the laccase enzyme covalently immobilized on nickel nanoparticles (NiNPs) MWCNT/PANI composite electrodeposited on the Au electrode surface, an excellent sensitive platform was produced with a detection limit of 0.05 mM and a wide linear range of 0.1-10 M (lower concentration range) and 10-500 M

(higher concentration range). Kunar et al. (2017) developed an ammonia (NH_3) gas sensor using polyaniline film deposited on a flexible PET (polyethylene terephthalate) substrate through a simple polymerization method. Upon evaluation across a range of 5-1000 ppm, the PANI film exhibited a significant change in resistance with observed variations of approximately 110 and 520 times for 200 ppm and 1000 ppm NH_3 exposure, respectively, compared to ∼5 ppm NH_3. They demonstrated that the developed PANI film shows promise for portable on-site ammonia detection applications (Kumar et al., 2017). A colorimetric-based PANI indicator was developed to access the freshness of fish which was further correlated with TVB-N values (Wang et al., 2018). An ammonia gas sensor based on flexible polyaniline to detect meat spoilage was developed by Matindoust et al. (2017) The developed sensor was based on the ammonia diffusion mechanism with the PANI film and showed the stable linear response of ammonia ranging from 50-150 ppm. Zohrevand et al. (2022) developed an electrochromic (EC) sensor using conducting polyaniline (PANI) film modified on fluorine-doped tin oxide (FTO) to detect amines as model analytes and employed to monitor the spoilage of beef and fish samples in the gaseous phase. They found that amines can influence the EC behavior of PANI, enabling the indirect determination of amines based on changes in PANI's EC response at wavelengths of 420 nm, 620 nm, and 750 nm. The sensor demonstrated detection of triethylamine in a linear range of 0.10-7.01 μmol L^{-1} with a limit of detection of 0.06 μmol L^{-1}. Several research on the application of polyaniline as a gas sensor to detect gases such as ammonia, histamine, hydrogen sulfide (H_2S), etc. has been studied (Crowley et al., 2008; Matindoust et al., 2017; Shoaie et al., 2019). Kuswandi et al. (2012) have developed a colorimetric approach based on PANI film for the development of smart packaging. They also stated that PANI film is a low-cost sensor that works well with smart packaging sensors. Chen et al. (2017) offered a low-cost method of food condition monitoring by reusing the barcode of the product as a colorimetric sensor array. For a quantitative assessment of food aging and quality, the smartphone camera decodes color information from the sensor barcode. Several studies reported the flexibility and transparency of the use of PANI-based sensors on ammonia vapor (Jin et al., 2001; Asijati et al., 2005, Wang et al., 2018).

2.3.2.2. *Dye-based sensor*

Concerning the growing need for non-destructive methods and sensors for monitoring food deterioration. Morsy et al. (2016) assessed 16 chemo-sensitive chemical compounds that were integrated into an array for colorimetric detection of spoilage compounds. The color changes were then characterized in response to compounds that were present in fresh products. They noticed that the sensitivity of the chemo-sensitive chemicals relies on the spoilage condition and discovered a linear correlation with the conventional spoilage measuring method (Morsy et al., 2016). Wells et al. (2019) created a colorimetric polymer film that included the pH-sensitive dye bromophenol blue (BPB) as a spoilage indication for packed fresh fish. Using RGB color analysis in digital photography and absorbance spectroscopy, they examined the film's reaction to various TMA concentrations. According to the study, the indicator was evaluated as a fish spoilage indicator at 22 °C and 4 °C, and color shift was correlated with bacterial colony-forming units on the fish. In another study, a low-cost smart diagnostic for food using a colorimetric sensor array was developed by Chen et al. (2017). They developed a barcode using Nile red, Zinc Tetraphenylporphyrin (Zn-TPP), and Methyl for sensing gases from the chicken meat, and a smartphone camera is used to read color information from the sensor barcode for quantitative estimation of the food aging and quality. Using different sensing dyes, rapid, easy-to-use sensors for monitoring food freshness have been carried out in several studies (Zhang et al., 2016, Zaragozá et al., 2015; Majdinasab et al., 2018; Wells et al., 2019; Koxmak et al., 2019). A colorimetric sensor layer using pH indicator dye 2-fluoro-4-[4-(2-hydroxyethanesulfonyl)-phenylazo]-6-methoxyphenol that was covalently immobilized onto cellulose microparticles with subsequent embedding into food-grade silicone for selective detection of amines. The developed sensor undergoes a color change from green to red upon exposure to amines exhibits a response time of 1.5 hours and reverses after 20 hours, suitable for monitoring spoilage processes (Schaude et al., 2017). Rakow et al., (2002) introduced a colorimetric sensor array utilizing metalloporphyrins as sensor indicators for both qualitative and quantitative detection of volatile substances. Colorimetric sensor arrays were created by Pacquit et al. (2004) that mimic human olfactory receptor cells, capable of reacting with different volatile substances, including those used to assess fish freshness. These sensitive colorimetric dyes have been employed successfully in monitoring fish freshness and decay. For instance, porphyrin compound-based sensors effectively detected initial fish spoilage

(Alimelli et al. (2007). Similarly, Dini et al. (2015) reported that a colorimetric sensor array consisting of acid-base indicators and porphyrin compounds was utilized to monitor the freshness of frozen cod fish slices during storage at room temperatures. Furthermore, sea bream's shelf life was evaluated using a colorimetric sensor array comprising pH indicators, Lewis's acids, and oxidation-reduction indicators during cold storage, demonstrating improved effectiveness (Zaragozá et al. 2015). Lv et al. (2019) produced a colorimetric sensor array (CSA) to detect volatile organic compounds (VOCs) for monitoring fish spoilage using chromogenic sensitive material (metalloporphyrins and protoporphyrin). Density functional theory (DFT) was utilized to investigate the reaction between chromogenic sensitive materials and characteristic gases emitted by spoiling fish. Gas chromatography-mass spectrometry (GC-MS) was employed to confirm the presence of specific gases, such as trimethylamine (TMA), during mackerel spoilage (Lv et al., 2019). Over the past decade, various colorimetric sensors have been developed for detecting volatile gases, food aging and safety, and other chemicals (Lee et al., 2013; Huang et al., 2014; Majdinasab et al., 2018; Zaragozá et al., 2015; Salinas et al., 2014; Xing et al., 2020; Chen et al., 2017). These studies highlight the versatility and potential applications of colorimetric sensor technology in food quality monitoring and safety assessment.

2.3.2.3. *Smartphone-based sensor*

Smartphones, equipped with a combination of sensors for data collection and software for data processing, serve as effective tools for monitoring and studying spoilage. Their advanced sensing capabilities make them ideal for detecting spoilage in various settings. Previously research implemented on poly (styrene-co-maleic anhydride)-based, miniature (2 cm × 2 cm) sensor has been developed to check the spoilage scrutiny via mobile phones in chicken and beef packages under different storage conditions (Istif et al., 2023). Different toxins, allergens, contaminants, and pathogens like zearalenone, *Escherichia coli*, and *Salmonella typhimurium* have been detected with the already-developed biosensors in combination with smartphones (Nnachi et al., 2022). Utilizing mobile phone technology for monitoring food quality is emerging as the growing significance of communication and data within the Internet of Things (IoT). Due to remarkable advancements in both hardware and software, smartphones have evolved into essential devices, offering a blend of convenience and computational capability

comparable to traditional methods of analysis. The rapid and continuous expansion of smartphone applications has been particularly evident, with a notable focus on their integration into analysis in the food sector. These smartphones now serve multifunctional roles, functioning not only as detectors and data processors but also as signal initiators. This versatility is often facilitated by specialized cradles or attachments, purposefully designed to accommodate various small optical and other components required for various applications (Meng et al., 2017; Xu et. al., 2017; Geng et. al., 2017). A CMOS (Complementary Metal Oxide Semiconductor) based fish spoilage detector for IoT application in fish markets was developed by Chiang et al. (2017) using analog circuits with freshness detection time ranging from 0-50 min, corresponding to an output period range of 107.51-14.66 μs for determining the freshness of swordfish (Chiang et al., 2017). Smartphone embedded sensors for fish spoilage detection are now emerging. Research work related to this is progressively going on by researchers (Mastnak et al, 2023; Doğan et al., 2024).

2.3.2.3.1. *Optical-Based Sensor*

A smartphone-based optical sensor refers to the integration of optical sensing capabilities within a smartphone device. Smartphones lead the way in innovation for analytical applications that can be largely attributed to their robust computing power and integrated sensors. It utilizes the existing hardware components, such as the camera, display, and built-in sensors, to perform optical measurements or analyses. Notably, smartphone cameras serve as optical sensors, particularly those equipped with high-resolution complementary metal-oxide semiconductor (CMOS) and Charge-Coupled Device (CCD) sensors. These sensors facilitate optical sensing across various aspects like color, imagery, and spectrum, encompassing colorimetric analysis, optical imaging, and spectroscopy techniques.

2.3.2.3.1.1 *Colorimetric analysis*

A smartphone-based colorimetric sensor for food analysis is a portable and easily accessible technology that uses a smartphone's camera and processing capabilities to assess color changes in food samples as an indicator of various properties such as freshness, ripeness, or the presence of specific contaminants. Typically, this novel approach entails attaching or placing a sensor or testing strip on or near the food sample

of interest. When the food meets the sensor, it causes a color change that is captured by the smartphone's camera. The image processing software on the smartphone analyses color changes and provides quantitative or qualitative information about the food sample, such as its pH level, sugar content, or the presence of allergens or pathogens (Geng et al., 2023). The concept of colorimetric measurements through a smartphone is achieved by directly capturing color information using the red, green, and blue (RGB) model from sample images taken with the rear camera of a smartphone. Subsequently, this color information is interpreted either through smartphone applications or computer software, involving data transfer to a computer for analysis. This methodology applies to diverse samples, including solutions, paper-based test strips, or microfluidic devices, where color changes can be quantified using the smartphone's camera (Di Nonno et al., 2021). The technology offers several advantages, including affordability, portability, and ease of use, making it a valuable tool for food quality control, safety assessments, and even on-the-spot consumer testing, ultimately contributing to improved food safety and quality assurance. Smartphone-based colorimeters are useful in a variety of fields, including health care, environment, food quality control, and others (Di Nonno et al., 2021).

A smartphone-based method for analyzing the color changes of pH-responsive inverse opal photonic gel (IOPG) sensor was presented by Park et al. (2021). The Hue (h) parameter was specifically measured in the HSV (Hue, Saturation, Value) color space, which is more related to visible light wavelengths than RGB coordinates. This method proved to be reliable under various lighting conditions and with different smartphone brands. This study of the IOPG sensor using smartphone-based hue analysis was found to be reasonably accurate under common illuminations, such as LED, fluorescent light, or a smartphone's built-in flash (Park et al., 2021). Domínguez-Aragón et al. (2018) established a colorimetric method to monitor the freshness of tilapia (*Oreochromisni loticus*) fillets using a copolymer of ortho-phenylenediamine and aniline, which changes color in the presence of alkaline vapors, including the volatile amines (TVB-N) produced during fish spoilage. In tilapia fillets refrigerated at 10°C, the levels of TVB-N and microbial growth were correlated with the copolymer's color change. Studies have shown that smartphones have been integrated with colorimetric sensors to detect Saxitoxin (STX), a dangerous toxin found in shellfish. The method uses a portable colorimetric analyzer (SBCA) and a cost-effective enhanced gold nanoparticle-based

ELISA (EGNB-ELISA) (Zhong et al., 2019). Colorimetric films (FG-UV-CD 100) and a specially created smartphone app (Smart Food) were created by Kilic et al. (2022) to be used with the films. A common indicator of food spoilage, i.e. ammonia vapor, was used to test the kinetic colorimetric responses of the films. They discovered that the films with 100 mg/L of CDs (FG-UV-CD100) had the best colorimetric response to ammonia vapor. They also found that the difference in color of FG-UV-CD100 films correlated well with microbial growth and TVB-N release (a measure of freshness) in skinless chicken breast samples (Kilic et al., 2022).

2.3.2.3.1.2. *Optical imaging and Spectroscopy techniques*

Recent years have witnessed significant advancements in optical smartphone sensor platforms. By integrating CMOS cameras, these sensors are designed as either colorimetric-based or spectrum-based biosensors. Detection techniques within optical sensors extend into two primary categories: (i) imaging-based and (ii) spectrometry-based. Spectral imaging sensors are advanced imaging systems that capture and analyze data across a range of wavelengths, allowing for the acquisition of both spatial and spectral information about a scene or object. These sensors go beyond traditional RGB (Red-Green-Blue) imaging by enabling the acquisition of data in multiple narrow spectral bands or continuous spectral information. Imaging techniques utilize microscopes to capture microscopic details, while spectrometry-based methods involve analyzing molecular reactions or changes (Geng et al., 2017). Imaging-based techniques on smartphones often involve the use of microscopes to capture microscale details and analyze them using the smartphone's camera. On the other hand, spectrometry-based techniques are employed to detect changes in molecular reactions. Smartphone cameras can be used to capture and analyze the absorption or transmission of light in samples. This allows for spectrophotometric measurements, such as determining the concentration of specific substances or analyzing the characteristics of materials. This can involve probing changes in spectral properties, such as absorption or emission of light, which can deliver valuable information about the composition and characteristics of the samples. There are spectroscopy methods that utilize smartphones to perform analysis, making spectroscopy more accessible and portable. One such method is called smartphone spectroscopy or mobile spectroscopy. It involves using the built-in camera and the processing power of a smartphone to capture and analyze spectral data. The combination

of imaging and spectrometry techniques in smartphone-based platforms has made it feasible to develop portable and affordable devices for a wide range of applications, including point-of-care diagnostics, and environmental, and food safety.

A study was reported in which (Semeano et al., 2018) gas sensing is used to monitor Tilapia fish spoilage. An intriguing method of keeping track of fish freshness is to analyze the headspace of fish specimens using gas sensing. Here, they present a gas-sensing technique based on the application of a single gas-sensitive gel material for monitoring Tilapia fish spoilage. This study shows that gas sensing can be used to detect fish spoilage, and this technology could potentially be adapted for use in a smartphone-based optical sensor. Liu et al. (2022) developed thermochromic microcapsules which could switch the color irreversibly when subjected to certain conditions, such as high temperature or humidity. Fang et al. (2016) developed a smartphone-based diagnostic platform for on-site analysis of okadaic acid (OA) and saxitoxin (STX). The platform uses a 3D-printed accessory to fix the test strips and a homemade app for data processing (**Fig. 2.3**). The detection limits for OA and STX are 2.80 ng/mL and 9.808 ng/mL, respectively. In another study, Liang et al. (2014) developed a smartphone biosensor to detect microbial contamination in ground beef. The sensor exposes the ground beef to an 880 nm near-infrared LED and measures the scatter signals at various angles. The amount of *E. coli* in the ground beef affects the angle that maximizes the scatter signal. Wu et al. (2018) introduced a novel application of Vis/NIR spectroscopy, combined with the SDAE-NN (Double Sacked Deionizing Autoencoder) algorithm, to predict the cold storage time of salmon meat and skin. Notably, they achieved impressive results without the need for spectral pre-processing. When compared to alternative data processing models, the SDAE-NN demonstrated high effectiveness in assessing salmon meat and skin, yielding RMSEP values of 0.93 days and 1.75 days, respectively. This underscores the Vis/NIR spectroscopy approach as a more rapid, simple, and non-destructive method for evaluating fish freshness compared to traditional techniques.

Kilic et al. (2018) and Di et al. (2021) provided an overview of smartphone-based spectrometry and colorimetry techniques for the analysis of fish samples. Chen et al. (2021) discusses the applications concerning smartphone-based optical sensors for bio samples, including colorimetric sensing, which can be used for fish analysis. Hussain et al. (2021) review the development of smartphone systems for optical spectroscopy and

highlight their potential for point-of-care diagnostics, which could be useful for fish analysis in resource-constrained environments.

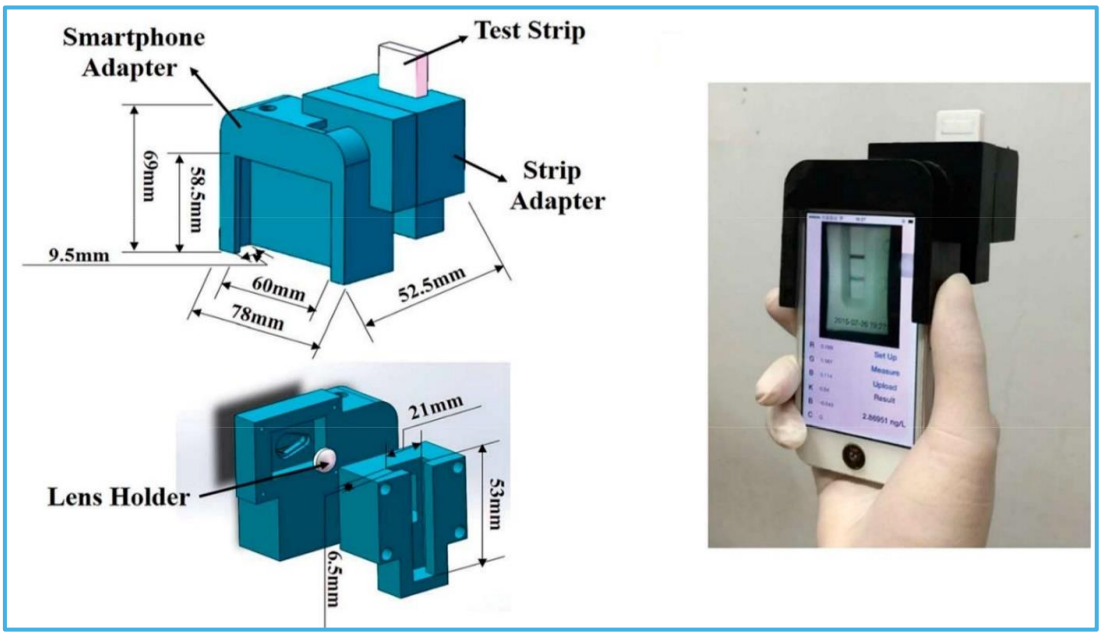


Figure 2.3. The diagnostic adapter for on-site marine toxins created by Fang et al. (2016).

2.3.2.3.2. *Fluorescence-based sensor*

When light is absorbed by a fluorophore, it excites the fluorophore from its ground state to an excited electronic state and emits light equivalent to the energy gap between the two states. This process is known as fluorescence, which is a photo-physical phenomenon (Bertocchi et al., 2013). For specific purposes, fluorescence analysis offers the lowest background levels and smallest detection limits and is widely available in most laboratories.

Fluorescence imaging mainly comprises tagging fluorescent dye labels on chemical or biological molecules that are to be examined. It aids in a variety of experimental observations, including where proteins are expressed, how genes are expressed, and how molecules interact with one another in tissues and cells (Rateni et al., 2017). Recently, biological targets, including viral and bacterial antigens, nucleic acids, mycotoxins, and proteins have been identified using fluorescent-based detection, which can be used for chemical targets (Ming et al., 2015). A fluorescent marker is attached to a biological molecule of interest so that it can bind to the target molecule. Mobile devices often use fluorescence assays. The fluorescence imaging setup uses a monochromatic light source,

typically a UV LED, to excite the dye. The fluorescence intensity is then measured using a smartphone camera as a detector (Rateni et al., 2017). This setup proposed can be used in specific detection applications using different specific molecular dyes which can be applied to other targets of interest.

This sensor helps to decay the target-fluorophore complex by detecting light that is emitted on radioactive excitation. By amplifying the signal response obtained for the photons that are present in a relatively narrow range of wavelengths with maximum emission, it becomes possible to detect even lower levels of target concentration. Unlike colorimetric assays, the spectrum and intensity of sensor illumination can be adjusted to decrease the limit of detection, since the fluorescence is directly proportional to the intensity of the excitation source. The targets are fluorescently tagged in the single sample, and it multiplexes, the strong and narrow peaks that are produced. Long pass filters are inserted between assay platforms in smartphones to separate emitted light before it reaches the CMOS array, which cannot fully distinguish between adjacent wavelengths.

Various designs have been developed to combine novel, high-performance filters, and materials with mobile assays. These strategies aim to improve light segregation, ensuring that light outside the target emission wavelength does not interfere with detection. Additionally, these strategies aim to spatially control the scattering and diffraction of emitted light. Various advanced filters and surfaces have been developed for their usage in smartphone fluorescence microscopy such as silo filters, multilayered photonic crystal surfaces, and polarizers. To improve spatial resolution for sensing individual cells, the silo filter uses a CMOS array to hold a grid of light-absorbing cells in place (Lee et al., 2013). Another advanced surface that has been developed is a multilayered photonic crystal surface, it allows the usage of collectors with lower numerical aperture which is potentially less expensive and even reduces residual aberrations in the signal. The advantage of detection is that it gives 40 times lower detection limits (Ricciardi et al., 2015). Using a tablet computer screen as an excitation source through rear-illumination of wells containing collagenase and trypsin with fluorescein dye, along with a smartphone camera detector, a polarizer can be utilized to disintegrate, excite, and emit light. Despite the intense proximal excitation light from the tablet computer, the polarizer enables low limits of detection (3.75 g/mL collagenase and 3.72 ng/mL trypsin) (Wargocki et al., 2015).

Fluorescence spectroscopy is an effective analytical technique used in monitoring the properties of several food products. Food authenticity and quality have been analyzed using fluorescence spectroscopy and it has been published in a few papers and citations have increased exponentially over the last decade. The fluorescent compounds found in food samples are highly sensitive to the environment; therefore, fluorescence can be used to identify conformational changes that take place under various production and storage conditions (Shaikh et al., 2017). The intrinsic fluorescence spectra of fish samples, encompassing both physicochemical and structural details, serve as a valuable tool in distinguishing between fresh and aged fillets (Hassoun et al., 2017). Fluorescence excitation-emission matrices (EEM), alternatively referred to as fluorescence landscapes, were employed to extract fluorescence-related information indicative of fish freshness (Elmasry et al., 2015). In a related study, Elmasry et al. (2016) investigated the distinctions between whole and filleted horse mackerel during frozen storage. They utilized fluorescence spectroscopy in conjunction with EEM, focusing on 8 specific wavelengths to calculate K values for the assessment of freshness differences. The freshness of fish was evaluated using the 'K value', a standard index calculated through paper electrophoresis from adenosine triphosphate concentration and its breakdown products. A study was conducted on Red Sea bream (*Pagrus major*) to monitor changes in fluorescence spectra and K values over time. The standard K value and uric acid fluorescence signal were plotted. The concentration of this indicator was determined using paper electrophoresis and adenosine triphosphate breakdown products. The study found that the fluorescence intensity ratio of the emission peak at 420 nm to the peak at 310 nm increased linearly during storage (R^2 = 0.95) and served as a non-destructive indicator of fish freshness. (Liao et al., 2018). Furthermore, Shibata et al. (2018) highlighted the suitability of ATP content as a reliable indicator for analyzing the postmortem freshness of frozen horse mackerel samples. Importantly, this analysis could be conducted without the need to thaw the samples, utilizing six discrete excitation-emission wavelength pairs, and determining ATP content through the HPLC method.

2.3.2.3.3. *Electrochemical sensor*

Smartphone-based electrochemical sensors have emerged as a revolutionary tool for food analysis in recent years. These sensors leverage the computational power and connectivity of modern smartphones to make food testing more accessible and efficient.

Electrochemical sensors detect and measure electrochemical reactions using electrodes, which are caused by the interaction between the sensing surface and the analytes (substances being measured). This responsive information is then converted to qualitative and quantitative electric signals which can be based on amperometry, potentiometry, and conductometry measurements. The connection between the electrode and smartphone is established through Bluetooth or USB connection to control the electrochemical measurements and displaying the results. By integrating miniaturized electrochemical components with user-friendly smartphone apps, these sensors can detect a wide range of food contaminants, such as pathogens, pesticides, and heavy metals, in real-time. Users can simply connect the sensor to their smartphone, apply a small sample of the food or beverage of interest, and receive instant results and data analysis through the app. According to Ji et al. (2022), electrochemical systems, including screen-printed and interdigital electrodes, along with smartphone technology, were utilized for in situ detection. These systems were modified with biomaterials, nanomaterials, and chemicals to be used as biosensors and biodetectors. The modified electrodes, when combined with the smartphone-based impedance system, were able to detect biomolecules with high accuracy. This smartphone-based electrochemical system provides a portable alternative for diagnosis and shows promise for point-of-care testing (POCT) in biosensors and biodetectors (Ji et al., 2022). Moreover, Fu et al. (2019) discussed an overview of the recent development of electrochemical sensors for fishery drug detection. An electrochemical sensor for histamine (HIS) detection in fish has been developed by Serrano et al. (2020). The sensor is based on molecularly imprinted polymer (MIP) film that is electropolymerized on a gold screen-printed electrode (Au-SPE). This allows the sensor to selectively detect HIS in the presence of other compounds. The sensor was shown to be selective for HIS against tyramine, another amine found in fish, and has a linear response from 500 nM to 1 mM with a limit of detection of 210 nM (Serrano et al., 2020). In a very recent study, Huang et al. (2023a, b) developed a portable wireless sensor (having a fast response and 78.5 % accuracy in predicting the storage time of meat) for real-time monitoring of meat by sensing NH_3 gas. They have developed MOF@SnS_2 PN heterostructure to sense NH_3 to evaluate the degree of meat spoilage. They have optimized the combination ratio of MOF and SnS_2 to achieve an optimal gas sensing response and the system works in terms of drop in resistance when it is exposed to an environment containing NH_3 gas. As a result, they

reported that their sensing system has 4 times higher sensitivity (between 500 ppb and 10 ppm) than the common pristine MOF with a very low LOD value of 9.84 ppb. The mechanism behind the increased sensitivity is governed by the higher charge transfer and the Schottky barrier formed in the PN structure. The author has also claimed that the sensing system is also capable of detecting very small changes in NH_3 concentration which ensures precise and real-time monitoring of food spoilage. With this concept, this study can be performed in real-time monitoring of fish spoilage as fish spoilage causes the development of NH_3 and low-grade amines. A smartphone-based portable sensing system can also be developed. Giordano et al. (2016) developed a point-of-use platform for multivariate analyses using a potentiostat and a smartphone. The system is simple, low-cost, portable, and has a long battery life. It can perform three types of electrochemical measurements (linear sweep, cyclic, and square wave voltammetry) and the data can be processed on the smartphone using the Tounge Matrix App (**Fig**. **2.4 (ii), (iii), (iv) and (v)**). This makes it ideal for in-situ assays, as the entire analytical measurement can be performed in real-time at remote locations.

Smartphone-based electrochemical sensor technology holds immense promise in enhancing food safety, quality control, and traceability, enabling consumers to make informed choices about the products they consume. Additionally, the affordability and portability of smartphone-based electrochemical sensors make them particularly valuable for both consumers and professionals in the food industry, facilitating faster and more cost-effective food testing and monitoring.

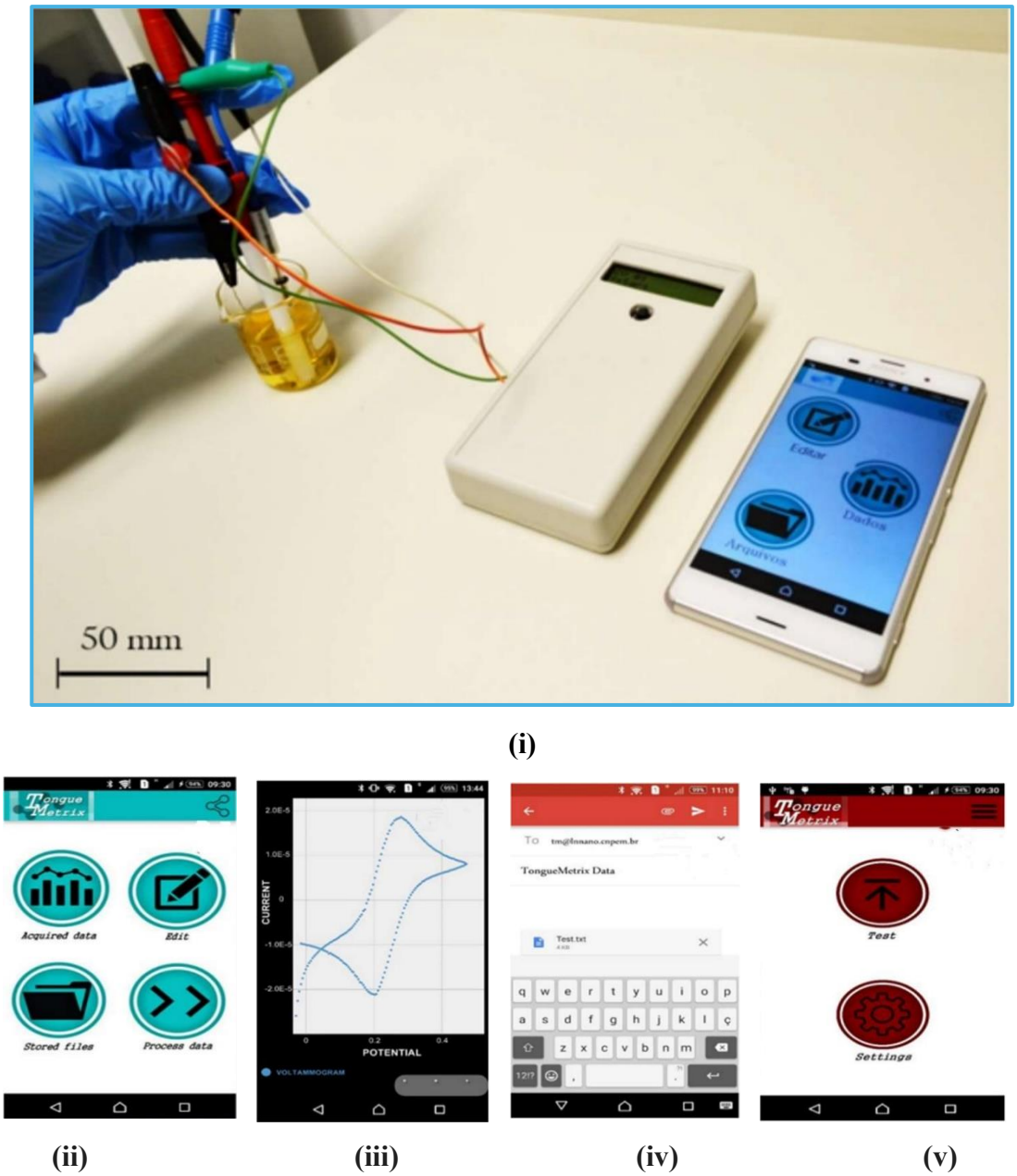


Figure 2.4. (i) A portable electrochemical platform deployed for point-of-use analyses; **(ii)** Smartphonescreenshots showing the Tongue Metrix Android App- Initial screen, **(iii)** typical cyclic voltammogram for the Fe (CN)6 4-/3- redox probe, **(iv)** optional data transmission by e-mail, and **(v)** interface screen for PCA treatment (adapted from Giordano et al., 2016).

2.3.2.3.4. *Label-free smartphone sensor*

Optical-based sensors are generally classified into label-based and label-free sensors. In label-free detection, the detected signal is generated with the interaction of the on-site

analyte and respective transducer. The label-free mode demands less cost and effort to develop an assay, especially by removing experimental things such as shelf-life, quenching, and background fluorescence, and not much expertise is required. An antigen-antibody interaction involves a label, which is conjugated with one of its reactants. Thereby, it alters binding properties and introduces systematic error to the bio-sensing system. As a result, the detection of biological analytes without labels was more advantageous because it reduced the cost and complexity of the assay while delivering higher throughput and more quantitative data. As it is devoid of label-free detection, it even removes experimental uncertainties that are commonly encountered due to a label that has been bound to a molecule's conformation, an active binding epitope that is obstructing the label, steric obstruction, or the lack of an appropriate label that works equally well for all the molecules in an experiment. Recent developments in label-free single molecule detection have largely relied on plasmonic nanostructures and high-Q resonators. Optical signals receive from a molecule produces without any fluorescence labels, makes a significant contribution to the fundamental research studying the behavior of biomolecules. (Zanchettaet al., 2017). Huang et al. (2023 a) reviewed recent advances in capacitive biosensors for the detection of biomarkers, with a focus on the common types of bioreceptors (antibodies and aptamers) using different transducers (CEs, AuEs, and IDEs). These capacitive biosensors enabled the label-free and ultrasensitive detection of biomarkers based on electrical double layer theory with reduced cost and higher sensitivity (Huang et al., 2023 b).

In label-free detection studies, various well-established instruments that can characterize specific molecular interactions or screen different constituents based on their interaction with their molecular conjugates have recently been used (Rich et al., 2011). This type of detection reduces the measurement process, implying a lack of additional secondary probes or reagents to create a small, quick, and ideal sensor with repeatable, affordable, and semi-automatic detection. A well-established approach in label-free sensing is the use of smartphones to collect and process signals. An LED torch and the phone's camera are both used to calculate the image of the light reflected by a prism made of perfluorinated plastics, which has a refractive index like water (Giavazzi et al., 2014).

Amongst the biosensors, label-free biosensors are characterized by direct detection of the analyte of interest, which can be easily employed using the impedimetric transduction

technique. Electrochemical Impedance Spectroscopy (EIS) is widely employed as it is a powerful and non-destructive method used in the biosensor field to analyze the electrical properties of sensing device interfaces and trace reactions on them (Malvano et al., 2020). This technique has made it more convenient and accessible to detect without the use of labels. It has revolutionized label-free detection technology. EIS is an analytical technique used to create label-free sensors by directly monitoring the interaction between a bio-receptor and its target. This technique offers significant advantages over labeled sensors, which are time-consuming and costly. The use of labeled sensors can reduce the affinity between the labeled receptor and its target, leading to lower sensitivity, reproducibility, and selectivity. On the other hand, label-free monitoring helps to reduce biosensor costs and allows for faster analysis. Now, real-time food biosensor analysis is possible using the EIS transduction technique. It involves studying the change in electrical properties of the electrode surface, which is solely dependent on the binding interaction between the receptor and its analyte.

A very limited study on smartphone-based label-free sensors in food materials is found. Particularly studies related to fish are rarely seen using label-free sensors, henceforth it can be considered as a research gap and can be utilized for further research. Ma et al. (2023) conducted a study on the development of a sensing platform for detecting Aflatoxin B1 (AFB1) using smartphone-based chemiluminescence via labeled and label-free dual modes. The schematic representation of a smartphone-based label-free sensor is in **Fig 2.5**.

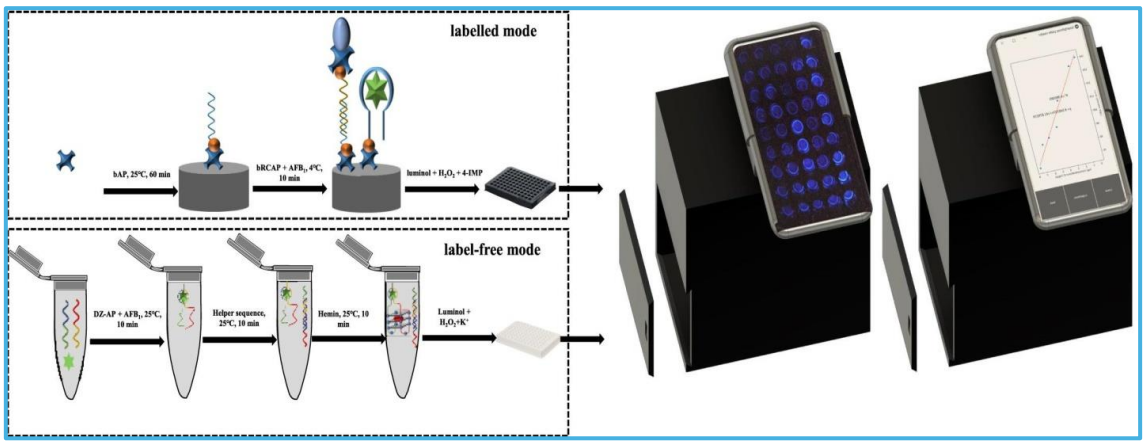


Figure 2.5. Smartphone-based Labelled and Label-free mode dual-sensing system or onsite determination of Aflatoxin B_1 (AFB_1) contamination in foodstuffs (adapted from Ma et al., 2023).

2.3.2.4. *Application of smartphone-based sensor in fish freshness analysis*

Smartphone-based sensor (SPBS) in food analysis is a kind of material that can fit itself in this "chip-in-a-lab" era because of their characteristic features. The implementation of the point of care (POC) concept is quite difficult without assessing certain lab equipment and is accelerated in this chip-in-a-lab era by holding the hands of smartphones (Tsagkaris et al., 2021). The accurate and rapid analyzing capability of SPBS draws the attention of the world towards food quality monitoring. Some of the advantages like ease of operation, portable, on-site sensing, etc. play a significant role in the rapid acceptability and growth of SPBS in the modern world. The revolutionary development of SPBS happened due to the engagement of biosensors with smartphones. SPBS can be applied to serve various purposes (foodborne pathogens, contaminants, toxins, spoilage detection, nutritional value, shelf-life monitoring, etc.) in food systems (Lu et al., 2019). The specific application of SPBS is solely related to the biosensors present in the setup. Monitoring the quality changes in foodstuff is very challenging, especially for fish which degrades fast. Moreover, the odor of fish helps to indicate its freshness. The chemical compounds produced during spoilage may act as the target substances of the biosensors fitted in the SPBS system. These chemicals can be detected by following different mechanisms like colorimetric sensing, fluorescent sensing, electrochemical, etc. In this section, the application of SPBS in the context of the detection mechanism of fish freshness is summarized in detail.

2.3.2.4.1. *Colorimetric method of fish spoilage detection using SPBS*

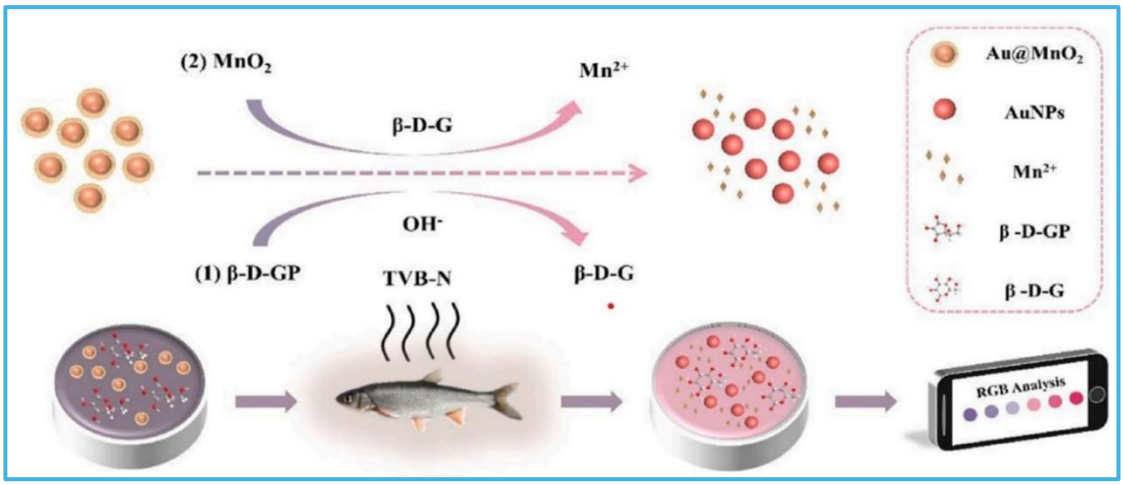
The colorimetric method is one of the simplest methods of fish spoilage detection among all the detection methods. This method is advantageous in terms of its simplicity, economy, and time of operation. The detection can be performed in less than half an hour in most cases. Visual and photoelectric colorimetry are the two most used techniques under the colorimetric method of detection. The spoilage compounds detected by the chemo-sensitive compounds (Cresol Red, Alizarin, Phenol Red, Bromocresol Green, Curcumin, Bromocresol Purple, Methyl Red, Bromophenol Blue, Xylenol Blue, etc.) incorporated in detection system and characterized through change in color with response to spoilage compounds (ammonia, putrescine, dimethylamine, trimethylamine, cadaverine, etc.) present in fresh products (Morsy et al., 2015). To evaluate fish spoilage,

colorimetric sensors can be devised with a single chemo-sensitive compound or an array of such compounds (Morsy et al., 2015).

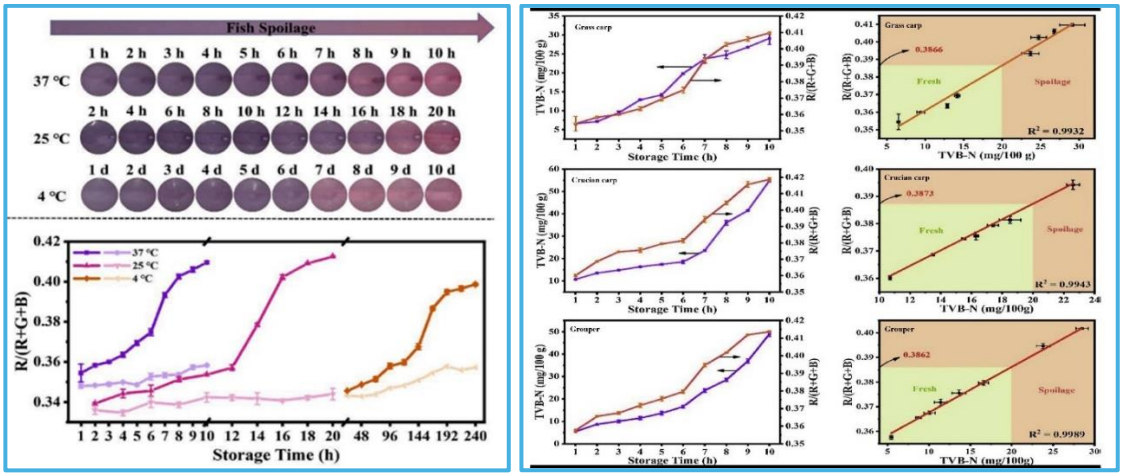
The most widely used index for the assessment of fish freshness is TVB-N content. Zhang et al. (2021) have established a colorimetric sensor for TVB-N that can be integrated into smartphones (**Fig. 2.6(i)**). The sensor is based on a special hydrogel that contains core-shell nanocomposites of Au@MnO_2 and β-D-glucose pentaacetate (β-D-GP). Au@MnO_2 functions in this hydrogel as a color-changing substance and β-D-GP functions as a precursor to a reducing agent. The hydrogel is permeable to TVB-N molecules, which produce an alkaline environment that causes the hydrolysis of β -D-GP and the production of β -D-glucose. As a result, the MnO_2 layer of the Au@MnO_2 is etched away due to a redox reaction between MnO_2 and β -D-glucose. By observing the change in the color of the gold nanoparticles (AuNPs) from purple to red, which is easily distinguishable to the naked eye due to the alteration of localized surface plasma resonance (LSPR) effect, the presence of TVB-N can be detected. The color information is conveniently converted into digital data using a smartphone with RGB analysis. The hydrogel-based sensing platform has proven to be effective in real-time monitoring of fish spoilage (**Fig. 2.6(ii)**). To further validate the accuracy of the method, it was compared to a widely used technique known as the semi-micro-Kjeldahl method, which is also recognized as a national standard in China (GB5009.228-2016). The semi-micro-Kjeldahl method was employed to measure the changes in the TVB-N value resulting from the spoilage of three types of fish (grass carp, crucian carp, and grouper) over 10 h at a temperature of 37 ℃. Additionally, the proposed sensing hydrogel was utilized to investigate the variations in the R/ (R + G + B) value. Based on the Chinese national standard (GB2733- 2015), freshwater fish and shrimp products must not exceed a TVB-N content of 20 mg/100 g sample. However, the measurements indicate that the TVB-N content exceeded this limit for the different fish types. After 6 h, the TVB-N content in grass carp reached 20.56 mg/100 g, while for crucian carp it was 22.6 mg/100 g at 7 h, and for grouper, it was 23.8 mg/100 g at 7 h. These measurements indicate that the fish fillets had started to degrade by those time points (**Fig. 2.6(iii)**). Based on the proposed method, a sensing hydrogel was used to determine the freshness of different fish species. The corresponding RGB ratio value was found to be approximately 0.38. A higher RGB ratio reading from the sensing hydrogel indicates that fish fillet spoilage has occurred.

These results suggest that the sensing hydrogel can be a useful tool for determining the freshness of various fish species.

Sensing of biogenic amine vapors and comparison with the existing detection techniques like a sensory test, total volatile basic nitrogen (TVB-N), microbiological assay, and high-performance liquid chromatography (HPLC) was performed by Gonz´alez-Ceballos et al. (2020). The response of smart-coated fibers against amines (β-ethylenediamine, Spermine, Spermidine, Cadaverine, Histamine, Trimethylamine, Morpholine, Piperazine, Tryptamine, Tyramine, β-phenylenediamine, and Putrescine) was observed by the researcher. The authors claimed that the concomitant generation of biogenic amines from fish meat during spoilage leads to color change in the label that can be observed visually as well as by the B-value (blue value of RGB data obtained from the images). The cost of the label was nearly 0.02 euros and was economical. Moreover, there is no need for much time and trained personnel to carry out the analysis. Mastnak et al. (2023) created a new smartphone-based system to validate color-changing sensor receptors (CSRs) using layers, allowing easy detection of color changes using a standard color sensor (**Fig. 2.7(i)**). They used a food-safe CSR model made from black carrot extract and ethyl cellulose. This method effectively measured color changes in response to NH_3, dimethylamine (DMA), and trimethylamine (TMA) concentrations, showing faster reactions for NH_3. The detection limits were similar for NH_3, DMA, and TMA (1.48 mg/L, 1.55 mg/L, and 1.58 mg/L, respectively). They demonstrated the CSRs' usefulness across various muscle food types, particularly frozen hake fillets, correlating the color changes with total volatile basic nitrogen (TVB-N) and microbial counts (**Fig 2.7(ii) & (iii)**). In another recent study, a color-changing FG-UV-CD100 film using red cabbage extract and carbon dots was developed by Dogan et al. (2024) for detecting food spoilage in real-time. By capturing color changes with smartphones under various light sources, a dataset was built and machine learning (ML) to achieve 98.8 % accuracy in classifying ammonia vapor concentrations. This ML classifier was embedded into an Android app, ‘SmartFood++’, allowing quick analysis without internet access, unlike cloud-based systems (**Fig. 2.8**). Testing on a real fish sample showed 99.6 % accuracy, proving it as a powerful tool for real-time, on-site food spoilage monitoring by non-experts.

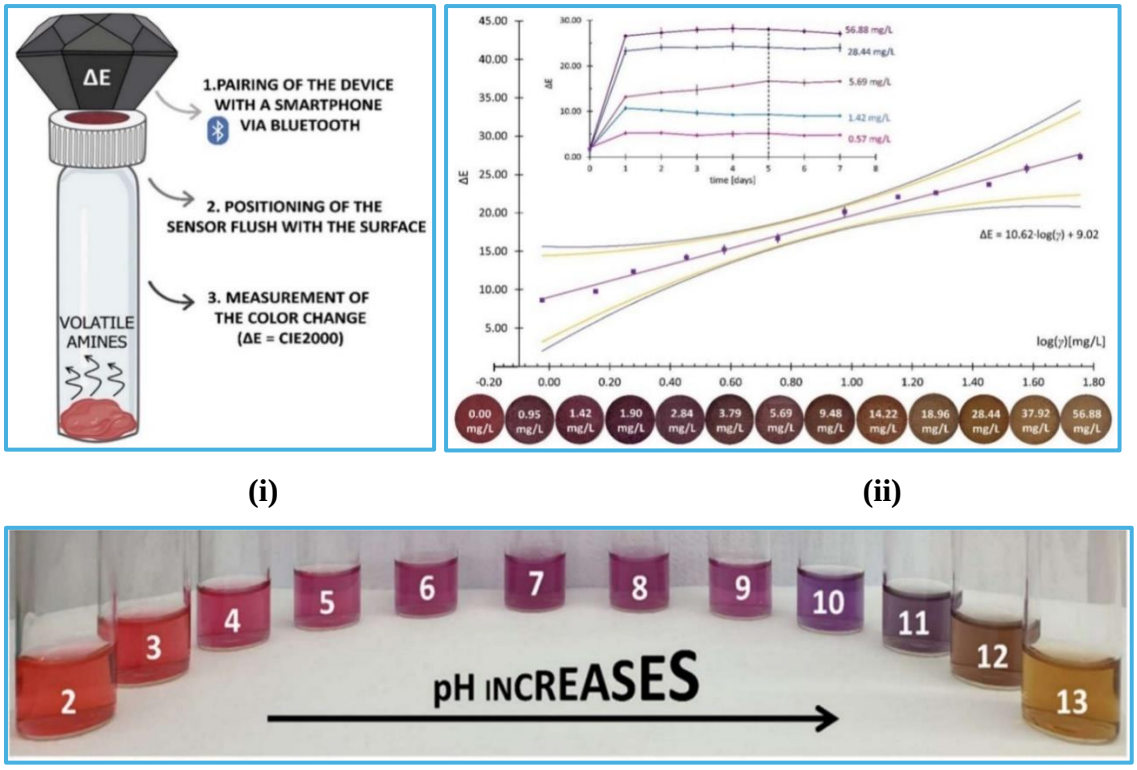


(i)



(ii) **(iii)**

Figure 2.6. (a) (i) Schematic of TVB-N sensing for fish freshness monitoring by the smartphone-integrated hydrogel sensing platform, **(ii)** Real-time monitoring of fish freshness stored at 37, 25, and 4 ℃, respectively by the sensing hydrogel with color evolution photographs as a function of time, and corresponding ratio value changes of R/(R + G + B), and **(iii)** The changes of TVB-N detected by the hydrogel sensing method and the semimicro-Kjeldahl method of fish (grass carp, crucian carp, and grouper) stored at 37 ℃ and correlation of the results between the semimicro-Kjeldahl method and the hydrogel sensing method in monitoring the freshness of three kinds of fish. Reproduced with permission (*adapted from Zhang et al., 2021*).



(i) **(ii)**

(iii)

Figure 2.7. (i) A schematic illustration of the color measurement technique, **(ii)** The calibration curve with 90 % and 95 % confidence intervals for the NH_3 analyte with the corresponding color changes and changes in the value of DE at different g with increasing time, and **(iii)** Color changes of the black carrot extract (BCE) in solutions with increasing pH (*adapted from Mastnak et al., 2023*).

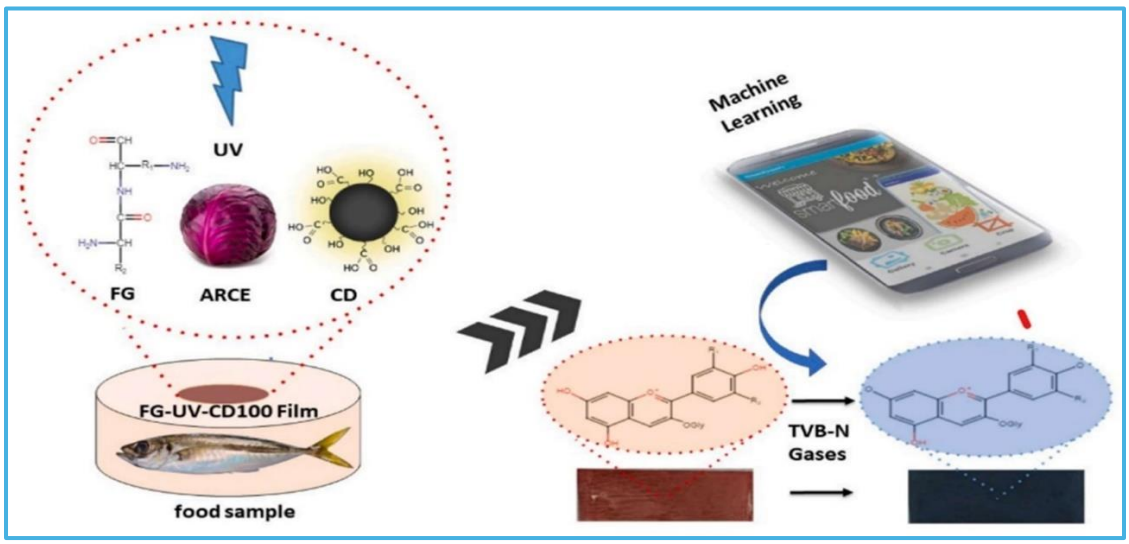


Figure 2.8. Schematic representation of the FG-UV-CD100 films using red cabbage extract and carbon dots sensing system with smartphone-embedded machine learning (*adapted from Dogan et al., 2024*).

2.3.2.4.2. *Electrochemical method of fish spoilage detection using SPBS*

The on-site detection and analysis capabilities of electrochemical biosensors have been significantly enhanced, leading to a wide range of applications. An enzyme-based cost-effective paper biosensor was developed by Mustafa et al. (2020) to detect the freshness of fish. The enzyme converts hypoxanthine or xanthine to hydrogen peroxide (H_2O_2), uric acid in the presence of molecular oxygen. The amount of xanthine is measured by measuring the amount of consumed O_2 or by measuring the produced H_2O_2 or uric acid by colorimetric or electrochemical means. The electrochemical biosensor involves electrodes whose characteristics are impacted by their excellent electro-catalytic ability, facilitating redox reactions associated with the chemical compounds formed during fish deterioration. Chang et al. (2017) introduced an electrochemical sensor that rapidly detected gases like NH_3, TMA, and DMA within 60 seconds in raw fish (Tilapia, Mackerel, and Beltfish). This enzyme biosensor proves to be a quick and reliable tool for assessing fish freshness by determining metabolites produced during ATP decomposition and vapor release after fish is slaughtered. Apetrei et al. (2016) developed an enzyme biosensor comprising a thick film, utilizing DAO Pt/GPH/Chitosan/CSPE, for the analysis of histamine in postmortem freshwater fish. In another study, Borisova et al. (2016) constructed a Xanthine Oxidase (XOD)-based electrode to establish the correlation between steady-state current and Xanthine concentration. The results closely resembled those measured by HPLC. Many studies related to fish quality evaluation by electrochemical biosensors have already been performed by several groups of researchers (Lin et al., 2018; Yazdanparast et al., 2019; Sharma et al., 2021; Draz et al., 2021; Nurlely et al., 2021) however, this technique in conjunction with a smartphone is a good scope for the researcher.

2.3.2.4.3. *Fluorescent method of fish spoilage detection using SPBS*

The biomarkers of fish spoilage are basic amines, TVB-N, cadaverine, etc. which can be determined by fluorescent sensing method to indicate the level of freshness of fish (Kilic et al., 2022; Liu et al., 2022; Jiang et al., 2022). This sensing method is a combination of the fluorescent method and colorimetric method. Jiang et al. (2022) have detected cadaverine by fluorescent method to evaluate fish freshness. In this method, the ratiometric fluorescent probe (PTCN) was developed for highly sensitive (LOD = 46 nM), selective, and fast (<15 s) detection of cadaverine. The PTCN compound contains

the arylidene malononitrile moiety, which undergoes a reversed Knoevena gel condensation reaction with cadaverine. This reaction is followed by an aldehyde-amine condensation, resulting in the formation of a Schiff-base product. The resulting product demonstrates a noticeable colorimetric and fluorescence reaction when exposed to cadaverine. PTCN displays a remarkable fluorescence transition, changing from orange to green rapidly upon detection of cadaverine. This response is characterized by its quick reaction time, exceptional selectivity, and excellent sensitivity. The authors have developed a smart portable sensing tag/kit based on the mechanism for onsite detection of fish spoilage. The fluorescence behavior of PTCN towards cadaverine is captured and studied by plotting chromaticity against different cadaverine concentrations using the CIE chromaticity diagram. These data points are then processed digitally using the CIE program stored in a typical cell phone (**Fig. 2.9**). After being exposed to various concentrations of cadaverine vapors, the PTCN test strips exhibit diverse fluorescence colors. Notably, an extraordinary change in fluorescence color from pink to green is seen when the cadaverine vapor exposure of the PTCN test strips is increased. The authors reported that PTCN has a low detection limit of 8.65 ppm and the presence of cadaverine can be visually detected by the change in color from red to green of the smart sensing tags. Jiang et al. (2022) have also studied the selectivity of the tag towards cadaverine in the presence of other analytes, but it shows no change in color (pink) in response to other analytes. Liu et al. (2022) developed a package fluorescent label based on an aggregation-induced emissive (AIE) polymer for visual and real-time detection of fish spoilage. Poly-methacrylic acid (PMAA) (stimuli-responsive polymer) and tetraphenylethylene (TPE) (AIE molecule) were used to prepare an AIE polymer and coated onto filter paper with rhodamine B (Rh B) as an internal reference to develop the sensing label. The smart label was attached to the package, and it contains the indicator portion (TPE/PMAA/Rh B) and reference color portion. Consumers and retailers can check the color of the indicator portion by projecting a portable UV light (**Fig. 2.10.**). The authors claimed that the color change of the indicator portion from pink to blue reflects spoilage of the fish contained inside the package. The authors have also correlated this change in fluorescence (color change) with the TVB-N value and they found it congruent. However, from the color image, it is difficult to determine the value of TVB-N or other biomarkers. To overcome this, researchers have developed smartphone-based applications associated with the calibration equation of biomarker and color parameters (Jiang et al., 2022; Liu et al.,

2022). This application can easily interpret the color image data and results in a quantitative value of the biomarker. Therefore, the freshness of fish can be easily accessed by simply capturing an image with a smartphone camera. **Table 2.4** gives a summary of recent smartphone-based sensors for fish freshness detection.

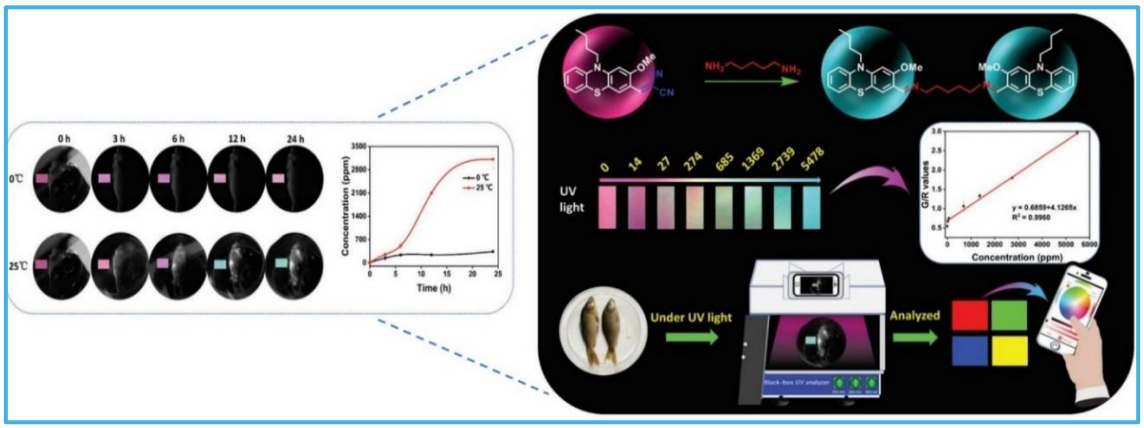


Figure 2.9. Schematic illustration of the working mechanism and application of smartphone-adaptable fluorescent sensing tag for monitoring the freshness of fish stored under different conditions (adapted from Jiang et al. (2022)).

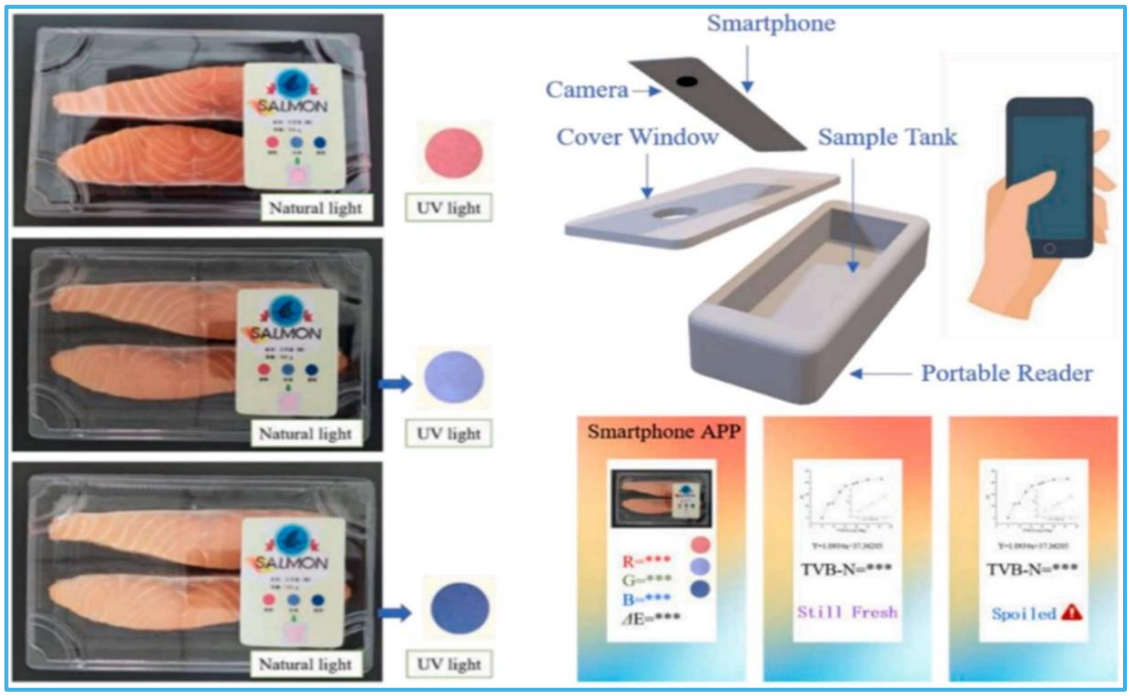


Figure 2.10. Application of the ratiometric fluorescent sensing label based on aggregation-induced emissive (AIE) polymers in salmon samples, and a conceptual design of smartphone-based reader and smartphone applications. Reproduced with permission (adapted from Liu et al. (2022)). Copyright 2022, Elsevier Ltd.

Table 2.4. Summary of the recent smartphone-based sensor used for the detection of fish freshness

Species	Mode of detection	Sensing material use	Detection target	LOD	Smartphone use	Reference
Fish (horse mackerels)	Colorimetric sensor	FG-UV-CD100 films (red cabbage extract and carbon dots)	TVB- N gases	-	Phone's camera and costume design Android App. 'SmartFood++'	Dogan et al., 2024
Fish (Frozen hake fillets)	Colorimetric sensor	Color-changing sensor receptors (CSRs) model made from black carrot extract and ethyl cellulose	NH_3, dimethylamine (DMA), and trimethylamine (TMA)	NH_3-1.48 mg/L, DMA-1.55 mg/L, TMA- 1.58 mg/L	Phone's camera and Bluetooth	Mastnak et al. 2023
Shrimp	Solid-state fluorescent based sensor	Fluorescent probe (PAA) nanoparticles and filter paper derived from cotton	NH_3	0.5 ppm	Color recognizing smartphone software	Chen et al., 2022

Fish	Fluorescence sensor	Ratiometric fluorescent probe PTCN (sensing tag)	Cadaverine	8.65 ppm	Phone's camera and a color-picker application	Jiang et al., 2022
Salmon fish	Fluorescent label based	AIE polymer (poly-methacrylic acid (PMAA) and tetraphenylethylene (TPE)) coated on to filter paper with rhodamine B (Rh B)	TVB-N	-	Phone's camera with portable UV light and color model application	Liu et al., 2022
Fish	Paper-based biosensor	Chitosan immobilized with XOD/HRP enzyme and 4-AAP/TBHBA chromogenic agents	Hypoxanthine	-	Phone's cameraand 'ON Color Measure' app	Tirigil et al., 2022

Fish (grass carp, crucian carp, and grouper)	Colorimetric method	Hydrogel loaded with Au@MnO_2 core-shell nanocomposites and β-D-glucose pentaacetate (β-D-GP)	TVB-N	-	Phone's camera	Zhang et al., 2021
Tilapia fish fillet	Biosensor	Paper based sensor fabricated with CeNPs and XOD	hypoxanthine(HX)	15 μM and 89 μM	-	Mustafa et al., 2021
Fish Atlantic horse mackerel (*Trachurus trachurus*)	Colorimetric sensor	Cotton fibers coated with sensory polymers (bromonaphthalimide motifs)	Biogenic amines	-	Phone camera and color model was obtained with Photoshop Software	Gonz´alez-Ceballos et al., 2020
Shellfish (Mussel, Oyster, Scallop)	Colorimetric sensors	Gold nanoparticle-based ELISA (EGNB-ELISA)	Saxitoxin (STX)	0.4 ng/mL	Phone's camera and external parts	Zhong et al., 2019

Fish	Electrochemical sensor	Molecularly imprinted polymer (MIP) on gold screen-printed electrode (Au-SPE)	Histamine	210 nM	-	Serrano et al., 2020
Tilapia, Beltfish, Mackerel	Organic gas sensor	P3HT (poly(3hexylthiophene)) based organic semiconductor chemical sensor	Volatile ammines (TVB-N)	0.1 ppm	-	Chang et al., 2017
Shellfish	Biosensor	Antigen-antibody test strip	OA (okadaic acid) and STX (saxitoxin)	2.800 ng/mL for OA and 9.808 ng/mL for STX	Phone's camera with 3D printed accessory and custom design app.	Fang et al., 2016
Carp, Prussian carp, Tench, Wels catfish, European perch	Amperometric enzymatic biosensor	Modified carbon screen-printed electrode with diamineoxidase, graphene and platinum nanoparticles	Histamine	25.4 nM	-	Apetrei, I. M., & Apetrei, C., 2016

2.3.2.5. *Keyword network analysis (KNA) of smartphone-based sensors application in fish spoilage*

The KNA diagram is created based on 9 articles published between 2019 and 2023 (**Fig. 2.11**). Web of Science is used as the database of all the articles used for constructing this diagram. The keyword used for searching was 'Smartphone based sensor and fish spoilage'. The articles consist of smartphone-based sensors for fish freshness monitoring. Additionally, these articles were chosen based on their applicability to the subject of this review. The bibliographic information for those articles was used to create the diagram. This KNA diagram's main goal is to outline the status of a smartphone-based sensor app for tracking fish spoilage. Moreover, it is important to know about the trends of smartphone-based sensor applications in fish spoilage monitoring in the last five years. In the **Fig. 2.11.**, the linkage between colored circles represents the interconnection between keywords related to this study. The size of the circle varies depending on how frequently a particular keyword appears.

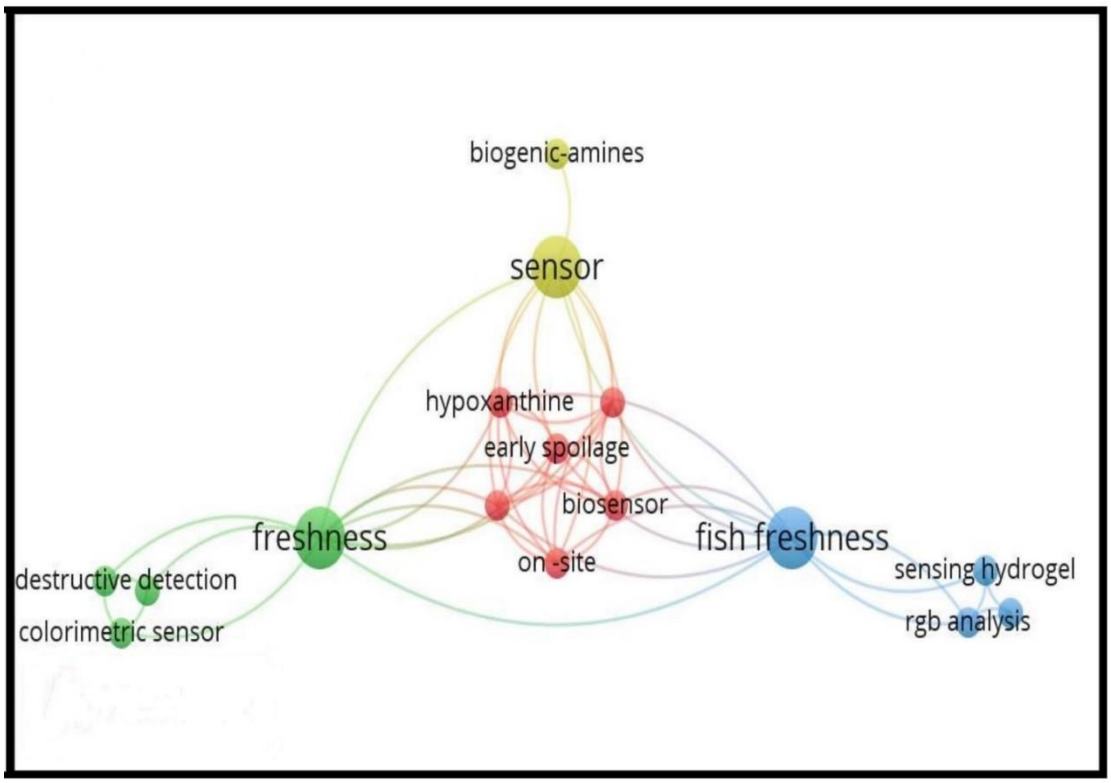


Figure 2.11. Keyword network analysis (KNA) of smartphone-based sensors application in fish spoilage.

2.3.2.6. *Advantages and limitations of smartphone-based sensors over analytical instruments*

Compared to traditional instruments, smartphone-based sensing offers advantages in speed, control, affordability, and accessibility, with improved data management (Rateni et al., 2017). SPBS are portable, easy to use, cost-effective, and provide real-time data collection and monitoring. SPBS can also be integrated with software applications for data analysis and interpretation and can be accessed remotely. Additionally, they can be scaled to accommodate the needs of different-sized operations. Firstly, their high portability allows them to be effortlessly transported to different locations, including fish processing plants, fishing vessels, and retail stores. This accessibility facilitates on-the-spot testing, eliminating the necessity to transport samples to a central lab for analysis. Secondly, their ease of use is a notable advantage, given the widespread familiarity with smartphones. These sensors typically feature user-friendly interfaces and simplified workflows, enabling even non-experts to conduct tests. The development of an integrated exogenous antigen testing (iEAT) system, designed for on-site food allergen detection comprising an electronic reader, a single-use allergen extraction device, and a smartphone application (Lin et al., 2017) is one such example. The iEAT system facilitates quantitative allergen detection within a brief and practical timeframe (specifically, less than 10 minutes for the complete assay) at a minimal cost (below $4 per assay). The system uses smartphone's Bluetooth to wirelessly send test results and information to a cloud server, enabling web-based data collection and sharing among users. This streamlines decision-making processes and reduces reliance on trained personnel (Fu et al., 2019; Lu et al., 2019; de Vargas et al., 2020; Ji et al., 2022; Pallavi et al., 2022). Thirdly, real-time monitoring and cost-effectiveness are key benefits. Traditional analytical instruments can be excessively expensive to acquire, operate, and maintain. Smartphone-based sensors leverage existing smartphone infrastructure, substantially reducing testing costs and allowing their use as point-of-care (POC) applications. This could be particularly advantageous for smaller businesses in the fish industry with limited financial resources (Sowoidnich et al., 2012; Rateni et al., 2017; Choi et al., 2019; Grazioli et al., 2020; Feng et al., 2020; Park et al., 2021). This ensures that food products remain within safe and optimal conditions during transportation, storage, and processing. Fourthly, wireless connectivity inherent in smartphones, such as Bluetooth and Wi-Fi, enables seamless data transfer to cloud-based platforms or central

databases (Ahmad et al., 2014; Jedermann et al., 2014; Javadi et al., 2020; Kalinowska et al., 2021). In addition, a lot of smartphone-based sensors are flexible and can measure several different things at once, including temperature, pH, and ammonia levels. A complete picture of the product's condition is provided by this extensive data. Additionally, these sensors are compatible with software programs that analyze and interpret the data they collect, making it easier to spot trends, patterns, and anomalies over time. This supports efforts to improve the quality and enables wise decision-making. Furthermore, remote accessibility is a useful feature because it enables stakeholders like managers, quality control teams, and regulators to keep an eye on conditions and data even when they are physically far from the location.

Despite having a number of advantages, the development of smartphone-based platforms is still in its early stages. The analytical performance of smartphone-based sensors is constantly being improved. Recent advances have made it possible to detect smaller and lower concentrations of molecules. In the fish industry, these advantages can contribute to improved product quality, reduced waste, enhanced food safety, and streamlined operations. However, it's important to note that while smartphone-based sensors offer numerous benefits, they may not replace all analytical instruments, especially for highly specialized tests that require advanced equipment and expertise. Therefore, a hybrid approach that combines both traditional instruments and smartphone-based sensors could be optimal in some situations. Despite these advantages, it's important to note that smartphone-based sensors might have limitations in terms of accuracy, precision, and sensitivity compared to highly specialized analytical instruments. The choice of a particular platform will depend on the specific application and the needs of the user. However, all these platforms offer the potential to make healthcare, environmental monitoring, and food safety more accessible and affordable.

Variability in sensor quality, calibration, and environmental conditions can affect the reliability of measurements, which could be particularly problematic when ensuring food safety and compliance with quality standards. Smartphones are versatile devices that emit electromagnetic signals and experience various levels of interference. This can introduce noise and affect the accuracy of sensor readings, especially in noisy or high-EMI (electromagnetic interference) environments. In regulated industries such as food and healthcare, using smartphone-based sensors might present challenges in terms of meeting

regulatory standards and obtaining necessary certifications for accuracy and reliability. Smartphone-based sensors often require data to be transmitted to cloud servers or remote databases for analysis. This raises concerns about data security, privacy, and the potential for sensitive information to be intercepted or compromised. Regular calibration and maintenance are crucial for accurate measurements. They might not have the same level of calibration and maintenance support as dedicated instruments, potentially leading to a drift in accuracy over time. Zhong et al. (2019) discuss the challenges of using smartphone-based sensors for food analysis, including the complexity of food matrices can make it difficult to obtain accurate and reproducible results. The limited sensitivity of smartphone-based sensors can make it difficult to detect low levels of analytes. The small size of smartphone-based sensors can make it difficult to accommodate all the components necessary for a complete analytical system. The lack of standardization in the development and validation of smartphone-based sensors can make it difficult to compare results between different studies. Some of the challenges are the need for further research to develop more robust and sensitive smartphone-based sensors, to develop standardized protocols for the development and validation of smartphone-based sensors, and to educate users about the limitations of smartphone-based sensors (Zhang et al., 2021). Considering these limitations, it's important to conduct a thorough assessment of the specific needs and challenges of the fish industry or any other sector before deciding to implement smartphone-based sensors. As this technology continues to develop, these limitations will likely be overcome.

The reviewed smartphone-based sensing methods are tailored to specific phone models used in the analysis. Calibration of each phone is essential due to hardware differences, such as varying camera spectral responsivities, lamp emissions, and digitizer elements. Image format is also important, with RAW format preferred for scientific imaging because it retains all sensor data and allows post-processing. Sample preparation remains a challenge in mobile food diagnostics, as non-expert users may introduce contamination, affecting measurement accuracy. Commercial systems address this with user-friendly designs and guidelines. All discussed methods indirectly measure target substance concentrations and require calibration with reference standards. Calibration involves maintaining identical conditions for standards and unknowns. Machine learning-based systems hold promise for self-calibration, leveraging large datasets generated by smartphone-based diagnostics for meaningful information extraction.

2.3.2.7. *Challenges and Future Trends*

Detecting fish spoilage through smartphone-based sensors is an interesting and promising area, but it comes with its own set of challenges. Fish spoilage indicators can vary based on species, storage conditions, and other factors. Developing a universal detection method that accommodates this variability is a challenge. Ensuring that smartphone sensors provide accurate and sensitive measurements for detecting specific spoilage indicators is crucial. Calibration and validation processes need to be robust. Achieving real-time monitoring capabilities is essential for timely intervention. Reducing the time delay between spoilage occurrence and detection is a significant challenge (Doğan et al., 2024). Integrating the detection system seamlessly into existing smartphone platforms and ensuring compatibility across various devices and operating systems can be challenging. Finding the right balance between the cost of using sensor technology and how well it works is crucial for broad acceptance, especially in places with limited resources. It is difficult to ensure that the sensors function properly in a variety of environmental circumstances, such as variations in humidity and temperature.

Machine learning algorithms may improve the accuracy and adaptability of sensors. These algorithms can learn from diverse datasets and continuously improve detection capabilities. Complementary data from other sensors may be captured, allowing for a more thorough analysis. Aiming to create more portable, smaller sensors that are easily integrated into different types of storage containers, allowing them to be used in a variety of scenarios. Improving the energy efficiency of the sensors to extend battery life, especially in scenarios where continuous monitoring is essential. Further, more research is still needed to combine artificial intelligence, data mining cloud computation, and other cutting-edge relevant technologies for uplifting the quality management of the fish processing sector.

2.4. Conclusion

In this chapter, we have highlighted the literature review on the physiology and composition of fish, the causes of fish spoilage, its physiological, biological, and chemical changes during spoilage, and the freshness indicator parameters like TVB-N, pH, and microbial load. It further highlighted the existing traditional methods and advanced technologies for the determination of fish spoilage focusing on their advantages and limitations. Alongside, characteristics of polyaniline and colorimetric dye-based sensors for spoilage detection and, the antimicrobial effect of essential oil on the storage stability of fish have been focused on detail. Lastly, recent advancements in smartphone-based food diagnostics focusing on fish analysis are briefly discussed. Smartphone-based sensors as an emerging field hold great promise with significant scientific and commercial impact. Progress in various disciplines like healthcare, environment, and food engineering has led to more portable, cost-effective, and user-friendly diagnostic platforms compared to traditional lab-based methods.

Researchers have explored different approaches to harness the potential of smartphones. While all the approaches have their advantages, they also face limitations. Fortunately, advancements in optical sensor fabrication, miniaturization, and cost reduction, along with sophisticated machine learning algorithms and cloud computing capabilities, are driving significant developments in mobile diagnostics. This extends beyond food monitoring to encompass environmental and biomedical sensing, promising exciting progress in the field. However, limited review and research were encountered for smartphone-based sensors for fish analysis. This opens the possible areas for researchers for the detection of fish spoilage. Therefore, the focus of the research is developing a portable smartphone-based device using optical components that works on the measurements by recording the sharp color change of polyaniline labels using mobile, as a marker for ascertaining fish freshness. The developed device can be a commercially feasible miniaturized device that can be integrated with a mobile phone for wider use by people in the food supply chain.

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