

Research Article

Cost-effective and Sensitive Anthocyanin-Based Purple Cabbage (*Brassica Oleracea* var. *Capitata* L) Extract Biosensor for Diagnostic Spoilage Fish Product

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Article Info	Abstract
Article History	Purple cabbage (<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>rubra</i>) contains anthocyanins that exhibit pH-dependent colour changes and can function as visual indicators of food spoilage through their response to volatile basic compounds. This study developed a simple colourimetric indicator label using purple cabbage extract and evaluated its suitability for monitoring milkfish (<i>Chanos chanos</i>) spoilage under different storage temperatures. Extracts were prepared at three levels (30 g, 50 g, and 70 g of cabbage per 100 mL of solvent) and used to fabricate indicator labels, which were characterised using UV–Vis spectroscopy and FTIR to confirm anthocyanin-related spectral features and functional groups. The labels were tested in sealed 100 mL bottles containing 10 g of milkfish, with the indicator positioned 4 cm above the sample to enable headspace exposure without direct contact. Storage was conducted at 15 °C, 25 °C, and 40 °C. Colour changes were recorded at 2, 10, 24, and 32 h and quantified using image analysis. The indicator labels exhibited progressive colour shifts over time, consistent with the accumulation of volatile bases associated with spoilage. Temperature strongly influenced the response rate, with slower changes at 15 °C and faster, more pronounced changes at 25 °C and 40 °C. These findings indicate that the proposed purple-cabbage-based label has potential as a low-cost, non-destructive tool for monitoring milkfish freshness across practical storage conditions.
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1. Introduction

Assessing the freshness of food products has traditionally relied on sensory evaluation, including appearance, texture, smell, and colour [1]. While such methods provide initial insights, they are subjective

and often insufficient without information such as expiration dates. Consumers not only need to determine the safety and suitability of food for consumption, but also to understand changes in taste, appearance, and nutritional content [2]. To address these limitations, alternative freshness detection approaches have been developed, including chemical sensors that detect gases produced during spoilage. Among these, ammonia gas sensors have attracted significant attention due to their ability to monitor volatile compounds released during protein degradation [3, 4].

Meat and fish contain proteins that undergo hydrolysis, breaking down into free amino acids and subsequently into simple compounds such as CO_2 , H_2O , NH_3 , and H_2S . The release of these compounds, particularly ammonia (NH_3), provides a reliable indicator of spoilage. Freshness indicators based on acid-base responses exploit this property, as the accumulation of volatile amines corresponds to increased alkalinity. Detecting NH_3 release enables distinguishing between fresh and spoiled food products [3]. In this study, the indicator labels were evaluated using milkfish headspace during storage at different temperatures. The observed colour response is attributed to the accumulation of volatile basic nitrogen compounds (e.g., ammonia and biogenic amines) generated during spoilage.

Anthocyanins are a group of red-to-blue pigments widely distributed in plants and serve as natural pH indicators, as they change colour under different acidic or alkaline conditions [5]. Purple cabbage was selected as the anthocyanin source because it is abundant, inexpensive, easy to extract, and has been shown to provide relatively stable colour with a wide transition range when incorporated into food packaging and smart indicator films [6]. The purple cabbage extract was then immobilised onto cellulose paper to form colourimetric labels, in line with previous reports of anthocyanin-based sensors immobilised on cellulose or paper substrates for the detection of basic analytes such as ammonia [7].

In the context of fish freshness monitoring, these labels are designed to respond to NH_3 and other volatile amines released during spoilage, producing a distinct, visually observable colour change that has been demonstrated to be effective in several anthocyanin-based freshness indicators for fish and seafood products [6, 8].

Despite the advantages of purple cabbage anthocyanins as natural pH-sensitive dyes for colourimetric labels, most existing studies have focused on general food packaging or other fish species rather than on milkfish freshness. Research specifically targeting low-cost, portable biosensors for monitoring milkfish spoilage remains scarce, and there has been little systematic evaluation of how anthocyanin loading and

storage temperature jointly affect the colour response and stability of such indicators [1, 9]. Building on the use purple cabbage extract immobilised on cellulose labels for detecting volatile amines, this study aims to develop a simple and affordable biosensor tailored for milkfish freshness monitoring by optimising the extract concentration, examining its performance under different storage conditions, and characterising the anthocyanin functional groups using Fourier-transform infrared (FTIR) spectroscopy to validate the presence of functional groups associated with natural dyes.

2. Literature Review

2.1. Anthocyanin-Based Colourimetric Biosensors for Food Freshness

The growing concern about food quality and safety has driven the development of simple tools for near-real-time monitoring of the freshness of fish and seafood. One important spoilage indicator is the formation of biogenic amines and other volatile nitrogenous compounds, such as ammonia, produced during the degradation of proteins in fish products [9, 10]. Paper-based colourimetric biosensors employing anthocyanin dyes have therefore been widely explored, as pH changes induced by these basic volatiles are translated into visible colour shifts that can be evaluated without instrumentation [5]. Intelligent films and labels incorporating anthocyanins from purple cabbage and other plant sources have been used to detect ammonia or total volatile basic nitrogen in food, providing clear colour responses but often suffering from limited colour stability during prolonged storage and at elevated temperatures [6, 11]. Other paper- or cellulose-based sensors immobilise anthocyanins to monitor seafood spoilage and show good sensitivity to amine vapours, yet they typically investigate only a single dye loading or storage condition, which restricts optimisation for practical applications [4, 12]. Building on these approaches, the present study focuses on a purple-cabbage-based paper biosensor specifically designed for milkfish, systematically varying extract concentration and storage temperature to improve colour stability and the response to volatile amines released during spoilage.

Among various sensing mechanisms, colourimetric biosensors have attracted attention for their easy interpretation via visible colour changes. Anthocyanins, natural pigments with strong pH sensitivity, are widely used in such systems due to their responsiveness to spoilage-related gases, such as ammonia [6, 11, 12]. Purple cabbage (*Brassica oleracea* var. *capitata* L.) is particularly rich in cyanidin-based anthocyanins, which exhibit a visible colour shift with pH [13]

Prior studies have employed anthocyanin-based sensors for spoilage detection in fish and meat products [14, 15]. For example, Agunos, et al. [4] developed anthocyanin strips to detect amines, while [13] designed colourimetric swabs and immobilised fibres for fish freshness monitoring. These sensors respond to the accumulation of volatile bases as fish decomposes [3]. The performance of these indicators depends on anthocyanin stability, which is influenced by pH, light, and storage temperature [16, 17]. Therefore, we offer a similar sensor from purple cabbage, which is rich in cyanidin-based anthocyanins that exhibit a visible colour shift with pH.

2.2. Recent Applications for Anthocyanin-Based Test Strips for Spoilage Detection

Recent innovations have focused on fabricating independent biosensor strips that detect spoilage gases via visual colour change, mimicking the ease of use of devices like pregnancy tests. developed a purple cabbage-based strip capable of detecting volatile amines in headspace gas from spoiled fish samples. Similarly, [13] mmobilised anthocyanins on cellulose swabs and fibres for label-type biosensors that react with gaseous ammonia. These studies demonstrate the feasibility of simple strip-based devices that can detect spoilage in real time without the need for expensive analytical instruments.

Typically, such test strips are prepared by applying an anthocyanin extract to filter paper or cellulose matrices, providing a portable, contactless detection format [18]. The response speed and clarity are influenced by several factors, including pigment concentration, storage temperature, and exposure time to gases [19]. In addition, researchers have adopted smartphone cameras and software such as ImageJ to perform semi-quantitative analysis of colour changes, thereby reducing subjectivity and making results more accessible [20, 21]. Other directions in this field include the use of biodegradable or biopolymer-based substrates, which not only maintain sensor stability but also improve sustainability [14, 22, 23]. Structural and functional validation of anthocyanins in these biosensors has been achieved using FTIR and UV-Vis Spectroscopy, confirming the chemical groups responsible for the pH-sensitive colourimetric response [24-26]. These advances highlight the growing scientific interest in anthocyanin-based freshness monitoring across different food systems.

Despite this progress, anthocyanin biosensors designed specifically for milkfish remain underdeveloped. As milkfish is both a staple food and an economically important commodity in Southeast Asia, this gap calls for more focused research. The present study, therefore, proposes the fabrication of a

portable test strip biosensor using purple cabbage extract as the anthocyanin source. Purple cabbage is known for its high anthocyanin content, which reacts to volatile compounds such as ammonia released during spoilage, producing a visible colour change. This property enables the design of simple, low-cost labels that can be applied directly to milkfish without requiring advanced instrumentation or trained personnel.

Recent reviews further suggest that the future of biosensors lies in integrating digital tools, such as smartphone-based image analysis, to allow users to capture and interpret colour changes more consistently. However, very few studies have applied these innovations to traditional fish commodities, such as milkfish. For this reason, the present work not only aims to provide an affordable diagnostic tool but also to investigate how extracted concentration and storage temperature affect biosensor performance. Understanding these factors is essential to improve both sensitivity and reliability. In doing so, this study aims to bridge the gap between earlier anthocyanin biosensor research and its practical application in milkfish spoilage monitoring, demonstrating real benefits for both industry and consumers.

3. Methodology

3.1. Materials

The chemical materials used in this experiment were absolute ethanol (purchased from JT Baker, 99.99%), sodium hydroxide (NaOH, 99.0%, purchased from Merck), and hydrochloric acid solution (HCl, 37%, purchased from Merck). Cellulose-based substrates consisted of Whatman filter paper (Grade 41, pore size 20-25 μm) cut into strips and used as the support for the indicator labels. For each formulation, purple cabbage extract concentrations of 30 g, 50 g, and 70 g were prepared by mixing 70 mL of ethanol with 30 mL of distilled water.

3.1.1. Extraction of Purple Cabbage

Freshly chopped purple cabbage (*Brassica oleracea* var. *capitata* L.) at 30 g, 50 g, and 70 g was blended with a solvent mixture of 70 mL ethanol and 30 mL distilled water until the total volume reached 100 mL. The mixture was acidified by slowly adding hydrochloric acid (HCl) until the pH reached 2. The acidified solution was transferred into a dark bottle and stored at 5 °C for 24 h. After 24 h, the solution mixture was filtered using Whatman no. 41 paper to separate the extract and remove any remaining particulates. The extract was stored in a refrigerator at 4 °C and protected from direct sunlight until used in

the next stage, namely the characterisation stage, and for biosensor applications. The extract was further investigated by measuring its absorbance spectra in the UV-Visible region using a UV-Vis spectrophotometer (Thermo Fisher Scientific) and Fourier Transform Infrared (FTIR) spectroscopy (Thermo Fisher Scientific). For UV-Vis analysis, spectra were recorded from 200 to 800 nm using a quartz cuvette (1 cm path length), and samples were diluted to keep absorbance within the linear range. For FTIR analysis, spectra were collected over 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans. All spectra were baseline-corrected before interpretation.

3.1.2. Indicator Labelling

The indicator labels were prepared from a purple cabbage dye extract and a cellulose paper matrix, cut into rectangular strips measuring 1.0 cm $\times$ 4.0 cm (width $\times$ length). Each strip was immersed in 5.0 mL of purple cabbage extract solution adjusted to pH 2 for 30 s until the entire surface was saturated, and the excess solution was allowed to drain off. The coated strips were then dried at room temperature (approximately 25 °C) for 24 h in the dark until completely dry and subsequently stored in a sealed container at room temperature until use.

3.1.3. Testing of Biosensors on Food Products

In this study, fresh Chanos (milkfish) was purchased from a local fish market, transported to the laboratory, and immediately prepared for the spoilage test. For each trial, 10 g of milkfish was placed into a 100 mL transparent glass bottle. The indicator label (prepared with purple cabbage extract at 30 g, 50 g, or 70 g formulations) was attached to the inner side of the bottle cap and positioned so it did not contact the fish. The distance between the indicator label and the fish sample was maintained at approximately 4 cm. The bottle was then tightly sealed, and $t = 0$ was defined as the time of sealing. Bottles were stored at 15 °C (refrigerator), 25 °C (room temperature), and 40 °C (temperature-controlled oven/incubator). For each extract formulation and storage temperature, experiments were performed using $n = 3$ independent bottles (biological replicates), each prepared using a different fish portion. In addition, for each formulation, a blank control bottle (indicator label without fish) was stored under the same conditions to confirm that any colour change was driven by spoilage volatiles rather than temperature or humidity alone. Colour changes in the indicator labels were photographed with a digital camera at 2, 10, 24, and 32 h, and the images were analysed using image analysis software to quantify colour variation over time.

3.1.4. Image Acquisition and Colour Analysis

All photographs were captured using the same camera model in a fixed setup. Images were taken at a constant distance (25 cm) under controlled lighting. A white reference card was included in each image to minimise lighting variation. Colour change was quantified using Fiji (ImageJ, version 1.54 or later; National Institutes of Health, USA). For each indicator strip, a consistent region of interest (ROI) was selected at the centre of the strip (avoiding edges). Mean colour values were extracted in the RGB colour space, and results are reported as mean $\pm$ standard deviation across replicates. For a single summary metric, colour difference was calculated as $\Delta\text{RGB} = \sqrt{[(R-R_0)^2 + (G-G_0)^2 + (B-B_0)^2]}$, where R_0 , G_0 , and B_0 correspond to $t = 0$.

3.1.5. Statistical Analysis

This study is presented as a descriptive evaluation of colour responses under different storage conditions. Results are reported as representative observations across three independent bottles per condition ($n = 3$). Quantitative colour values (RGB) were summarised as mean $\pm$ standard deviation to describe trends over time. No inferential statistical testing was performed.

4. Results and Discussion

4.1. Fourier Transformation Infra-Red and UV-Spectrometer Analysis

Figure 1 shows the broadband at 3395.19 cm^{-1} , 3382.12 cm^{-1} , and 3368.39 cm^{-1} in the spectra of extractions with 30 g, 50 g, and 70 g, respectively, which are attributed to the O-H bond, as all materials contain hydroxyl groups. The peak at 2931.67 cm^{-1} appeared for all samples and corresponded to a medium C-H bond, corresponding to C-H stretching vibrations (aliphatic groups). The bands at 1739.67 cm^{-1} were attributed to the C=O solid bond. The band at 1651.41 cm^{-1} is assigned to C=C stretching vibrations of conjugated/aromatic structures, which is consistent with anthocyanin-containing plant extracts. Moreover, the vibrational mode of angular deformation of CH_3 was observed at peak 1223.14 cm^{-1} .

In addition, the absorbance spectra were obtained for purple cabbage extracts at various concentrations, as shown in Figure 2. The peak absorbance was observed at 527 nm for all biosensors, with no shift in the peak positions. However, the absorbance was much higher at 70 g than at 30 or 50 g. The most commonly found anthocyanins are cyanidin and peonidin. In purple cabbage, the anthocyanin present is cyanidin, as the functional groups are filled with -OH and -H. The anthocyanins in purple cabbage are

unstable. The smaller the anthocyanin, the greater the transmittance, while the higher the anthocyanin content, the greater the absorbance.

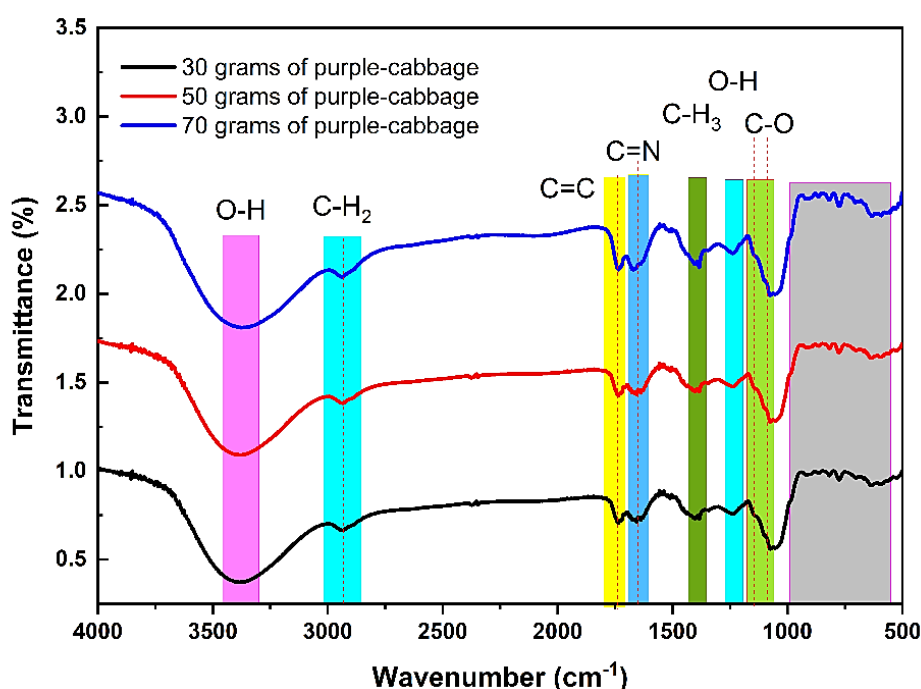


Figure 1. Fourier Transform Infra-Red (FTIR) with the variation of concentration of cabbage extract

Table 1. FTIR result of purple cabbage for different concentrations

FTIR Wave Number					
No	Purple cabbage (g)			Literature	Functional Group
	30	50	70		
1	1050.05	1065.45	1072.72	1000-1230	C-O
2	1369.89	1401.81	1363.63	1350-1470	C-H
3	1652.72	1661.81	1656.65	1450-1650	C=C

4.2. Biosensor for Diagnostic Spoilage

The biosensor in this study is an indicator label that can detect the freshness level of milkfish quality by utilising the anthocyanin contained in purple cabbage extract. The indicator label is made of Whatman paper measuring 1 x 4 cm and has been dipped into purple cabbage extract at concentrations of 30, 50, and 70 g. Milkfish storage conditions at the time of testing were three: 15 °C (refrigerator), 25 °C (room), and 40 °C (oven). Biosensor testing was carried out for 32 h at each temperature. Milkfish spoilage was inferred from the time-dependent colour response of the indicator strips to volatile bases accumulating in the bottle headspace. In this study, TVB-N was not directly measured, and the discussion therefore refers to TVB-N as a widely accepted spoilage indicator reported in the literature rather than as a measured outcome [14].

Biosensor testing at 40 °C with varying concentrations shows a noticeable difference. Visually, the colour change was most evident in the biosensor at storage temperatures of 15 °C and 25 °C. The variation of storage temperature at 25 °C and 40 °C showed that the biosensor's colour changed after 24 h. This accelerated colour change at higher temperatures consists of faster microbial and enzymatic degradation, which increases the rate of volatile base release into the headspace. Colour change at 25 °C and 40 °C was comparable; however, the response occurred earlier at 40 °C, consistent with faster spoilage kinetics at higher temperature. At refrigerator temperature (15 °C), the biosensor shows no clear visual change. It happens because low temperatures are an essential factor that maintains anthocyanin stability, preventing the balance from shifting and ultimately degrading [7, 27]. The results of the biosensor test on storage temperature showed that the lower the storage temperature, the more durable the fish would be and the slower it would decay.

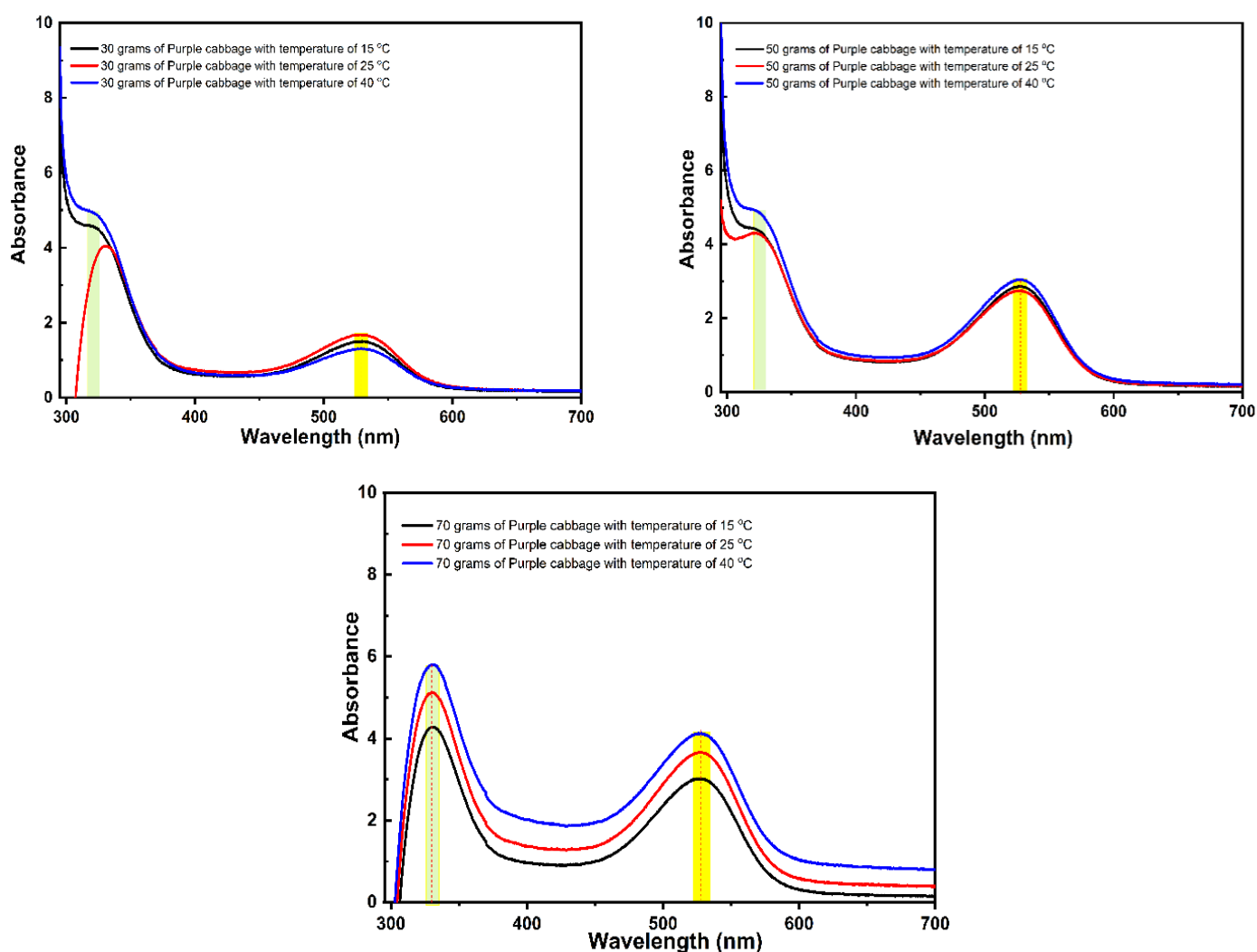


Figure 2. UV-Spectrometer with the variation of concentration of cabbage extract with the variation of concentration of cabbage extract

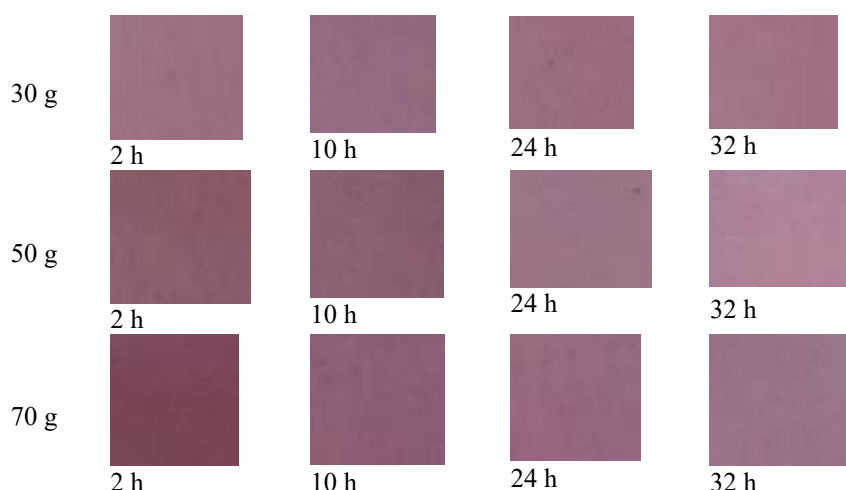


Figure 3. Biosensor colour changes when detecting spoilage of milkfish at 15 °C storage temperature with different purple cabbage concentrations

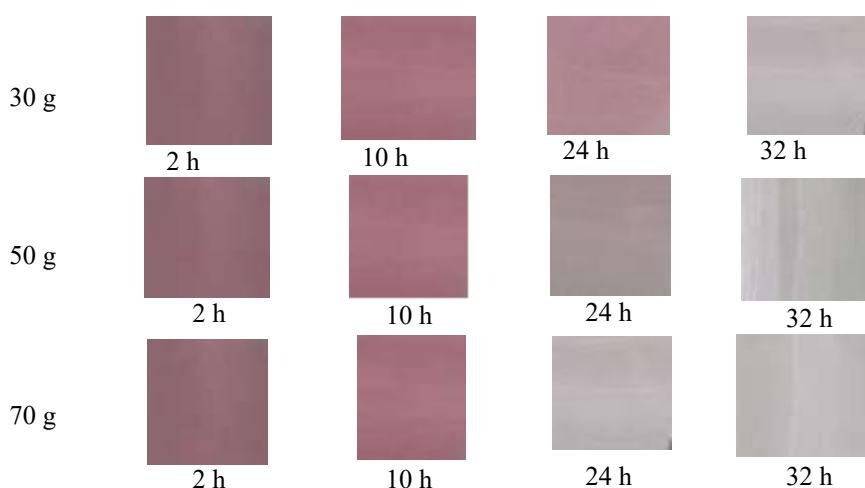


Figure 4. Biosensor colour changes when detecting spoilage of milkfish at 25 °C storage temperature with different purple cabbage concentrations

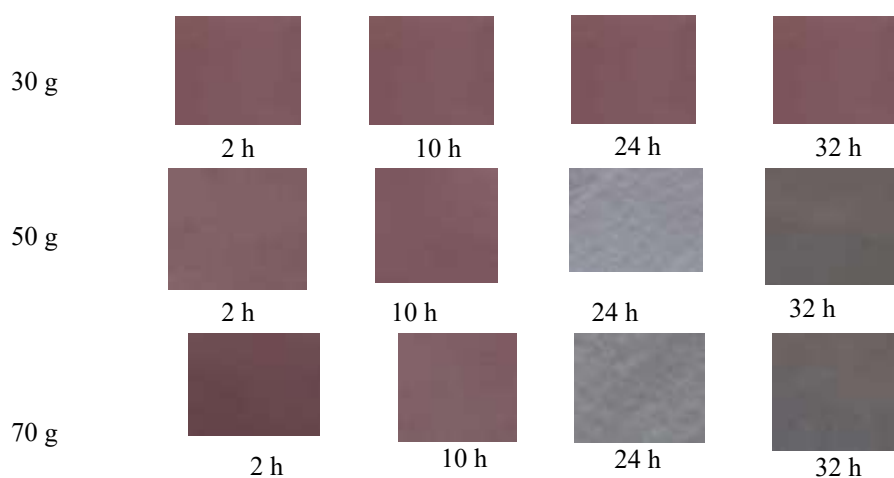


Figure 5. Biosensor colour changes when detecting spoilage of milkfish at 40 °C storage temperature with different purple cabbage concentrations

These findings confirm that anthocyanin-based biosensors are highly responsive to ammonia released

during fish spoilage. The pH-sensitivity of anthocyanins makes them particularly effective for detecting volatile amines, which accumulate as spoilage progresses. In this study, the biosensor successfully translated these chemical changes into visible colour shifts, supporting its potential as a visual freshness indicator for milkfish.

The observed increase in absorbance intensity at higher extract concentrations (70 g) indicates enhanced sensitivity due to greater anthocyanin content. This behaviour reflects improved interaction between anthocyanins and ammonia, thereby strengthening the visual response. Such a trend is consistent with previous studies that reported a positive correlation between pigment concentration and sensor response. These results suggest that optimising extract concentration is critical for maximising the biosensor's performance.

Furthermore, the faster and more distinct colour change observed at 25 °C and 40 °C aligns with known biochemical processes of fish degradation. At these higher temperatures, protein breakdown is accelerated, leading to the rapid release of volatile amines that interact with anthocyanins. The biosensor's responsiveness under these conditions highlights its potential for detecting spoilage in environments where refrigeration may be limited or inconsistent.

In contrast, storage at 15 °C maintained anthocyanin stability and delayed spoilage, demonstrating the biosensor's value for cold-chain monitoring. By comparing our findings with the existing literature, it is evident that the proposed biosensor demonstrates both sensitivity and practicality for real-world use in detecting milkfish freshness. These findings suggest that the proposed biosensor has potential as a practical freshness indicator for milkfish, particularly at 25 °C, where the colour contrast is most pronounced.

5. Conclusion

This study developed a simple, low-cost colourimetric indicator label based on purple cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) extract and assessed its potential for monitoring milkfish (*Chanos chanos*) spoilage through headspace exposure in sealed containers. UV–Vis and FTIR characterisation supported the presence of anthocyanin-related spectral features and functional groups in the extract used to fabricate the labels. When applied to milkfish stored at 15 °C, 25 °C, and 40 °C, the indicator labels exhibited progressive colour changes over time, reflecting the accumulation of volatile basic compounds associated with spoilage. The storage temperature strongly influenced the response rate, with slower, less

pronounced changes at 15 °C and faster, more pronounced shifts at 25 °C and 40 °C. Overall, the results indicate that purple-cabbage-based labels can provide a practical visual signal for freshness monitoring under different storage conditions. Future work should strengthen the evidence base by increasing the sample size, improving environmental control during imaging, and correlating colour metrics with standard spoilage indices (e.g., TVB-N, microbial counts, or sensory scores) to establish quantitative thresholds for decision-making in real packaging and supply-chain applications.

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