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Application of curcumin-based pH-sensitive film for freshness assessment of Indian carp species

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Abstract
The present study explore the application of a curcumin-based pH-sensitive colourimetric film as an intelligent indicator for freshness assessment of Indian carps species. The film was developed by incorporating curcumin, a natural polyphenolic pigment into biodegradable polymer matrix of sodium alginate, enabling visible colour transitions in response to pH changes associated with fish spoilage. Fresh Indian carps Rohu (*Labeo rohita*), and Mrigal (*Cirrhinus mrigala*) were stored under controlled conditions, and periodic analysis of physicochemical and microbial parameter, including pH, total volatile basic nitrogen (TVBN), and total plate count were conducted. Simultaneously, the colour response of the developed film was monitored and correlated with spoilage indicators. The film exhibited a distinct colour change from yellow to orange-red with increasing pH, corresponding to the accumulation of basic volatile compounds during storage. A strong correlation was observed between film colour variation and convectional freshness indices, confirming its reliability as real-time freshness indicators. The results demonstrate that the curcumin-based film is a cost effective, eco-friendly, and non-destructive tool for monitoring fish freshness. This study highlights its potential application in intelligent food packaging system to enhance quality control and consumer safety in the seafood industry.

Keywords: Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), fish, spoilage indicators, Indian carps

Introduction

Fish and fisheries products are well known for being extremely nutritious diets, due to their abundance of high-quality proteins, necessary amino acids, omega-3 fatty acids, vitamins, and minerals. Major Indian carps, such as rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (*Cirrhinus mrigala*), are among the freshwater aquaculture species that greatly contribute to fish production and consumption in India (Manam & Quraishi, 2024) [16]. However, these species are extremely vulnerable to quick spoiling during post-harvest handling, refrigerated storage, and shipping because to their high moisture content, almost neutral pH, and protein-rich muscle matrix.

Fish muscle deterioration is a complicated biochemical and microbiological process that includes microbial growth, lipid oxidation, and autolytic enzyme activity. Ammonia, trimethylamine (TMA), and dimethylamine (DMA) are among the volatile basic nitrogenous chemicals that are created when endogenous enzymes and spoilage microbes break down muscle proteins and amino acids during storage. These substances are commonly recognized as trustworthy markers of fish freshness and spoiling state and are measured collectively as total volatile basic nitrogen (TVB-N). According to recent research, a decline in the smell, texture, and sensory quality of packaged fish products is closely linked to an increase in TVB-N (Devarayan *et al.*, 2024) [8].

Traditional techniques for assessing fish freshness, like TVB-N estimation, total plate count, pH measurement, and sensory evaluation, are useful but frequently destructive, timeconsuming, labour-intensive, and laboratory-specific, which restricts their usefulness for realtime monitoring in commercial supply chains. Intelligent packaging technologies, especially colourimetric freshness indicators, have drawn a lot of attention recently as quick and nondestructive methods for monitoring aquatic product degradation. These methods allow for direct freshness evaluation without opening the packaging by responding to volatile spoilage metabolites generated during storage with observable colour changes (Zhang *et al.*, 2024) [13].

The principle of halochromism, which describes how p H-sensitive dyes or natural colours react to p H changes brought on by volatile amines released during fish spoiling, is typically how colourimetric films work. These films have been successfully used in aquatic products in a number of recent research. For instance, Devarayan *et al*. (2024) [8] created a cellulose-based halochromic sensor that demonstrated distinct colour shifts in packed fish during chilled storage that corresponded to TVB-N levels, p H variations, and microbial development. In a similar vein, Li *et al*. (2024) [13] found that using observable ΔE changes, natural pigment-based colourimetric indicators successfully tracked freshness variations in carp and other aquatic goods. More recently, hydrogel and cellulose-based visual indicators have been successfully applied for real-time freshness tracking in fish using natural pigments and nanocomposite systems (Wang *et al*., 2024) [29].

Even though intelligent freshness indicators for aquatic products have advanced significantly, there are still few studies that particularly address the use of colourimetric films in major Indian carps, despite their commercial significance in India. There is a glaring research deficit for Indian freshwater muscle meals because the majority of recent studies have concentrated on marine fish, salmon, seer fish, and generic carp species. Thus, the current study intends to examine the use of a colourimetric film sensor for real-time freshness and spoilage detection in major Indian carps during refrigerated storage and to correlate its visual response with traditional spoilage indicators like microbial load, pH, and TVB-N.

Materials and methods

Fresh Rohu and Mrigal fish samples were procured from a local fish market at Amravati and transported to the laboratory under iced conditions. The fish were washed, eviscerated, and cut into uniform fillet portions of approximately 100–150 g.

Development of colourimetric sensor

The pH indicating dyes that curcumin (Cu) was taken and mixed in ethanol solvent for solubilisation of natural dye curcumin. Separately sodium alginate (SA) was dispersed in distilled water to prepare the film-forming solution. The curcumin solution was then incorporated into the sodium alginate mixture and heated under continuous magnetic stirring to obtain homogenous mixture. Glycerol was added as a plasticizer and then mixture was stirred. The resulting sodium alginate – curcumin solution was poured on onto non-woven sheet. The coated non-woven sheet exhibited improved water resistance and was dried in a hot air oven until a stable film was formed (Tupuna-Yerovi *et al*.,2025) [28]

Preparations of the fish Samples and Storage Conditions

The fish were divided into sets. The set of samples was removed from the refrigerator every day until day 10 of storage time and analyzed for a chiller condition (4°C). The fish were prepared for analysis at each time point, up to day 10. Packing was realized in similar conditions for all sets of samples.

Application of Colourimetric Film

The colourimetric film was fixed to the inner side of the package headspace without direct contact with the fish fillets. This application strategy is consistent with reported intelligent packaging studies in carp and fish fillets. Each sample was packed in sterile PET trays and sealed. As on-package labels, all colourimetric films having size 2x5 cm were placed inside PET tray having size 6.3 x 4 inches having capacity 500 gm. into packaging material without contacting the packaged to fish samples, responding to the increasing of volatile amine released by fish. Two sample were examine for freshness indication ie. Labio rohita, *Cirrhinus mrigala*, stored at ambient temperature and refrigeration temperature. Examination was carried out for next day for refrigerated samples. Variation in colour changes as noted. Colour changing behaviour of a film observed visually and recorded with help of CIALAB hunter colourimeter recorded the change in colour of film.

Freshness Evaluation of fish

To date, sensory evaluation is the most important method for freshness evaluation of fishery products. Other approaches based on microbial methods (total viable count, TVC); physical measurements (e.g., odour, texture, and colour); and chemical methods (e.g., K and K1 values, total volatile basic nitrogen (TVBN), lipid oxidation) have also been described by Olafsdottir *et al*. (1997) [20] in an excellent review of these methods.

Detection of Biogenic amines in fish samples

The commonly employed approach for assessing the TVBN is the microdiffusion method‖ as outlined by Conway and Byrne in 1933.The assessment of total volatile nitrogenous bases is commonly employed in routine analysis to evaluate the extent of spoilage in fish samples. L. Rohita fish, C. Mrigala fish, samples were subjected to treatment with a strong alkali, followed by the distillation or diffusion of the volatile base into standard acid or boric acid. The initial step involves the separation of volatile bases through the precipitation of proteins utilizing a trichloroacetic acid (TCA) solution. The filtered solution, which contains the volatile substances, is treated with a strong alkali. The released ammonia is captured by the standard H2SO4 solution, and the amount absorbed is assessed by titrating the leftover acid with the standard NaOH.

Calculation

Calculate VBN content using the formula

$$\text{VBN (mg \%)} = \frac{14 \times N \times (x - y) \times 100 \times 100}{S}$$

- N = Normality of H_2SO_4;
- x = ml of H_2SO_4 required for titration of samples;
- y = ml of H_2SO_4 required for blank; and
- S = weight of sample taken.

pH Measurement of fish sample

The p H was assessed by evaluating the electrode potential between glass and reference electrodes, with the p H meter calibrated using standard p H buffers. The p H levels of fish samples (L. Rohita, C. Caprio) were assessed using homogenized samples diluted in distilled water at a ratio of 1:10 (w:v) with a p H meter. Immerse or embed the electrode, ensuring sufficient contact between the probe and the sample. Observe the meter reading when it remains consistent (Lim, P. Y., 1987) [14]. The p H meter underwent periodic calibration following the manufacturer's guidelines, utilizing buffer solutions of p H 7.0 and p H 4.0, and was adjusted in accordance with the temperature of the samples.

Microbial analysis of fish

For microbial enumeration, 0.1 ml samples from serial dilutions (1:10, diluent, 0.1% peptonewater) of homogenates (L. Rohita, C. Caprio) were spread on the surface of dry media, with the exception of the 1:10 dilution, where 1 ml of the sample was utilized. Decimal dilutions of the samples were prepared up to 108 cfu/ml, followed by the execution of microbiological inoculations. The petri plates inoculated on the same medium were incubated at 37 ºC on the Plate Count Agar (Oxoid® CM463) for a duration of 24-48 hours to assess total plate count and microorganism count.

After incubation, plates with 30–300 colonies were selected for counting. Colonies were counted using a digital colony counter.

Results were calculated and expressed as log CFU/g of fish tissue using the formula:

$$\mathrm{CFU/g} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated}}$$

Chromatic freshness index of colourimetric curcumin alginate film

Amount of TVBN produced and pH of fish and meat sample correlates with microbial analysis results of fish and meat sample. Also all these values are correlates with the change in colour of SA/Cu film. Among all the result of above it will be decided that chromatic index of colourimetric film which can be used for future study of muscle food and marker will set for freshness index of muscle food.

Results and Discussion

Detection of Biogenic amines of Fish

As trustworthy chemical markers of microbial deterioration and protein degradation, the identification of biogenic amines in muscle meals is extremely important.. Elevated levels of biogenic amines are an indication of deterioration and pose serious health risks, such as foodborne infections, headaches, and allergic reactions.

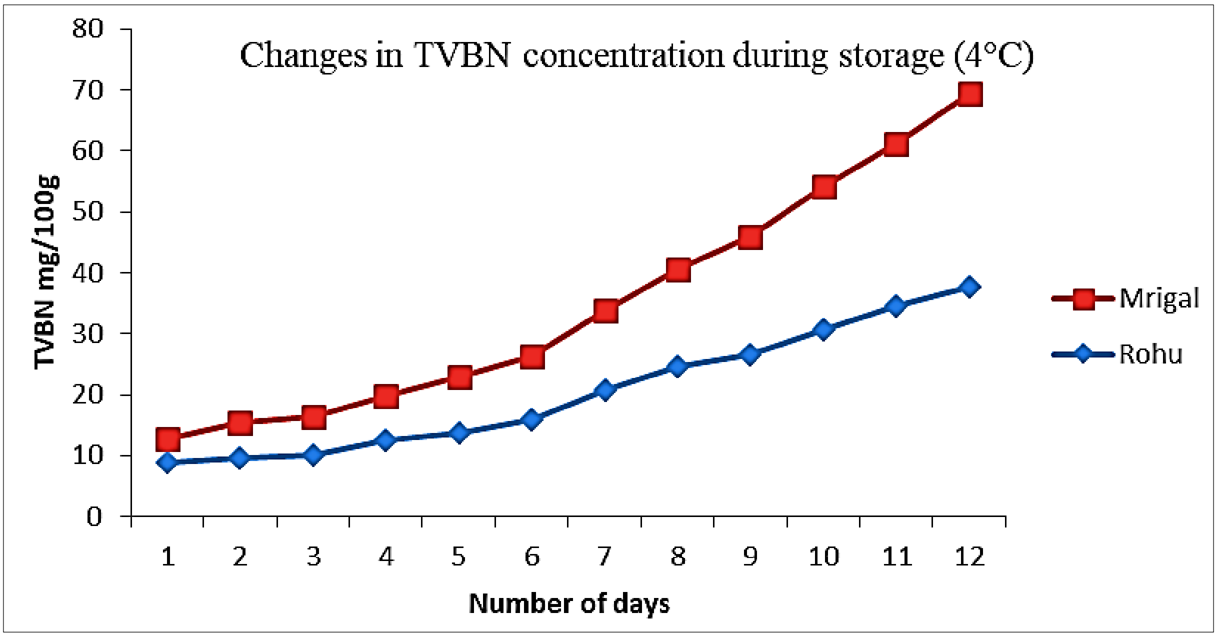


Fig 1: Change in TVBN concentration during storage at refrigerated temperature of Indian major carps Rohu, Mrigal

The Rohu sample demonstrated the most significant rise in TVBN concentration, in Table no. 4.9 shows that increasing from 9.4 mg/100g on day 0 to 21.2 mg/100g by day 15. Mrigal began with the lowest initial concentration of 6.5 mg/100g, but demonstrated a steady increase, achieving 18.7 mg/100g by day 15. Comparable findings were reported by Chakrabarti (1984) [4] in Indian major carps, Magnússon and Martlnsdóttlr *et al*. (1995) [15] in cod and perch fillets, Sarma *et al*. (1998) [26] in pink perch and oil sardine, Raju *et al*. (1998) [24] and Asgharzadeh *et al*. (2010) [3] in silver carp, and Orak and Kayisoglu (2008) [21] in *Gadus euxinus*, *Mugil cephalus*, and Encrasicholus.. Mrigal fish, the second largest Indian carp, showed a steady rise in spoiling, starting at the lowest concentration and progressing more slowly than Rohu. Ababouch *et al*. (1996) [1], Köse and Erdem (2004) [12], Ola and Oladipo (2004) [19], and Adoga *et al*. (2010) [2] all reported similar results in their investigations on anchovies. This study shows a relationship between bacterial proliferation and TVB-N concentration. In particular, TVB-N levels increase with decreasing temperatures because lower temperatures prevent the growth of bacteria.

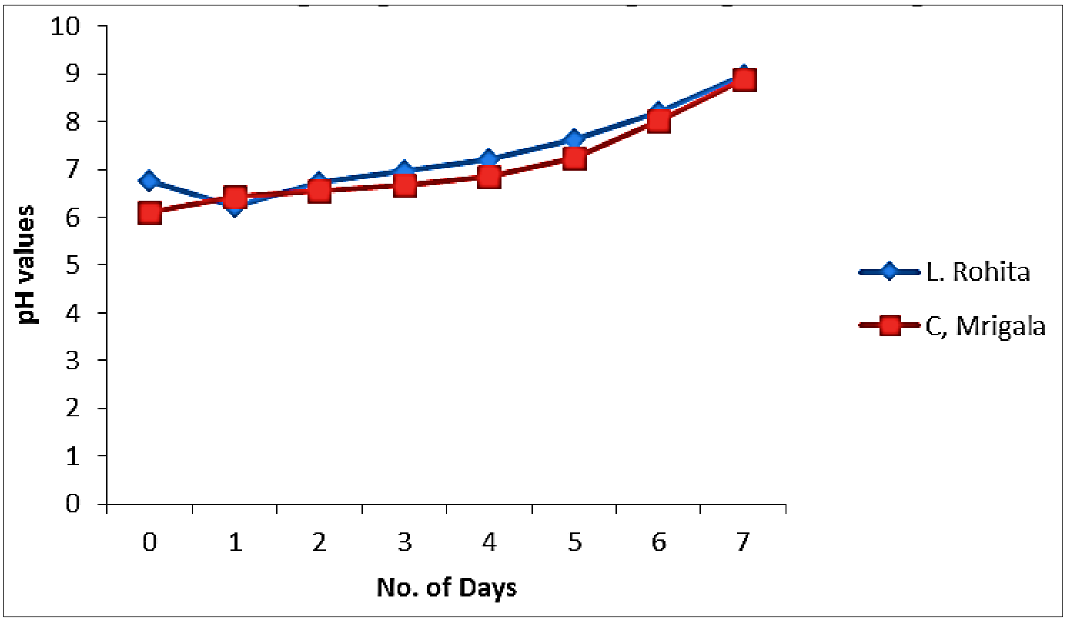


Fig 2: Change in pH during refrigeration temperature in Rohu and Mrigal

The p H variations of *Cirrhinus mrigala*, and *Labeo rohita* and chicken over a seven-day period under refrigeration are depicted in the graph above. L. rohita: The p H is roughly 6.8 on day 0. Table No. 4.14 shows a small decline on day 1 (approximately 6.2), followed by a consistent rise to roughly 8.0 by day 7. The p H of C. mrigala begins at about 6.2 and gradually rises over the course of seven days, reaching about 7.5 every day. Seven. Meat: starts out at a lower p H of about 5.5 and gradually rises to about 6.7 over time. 7. The early reductions in p H may correlate with lactic acid production via anaerobic glycolysis and the release of inorganic phosphate from ATP degradation. Conversely, the subsequent increases in p H during later storage stages could be linked to the buildup of basic compounds, including ammonia and trimethylamine, arising from autolytic and microbial processes (Delbarre-Ladrat *et al.*, 2006) [7]. Múgica, *et al.*, (2008) [18] found that the p H of superchilled (1.5 °C) and iced whole rays rose over a 10-day storage period. L. rohita has the highest final p H, which may indicate a larger microbial load or a faster rate of spoiling. Under the same circumstances, C. mrigala exhibits a slight growth, indicating comparatively better stability or slower spoiling. Low initial p H may associated with higher stress before slaughtering. This was caused by depletion of energy reserves, mainly glycogen. Low p H also promotes of oxidation of myoglobin and p H (Ozogul, 2010) [22].

Microbial analysis of Fish

Understanding the proliferation of biogenic amine-producing micro flora during storage required the use of specific microbiological techniques to examine fish flesh, including total plate count (CFU/g). Fish freshness deterioration, biogenic amine accumulation, and microbial proliferation are all directly correlated when their population dynamics are monitored. Because it verifies that the observed chemical or colourimetric changes are microbiologically driven, this analysis was essential for validating spoilage indicators and intelligent sensors, guaranteeing a trustworthy evaluation of the safety, quality, and shelf life of fish.

Table 1: Microbial analysis of Indian major carps stored at 4 °C

No. of Days	Rohu			Mrigal		
	TPC	Pseudomonas	Enterobacteriaceae	TPC	Pseudomonas	Enterobacteriaceae
1	10.70x 10^2	1.42x 10^1	1.68 x 10^1	7.70 x 10^2	Est ≤ 1	2.41 x 10^1
2	10. 72x 10^2	1.46 x 10^1	1.69 x 10^1	7.79 x 10^2	Est ≤ 1	2.68 x 10^1
3	10. 72 x 10^2	1.49 x 10^1	1.77 x 10^1	7. 82 x 10^2	Est ≤ 1	2.82 x 10^1
4	10. 73 x 10^2	1.50 x 10^1	1.82 x 10^1	7. 88x 10^2	Est ≤ 1	3.12 x 10^1
5	10. 72 x 10^2	1.59 x 10^1	1.97 x 10^1	8.06x 10^2	0.21 x 10^1	3.48 x 10^1
6	10. 79 x 10^2	1.68 x 10^1	2.11 x 10^1	8.36x 10^2	0.43 x 10^1	3.59 x 10^1
7	11.03 x 10^2	1.82 x 10^1	2.23 x 10^1	8.73x 10^2	0.65 x 10^1	3.67 x 10^1
8	11.10 x 10^2	2.21 x 10^1	2.35 x 10^1	8.71x 10^2	0.94 x 10^1	3.77 x 10^1
9	10. 63x 10^2	2.40 x 10^1	2.43 x 10^1	9. 42 x 10^2	1.2 3 x 10^1	3.80 x 10^1
10	9. 85x 10^2	2.51 x 10^1	2.67 x 10^1	9. 22x 10^2	1.72 x 10^1	3.93 x 10^1

Fish samples' microbial presence can be broadly gauged using the Total Plate Count (TPC). Table No. 1 showed that the TPC was 10.70 × 102 cfu/g on Day 1; it increased slightly to a peak of 11.10 × 102 cfu/g by Day 8, but then decreased to 9.85 × 102 cfu/g on Day 10. The TPC of L. rohita meat in ice significantly increased after ten days of storage. TPC levels in native carps increase after 12 days of ice storage, according to Mehta and Shamasundra (2015). Species of pseudomonas. Over the course of the 10-day storage period, the number of Pseudomonas increased steadily, beginning at 1.42 × 101 cfu/g and peaking at 2.51 × 101 cfu/g by Day 10. From 1.68 × 101 cfu/g on Day 1 to 2.67 × 101 cfu/g by Day 10, the Enterobacteriaceae count showed a steady increase. Over the course of the 24-day storage period, Ozogul *et al*. (2011) [23] reported a significant statistical increase in the total viable counts of entire gutted common sole (*Solea solea*). Gandotra *et al*. (2016) [10] found in a related investigation that *Cyprinus carpio* raw samples kept at -120°C,, the total plate count rose from 1.10 ± 0.04 log cfu/g to 12.41±0.17 log cfu/g.

The total plate count (TPC), of Mrigal fish which increased steadily from 7.70 × 102 cfu/g on Day 1 to 9.22 × 102 cfu/g on Day 10. In the tests using un-gutted silver carp (*Hypophthalmichthys molitrix*), comparable outcomes were obtained. Ehsani A. *et al*. (2012) [9] state that Pseudomonas species were not detected (estimated at ≤1 cfu/g) until Day 5, at which point their concentrations significantly increased to 1.72x101 cfu/g by Day 10. Huang *et al*. (2022) [11] identified Pseudomonas as one of the dominant spoilage bacteria during chilled storage of silver carp products. The constant detection of Enterobacteriaceae revealed a rise from 2.41 × 101 to 3.93 × 101 cfu/g.

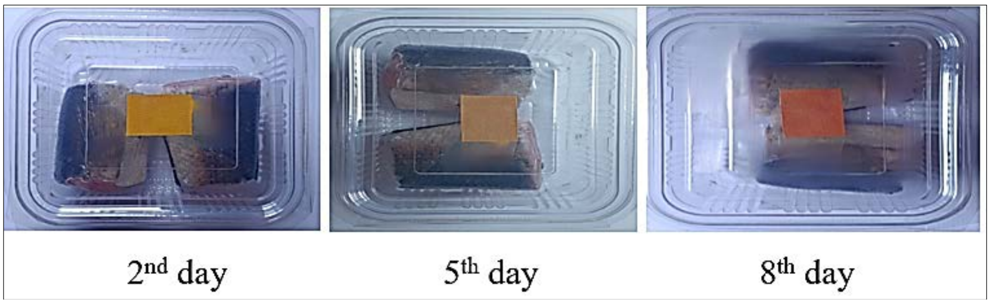
A colourimetric sensor's ability to provide quick, non-invasive, and on-going deterioration assessment made its use as a freshness indicator for muscle meals interesting. The visual reaction makes it possible to distinguish between fresh and ruined states since it is directly related to microbial growth, elevated pH levels, and the build-up of total volatile basic nitrogen (TVBN).

Table 2: Colour analysis of Indian major carps Rohu, Mrigal on application of SA / Cu film

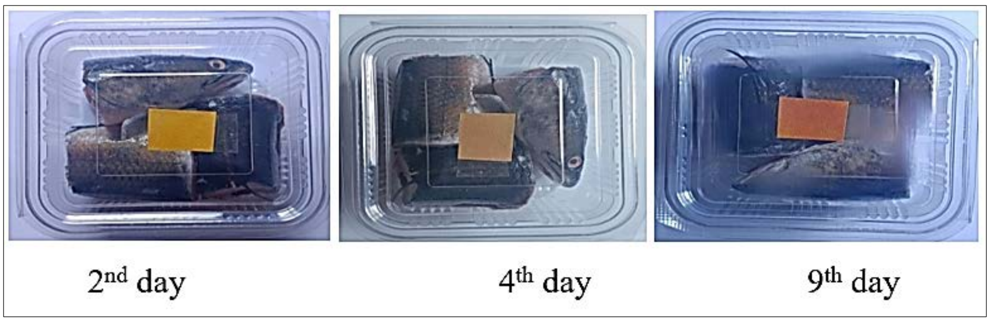
Sample type	Period	Colour value		
		L*	**a***	**b***
Labio Rohita	0th day	65.47 ±0.07	22.61 ±0.03	60.51 ±0.04
	4th day	37.35±0.02	23.84±0.07	36.12±0.06
	7th day	33.57±0.02	32.77±0.03	24.30±0.04
C. Mrigala	0th day	65.47 ±0.07	22.61 ±0.03	60.51 ±0.04
	4th day	56.41±0.07	23.04±0.04	41.18±0.05
	9th day	27.67±0.07	30.87±0.05	23.04±0.07

Table no. 2 indicates that on the first day, L. rohita showed a high degree of lightness (L ast = 65.47), along with moderate redness (mathsf a = 22.61) and significant yellowness (b = 60.51), indicating a fresh condition. But by the fourth day, L had significantly decreased to 37.35, indicating significant darkening. This finding is consistent with the oxidative discolouration and deterioration of muscle pigment observed in fish kept in cold storage. as detailed by Sivertsen *et al*. (2021) [27]. Furthermore, the increase in the mathtt a value (23.84) suggests a minor development of red hues, potentially attributable to the formation of met myoglobin and biochemical alterations associated with microbial proliferation. As evidenced by more darkening (L = 33.57), a notable rise in mathtt a (32.77), and a noticeable drop in mathfrak b (24.30) by the seventh day, spoiling was obvious.

The sensor correctly identified L. rohita spoiling. On the first day, fresh C. mrigala showed comparable baseline values (L ast = 65.47). Lightness dropped to 56.41 on the fourth day indicating initial quality degradation, though at a slower rate compared to L. rohita. The start of lipid oxidation and microbial multiplication is indicated by the rise in mathtt a (23.04) and fall in mathfrak b (41.18). L showed a significant drop (27.67) on the ninth day, indicating noticeable discolouration. Advanced spoiling and a strong interaction between basic volatile nitrogen and the curcumin sensor are shown by the notable increase in a\ (30.87) and the continuous reduction in b* (23.04). By day nine of chilled storage, Das *et al*. (2020) [6] found that freshwater fish had severe colour oxidation and sensory rejection. The sensor's reaction validates its dependability by showing an increase in pH and TVB-N buildup.



L. Rohita Fish



C. Mrigala Fish

Fig 3: Colour index of SA/Cu film during storage of Muscle food (Indian major carps Rohu, Mrigal)

Chromatic freshness index of Muscle food (Indian major carps Rohu, Mrigal)

The developed film provides a dependable non-destructive freshness monitoring solution for intelligent packaging applications, as evidenced by the good agreement between visual/quantitative sensor response and the biochemical spoiling markers. A quick and easy way to evaluate food quality is through the tier-based interpretation of ΔE values, which guarantees precise decisions about product safety during storage.

The sensor showed a discernible chromatic change (ΔE 5–12) from Day 3–5, signifying the stage of borderline freshness. With TVB-N rising to 25–35 mg/100 g, ammonia levels rising to 5–15 ppm, and microbial loads reaching 5–7 log CFU/g, the biochemical results corroborated this transition. The sensor's ability to identify the beginning of degradation prior to full spoiling is demonstrated by the tight agreement of intermediate ΔE values with spoilage indices.

The spoilt quality tier was indicated by the indicator's severe and obvious colour change (ΔE > 12) starting on Day 6. At this point, microbiological and biochemical degradation were confirmed by spoilage indices exceeding safe limits (TVB-N > 35 mg/100 g, ammonia > 15 ppm, TVC > 7 log CFU/g).The strong correlation between ΔE and all spoilage parameters demonstrates that volatile amine production — rather than time alone — triggers the colour transition. Fish muscle has rapid microbial growth and considerable increases in ammonia and biogenic amines during the spoiling phase. This leads to notable changes in pH and levels of total volatile basic nitrogen (TVBN) exceeding 30 mg/100 g. Decomposition is shown by the colourimetric sensor's clear and permanent change to an orange-reddish brown hue. The ΔE thresholds used to classify freshness stages (Fresh < 5, Borderline 5–12, Spoiled > 12) are scientifically validated because they correspond directly to established quality limits for TVB-N and microbial counts. Therefore, the developed indicator can be effectively applied in intelligent packaging for monitoring the freshness of muscle foods

Conclusion

In conclusion, the study successfully developed and applied a curcumin-based pH-sensitive colourimetric film as an intelligent freshness indicator for monitoring the spoilage of Indian carps, Rohu and Mrigal. The colour change in the film, from yellow to orange-red, was directly correlated with the increasing pH and biochemical markers associated with spoilage, such as total volatile basic nitrogen (TVBN) and microbial load. The film proved to be a cost-effective, eco-friendly, and non-destructive method for real-time freshness assessment, offering a valuable alternative to traditional, often destructive, freshness evaluation techniques. The correlation between the film's colour response and conventional spoilage indicators supports its potential for use in intelligent food packaging systems, thereby enhancing food safety and quality control in the seafood industry. This innovative approach provides a promising solution for improving freshness monitoring in commercial supply chains, ensuring better consumer safety and reducing waste in the seafood industry.

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