Chapter 8
Summary and Conclusion

8. Summary and Conclusion

Evaluation of fish freshness is challenging due to its perishable nature, and current advanced techniques are often expensive, bulky, and time-consuming, limiting their use to fishermen, retailers, and buyers.

Chapter 1 presents a brief introduction to the concept of fish spoilage alongside discussions on polyaniline and dye-based sensors, smartphone-based sensor applicability, and different methods used to detect fish spoilage, emphasizing the major limitations of existing destructive and non-destructive methods. Additionally, the chapter covers the effect of pomelo peel essential oil on storage stability and highlights the challenges associated with detecting fish spoilage while addressing the research gap and justifying the study's necessity.

Chapter 2 summarizes the literature review on 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. Also, recent technologies developed based on smartphones to detect fish spoilage are briefly discussed.

Chapter 3 findings revealed the successful synthesis of PANI, confirming the presence of benzenoid and quinonoid groups and highlighting its active characteristics within the 2300-2800 cm^{-1} range. The PANI exhibited a microcrystalline nature with 31.42 % crystallinity and a mixed morphology of granular and agglomerated nanofibrous structures. When exposed to ammonia solutions up to 400 ppm, the PANI label showed a responsive peak at 530 nm within 0 to 5 min., demonstrating high sensitivity. The spectrophotometric response produced a strong linear equation (regression coefficient 0.98), indicating reliability in detecting ammonia levels. The transmitted spectra during fish storage highlighted its potential for qualitative and quantitative analysis of fish spoilage, confirming the PANI label's utility as a leach-free sensor for monitoring volatile amines.

Chapter 4 focused on developing a sensor based on bromocresol purple dye (BPD). The deposition of 1 mL of dye showed superior results in reacting to ammonia vapor, leading to its selection for further analysis. The dye label had a rapid response time ranging from 0 to 1 min. Its spectrophotometric response to ammonia vapor yielded a linear equation with a regression coefficient of 0.96, indicating its potential for detecting volatile amines in fish spoilage. However, despite its quick response, the BPD label faced dye-leaching issues, compromising its efficiency and reliability. Consequently, the developed BPD label was deemed unsuitable for consistent detection purposes.

Chapter 5 outlines the development of a smartphone-based optical sensing system, designed with engineering drawing software and a 3D printer. The smartphone sensor's laser light had a wavelength of 391.84 nm, with a resolution of 0.21 nm per pixel over a range of 391.84 to 633.54 nm, covering approximately 1139 pixels. Calibration of the smartphone sensing system and the PANI label with ammonia vapor (0-400 ppm) produced a linear equation with a regression coefficient of 0.97. These results confirmed the system's readiness for experimental analysis of fish samples.

Chapter 6 focuses on the calibration and validation of the developed sensor for fish spoilage detection. The calibration process involved exposing the PANI sensor to various concentrations of standard ammonia solution, resulting in a linear equation that correlates grayscale intensity with ammonia concentration. This equation was applied to monitor volatile amines from fish samples during spoilage. The sensor demonstrated a detection limit of 3.83 ppm, with minimal bias (0.14 %) and variation (1.87 % RSD) and achieved a recovery rate of 94-108 % for standard ammonia solutions. Testing with freshwater fish fillets at different temperatures and validating the sensor against a spectrophotometer showed spoilage detection deviations within -9.97 to 9.66 %. The sensor effectively identified the fish rejection threshold (10^7 Cfu/g microbial population and 30 mg/100 g TVB-N value) at both ambient and refrigeration temperatures. This data supported the development of a web application for rapid fish spoilage detection, allowing users to upload an image and receive results within seconds. The sensor maintained stability at 60-70 % relative humidity and showed minimal impact from temperature changes. Its regeneration capability was tested through repeated cycles, showing only a slight decrease in intensity after the fourth cycle, which did not significantly affect performance, demonstrating the sensor's excellent regeneration ability.

In **Chapter 7**, the essential oil was successfully extracted from pomelo peels through hydro distillation, yielding 2.66 %. GC-MS analysis revealed a composition dominated by D-limonene, terpinyl acetate, α-pinene, β-pinene, and terpinolene among twelve major compounds. The extracted oil exhibited potent antioxidant properties, with a 65 % DPPH* scavenging activity and a high phenolic content (6.68 mg GAE/g), attributed to key constituents like limonene, pinene, and citral. The oil showed significant antimicrobial efficacy, particularly against *C. albicans* and *B. cereus*, with inhibition zones of 23 mm and 21 mm, respectively, and it outperformed gentamicin against *L. monocytogenes*. Moderate activity was also observed against *Yersinia pestis, E. coli, S. aureus, and P. aeruginosa.* In fish storage experiments, oil-treated fillets were significantly more effective in maintaining quality than untreated ones over 15 days, showing significant differences ($p \leq 0.05$). The developed sensor, when tested on these oil-treated fish fillets at refrigeration temperature, successfully correlated with various fish spoilage determination methods. It effectively identified the fish rejection threshold during storage, consistent with microbial population and TVB-N value benchmarks (10^7 CFU/g microbial population and 30 mg/100 g TVB-N value).

These objectives collectively emphasize the innovative sensor technology's practical application in detecting fish spoilage while presenting a promising natural preservative option in the essential oil extracted from pomelo peels.

Contribution to the knowledge

1. The developed sensor is helpful in the rapid determination of spoilage in varieties of fish, including fresh and frozen storage.
2. The developed sensor is also helpful in effectively detecting preservative-treated fish fillets.
3. The developed PANI-based smartphone sensor can determine the degree of spoilage of fish during storage within 3-5 min with a limit of detection at 3.89 ppm.
4. PANI label can be reutilized up to 4 cycles for detection without any significant change in the stability of the sensor.

5. It is cost-effective in comparison to previously developed sensors or instruments.
6. Determining and measuring the level of deterioration assures both the supply chain and consumers regarding food safety. Additionally, it serves as an alert for implementing preventive measures to minimize food loss.
7. It can also be used in field applications to monitor fish freshness and may reduce post-harvest loss.
8. There is a potential for its future utilization as a platform test to assess the acceptance or rejection of fish samples within the fisheries industry.
9. Both fishermen and the public can use the developed sensor without requiring specialized expertise. It will be beneficial in supplying fresh products to remote and challenging areas, particularly for defense personnel.
10. The sensor could be effectively utilized by all sectors in the fish supply chain which further boosts the economy and reduces food loss in fish industries.