Chapter 6

To calibrate and validate the developed cradle for development of a mobile application for monitoring of chemical spoilage of fish during storage

6.1. Introduction

Global fish production has been increasing in the last few decades with an estimation of about 179 million tons in 2018 with an estimated loss of 30-35 %. However, around the world, fish consumption is evaluated to arrive at 21.4 kg per capita in 2031, from the base period per capita of 20.5 kg (average 2019-2021) (Rezaei et al., 2018; Tilami et al., 2018, FAO, 2020, FAO, 2022). Yet, fresh fish are highly prone to microbial spoilage and biological changes due to high moisture content, low acid, reactive endogenous enzymes, and enriched nutrient value. The deterioration in the quality of fresh fish leads to the breakdown of proteins and non-protein nitrogenous substances, which is mostly driven by the action of microorganisms, resulting in volatile amines. As the fish tissue is degraded by microorganisms the compound trimethylamine oxide (TMAO) converts into TMA (trimethylamine), DMA (dimethylamine), and ammonia. These compounds that are produced during spoilage are collectively known as TVB-N (total volatile basic nitrogen) and considerably produce unpleasant off-odor. Sensory evaluation such as appearance, odor, and color has always played a key role in the fish industry, and pH, TVB-N value, and microbial count of the fish is the sensory characteristic that concerns their freshness conditions during storage (Abbas et al., 2008; Yerlikaya et al., 2015; Cai et al., 2015; Chen et al., 2017; Hao et al., 2021; Tonezzer 2021). Although the TVB-N value and microbial count are the standards for indication of fish freshness, it is a time-consuming process and involves multiple complicated steps for analysis (Abbas et al., 2008). Therefore, a customer-friendly rapid technique is required for the freshness determination of fish during storage. To monitor the deterioration of fish during storage, we created a PANI label and smartphone-based sensor in which the change in color of the PANI label was observed through the designed cradle and the rear camera of the smartphone. We focused on the steps for embedding optical components as an alternative to a spectrophotometer onto a mobile phone. Utilizing a 3D printed compartment, it is possible to use the broadband light source without depending on the availability of ambient light and it minimizes the cost as compared to the benchtop commercial instruments. Subsequently, we created a financially feasible scaled-down sensing system that can be coordinated with a smartphone for more extensive use by individuals in the food production network, particularly for affirming the quality and freshness of fish. In Chapter 6, three different

varieties of fish fillet i.e., rohu (*Labeo rohita*), mullet (*Mugil cephalus*), and common carp (*Cyprinus carpio*) is used to monitor the performance of the sensor and to determine the chemical spoilage of fish. The *Labeo rohita* belongs to the carp family having a large, silver-colored typical cyprinid shape, with a noticeably arched head. It is extensively used in aquaculture being an important aquaculture freshwater species in South Asia. It occurs throughout the rivers of northern, central, and eastern India, Pakistan, Bangladesh, Nepal, and Myanmar. It is commonly known as rohu in Hindi, Rohiti or Rui in Assamese, Rou in Manipuri, and Tambadamassa in Marathi. The *Mugil cephalus* are commercially valuable schooling fishes of the family Mugilidae. It is found worldwide in coastal temperate and tropical waters, and some species in fresh water. *Cyprinus carpio* is a freshwater fish species in the family Cyprinidae and belongs to the Class Osteichthyes (the bony fishes). Common carp fish is a fish from temperate areas of Asia. This fish can be found in a large number in south China. They live generally in large rivers and lakes. Common carp fish are very suitable for farming with other species of carp fish in ponds. The observation of the sensor was also validated with other existing conventional techniques. A customer-friendly web application is also developed using the findings of the present investigation to evaluate the freshness of fish.

6.2. Materials and Methods

PANI label, and smartphone sensor developed in Chapters 3 and 5 respectively were used. Ammonia solution, nutrient agar, and cetrimide agar were obtained from Merck-Sigma, India. Fresh fish samples were procured from Tezpur's local market near Tezpur University.

6.2.1. *Response of PANI label using the developed smartphone sensor*

The response of the PANI label using the developed smartphone sensor was tested by exposing the label to ammonia vapor at a concentration of 400 ppm for varying durations (0-25 min) and the response was recorded as discussed in Chapter 3, section **3.2.3**.

6.2.2. *Sample preparation and performance evaluation of the developed sensor*

Three different varieties of live fish i.e., rohu (*Labeo rohita*) (**Fig. 6.1a**), mullet (*Mugil cephalus*) (**Fig. 6.1b**), and common carp (*Cyprinus carpio*) (**Fig. 6.1c**) were

collected for the experiment. 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 at ambient room temperature (29±1 ℃), and the polyaniline label was attached inward to the lid keeping a headspace of 2 cm. The response of the label was evaluated using the developed smartphone sensor with an interval of 2 h (0 h, 2 h, 4 h, 6 h, 8 h, 10 h) in an ambient room and with an interval of 2 days (0-15 days) at refrigeration temperature and validated using a UV-visible spectrophotometer and other conventional techniques. The graphical presentation of the developed smartphone-based sensor equipped with PANI sensor for determination of fish spoilage is presented in **Fig. 6.2**.

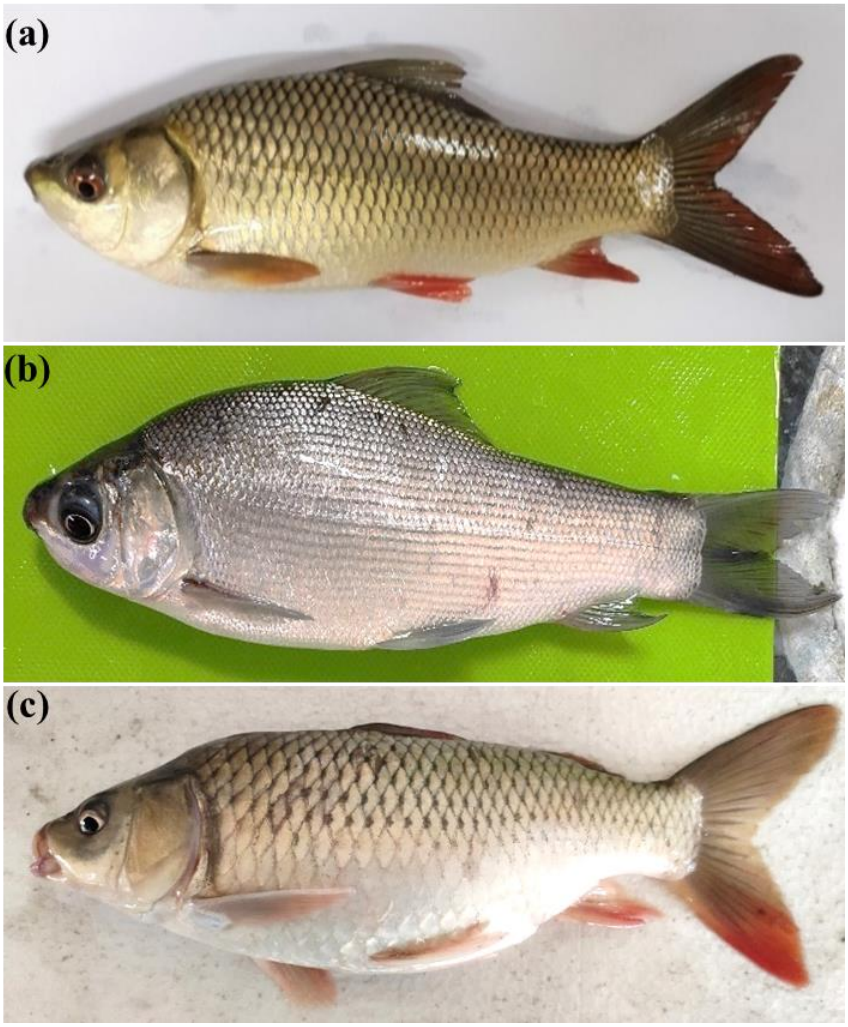


Figure 6.1. (a) Rohu (*Labeo rohita)*, **(b)** Mullet (*Mugil cephalus)*, and **(c)** Common Carp (*Cyprinus carpio)*.

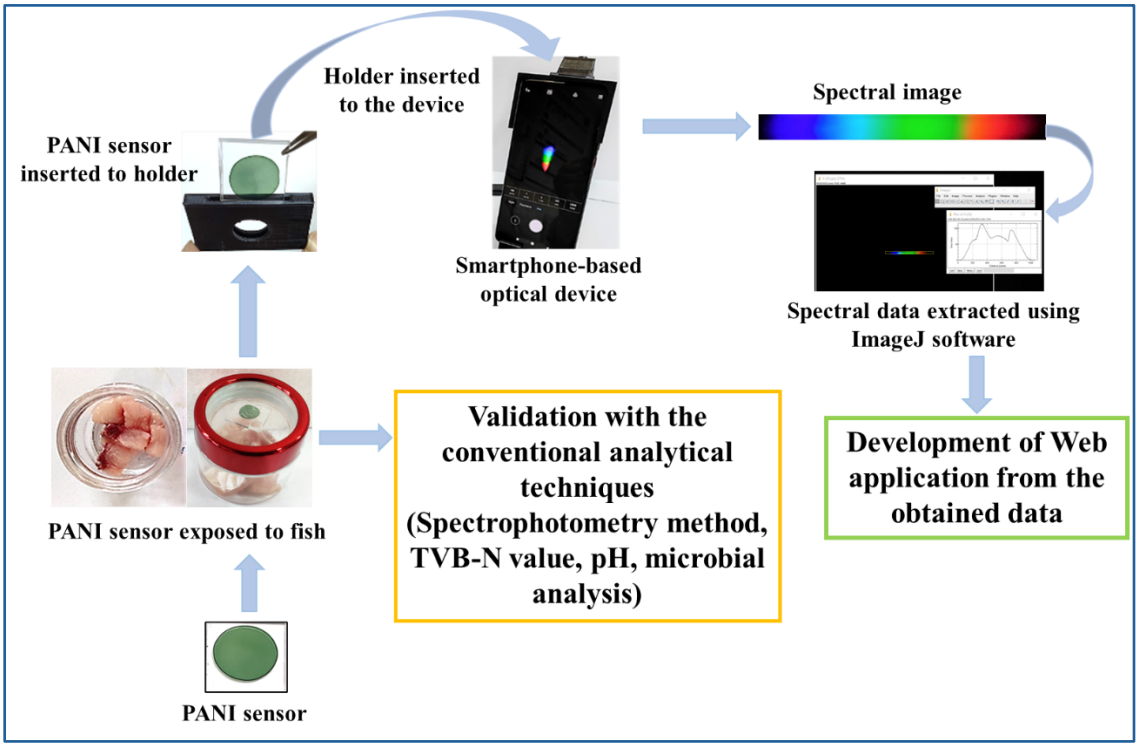


Figure 6.2. Graphical representation of developed smartphone-based sensor equipped with PANI sensor for determination of fish spoilage.

6.2.3. *Sensor validation*

6.2.3.1 *Using UV-Vis's spectrophotometer*

For validation of the sensor, the developed PANI label without exposure to the ammonia vapor was scanned with the UV visible single-beam spectrophotometer (Cary 60 UV–Vis Spectrophotometer, Agilent Technologies, Germany) to find out the maximum transmittance wavelength. Then the PANI label was exposed to the vapor of ammonia solution (0-400 ppm) for 5 min and the % transmittance of the label was observed at 550 nm. A graph between ammonia concentration and % transmittance was plotted to find out the concentration of the unknown sample. The findings of the spectrophotometer and the sensor were compared to validate the findings of the sensor.

6.2.3.2. *Measurement of TVB-N and pH*

The measurement of TVB-N (Total Volatile Basic Nitrogen) in the fish sample was conducted using an ion-selective electrode specifically designed for detecting

ammonia (NH_4^+ ISE) and the pH was measured using the pH meter, as discussed in Chapter 3, section **3.2.7**.

6.2.3.3 *Microbial analysis of fish*

The microbial load of the fish sample was evaluated by total viable count (TVC) during storage. 5 g of fish samples were homogenized with 45 mL of 0.1% peptone water in a sterile grinder. A ten-fold dilution was prepared, and 0.1 mL aliquots were spread on nutrient agar plates for microbial enumeration. After 48 hours of incubation at 37 °C, the total viable count (TVC) was calculated and reported as log CFU/g (Giarratana et al., 2016).

6.2.3.4. *Texture Analysis*

The texture parameters of the fish fillet with 3 cm × 3 cm in dimension were measured using texture analyzer TA-XD Plus (Stable microsystem, UK) with a 30 kg load cell. The TPA test involved a p/5 cylindrical probe to compress the fish fillets which were compressed twice to 30 % at a compression speed of 1.0 mm/s. The pre-test and post-test speeds were set at 5 mm/s and 10 mm/s respectively. For each sample in the same lot, six replicates were taken, and the average value for each parameter was recorded. The hardness, springiness, cohesiveness, and gumminess were calculated as per the method given by Wiles and Green (Wiles et al., 2004).

6.2.4. *Stability and reproducibility test of polyaniline label*

For the stability test of the polyaniline label, the labels were stored at different relative humidity (60 %, 70 %, and 85 %) and two different temperatures (ambient room temperature and refrigeration temperature). The optical image spectrum of the label was analyzed at a predetermined time point for 25 days with 5 days interval.

The reproducibility test of the polyaniline label was done by analyzing the optical image spectrum of the exposed label to 400 ppm ammonia vapor for 5 min using the developed smartphone sensor. The exposed label was kept in an acidic environment for 30 min for regeneration of the label through the doping of the deposited PANI label. The regenerated label was further exposed to the ammonia solution to find out the efficiency of the label for recycling. The same procedure was repeated 4-5 times after keeping the

same label in an acidic environment for 30 min to regenerate. Five sensors were used for the observation and the average was recorded (**Fig. 6.3**).

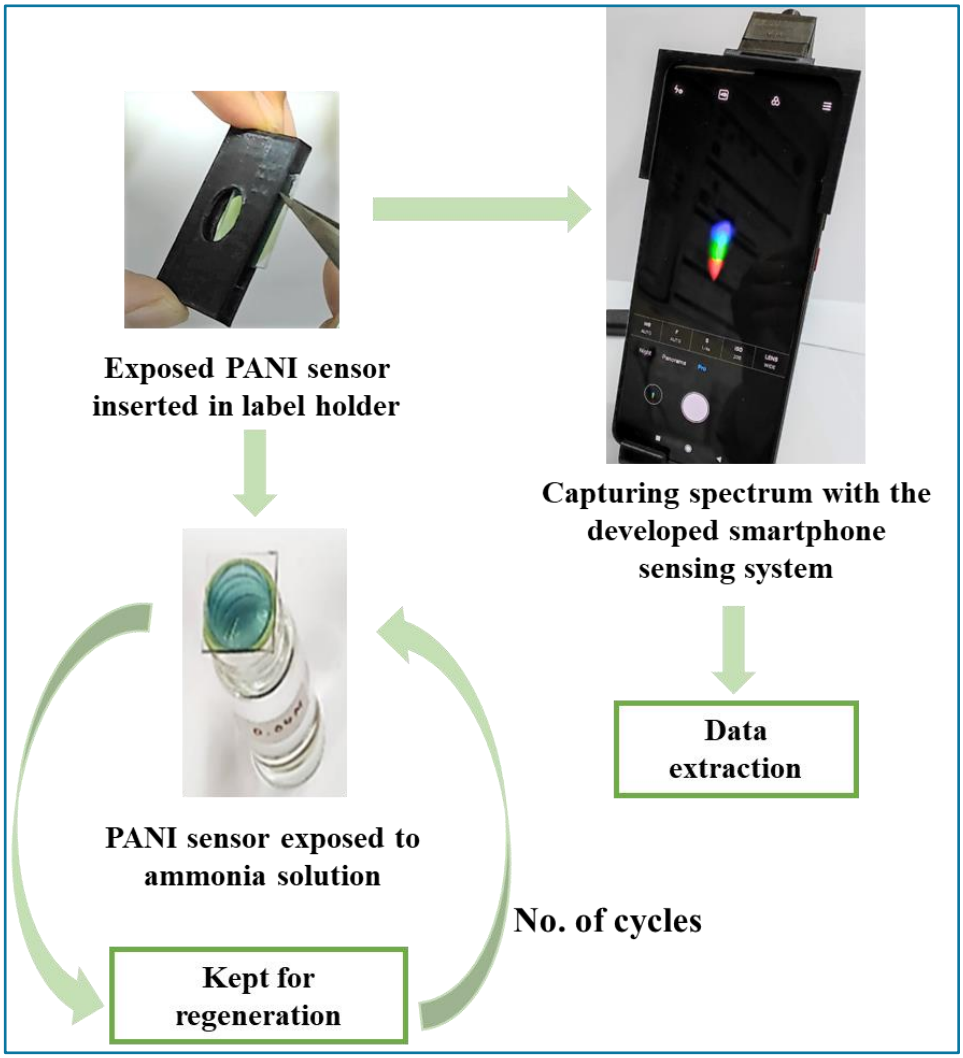


Figure 6.3. The PANI label regeneration process.

6.2.5. *Statistical analysis*

Statistical analyses for all experiments were performed using one-way analysis of variance (ANOVA) through Duncan's multiple range test ($p < 0.05$) in IBM SPSS Statistics 21 software for Windows. Results were presented as mean ± standard deviation (SD). Origin 2021 was employed for graph plotting. All experiments were conducted in triplicate (n=3).

6.3. Results and discussion

6.3.1. *Response of PANI label using smartphone sensor*

The PANI label showed response within 0-5 min at 549 nm when exposed to 400 ppm of ammonia solution (**Fig. 6.4**). This demonstrates the efficiency and sensitivity of the developed smartphone sensor equipped with the PANI label towards the ammonia concentration changes which are in corresponding to the response using UV-visible spectrophotometer (Chapter 3, section **3.3.2**.)

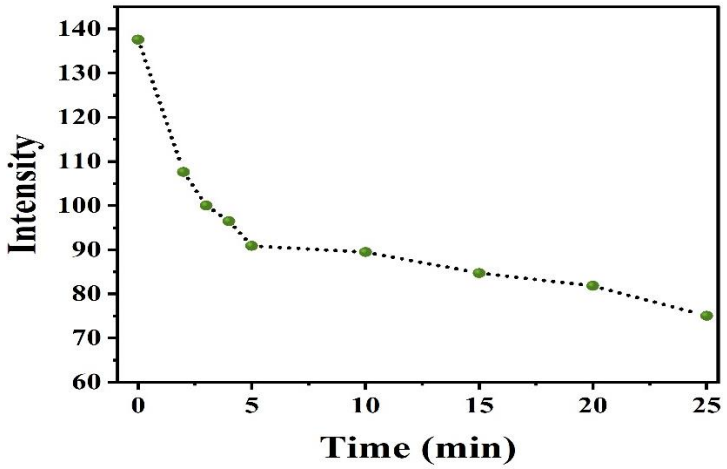


Figure 6.4. PANI label response using smartphone sensor.

6.3.2. *Performance evaluation of sensor using fish sample*

The freshness of the stored fish fillets of various varieties was tested. The exposed PANI label with stored fish fillets was removed from the lid and the spectrum of the label was recorded with the smartphone-based sensor to detect the degree of spoilage of fish. The retrieved spectrum was used to create a graph between wavelength and grayscale intensity and the percent intensity reduction was calculated at 549 nm. For the fish sample stored at ambient temperature, a noticeable intensity reduction was observed after 4 h of storage in rohu fillets whereas, in the case of mullet and common carp, it was noticed after 6h **(Fig. 6.5a)**. During the first few hours of storage, the percent intensity was lower. Between 4 and 8 h, it went up by 5 % in mullet and common carp fish fillets and by 10 % in rohu fish fillets. But, as the storage time goes on, the % decrease in intensity of all types of fish gradually increases.

In all the tested fish fillets no significant difference in the reduction in intensity could be noticed till 4 h of storage at room temperature. For fish fillets stored at refrigeration temperature the reduction in intensity was observed from the 3 days of storage for both rohu and common carp fish fillets. However, 25 % and 30 % intensity reduction were observed in rohu and common carp fillets, respectively, at 6 days and gradually increased during the storage, reaching 40-45 % at 15 days (**Fig 6.6a**). Since polyaniline reacts with ammonia and transforms into emeraldine base, the PANI base label was found to be specific for the detection of fish freshness. When aniline is oxidatively polymerized in an acid solution with a strong oxidant, it forms a green polymer (emeraldine salt) that could be changed to blue polymer (emeraldine base) when treated with ammonium hydroxide (base), which is regarded as the most applicable form of polyaniline. This property of polyaniline lends itself to use as a sensor to detect the level of TVB-N and to determine the degree of fish spoilage during storage (Kumar et al., 2017). We successfully developed a smartphone-based optical sensor to detect fish freshness or fish spoilage quantitatively. Rohu fish fillet was reported to be spoiled after 5-6 h, mullet, and common carp at 8 days of storage at ambient temperature and 6-7 days in both the varieties of fish at refrigeration temperature using the developed sensor. The obtained findings were further correlated with the conventional techniques.

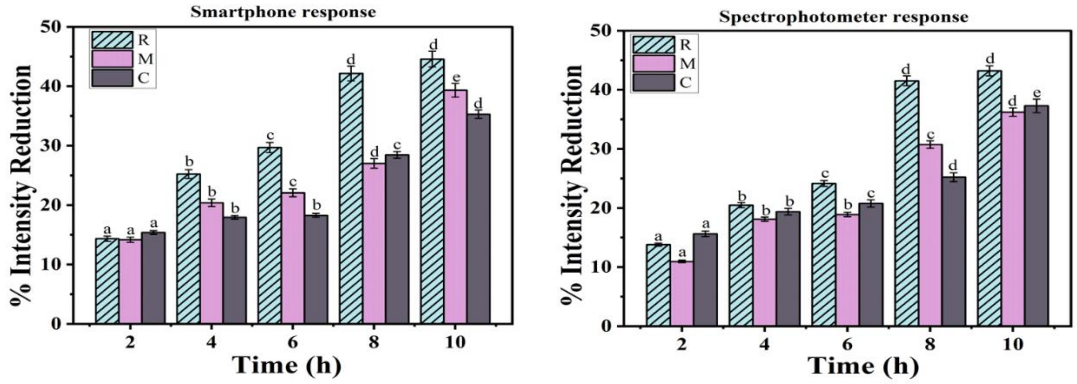
6.3.3. *Sensor validation*

6.3.3.1. *Using UV-Vis's spectrophotometer*

The PANI label was exposed to ammonia vapor as the method described in Chapter 3, section **3.2.4**. The maximum transmittance of the virgin PANI label without exposure to ammonia vapor, when scanned to a UV visible spectrophotometer from 400-800 nm, was found to be 530 nm, and a calibration curve was plotted against % transmittance and ammonia concentration. The calibration curve of the spectrophotometer response was discussed in Chapter 3, section **3.3.2**.

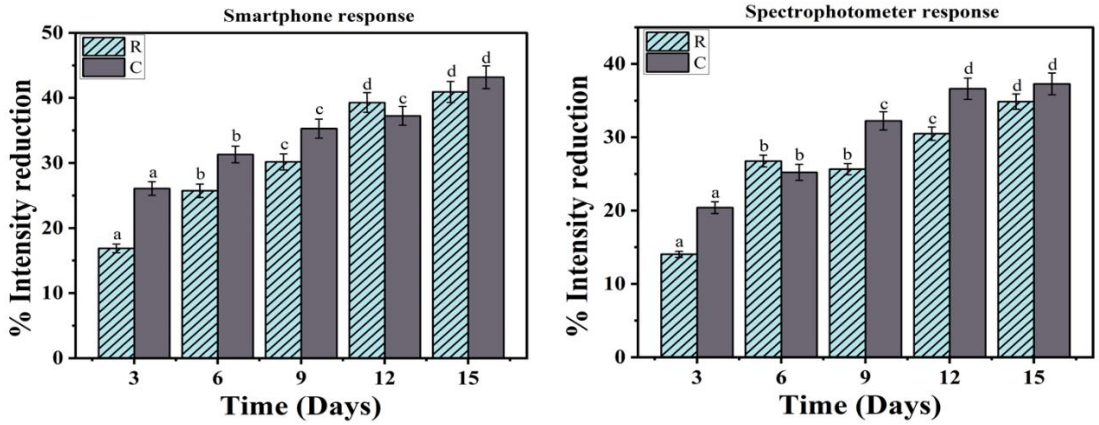
For the fish sample, as the exposure time of the PANI label increased, the shift in the spectra resulted in an intense transmittance reduction during storage which indicated the degree of spoilage in the fish during storage. From the information recorded using a smartphone sensor and spectrophotometer of the fish sample during storage at ambient and refrigeration temperatures, the ammonia concentration of the fish was estimated and

shown in **Tables 6.1** and **6.2**. The slight deviation in the values may be due to the in-built optical filter embedded in the CMOS camera sensor of the smartphone (Kalinowska et al., 2021). However, the resulting values obtained from the smartphone sensor and spectrophotometer are comparatively good for all the different varieties of fish showing that the ammonia concentration of the fish increases with time of storage. When the fish tissues are degraded by microorganisms, the odorless chemical compound trimethylamine oxide present in the fish is converted into trimethylamine or TMA and dimethylamine or DMA. At the same time, ammonia is produced by the decomposition of urea and amino acids by bacteria. These volatiles are collectively referred to as total volatile basic nitrogen (TVB-N) and their concentration is believed to be a good indicator of fish freshness (Wojnowski et al., 2017). It is clearly shown that as storage time progresses, the transmission peak at 530 reduces, and a sharp reduction is observed after 4-6 h **(Fig. 6.5b)** which also follows the intensity reduction of the smartphone sensor after 4 h **(Fig. 6.5a)**. From the estimated ammonia concentration, the degree of spoilage occurs after 4 h for rohu fish and after 6 h for mullet and common carp fish fillet (**Table 6.1)**. Variations in both the spectrophotometer and sensor were observed in the range of -9.97 to 9.66 % which indicated that the findings of the sensor were comparable with the findings of the spectrophotometer. Similar trends were observed in the fillets stored at refrigeration temperature when compared to the spectrophotometer response (**Fig. 6.6b**) and developed smartphone sensor (**Fig. 6.5b**). The difference in the degree of spoilage between the fish varieties may be due to its composition, habitat, temperature, and the condition of the fish during harvesting and post-harvesting which ultimately affect the freshness of the fish. A similar trend was detected in the relative correlation between the smartphone sensor and the spectrophotometer, showing that the PANI label created with the smartphone sensing system is adequate for determining fish spoilage.



(a) **(b)**

Figure 6.5. (a) Percent (%) intensity reduction of smartphone sensor response, and **(b)** Percent (%) intensity reduction of spectrophotometer response at ambient room temperature**.** Different letters indicate significant differences ($p < 0.05$) in % intensity reduction with storage time. (**Note: R:** Rohu fish fillet, **M:** Mullet fish fillet, **C:** Common carp fish fillet).



(a) **(b)**

Figure 6.6 (a) Percent (%) intensity reduction of smartphone sensor response, and **(b)** Percent (%) intensity reduction of spectrophotometer response at refrigeration temperature. **(Note: R:** Rohu fish fillet, **C:** Common carp fish fillet). Different letters indicate significant differences ($p < 0.05$) in % intensity reduction with storage time.

Table 6.1. Estimated ammonia concentration (ppm) of the rohu, mullet, and common carp fish stored at ambient temperature using the smartphone sensor and spectrophotometer

Sample	Time (h)	Ammonia concentration equivalent using smartphone (ppm)	Ammonia concentration equivalent using spectrophotometer (ppm)	% Deviation of smartphone response from spectrophotometer
Rohu	0	77.82± 0.45	85.84± 0.63	9.34
	2	205.42± 1.00	222.66± 1.50	7.74
	4	307.40± 0.40	289.00± 0.80	-6.37
	6	349.46± 1.70	325.40± 0.73	-7.39
	8	466.34± 1.47	497.74± 0.25	6.31
	10	489.13± 0.90	514.45± 0.97	4.92
Mullet	0	67.51± 1.70	62.97± 2.79	-7.21
	2	189.44± 0.24	174.08± 0.71	-8.82
	4	257.34± 0.81	246.73± 1.05	-4.30
	6	273.00± 0.15	254.57± 0.51	-7.24
	8	335.08± 0.60	374.94± 1.55	10.63
	10	433.86± 1.37	430.49± 0.36	-0.78
Common Carp	0	56.16± 0.23	52.29± 0.77	-7.40
	2	202.82± 0.89	212.54± 0.44	4.57
	4	227.05± 0.10	251.00± 2.51	9.54
	6	239.34± 0.19	265.30± 1.20	9.79
	8	327.28± 1.86	310.83± 1.19	-5.29
	10	434.47± 0.48	434.76± 1.48	0.07

Table 6.2. Estimated ammonia concentration (ppm) of the rohu and common carp fish stored at refrigeration temperature using the smartphone sensor and spectrophotometer

Sample	Time (Days)	Ammonia concentration equivalent using smartphone (ppm)	Ammonia concentration equivalent using spectrophotometer (ppm)	% Deviation of smartphone response from spectrophotometer
Rohu	0	83.79± 0.84	90.29± 0.52	7.20
	3	239.85± 1.42	234.76± 1.67	-2.17
	6	321.78± 2.60	365.69± 0.41	12.01
	9	362.93± 0.92	354.40± 1.35	-2.41
	12	447.35± 1.41	404.06± 0.76	-10.71
	15	462.44± 0.01	449.21± 0.65	-2.95
Common Carp	0	83.79± 0.18	90.29± 0.50	7.20
	3	325.02± 1.36	300.23± 0.12	-8.26
	6	373.48± 0.29	349.89± 0.89	-6.74
	9	410.23± 0.54	422.12± 1.14	2.82
	12	428.46± 0.94	467.27± 2.88	8.31
	15	483.40± 0.16	474.04± 0.31	-1.97

6.3.3.2. *Using TVB-N and pH*

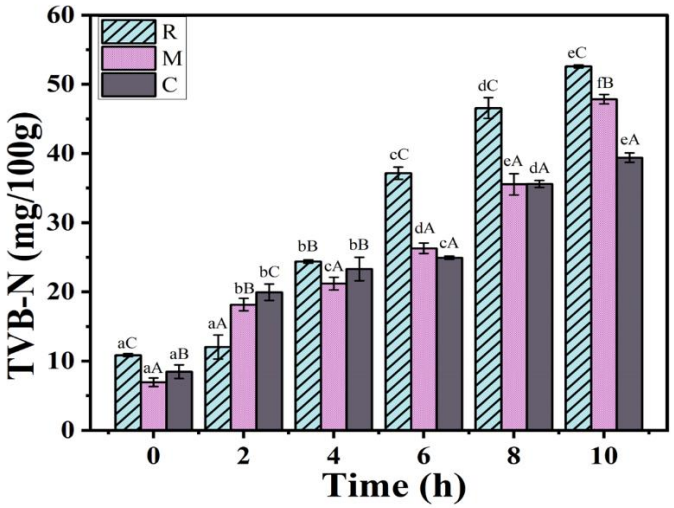
The TVB-N produced during fish degradation is alkaline so, a standard ammonia solution is used in terms of these volatiles' amines. The TVB-N levels in all the fish stored at ambient and refrigeration temperatures were measured and shown in **Fig. 6.7a** and **Fig. 6.8a.** For fish fillets stored at ambient temperature, the TVB-N value was observed to be below 25 mg/100 g during the initial period of storage (0-4 h) for all the tested fish. The TBV-N level of mullet and common carp fish were below 30 mg/100 g till 6h of storage and was found acceptable, however, the level of TVB-N of rohu fish crossed the recommended safe level (as per the European Union Regulation (EC) no. 2074/2005) at 6 h of storage. For fish fillets stored at refrigeration temperature, the TVB-N value was observed to be 28-29 mg/100 g at 3 days for all the tested fillets. However, the level was observed to be 35 mg/100 g at 6 days and increased during the storage (**Fig. 6.8a**). The TVB-N values increased significantly with the storage time and showed a close correlation with the developed smartphone sensor in both temperatures. A sharp intensity reduction after 4 h and 6 days of storage at ambient and refrigeration temperatures indicated the poor quality of rohu fish which is in line with the results of the TVB-N values where the acceptable range of TVB-N was observed till 4 h and 6 days of storage. As per the European Union Regulations fish having 35 mg/100 g TVB-N or above is considered as spoiled. The developed sensor is calibrated to 400 ppm, and the recovery of ammonia was in the range of 94 to 108 % therefore the developed sensor was found to be effective to meet the market demand effectively.

The relationship of pH value towards the freshness of fish stored at ambient and refrigeration temperatures has been shown in **Fig. 6.7b** and **Fig. 6.8b**. It has been found that the pH value of the fish sample in both temperatures was initially low and gradually increased during storage. The relation of pH with the degree of spoilage was briefly discussed in Chapter 3, section **3.3.4**. The rise in pH could be attributed to alkaline chemicals created by microbial action during fish deterioration, because of the increase in TVB-N. This evidence that the developed smartphone sensor is per the values of TVB-N and pH of the fish.

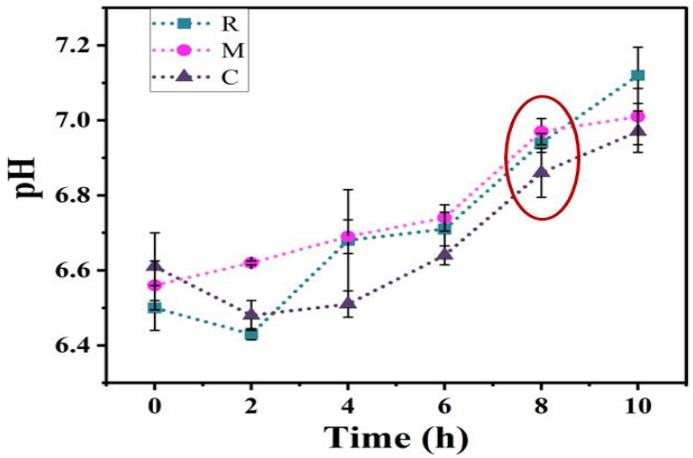
6.3.3.3. *Microbial analysis of stored fish*

The observed microbial population in the varieties of fish samples stored at ambient and refrigeration temperatures is shown in **Fig. 6.7c** and **Fig. 6.8c, d.** During the initial stage

of storage, there was no sign of microbial growth but, the TVC (total visible count) of spoilage organisms increased gradually through the storage time of all the varieties of fish reaching 6.6 log CFU/g, 7.3 log CFU/g, and 8 log CFU/g at 6 h, 8 h, and 10 h respectively for rohu fish. The microbial count of mullet and common carp fillet at 10 h was observed to be 7.4 log CFU/g and 7.2 log CFU/g respectively **(Fig. 6.7c)**. Fish is spoiled when the microbial count of specific spoilage organisms reaches the level of 10^6-10^7 CFU/g (Chun et al., 2014; Giarrata et al., 2016; Hao et al., 2021). The limit for the total aerobic count in fish and frozen fish is set at 10^7 CFU/g, as per the International Commission on Microbiological Specification for Food (ICMS) (ICMS, 1986) as discussed in Chapter 3, section **3.3.4**. In the present observation, the TVC was sharply increased to 10^7 CFU/g at 6 h and 6 days in rohu fish which is in parallel with the PANI label response obtained by the developed smartphone sensor. The response of the constructed smartphone sensor reflects the rapid intensity decline during storage at 4-6 h **(Fig. 6.5a)** for the selected varieties of fish. The *Pseudomonas* count was found to increase significantly during storage at refrigerated conditions (**Fig. 6.8d**). The changes in the PANI label during storage in all the varieties of fish fillets indicate that the concentration of volatile gases accumulated in the container's headspace, and the degree of spoilage was easily determined by the smartphone sensing device, with the resulting data coinciding with the microbial count of the fish samples during storage at room temperature. Chun et al. (2014) also reported that the microbial count of fresh fish is influenced by the time and storage temperature.



(a)



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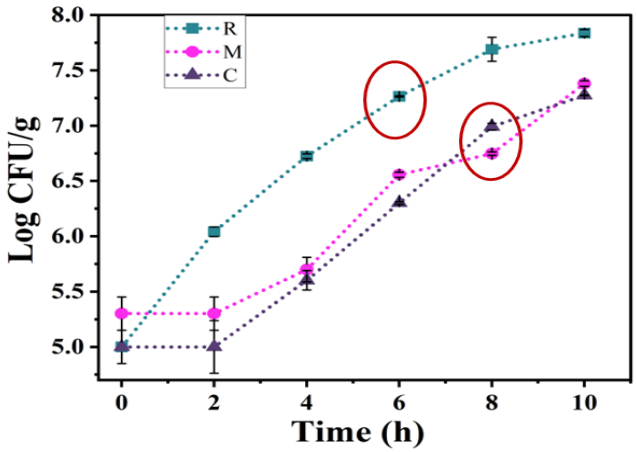


(c)

Figure 6.7. (a) TVB-N value (Different letters indicate significant differences ($p < 0.05$) in TVB-N of fish fillet during storage), **(b)** pH value, and **(c)** Total viable count (TVC)

of three different varieties of fish fillet stored at ambient temperature. (**Note: R:** Rohu fish fillet, **M:** Mullet fish fillet, **C:** Common carp fish fillet).

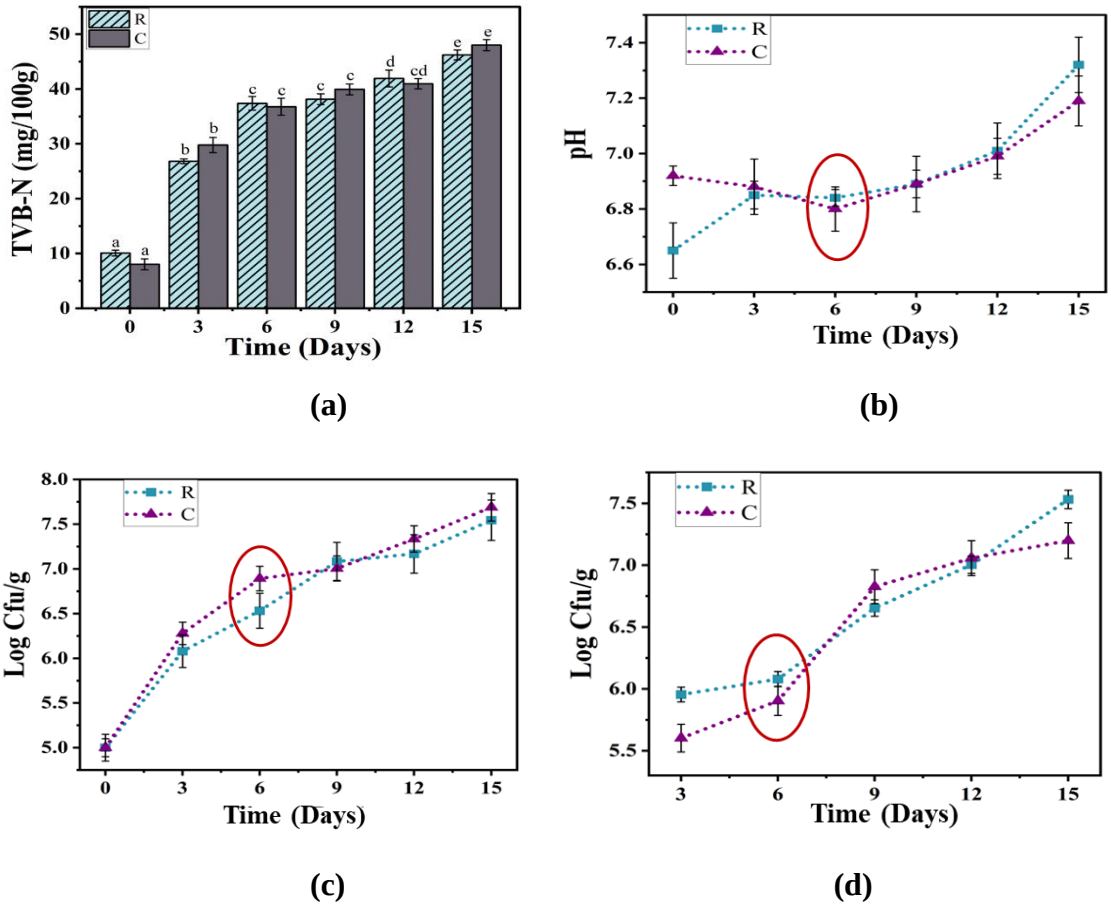


Figure 6.8. (a) TVB-N value (Different letters indicate significant differences ($p < 0.05$) in TVB-N of fish fillet during storage), **(b)** pH value, **(c)** Total viable count (TVC), and **(d)** *Pseudomonas* count of two different varieties of fish fillet stored at refrigeration temperature. (**Note: R:** Rohu fish fillet, **C:** Common carp fish fillet).

6.3.3.4. *Texture analysis*

The internal cross-linking of connective tissue and the detachment of fibers are important freshness quality attributes that depend on the texture and structure of fish muscle. The texture parameters of the three varieties of fish fillets during storage at ambient temperature are shown in **Fig. 6.9**. The hardness index of the fish fillet gradually decreases during 10 h of storage from 5.92 N to 2.51 N for rohu fish, from 9.38 N to 5.12 N for mullet fish, and 6.24 N to 3.75 N for common carp fish fillet **(Fig. 6.9 A)**. Overall, significant differences were observed in the hardness, springiness, cohesiveness, and

gumminess of the fish fillets as the storage period progressed **(Fig. 6.9**). A gradual decline in all the structure of the connective tissue greatly influences the textural properties of the fish (Cheng et al., 2014). Similarly, the texture parameters of two varieties of fish fillets stored at refrigeration temperature showed a gradual decrease over time (**Fig. 6.10**). For rohu fillets, the initial values for hardness, springiness, cohesiveness, and gumminess were 5.70 N, 0.93, 0.70, and 286.01, respectively. Raw data of few observation of texture analysis of fish are shown in **Annexure I.** After 15 days, these values decreased to 3.05 N for hardness, 0.76 for springiness, 0.24 for cohesiveness, and 110.70 for gumminess. The hardness index for common carp was 7.67 N initially and after 15 days of storage, this value decreased to 4.74 N. Fish at chilled storage has the potential to maintain its flavor, texture, and freshness due to low temperature to some extent. Yet, the quality of fish deteriorates with the rapid growth of psychrophilic microorganisms leading to off-odor and reduction in shelf life (Ding et al., 2013). Fish tissue is generally more perishable than other animal tissues, even when refrigerated. Bacteria that inhabit cold-blooded fish are adapted to the low temperatures, allowing them to thrive and continue growing under common refrigeration conditions. Fish muscle is very prone to becoming soft during postmortem conditions, which further affects the textural quality of fish. This causes variation in the textural parameters of the current study. The most significant component of fish muscle is collagen, which makes up between 3- 10 % of the protein and is crucial for preserving the integrity of the fillet and the cohesiveness of the muscle. Following the death of the fish, autolysis caused by collagenases and other proteases changes the collagen and disintegrates the muscle tissue, resulting in the release of total volatile basic nitrogen (TVB-N) (ICMS, 1986).

As a result, the texture profile of the current study reveals that the rate at which the muscles of fish fillets disintegrate begins from 0-6 h for storage at ambient room temperature and then gradually increases throughout storage in all the varieties of fish. After 6 h, the fish muscles completely disintegrated, which can be related to the TVB-N and pH values discussed in Chapter 4, section **4.7.2**. The degeneration of most of the fish protein was up to the desirable label until that time. The findings also support previous research that showed that increasing the pH of the fish reduces its hardness due to the depletion of fish muscle tissues by certain enzymes and microbes (Rhee et al., 1984; Jain

et al., 2007; Abbas et al., 2008). We successfully developed a web application using the findings of the present study to evaluate the degree of spoilage of fish.

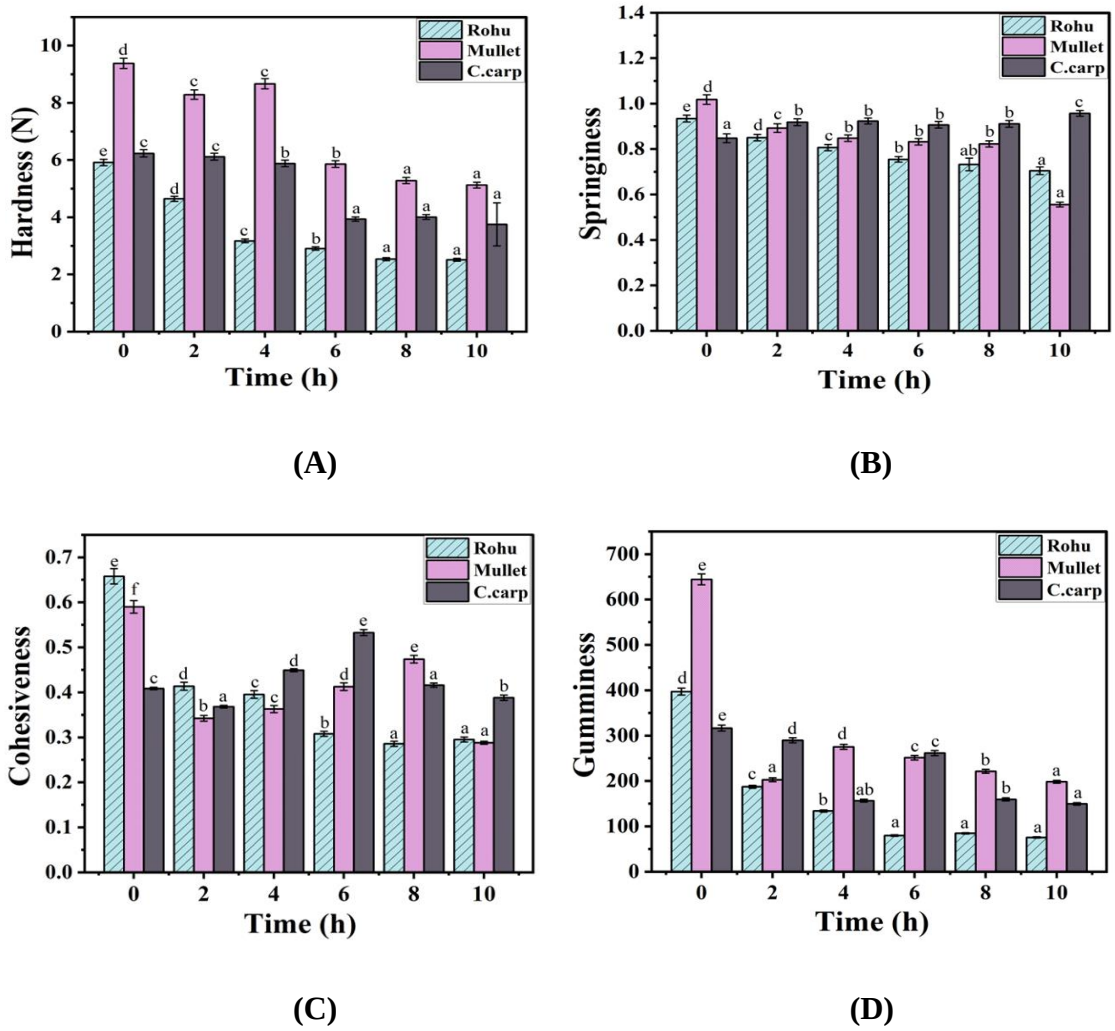


Figure 6.9 Texture profile analysis of **(A)** Hardness, **(B)** Springiness, **(C)** Cohesiveness, and **(D)** Gumminess of rohu, mullet, and common carp fish fillets during storage at ambient temperature. Different letters indicate significant differences ($p < 0.05$) in texture parameters during storage.

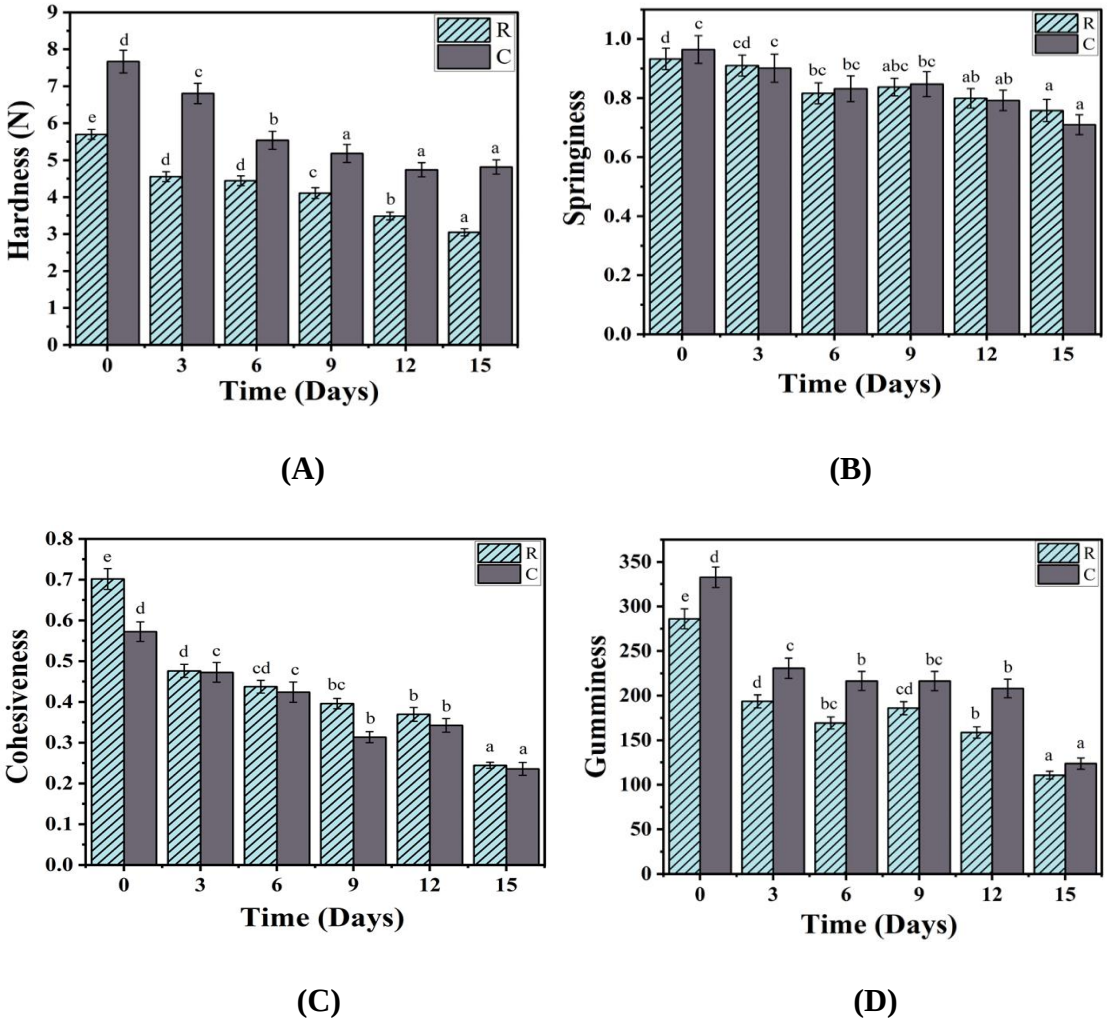


Figure 6.10 Texture profile analysis of **(A)** Hardness, **(B)** Springiness, **(C)** Cohesiveness, and **(D)** Gumminess of rohu and common carp fish fillets during storage at refrigeration temperature. Different letters indicate significant differences ($p < 0.05$) in texture parameters during storage. (**Note: R:** Rohu fish fillet, **C:** Common carp fish fillet).

6.3.4. *Stability and reproducibility test of polyaniline label*

Relative humidity (RH) above 75 % may affect the response of the sensor as it was observed that the intensity of the label was stable at 60 % and 70 % RH, however, significantly decreased at 85 % RH during storage **(Fig. 6.11)**. On the other hand, the temperature showed no significant effect on the intensity of the sensor. It was observed that the label could be used efficiently till three cycles without significant change in the intensity. However, after the third cycle, a drastic intensity reduction was observed during the fourth cycle of regeneration, which may be due to the morphological destruction of polyaniline nanoparticles during the repetition cycle, which affects its

ability to regenerate **(Fig. 6.12)**. A similar trend was observed in Wang's study, which found that quick restoration to the initial color at first three cycles demonstrated good reproducibility of PANI film (Wang et al., 2018).

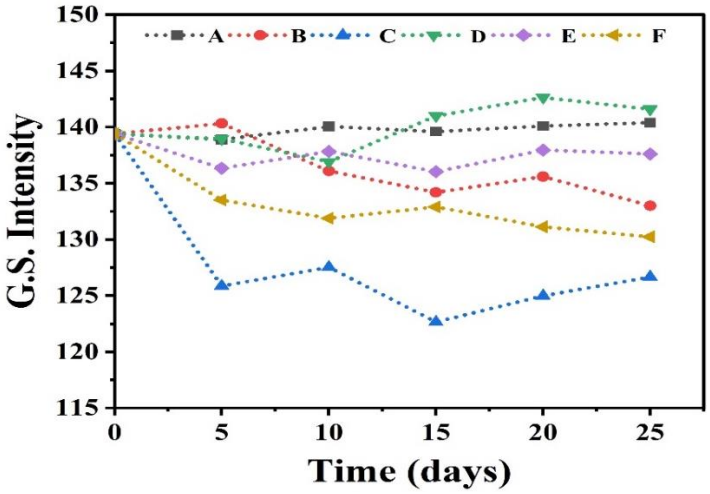


Figure 6.11. Response of PANI label stored at three different relative humidity (40- 60 %, 70 %, and 85 %) at room temperature (RT) and refrigeration temperature (RFT) (**Note: A**: 40-60 % RT; **B**: 70 % RT; **C**: 85 % RT; **D**: 40-60 % RFT; **E**: 70 % RFT; **F**: 85 % RFT)

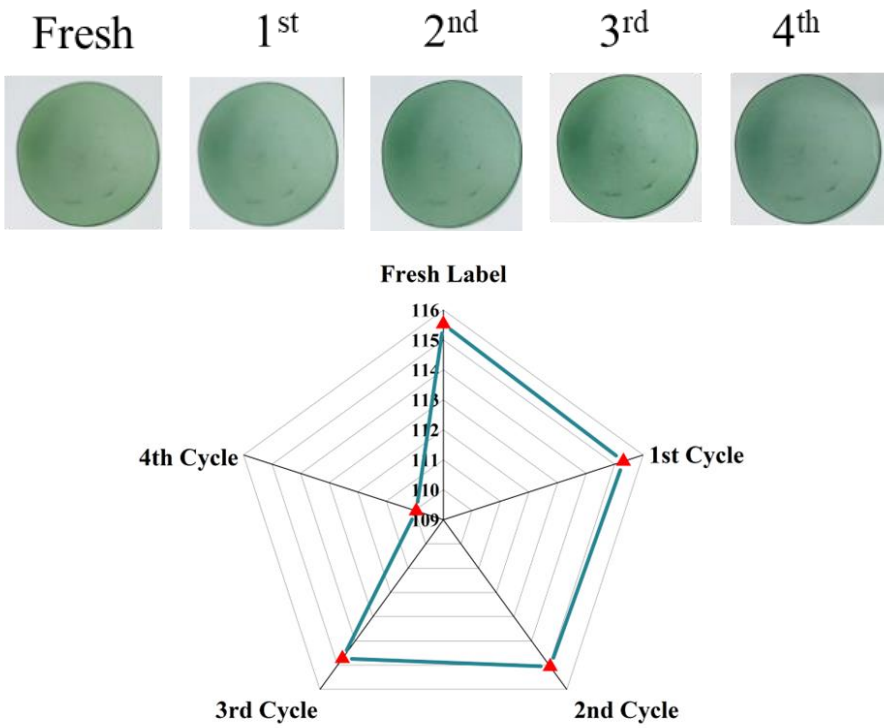
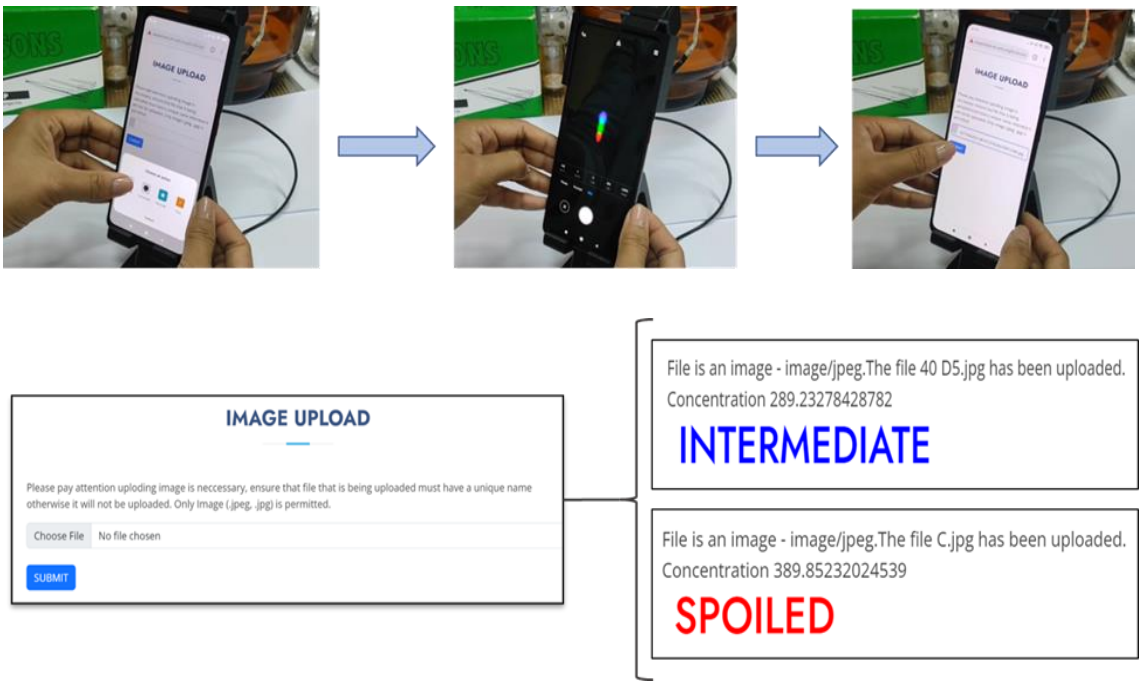


Figure 6.12. Regeneration study of PANI label.

6.4. Development of web application

A web application was developed based on the findings using the smartphone-based sensor to offer a user-friendly tool for assessing the food safety and quality of fish. To use the application, the user captures an image of the PANI label and accesses the weblink 'http://ampschool.in/webLengthCalculator/'. The captured image is then uploaded, and upon clicking the submit button, the application automatically processes the image. It provides a result indicating whether the fish is fresh, intermediate, or spoiled, along with a quantitative analysis showing the concentration of ammonia present (**Fig. 6.13**).



Web link: http://ampschool.in/webLengthCalculator/

Figure 6.13. Process of web application utilization.

6.5. Conclusion

A portable, cost-effective, user-friendly smartphone-based sensing device using a polyaniline (PANI) label that enables monitoring of the freshness of fish during storage was developed. The developed sensor was successfully calibrated and validated. The LOD, LOQ, % Bias, and % RSD of the sensor were 3.83 ppm, 12.96 ppm, 0.14 %, and 0.187 % respectively. Upon testing the sensor with freshwater fish fillets at different temperatures and validating its performance against a UV visible spectrophotometer for determining fish spoilage showed a deviation within -9.97 to 9.66 %. The sensor effectively detects the fish rejection threshold (10^7 CFU/g and 30 mg/100 g TVB-N value) during storage at both ambient and refrigeration temperatures. The findings of the sensor were compared and correlated with the findings of the spectrophotometer, TVB-N, pH, and microbial analysis. A strong correlation between the sensor and other conventional techniques was observed. The sensors showed stability within the range of 60-70 % relative humidity, and minimal impact from temperature changes. Regeneration capability was assessed through repeated cycles, revealing a slight decrease in intensity only after the fourth cycle. As communication and data are becoming important as a part of the Internet of Things (IoT), utilizing mobile phone technology for food quality can be an important tool. From the results & findings, a web application is developed successfully to evaluate the chemical spoilage of fish. The developed sensor represents an innovative and sensitive optical method via smartphones for detecting fish spoilage, which holds practical significance.

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