***Abstract***

**Design and Development of a Smartphone Sensing System for Determination of Freshness of Fish fillets During Storage**

## ABSTRACT

Fish is an important source of protein, providing essential nutrients and supporting livelihoods globally. However, its high perishability leads to postharvest losses that are 50-60 % higher than those of agricultural products. Globally, the estimated loss is 30-35 %, highlighting the need for early-stage spoilage detection in the fish supply chain. Physiological changes in fish, such as rigor mortis and enzyme activity, along with microbial growth, contribute to spoilage by releasing volatile amines like ammonia, dimethylamine (DMA), and trimethylamine (TMA). These changes ultimately affect the fish's texture, odor, and appearance. Assessing fish freshness is challenging due to its highly perishable nature, and current advanced techniques are often expensive, bulky, and time-consuming, making them impractical for fishermen, retailers, and buyers. To address these challenges and improve storage stability, the present research was formulated with five objectives:

Firstly, the polyaniline (PANI) was successfully synthesized and characterized for its functional properties using FTIR, XRD, and FESEM, confirming the presence of benzenoid and quinonoid groups at specific frequencies. The degree of crystallinity was found to be 31.42 %, indicating a microcrystalline nature. The PANI label was developed in a polystyrene sheet (20 mm ×20 mm), with a thickness of 0.25 mm, and exhibited a mixed morphology of granular and agglomerated nanofibrous formations, indicating a compact and interconnected structure. Its responsiveness to nitrogen-containing groups made it an excellent sensor for analyzing total volatile bases, important in assessing fish spoilage. When exposed to ammonia solutions of varying concentrations, the PANI label displayed a responsive peak at 530 nm within 0-5 min, showcasing its high sensitivity to changes in ammonia concentration. The resulting spectrophotometric response to different ammonia concentrations yielded a strong linear equation ($R^2$=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. The utility of the PANI label was further examined using the optical smartphone-based sensor.

Secondly, a dye-based label using bromocresol purple (BPD), a pH-sensitive dye, was developed for detecting fish spoilage. The 1 mL of dye deposition on an optically active polystyrene sheet displayed superior outcomes in reaction to ammonia vapor. The BPD label's response towards ammonia vapor shows a response peak at 629 nm with a response time of 0-1min. A linear spectrophotometric response was obtained when exposed to different concentrations of ammonia solution, resulting in a linear equation with a regression coefficient of 0.96. Despite its rapid response, the BPD label faced dye-leaching issues, compromising its efficiency, and making it unsuitable for detection purposes, therefore dye-based label was not further utilized for the development of the smartphone-based sensor.

Thirdly, for the development of a cost-effective, user-friendly smartphone-based sensor, an optical system, composed of five different parts (LED, pinhole, collimating lens, diffraction grating, and label holder, was designed using Google Sketch Up 2020 and ZW3D software, and the entire optical setup was 3D-printed using a biodegradable polymer (polylactic acid) to combine all the components. The optical device equipped with a developed label along with a smartphone (Redmi K20, Mi India) is used for further studies. The light emitted from the LED was directed by collimating the lens onto the label (that exposed to the sample) and grating, and then onto the rear camera of smartphone, set at 45° to capture the first-order diffracted output from the grating, illuminating the sensor. The image capture was analyzed and the spectrum was extracted using ImageJ software. While capturing the image the parameters of the camera were set at 100 ISO, its aperture at f/1.75 and the focal length of the camera was 4.77 mm. Before experimentation, wavelength calibration was performed using known red, green, and blue laser light wavelengths to align the smartphone camera sensor's pixel position with the spectrophotometer's wavelength. The smartphone sensor's laser light wavelength was determined to be 391.84 nm, offering a resolution of 0.21 nm per pixel within a range from 391.84 to 633.54 nm, utilizing approximately 1139 pixels. The wavelength calibration of the smartphone sensing system and the calibration of the PANI label with ammonia vapor (0-400 ppm) using the smartphone sensor which resulted in a linear equation with a regression coefficient of 0.97, affirmed the readiness of the smartphone sensing system equipped with the PANI label for experimental analysis with fish samples.

 

Fourthly, the performance of the smartphone-based sensor equipped with the PANI label was evaluated. The sensor exhibited a limit of detection of 3.83 ppm of ammonia with minimal bias (0.14 % Bias) and variation (1.87 % RSD). It achieved a recovery percentage of 94-108 % for standard ammonia solutions. Upon testing the sensor with freshwater fish fillets at different temperatures and validating its performance against the spectrophotometer determining fish spoilage showed a relatively low deviation range. 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. This data facilitated the development of a web application, allowing users to upload an image for rapid fish spoilage detection, providing results within seconds. 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. However, this reduction did not significantly affect the sensor's performance, showcasing its notable ability for regeneration.

Lastly, the essential oil from freshly ground pomelo peels was extracted using the hydro-distillation method, revealing a composition dominated by D-limonene, terpinyl acetate, α-pinene, β-pinene, and terpinolene among twelve major compounds when estimated by GC-MS. The extracted essential oil shows a potent antioxidant property, and high phenolic content, and exhibits significant antimicrobial properties against the various tested organisms. Fish fillets were treated with the extracted pomelo peel essential oil and stored at refrigeration temperature (4 °C±1 °C). Oil-treated fillets proved noticeably more effective than untreated ones throughout 15 days, demonstrating significant differences ($p \leq 0.05$) during storage. The developed sensor, when tested on oil-treated fish fillets at refrigeration temperature, displayed a successful correlation when validated against various fish spoilage determination methods. Additionally, it effectively identified the fish rejection threshold during storage, aligning with microbial population and TVB-N value benchmarks ($10^7$ CFU/g microbial population and 30 mg/100 g TVB-N value).