Chapter 3

To develop and characterize the polyaniline-based sensor for monitoring the freshness of fish during storage

3.1. Introduction

For many years fish has been an important part of the diet and nutrition of people from different backgrounds. Freshwater fish are commonly preferred in the northeastern region of India as a delicacy and for their rich source of nutrients. However, fish has the disadvantage of being highly perishable due to its high moisture content, and chemical and enzymatic reactions that lead to rapid microbial deterioration. During spoilage, the nutrients present in the fish decompose and result in various chemical reactions leading to undesirable off odor which sometimes may affect human health. The activity of spoilage organisms such as *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Staphylococcus aureus* (Giarratana et al., 2016) converts the natural chemical compound TMAO (Trimethylamine oxide) which is present in the fish to TMA (Trimethylamine), DMA (Dimethylamine), etc. and these volatile amines are collectively known as TVB-N (Total volatile basic nitrogen) which is consider as a major indicator of chemical spoilage of fish (Abbas et al., 2008; Yerlikava et al., 2015, Hao et al., 2021). Fish freshness monitoring has become increasingly important and is an area of growing research as consumers are more concerned about food nutrition and safety.

Polyaniline (PANI) is one of the conductive polymers that exhibit unique properties due to its simplicity in synthesis, stability in a different environment, and easy doping and dedoping. Polyaniline is chemically synthesized by oxidative polymerization of aniline in an acidic solution with a strong oxidant, resulting in the acid-doped, emeraldine salt form, which can then be dedoped with a base to yield the emeraldine base (Huang et al., 2006). Kuswandi et al. (2012) have proposed a colorimetric method using polyaniline (PANI) films to develop smart packaging. Kumar et al. (2017) have developed a highly flexible, sensitive, and reproducible PANI-based ammonia sensor that can detect ammonia vapor up to 5 ppm at room temperature with good response and reproducibility. The acid doping of PANI involves a protonation reaction. As a result, neutral PANI molecules gain protons and form chemical bonds, creating actively advantageous conditions. On the other hand, deprotonation occurs when the doped PANI interacts with ammonia where the ammonia molecules absorb protons from PANI resulting in the formation of ammonium ($NH^{4+)}$ that leads to the change in its properties. When the ammonia replacement is absent, the proton reverts to its state and the initial doped PANI is recovered. This process results in the effect of ammonia on PANI exhibiting reversibility (Kumar et al., 2017).

For fish freshness determination conventional techniques are time-consuming, lengthy, and destructive. Therefore, in the study, a non-destructive, low-cost polyaniline (PANI)-based sensor was developed for rapid detection of fish spoilage or freshness.

3.2 Materials and Methods

In the experiments, all chemicals of AR grade including aniline, ammonium peroxidisulphate, hydrochloric acid, and ammonia solution (25 %) were obtained from Merck-Sigma, India, and were used without any additional purification. Fresh fish samples were procured from Tezpur's local market near Tezpur University.

3.2.1. *Preparation of polyaniline (PANI) label*

The chemical polymerization method is used to synthesize polyaniline as described by Jarad et al. (2016) with slight modifications. Aniline was chemically polymerized with hydrochloric acid as a catalyst and ammonium peroxidisulphate as an oxidant. Aniline (2 mL) was mixed in 50 mL of 1M HCl and considered as solution 'A', and 5 g of ammonium peroxidisulphate ($(NH_4)_2S_2O_8$) was mixed separately in 50 mL of 1M HCl to prepare solution 'B'. Both solutions 'A' and 'B' were kept overnight at refrigeration temperature and the two solutions were mixed (1:1) rapidly in an electromagnetic stirrer for 3 h at 600 rpm and left in the dark for another 24 h for effective polymerization. After polymerization, the precipitate obtained was filtered, washed with distilled water until colorless, and centrifuged at 7000 rpm for 30 minutes. To standardize the PANI label, three different quantities of supernatant solution of synthesized polyaniline (0.2 mL, 0.5 mL, and 1 mL) were deposited on an optically inactive polystyrene sheet (20 mm × 20 mm) and dried in a desiccator with a relative humidity of 40-60 % at ambient temperature for 48 h and the thickness of the loaded PANI labels was 0.21 mm, 0.2 mm, and 0.25 mm respectively. The amount of polyaniline to be deposited is determined by scanning through the spectrophotometric spectrum (400-800 nm) and observing the response. The polyaniline label was kept in the dark for further experimental analysis. The schematic diagram of the synthesis of PANI and PANI labels is shown in **Fig. 3.1**.

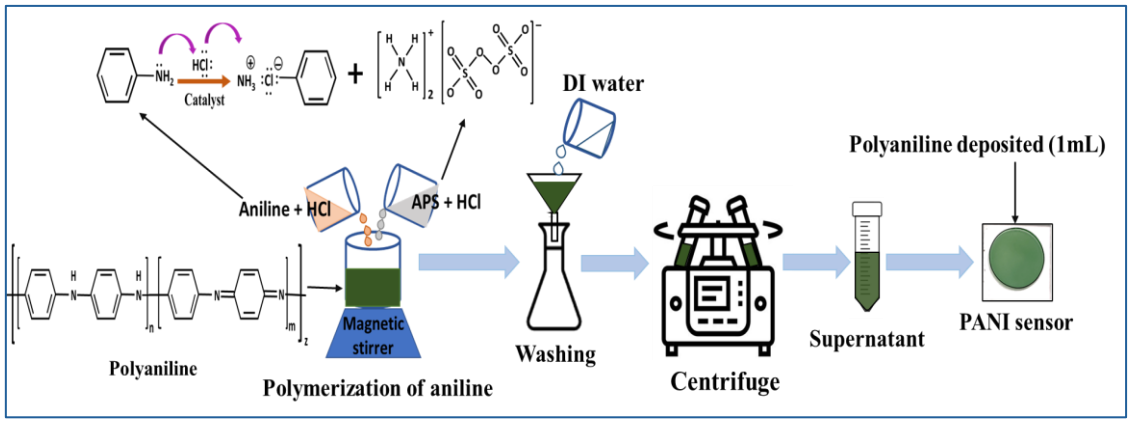


Figure 3.1. Synthesis of PANI and PANI label.

3.2.2. *Characterization of polyaniline (PANI)*

The functional groups of polymerized aniline and aniline were observed using FTIR (Fourier Transform Infrared) spectroscopy (Frontier, PerkinElmer, USA) between the frequency range of 400-4000 cm^{-1} at 4 cm^{-1} resolution using 64 scans (Sanches et al., 2013).

The X-ray diffraction (XRD) of the polyaniline was performed using an X-ray diffractometer (BRUKER AXS D8 FOCUS). The dried polyaniline sample was exposed to an X-ray beam at 100 mA and 50 kV. Powder diffraction patterns were obtained in step scanning mode, 2θ = 5-60°, a step of 0.02°, and 5 s/step. The area of the crystalline and amorphous region was determined by integrating the curve over 2θ between 10 and 80°. Then, the degree of crystallinity was calculated by the given **Eq. 3.1** (Sanches et al., 2013):

$$\% \text{ Crystallinity} = \frac{\text{Area of crystalline peak}}{\text{Total area under curve(crystalline + amorphous)}} \times 100 \quad (3.1)$$

Field emission scanning electron microscopy (FESEM) (JSM 7200F JEOL Ltd. 1-2 Musashino 3-chome Akishima Tokyo 196-8558 Japan) was performed to examine the morphology of the synthesized polyaniline label. The surface morphology was obtained at room temperature at an accelerating potential of 5 kV at 10,000X, 20,000X, 30,000X, and 40,000X.

3.2.3. *Response time*

The response time of the developed PANI label was examined by exposing the label to ammonia vapor (400 ppm solution) for different mins (5, 10, 15, 20, 25 min).

The response of the polyaniline label has been characterized by % transmittance spectra using a UV visible spectrophotometer in the visible region between 400-800 nm (**Fig. 3.2**).

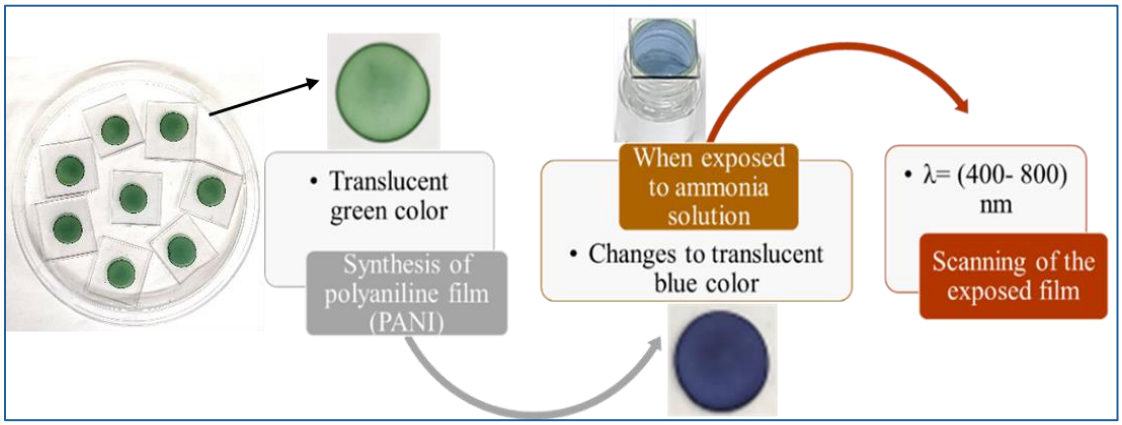


Figure 3.2. PANI label exposed to ammonia vapor.

3.2.4. *Calibration of the PANI label using UV-visible spectrophotometer*

The developed PANI label was calibrated without exposure to the ammonia vapor and was scanned with the UV visible single-beam spectrophotometer (Cary 60 UV–Vis Spectrophotometer, Agilent Technologies, Germany) to find out the response transmittance peak wavelength. Then the PANI labels were exposed to 0-400 ppm concentration of standard ammonia solution for 5 min (**Fig. 3.3**), and the % transmittance was recorded. A calibration curve between the % transmittance and ammonia concentration was plotted. The linear equation obtained was used for monitoring the volatile amines released from the stored fish sample **(Eq. 3.2)**:

$$\text{Ammonia Concentration} = \frac{(\text{Intensity} - \text{b})}{\text{S}} \qquad (3.2)$$

Where b and S represent the intercept and slope of the calibration curve.

100ppm **200ppm** **300ppm** **400ppm**

Figure 3.3. PANI labels exposed to different concentrations of ammonia vapor.

3.2.5. *Sample preparation*

Fresh live Tilapia *(Oreochromis nilotica*) fish were collected from the local market of Tezpur, Assam, India. Some species of fish in the Cichlidae family are known by their common name, tilapia. Fish that live in freshwater environments such as lakes, ponds, rivers, and streams are known as tilapia. They are not frequently seen in brackish water. It's also a long-time favourite dish in the Philippines, where people appreciate its mild flavour and affordable price (**Fig. 3.4**). The experiment was conducted on the same day the fish was harvested. The head, gut, scale, and middle bonds were removed and washed under tap water, and fish fillets of uniform size were prepared in a sterile environment. The fresh fillet (30 g) was stored in a sterile glass container and the polyaniline label was attached inward of the lid keeping a headspace of 2 cm (**Fig. 3.5**).

Figure. 3.4. Tilapia *(Oreochromis nilotica)* fish.

Figure 3.5. PANI label exposed to fish fillets.

3.2.6. *Performance evaluation of the label*

To check the performance of the label, the response of the label was evaluated using a UV visible spectrophotometer with an interval of 2 h (0 h, 2 h, 4 h, 6 h, 8 h, 10 h) at ambient and with an interval of 2 days (0, 3, 6, 9, 12, 15 days) at refrigeration temperature. The % transmittance spectra obtained were calculated to % intensity reduction over time during storage. The resulting findings were validated by conventional methods such as measurement of TVB-N, pH, and microbial count.

3.2.7. *Measurement of TVB-N and pH*

TVB-N (Total volatile basic nitrogen) in terms of volatile amines from the fish sample was measured by an ion-selective electrode for ammonia (NH_4^+ ISE). The fish sample (5 g) was partially dissolved and dissociated in an aqueous phase, the ammonia (NH_4^+) ion content in the water was measured and the output of the signal was then converted to mg/100 g (Kuswandi et al., 2012). A pH electrode was used to test the pH of the fish at the same time. The results were used to correlate the quality of the fish during storage.

3.2.8. *Microbial analysis of fish*

In a sterile mixer grinder, 5 g of fish sample was homogenized with 45 mL peptone water (0.1 %). A ten-fold dilution of the sample was prepared in 0.1 % peptone water, and 0.1 mL aliquots were spread on nutrient agar plates for the total visible count with control (nutrient agar plates without sample) for microbial enumeration. After incubating the plates at 37 °C for 48 h, the total visible count (TVC) was calculated (Giarratana et al., 2016) and reported as log CFU/g.

3.2.9. *Statistical analysis*

Statistical analysis of all experiments was conducted using the IBM SPSS Statistics 21 (IBM, Armonk, NY, USA) software application for Windows, and the means were separated using Duncan's multiple range test ($p \leq 0.05$). All the experiments were performed in triplicates (n=3) and data were expressed as mean ± standard deviation (SD) and Origin 2021 was used to plot all the graphs.

3.3. Results and discussion

3.3.1. *Polymerization and characterization of polyaniline*

The mechanisms of polymerization and the change of state of polyaniline are demonstrated in **Fig. 3.6.** Polyaniline is classified as a synthetic polymer where the transport of electrons and protons is involved in polymer doping. The three different oxidation states of polyaniline are leucoemeraldine (totally reduced formed of undoped polyaniline), emeraldine (intermediate oxidation state), and perningraniline (fully oxidized form of undoped polyaniline) (Cheng et al., 2013; Kumar et al., 2017). Polyaniline is ecologically and thermally stable, and it is unique as it may change its properties in response to structural changes by simply treating it with various chemicals. The structure may be changed by adding electrons and lowering the N atom, as well as eliminating the polaron-stabilizing acid (Silverstein et al., 2005; Sambasevam et al., 2015). Therefore, its response toward the nitrogen-containing group makes it specific for the analysis of the total volatile base produced by fish. The FT-IR spectrum of aniline and polyaniline is illustrated in **Fig. 3.7.** The characteristic bands obtained at 1584 cm^{-1}, 2920 cm^{-1} and 3424 cm^{-1} signify the functional group region where 3424 cm^{-1} characterizes the free asymmetrical and symmetrical N-H stretching vibration and 2920 cm^{-1} represents C-H stretching vibration **(Fig. 3.7)**. The peaks at 1584 cm^{-1} and 1497 cm^{-1} are attributed to the quinoid and benzenoid rings' stretching vibrations, which are the PANI backbone's telltale sign, while the intense peak at 1297 cm^{-1} indicated the strong aromatic C-N stretching vibration (Sambasevam et al., 2015). The peaks at 1117 cm^{-1} and 1132 cm^{-1} are due to aromatic C-H in-plane and the transmission band at 795 cm^{-1} exhibits C-H out-of-plane bending vibration that is related to the deformation pattern (Silverstein et al., 2005). The presence of $C=N^+$ sites with dopant ions on PANI is indicated by the peak in the range of 2300–2800 cm^{-1} (Dhivya et al., 2019). In doped PANI, a strong transmission peak at 2349 cm^{-1} was observed, indicating the existence of such sites. While in dedoped PANI, the absence of a peak was observed, except for a small, weak peak at 2309 cm^{-1}, suggesting that the dopant ions had been removed from the polymer matrix. Additionally, the stretching and bending vibrations between 1600 and 500 cm^{-1} were shifted, indicating that NH_4OH has removed the chloride ions from doped PANI **(Fig. 3.7)**. The obtained spectra show closely comparable values that were stated in the previous study (Khan et al., 2020; Jarad et al., 2016; Sambasevam et al., 2015; Dhivya et al., 2019; Abedelraheem et al., 2018). Slight variations in the band

stretching may be due to the alteration in the method of polyaniline synthesis. The oxidation of the monomers generated by the oxidizing agent (ammonium peroxydisulfate) results in the formation of cation radicals and when these radicals react with additional monomers, oligomers are formed. The mechanism of PANI synthesis and its form is explained in **Fig 3.6**.

The X-ray diffractograms of dried polyaniline powder were recorded at 10-80° (2θ) as shown in **Fig 3.8**. A sharp peak and a broad peak at around 33° and 25° crystal plane and some weak peaks (47°, 58°, 68°, and 77°) with low intensity were observed which revealed the degree of crystallinity. The degree of orientation of the polymer chains in that crystal plane is represented by the sharpness (width) of the peaks, and the intensity (peak height) represents the population of crystallites in that plane. The peak could be attributed to scattering from polyaniline chains in interplanar space. The presence of the benzenoid and quinoid groups in the polyaniline chain accounts for the sharp peak at 33°. The regular aniline monomers' repetition units give rise to a broad peak in the 23°-26° range, which is indicative of the tightly packed phenyl rings that give rise to a planar conformation (Feng et al., 2000; Bhadra et al., 2008). The degree of crystallinity was obtained by the ratio of the area under the curve to the total area of the curve and from the diffraction pattern, the degree of crystallinity of polyaniline was found to be 31.42 % which depicts microcrystalline nature. The degree of doping, oxidation states, and molecular weight of polyaniline all affect its sensing activity. According to the literature, polyaniline synthesized at low temperatures may have fewer defect sites, increased crystallinity, long polymer chains with numerous polarons, and maximum inter-and intra-chain interactions, which led to high active sites for sensing (Sambasevam et al., 2015). Similarly, the polyaniline used in this study was produced at a low temperature and followed the same pattern.

A mixed morphology and a compact structure with granular and agglomerated nanofibrous structures were observed **(Fig. 3.9)**. Interconnected, granular, and fibrous morphology illustrated the energy binding to integrate with the case of rapid chemical synthesis of polyaniline (Sai et al., 2020).

PANI is employed in many different fields because of its versatile structure, low cost of synthesis, and conductive nature (Abdolsattari et al., 2022). The previously

described spectral methods to assess fish spoilage (Zhu et al., 2013; Cheng et al., 2014) are species-specific, and a huge amount of data is needed to calibrate the sample.

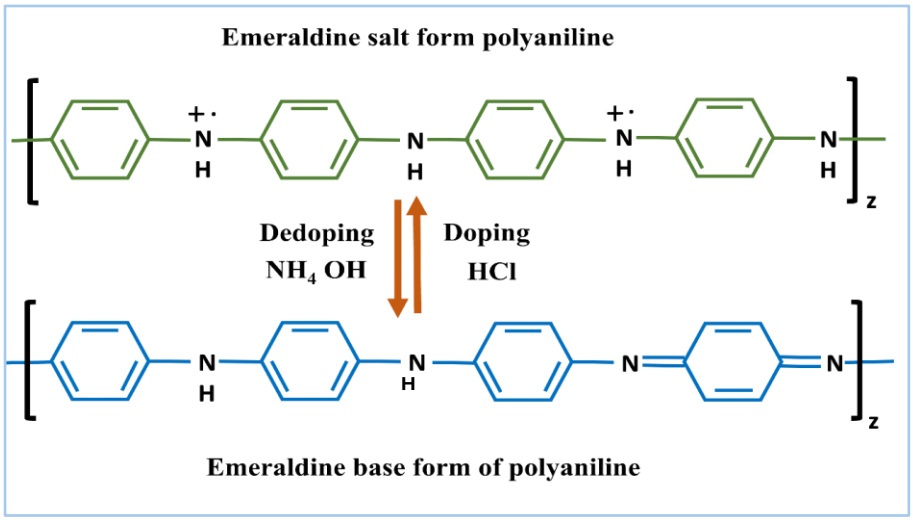


Figure 3.6. Mechanism of PANI synthesis and its forms.

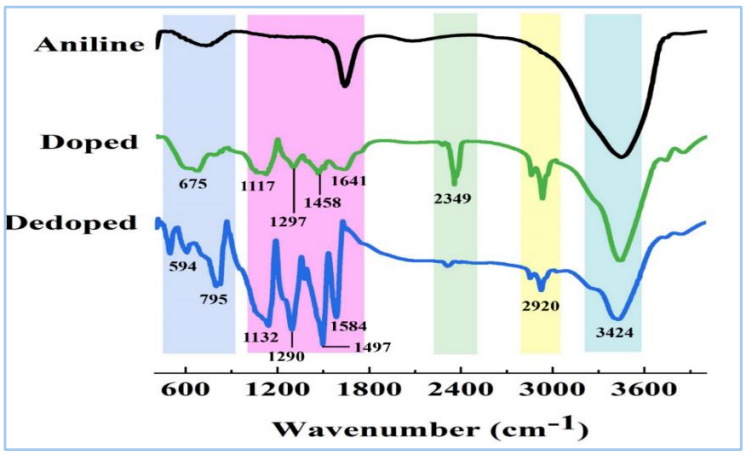


Figure 3.7. Fourier Transform Infrared (FTIR) spectra of aniline and PANI.

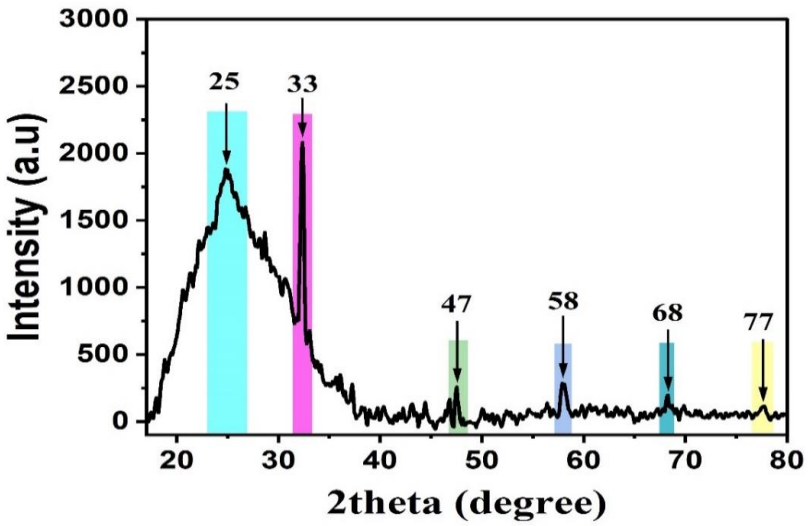


Figure 3.8. XRD of PANI.

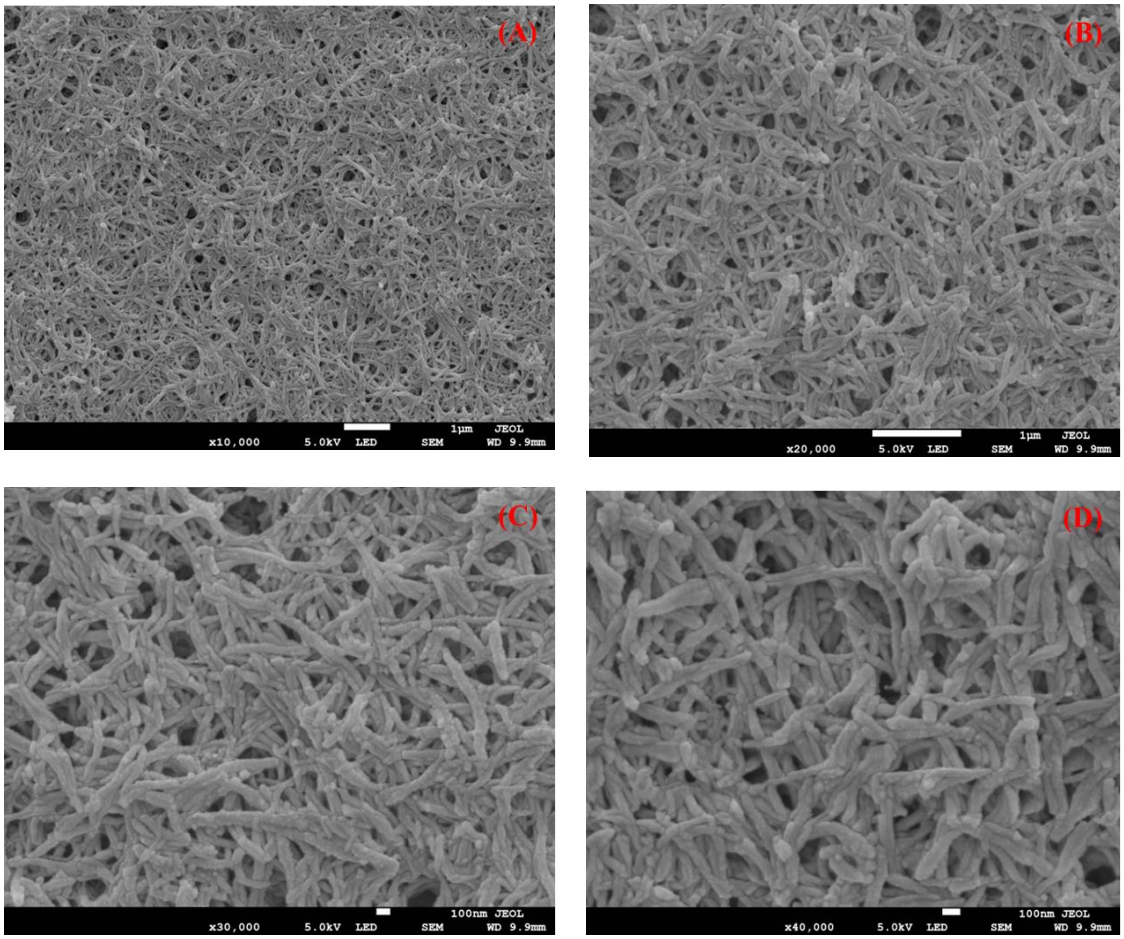
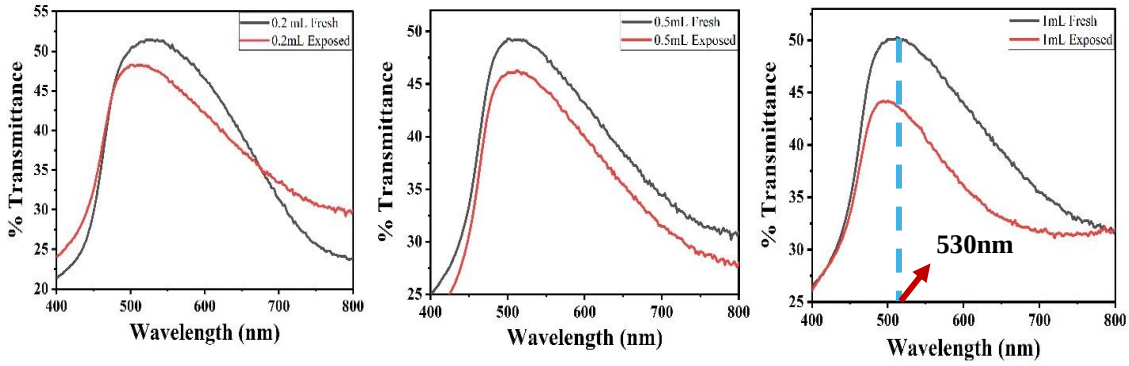


Figure 3.9. FESEM image of PANI label deposited with 1 mL **(A)** 10,000X, **(B)** 20,000X, **(C)** 30,000X, and **(D)** 40,000X.

3.3.2. *Response time and calibration of PANI label*

The label deposited with 1 mL polyaniline was found suitable in terms of stability and uniform distribution on a polystyrene sheet (**Fig. 3.10)** however, responses received with 0.2 mL and 0.5 mL of PANI solution deposited were significantly less and not reproducible**.** Therefore, 1 mL polyaniline solution with 0.25 mm thickness was selected for label development. The PANI label exhibited a response peak at 530 nm in 0-5 min when exposed to 400 ppm of ammonia solution, demonstrating its sensitivity to ammonia concentration changes (**Fig. 3.11**). The response of the PANI label to various concentrations of ammonia solution yielded a linear equation with a high regression coefficient of 0.98, affirming its reliability and consistency in detecting different ammonia concentrations (**Fig. 3.12**).



(a) **(b)** **(c)**

Figure 3.10. Response of fresh and exposed PANI label to 400 ppm ammonia vapor **(a)** 0.2 mL PANI deposited, **(b)** 0.5 mL PANI deposited, and **(c)** 1 mL PANI deposited.

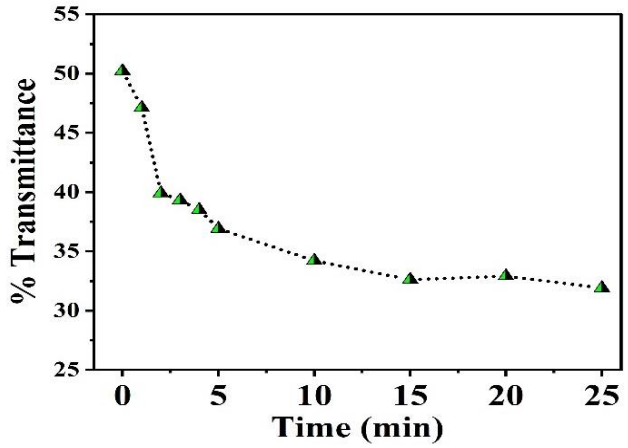


Figure 3.11. Response time of PANI label with 1 mL of PANI deposition.

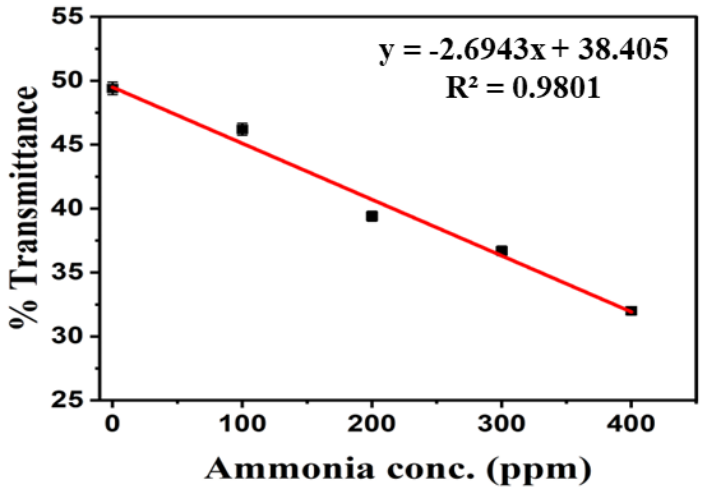
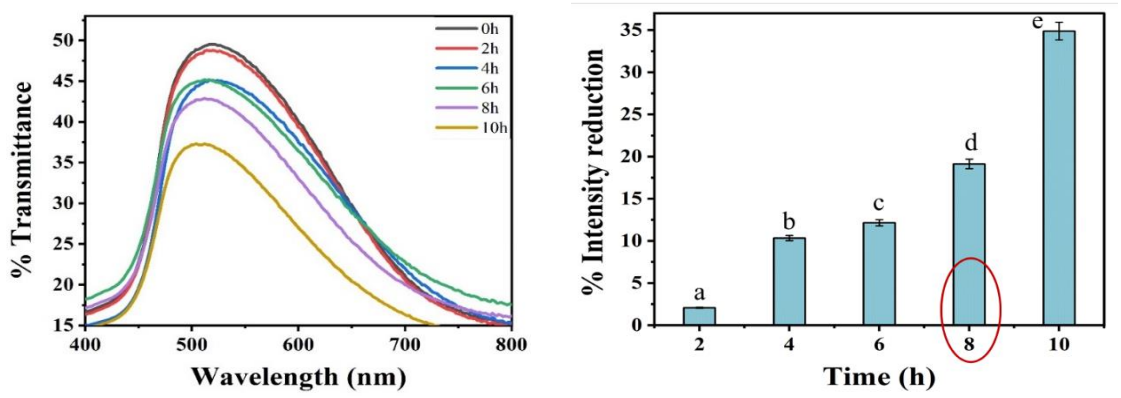


Figure 3.12. Calibration curve of PANI label response using UV-visible spectrophotometer.

3.3.3. *Performance evaluation of the developed PANI label*

The maximum transmittance of the fresh PANI label without exposure to ammonia vapor was determined at 530 nm when scanned using a UV-visible spectrophotometer across the range of 400-800 nm. A calibration curve correlating % transmittance to ammonia concentration was generated, as depicted in **Fig. 3.12**.

In analyzing the PANI label with fish samples, the exposure time of the PANI label proved crucial. As the exposure duration increased, the spectral shift indicated a significant reduction in transmittance during storage, reflecting the degree of spoilage in the fish over time, highlighting an increase in ammonia concentration with prolonged storage time. The reduction in intensity after 4-6 h (**Fig. 3.13a**) shows the initial stage of changes occurring in the fish tissues which progressively increased with the time of storage at ambient temperature. The % intensity reduction was calculated considering 0 h as 100 %, and a method was developed. The % intensity reduction of the fillets stored at ambient temperature was found to increase reaching 20 % and 35 % at 8 and 10 h respectively (**Fig. 3.13b**). During fish degradation, microbial activity leads to the conversion of trimethylamine oxide (TMAO) into trimethylamine (TMA) and dimethylamine (DMA), while bacteria decomposition of urea and amino acids generates ammonia. These volatile compounds collectively constitute total volatile basic nitrogen (TVB-N), serving as a reliable indicator of fish freshness. The % intensity reduction of fish fillets stored at refrigeration temperature was also found to increase with the storage time following a linear trend obtaining a 20 % intensity reduction at 6h (**Fig. 3.14**). Spoilage occurs naturally after the death of the fish and bacteria initiate to invade the tissue through the gills, blood vessels, the skin, and the lining of the abdominal cavity. In addition to bacteria, enzymatic reactions, and chemical changes involving oxygen from the air and fat from the flesh are the sources of spoilage. The degradation of chemical compounds during spoilage results in the release of total volatile basic nitrogen (TVB-N). This TVB-N reacts with the emeraldine salt form of PANI label and deprotonates to emeraldine base that results in the reduction of intensity when measured using a UV-visible spectrophotometer.



(a) **(b)**

Figure 3.13. (a) Spectrophotometer response of Tilapia fish, and **(b)** Percent (%) intensity reduction at ambient room temperature (Different letters indicate significant differences ($p < 0.05$) in % intensity reduction with storage time).

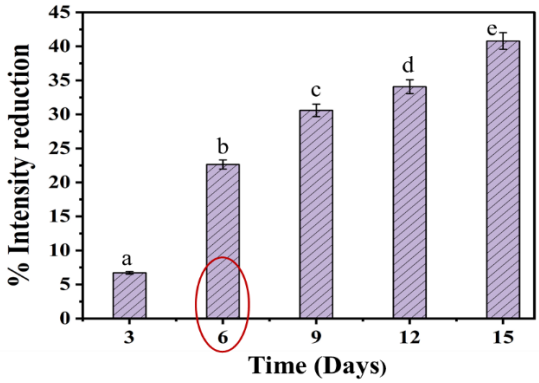


Figure 3.14. Percent (%) intensity reduction for spectrophotometer response of Tilapia fish at refrigeration temperature. Different letters indicate significant differences ($p < 0.05$) in % intensity reduction with storage time.

The decrease in spoilage time in the study may be due to the washing and cleaning of the fish before storage, since washing may reduce the surface microbial load. The degree of spoilage depends on the temperature; the higher the temperature, the faster the spoilage as the bacteria multiply using the dead fish flesh as their food, and at low temperatures, the action of bacteria can be stopped or slowed down. This is the reason why the fish fillet at refrigeration temperature can withstand up to 5-6 days as compared to fillets stored at ambient temperature.

3.3.4. *Measurement of TVB-N, pH, and microbial count*

The TVB-N levels of tilapia fish stored at ambient and refrigeration temperatures were evaluated, as depicted in **Fig. 3.15** and **3.17** respectively. Initially, within the storage period of 0-6 h at ambient temperature, the TVB-N value remained below 25 mg/100 g, which was deemed acceptable. However, the TVB-N level exceeded the recommended safe threshold from 8 h of storage (30 mg/100 g) followed by 10 h (38 mg/100 g). The TVB-N value of the fish fillets stored at refrigeration temperature was found to be 32mg/100g at 6 days, 37 mg/100 g at 9 days, and 48mg/100g at 15 days. With increasing storage time, TVB-N values significantly rose, exhibiting a close correlation with the response of the PANI label. According to European Union Regulations, the limit of acceptability of fish was reported to be 30-35 mg/100 g, and fish with 35 mg/100 g TVB-N or above are considered spoiled (European Union Regulation (EC) no. 2074/2005).

Fig. 3.16 and **3.18** illustrate the correlation between pH value and fish freshness when stored at ambient and refrigeration temperatures respectively. Initially, the pH value of the fish sample was low, gradually increasing over the storage period in both storage temperatures. This trend suggests the conversion of glycogen in the muscles to lactic acid, explaining the initial low pH. Subsequently, the pH value began to rise after 2h, continuing until 10h for storage at ambient temperature. Similarly, the pH value of the fillets stored at refrigeration temperature increased with the time of storage, indicating the progression of deterioration. This observation is consistent with findings by Kuswandi et al. (2012), who noted a gradual increase in pH with prolonged storage duration. During degradation, alkaline substances such as TMA (trimethylamine), DMA (dimethylamine), and ammonia accumulate in the fish flesh, leading to a rise in pH (Chun et al., 2014, Huang et al., 2018, Nazaruddin et al., 2021, Safitri et al., 2022). This increase in pH may be attributed to alkaline compounds generated by microbial activity during fish degradation, correlated with the increase in TVB-N which aligns with the response obtained from the PANI label upon exposure to fish sample.

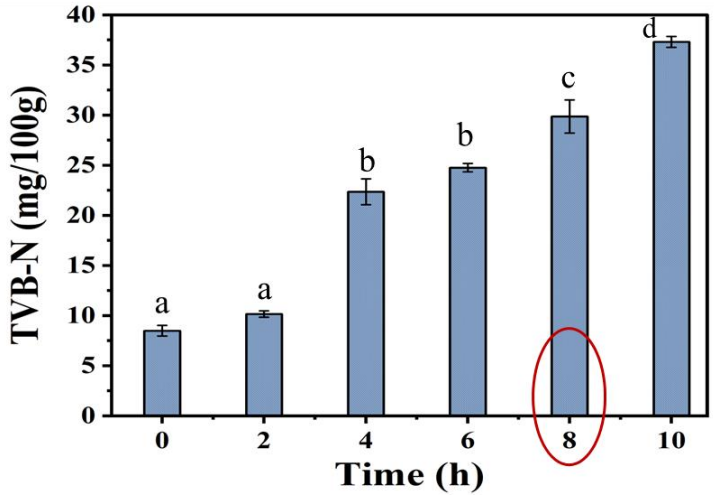


Figure 3.15. TVB-N value of the fish sample at ambient room temperature. Different letters indicate significant differences ($p < 0.05$) in TVB-N of fish fillets during storage.

The total viable count (TVC) of the tilapia fish sample at ambient and refrigeration temperatures was illustrated in **Fig. 3.16** and **3.18** respectively. There was no indication of microbial growth during the early stage of storage. However, the number of spoilage organisms gradually increased over the storage period, reaching TVC levels of 6.8 log CFU/g, 7 log CFU/g, and 7.1 log CFU/g at 6 h, 8 h, and 10 h, respectively, for fillets stored at ambient temperature. The TVC value of fillets stored at refrigeration temperature reaches 7 log CFU /g at 6 days and subsequently increases up to 7.5 log C CFU/g at 15 days (**Fig. 3.18**). Studies have shown that the microbial count of fresh fish is influenced by both time and storage temperature (Chun et al., 2014). Although lower temperatures slow the rate of microbial growth, the psychrophilic organisms actively participate in the degradation of fish tissue. As a result, the *pseudomonas* count of the fish fillet at refrigeration temperature shows a gradual increase during the time of storage (**Fig. 3.19**). Fish is deemed spoiled when the microbial count of specific spoilage organisms reaches levels between 10^6-10^7 CFU/g. According to the International Commission on Microbiological Specifications for Food (ICMS), the limit for total aerobic count in fresh and frozen fish is set at 10^7 CFU/g. Microbial activity is the primary factor determining the shelf life of fresh fish, thus, the estimation of total viable counts (TVC) serves as a reference for fish freshness assessment.

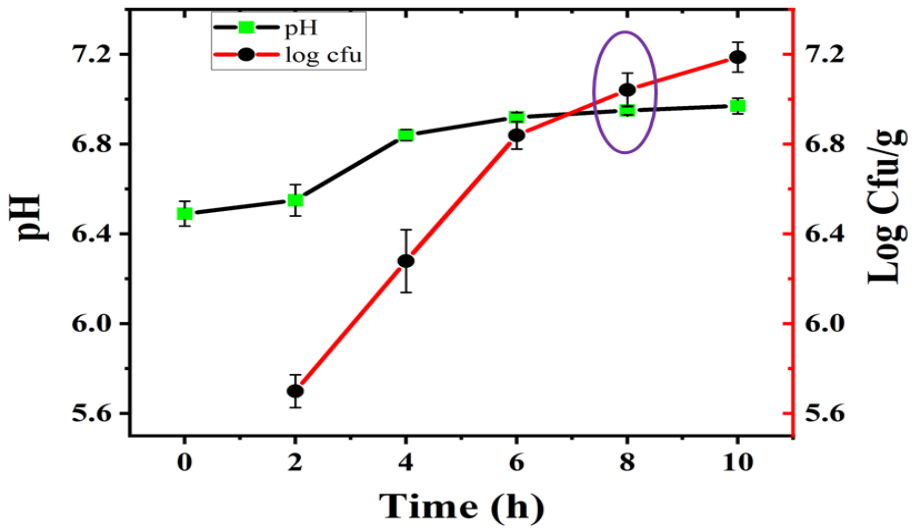


Figure 3.16. pH and TVC (total viable count) of fish sample at ambient room temperature.

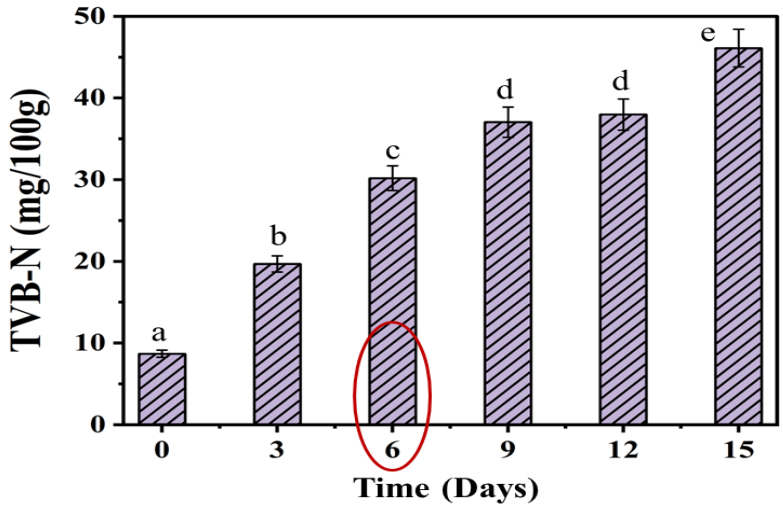


Figure 3.17. TVB-N value of the fish sample at refrigeration temperature. Different letters indicate significant differences ($p < 0.05$) in % intensity reduction with storage time.

In this study, the TVC sharply increased to 10^7 CFU/g at 8 h in fillets stored at ambient temperature and at 6 days in fillets stored at refrigeration temperature, aligning with the PANI label response obtained by the UV-visible spectrophotometer. A method was developed based on the observed response of the PANI label in corresponds to the measurement of TVB-N value and microbial count, where 0-10 % intensity reduction = fresh, 10-15 % intensity reduction = intermediate, and >20 % intensity reduction =

spoiled. The observed reduction in transmission peak at 530 nm over storage time aligns with the degree of spoilage of fish, that decline is particularly notable after 4-6 h at ambient temperature and at 6 h for refrigeration temperature. This coherent trend underscores the potential utility of PANI-based sensing systems for real-time monitoring of fish freshness, crucial for maintaining food quality and safety.

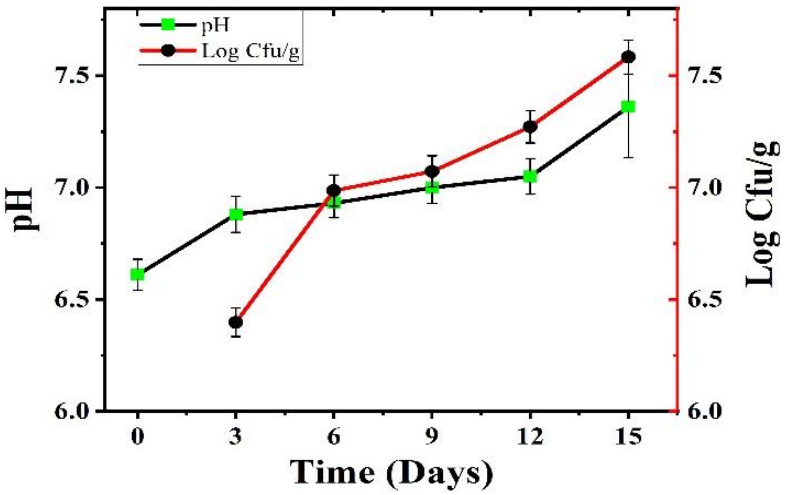


Figure 3.18. pH and TVC (total viable count) of fish sample at refrigeration temperature.

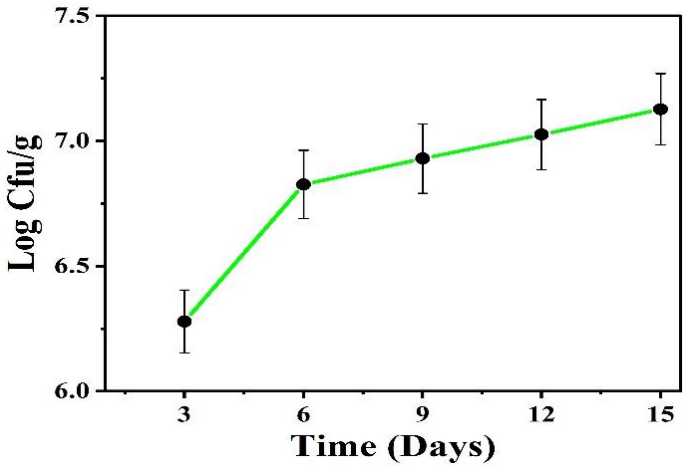


Figure 3.19. *Pseudomonas* count of fish sample at refrigeration temperature.

3.4. Conclusion

The polyaniline (PANI) was successfully synthesized and characterized for its functional properties using FTIR, XRD, and FESEM. The confirmation of benzenoid and quinonoid groups at specific frequencies highlighted its unique characteristics. Active sites on PANI were identified, emphasizing its active nature, within the range of 2300-2800 cm^{-1}. The degree of crystallinity was found to be 31.42 %, indicating a microcrystalline nature. The sensing activity of PANI is influenced by factors such as the degree of doping, oxidation states, and molecular weight. As per existing literature, PANI synthesized at lower temperatures tends to possess fewer defect sites, increased crystallinity, longer polymer chains with numerous polarons, and enhanced inter and intra-chain interactions resulting in a higher number of active sites for sensing. Consistently, in this study, the PANI used was synthesized at a low temperature, aligning with the pattern observed in the literature. Also, the PANI label, with a thickness of 0.25 mm, exhibited a distinct mixed morphology indicating a compact and interconnected structure, combining granular and agglomerated nanofibrous formations. Its distinct responsiveness to nitrogen-containing groups made PANI an excellent candidate for analyzing total volatile bases, crucial in assessing fish spoilage. When exposed to ammonia solutions of varying concentrations (up to 400 ppm), the PANI label displayed a responsive peak at 530 nm, reacting within a range of 0 to 5 min, showcasing its high sensitivity to changes in ammonia concentration. The resulting spectrophotometric response to different ammonia concentrations yielded a strong linear equation (with a regression coefficient of 0.98), setting its reliability in detecting various ammonia levels. The transmitted spectra obtained from the PANI label during fish storage demonstrated its potential for both qualitative and quantitative analysis of chemicals during fish spoilage. This development signifies the PANI label's utility as a leach-free label, specifically designed for monitoring volatile amines produced during fish spoilage.

3.5. References

95/149/EC. (1995). Commission Decision of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. *Official Journal*, L 97, 84-87 ELI: http://data.europa.eu/ eli/dec/1995/149/oj [legislation]

Abbas, K. A., Mohamed, A., Jamilah, B., & Ebrahimian, M. (2008). A review on correlations between fish freshness and pH during cold storage. *American journal of biochemistry and biotechnology*, *4*(4), 416-421.

Abdelraheem, A., El-Shazly, A. H., & Elkady, M. F. (2018). Characterization of atypical polyaniline nano-structures prepared via advanced techniques. *Alexandria engineering journal*, *57*(4), 3291-3297.

Abdolsattari, P., · Rezazadeh-Bari1, M., Pirsa, S. (2022). Smart Film Based on Polylactic Acid, Modified with Polyaniline/ ZnO/CuO: Investigation of Physicochemical Properties and Its Use of Intelligent Packaging of Orange Juice. *Food and Bioprocess Technology*, https://link.springer.com/article/10.1007/s11947-022-02911-3.

Bhadra, S., & Khastgir, D. (2008). Determination of crystal structure of polyaniline and substituted polyanilines through powder X-ray diffraction analysis. *Polymer Testing*, *27*(7), 851-857.

Cheng, J. H., Dai, Q., Sun, D. W., Zeng, X. A., Liu, D., & Pu, H. B. (2013). Applications of non-destructive spectroscopic techniques for fish quality and safety evaluation and inspection. *Trends in food science & technology*, *34*(1), 18-31.

Cheng, J. H., Sun, D. W., Pu, H., & Zeng, X. A. (2014). Comparison of visible and long-wave near-infrared hyperspectral imaging for colour measurement of grass carp (*Ctenopharyngodon idella*). *Food and Bioprocess Technology*, *7*(11), 3109-3120. https://doi.org/10.1007/s11947-014-1325-7

Chun, H., N., Kim, B., & Shin, H., S. (2014) Evaluation of a freshness indicator for quality of fish products during storage. *Food Science Biotechnol*, 23, 1719–1725 (2014).

Dhivya, C., Vandarkuzhali, S. A. A., & Radha, N. (2019). Antimicrobial activities of nanostructured polyanilines doped with aromatic nitro compounds. *Arabian Journal of Chemistry*, *12*(8), 3785-3798.

Feng, W., Sun, E., Fujii, A., Wu, H., Niihara, K., & Yoshino, K. (2000). Synthesis and characterization of photoconducting polyaniline-TiO2 nanocomposite. *Bulletin of the Chemical Society of Japan*, *73*(11), 2627-2633.

Giarratana, F., Muscolino, D., Beninati, C., Ziino, G., Giuffrida, A., & Panebianco, A. (2016). Activity of R (+) limonene on the maximum growth rate of fish spoilage organisms and related effects on shelf-life prolongation of fresh gilthead sea bream fillets. *International Journal of Food Microbiology*, 237, 109-113.

Hao, R., Roy, K., Pan, J., Shah, B. R., & Mraz, J. (2021). Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analysis. *Trends in Food Science & Technology*, 111, 175–190.

Huang, J., & Kaner, R. B. (2006). The intrinsic nanofibrillar morphology of polyaniline. *Chemical Communications*, (4), 367-376.

Huang, Z., Liu, X., Jia, S., Zhang, L., & Luo, Y. (2018). The effect of essential oils on microbial composition and quality of grass carp (*Ctenopharyngodon idellus*) fillets during chilled storage. *International Journal of Food Microbiology*, *266*, 52-59.

Jarad, A. N., Ibrahim, K., & Ahmed, N. M. (2016). Synthesis and characterization thin films of conductive polymer (PANI) for optoelectronic device application. In *AIP Conference Proceedings* (Vol. 1733, No. 1). AIP Publishing.

Khan, A., Munir, M. T., Yu, W., & Young, B. R. (2020). A review towards hyperspectral imaging for real-time quality control of food products with an illustrative case study of milk powder production. *Food and Bioprocess Technology*, *13*, 739-752.

Kumar, L., Rawal, I., Kaur, A., & Annapoorni, S. (2017). Flexible room temperature ammonia sensor based on polyaniline. *Sensors and Actuators B: Chemical*, 240, 408-416.

Kuswandi, B., Jayus, A. Restyana, A. Abdullah, Heng, L.Y., Ahmad, M., (2012). A novel Colorimetric Food Package label for fish spoilage based on polyaniline film. *Food Control*, 21, 184–189.

Nazaruddin, N., Afifah, N., Bahi, M., Susilawati, S., Sani, N. D. M., Esmaeili, C., Iqhrammullah, M., Murniana, M., Hasanah, U., & Safitri, E. (2021). A simple optical pH sensor based on pectin and Ruellia tuberosa L-derived anthocyanin for fish freshness monitoring. *F1000Research*, *10*.

Safitri, E., Omaira, Z., Nazaruddin, N., Mustafa, I., Saleha, S., Idroes, R., Ginting, B., Iqhrammullah, M., Alva, S., & Paristiowati, M. (2022). Fabrication of an immobilized polyelectrolite complex (PEC) membrane from pectin-chitosan and chromoionophore ETH 5294 for PH-based fish freshness monitoring. *Coatings*, *12*(1), 88.

Sai, S., & Kumar, A. (2020). Synthesis And Morphological Study of Polyaniline. *European Journal of Molecular & Clinical Medicine*, *7*(07).

Sambasevam, K. P., Mohamad, S., & Phang, S. W. (2015). Enhancement of polyaniline properties by different polymerization temperatures in hydrazine detection. *Journal of Applied Polymer Science*, *132*(13).

Sambasevam, K. P., Mohamad, S., & Phang, S. W. (2015). Enhancement of polyaniline properties by different polymerization temperatures in hydrazine detection. *Journal of Applied Polymer Science*, *132*(13).

Sanches, E. A., Soares, J. C., Mafud, A. C., Fernandes, E. G. R., Leite, F. L., & Mascarenhas, Y. P. (2013). Structural characterization of Chloride Salt of conducting polyaniline obtained by XRD, SAXD, SAXS and SEM. *Journal of Molecular Structure*, 1036, 121-126.

Silverstein, R., M., Webster, F., X., Kiewle, D., J. Spectrometric Identification of Organic Compounds, John Wiley & Sons Inc, 2005, pp. 72–108

Yerlikaya, P., Ucak, I., Gumus, B., & Gokoglu, N. (2015). Citrus peel extract incorporated ice cubes to protect the quality of common pandora: Fish storage in ice with citrus. *Journal of Food Science and Technology*, *52*, 8350-8356.

Zhu, F., Zhang, D., He, Y., Liu, F., & Sun, D. W. (2013). Application of visible and near infrared hyperspectral imaging to differentiate between fresh and frozen–thawed fish fillets. *Food and Bioprocess Technology*, *6*(10), 2931-2937.