

Sustainable Food Packaging: Butterfly Pea Anthocyanin-Loaded Beads for Monitoring Shrimp Freshness

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ABSTRACT

The growing demand for sustainable and intelligent food packaging has led to increased interest in the development of materials that are both environmentally friendly and capable of monitoring food freshness. This study explores the development and reuse of butterfly pea (*Clitoria ternatea*) anthocyanin-loaded calcium alginate beads as a sustainable visual indicator for shrimp freshness monitoring. The objective of this study is to focus on preparing and evaluating anthocyanin-loaded beads for intelligent packaging, aiming to explore their performance in both freshly prepared and recycled forms. The beads, prepared via ionic gelation using calcium chloride, encapsulated natural anthocyanins extracted through ultrasonic-assisted methods. When exposed to spoilage conditions, the beads exhibited colour changes from blue to green in response to pH shifts associated with volatile amine release. Spectral Fourier Transform Infrared (FTIR) analysis revealed minor structural changes in the anthocyanin-loaded beads over three reuse cycles, with the beads remaining reusable for up to two cycles (Cycles 1 and 2), during which the RGB values decreased by only 4–5% compared with the initial beads (R: 156.00–148.67, G: 176.67–165.67, B: 204.67–191.67). However, by Cycle 3, the RGB values declined markedly by approximately 23–26% (R: 119.67, G: 134.00, B: 162.00), which may compromise the effectiveness of spoilage indication. These findings highlight the potential of biopolymer-based beads as recyclable and effective components of smart food packaging while demonstrating the need to improve anthocyanin encapsulation to enhance colour stability and spoilage detection over multiple reuse cycles.

Keywords: Anthocyanin-loaded beads; butterfly pea; food freshness monitoring; pH responsive colourants; intelligent packaging system

INTRODUCTION

Ensuring food freshness is essential, especially for perishable products like seafood, which are highly susceptible to spoilage. Traditional methods for monitoring freshness, such as microbiological tests and chemical analysis, are effective but often time-consuming, expensive,

and require specialized equipment (Husin et al. 2020). With growing concerns over food safety and waste, there has been increasing interest in intelligent packaging systems that offer real-time freshness monitoring (Mohammadalinejad et al. 2023). Among these, colourimetric indicators based on natural pigments have gained attention due to their ability to provide a visual

signal of food quality changes (Mohammadalinejad et al. 2024).

Anthocyanins, naturally occurring pigments found in plants like *C. ternatea* (butterfly pea), have shown great potential as pH-sensitive indicators for food freshness (Zhao et al. 2022). Their ability to change colour in response to pH variations makes them particularly useful for monitoring seafood spoilage, where increasing pH levels indicate protein breakdown and microbial activity (Mohammadalinejad, 2024). However, despite their advantages, anthocyanins are sensitive to environmental conditions such as temperature, light, and oxygen, which can affect their stability and performance in packaging applications (Oancea, 2021; Xue et al. 2024). To overcome these challenges, encapsulating anthocyanins in protective biopolymer matrices, such as methylcellulose, calcium alginate, and pectin, has been explored as a way to improve their durability and effectiveness as freshness indicators (Ekaputra & Pramitasari, 2020). These biopolymers are biodegradable, renewable, and non-toxic, making them a sustainable alternative to conventional synthetic polymers that contribute to environmental pollution (Rani et al. 2023). Moreover, using butterfly pea extract, which is a natural, plant-based dye supports green chemistry principles by reducing reliance on petrochemical-based colourants in packaging.

Integrating these eco-friendly materials into freshness indicators not only enhances food safety and waste reduction but also contributes to the larger goal of developing sustainable food packaging solutions aligned with global environmental standards. This research aims to contribute to a more practical, sustainable, and consumer-friendly approach to food freshness monitoring.

METHODOLOGY

MATERIALS

Butterfly pea powder and raw shrimp were obtained from local suppliers. Sodium alginate ($C_6H_7NaO_6$) and calcium chloride ($CaCl_2$) were purchased from Stabilab Sdn. Bhd., Malaysia. Peptone water and ethanol (Merck KGaA, Germany) were supplied by Stabilab Sdn. Bhd., Malaysia.

MICROORGANISMS

Two bacterial strains, namely, *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 9001), procured from the Food Microbiology Laboratory, Universiti Kuala Lumpur – Malaysian Institute of Chemical and Bioengineering Technology (UniKL MICET), were used

in this study. The bacterial cultures were maintained in sterile nutrient broth and subcultured into fresh nutrient broth every two months to preserve their viability and purity prior to use in the experiments.

EXTRACTION OF NATURAL COLOUR

The extraction of natural pigment was carried out based on the method described by Husin et al. 2020, with minor modifications. Briefly, butterfly pea (*C. ternatea*) powder was mixed with 15 mL of distilled water and subjected to sonication in an ultrasonic water bath for 30 minutes to facilitate pigment release. The resulting mixture was centrifuged at 2000 rpm for 10 minutes. The supernatant was collected into a clean conical flask and filtered through a fine filter cloth to remove particulate matter. Subsequently, 25 mL of the filtered extract was transferred into a 50 mL conical flask and diluted threefold to achieve a final dilution factor of 3 times. Each diluted sample was analyzed using a Shimadzu UV-1800 UV-Vis spectrophotometer, with distilled water serving as the blank reference.

PREPARATION OF CALCIUM ALGINATE BEADS

The calcium alginate beads were prepared based on the method outlined by Dalponte et al. (2020), with minor modifications. In brief, 8 g of sodium alginate was dissolved in 350 mL of distilled water containing 15 mL of butterfly pea extract. The solution was stirred continuously using a magnetic stirrer at 200°C until a homogeneous and viscous mixture was achieved. In parallel, 8 g of calcium chloride ($CaCl_2$) was dissolved in 400 mL of distilled water to serve as the crosslinking medium. The alginate was then added dropwise into the calcium chloride solution using a 15 mL syringe, maintaining a flow rate of approximately one drop per second. Upon contact, spherical beads were formed and allowed to stabilize in the calcium chloride solution for 10 minutes. The beads were subsequently collected, weighed (3 g), and placed into a porous pouch (tea-filter bag) for application in freshness monitoring experiments.

APPLICATION OF BEADS ON SHRIMP SAMPLE AND COLORIMETRIC ANALYSIS

The application of anthocyanin-loaded beads for shrimp freshness monitoring was conducted based on the method by Mohammadalinejad et al. (2024), with minor modifications. A total of 10 g of shrimp was placed in a sealed container, and a porous pouch containing the prepared anthocyanin-loaded beads was suspended above the shrimp without direct contact. The container was stored

at ambient temperature (25°C) for 24 hours to stimulate spoilage conditions. Colour changes in the beads were measured throughout the three reuse cycles storage using a smartphone (Samsung A15, 50 megapixel) and a photo box (enclosed 30 × 30 × 30 cm cardboard box lined with matte white paper, equipped with two diffused 10 W cool-white LED strips (5500–6500 K) placed on the side walls, with a smartphone mounted 15 cm directly above the samples on a fixed stand using manually locked camera settings (fixed white balance, ISO 100, shutter speed 1/100 s) and a neutral 18% gray card included as a reference for subsequent RGB normalization) to ensure consistent lighting and imaging conditions.

The captured images were analysed using ImageJ Software (Image Processing and Analysis in Java - Java 8) to obtain the Red (R), Green (G), and Blue (B) colour values of each bead sample. The RGB data obtained were subsequently used to evaluate and quantify the colour changes of the anthocyanin-loaded beads under the tested conditions.

MICROBIAL COUNT AND ANTIBACTERIAL TEST

Microbial analysis was conducted following the method described by Mohammadlinejad et al. (2024), with minor adjustments. Shrimp samples were homogenized with 90 mL of sterile peptone water in stomacher bags and processed using a stomacher machine to ensure uniform mixing. The homogenate was then subjected to a serial dilution up to 10⁻⁶. For total viable bacteria enumeration, 0.1 mL from each dilution was spread onto plate count agar, and the plates were incubated at 37°C for 48 hours.

A 0.1 mL aliquot of each *Escherichia coli*, *Staphylococcus aureus*, and shrimp homogenate (10⁻⁵ dilution) sample was spread evenly onto nutrient agar plates using the spread plate technique. Four autoclaved anthocyanin-loaded beads were then aseptically placed at different locations on the surface of each agar plate to evaluate their antimicrobial activity. The plates were subsequently incubated at 37°C for 24 hours, after which the presence of inhibition zones around the beads was observed and recorded.

PH MEASUREMENT AND REUSABILITY OF BEADS

pH of shrimp was determined according to the method reported by Mohammadlinejad et al. (2024), with minor modifications. Briefly, 10 g of shrimp was homogenized with 90 mL of distilled water using a stomacher machine, and the pH of the homogenate was measured using a calibrated digital pH meter. Additionally, pH changes were

monitored using pH indicator strips to visually assess spoilage progression before and after storage.

The recyclability of the anthocyanin-loaded beads was evaluated by monitoring their ability to recover their original colour after exposure to shrimp spoilage. Following each detection cycle, the beads were removed from the shrimp samples and exposed to ambient air without contact with the spoilage source. During air exposure, the beads gradually reverted to their original blue colour as the volatile spoilage compounds dissipated. The beads were left under these conditions for 24 h and subsequently monitored for an additional 24 h to ensure that no further colour change occurred, indicating complete colour recovery.

The regenerated beads were then stored at 4°C until the next reuse cycle. This regeneration and storage procedure was repeated prior to each subsequent cycle, and the beads were reapplied to fresh shrimp samples under identical experimental conditions for a total of three consecutive reuse cycles.

FTIR ANALYSIS

The Fourier transform infrared (FTIR) spectra of the anthocyanin-loaded beads were obtained using the attenuated total reflection (ATR) technique over a wavenumber range of 500–4000 cm⁻¹. The FTIR spectra were recorded directly from the surface of the beads. Before each measurement, the instrument was calibrated, and a background air spectrum was collected to minimize baseline interference. The sample spectrum was then automatically corrected by subtracting the background spectrum. The ATR crystal was cleaned with ethanol and distilled water after each measurement to prevent cross-contamination (Nesakumar et al. 2018).

STATISTICAL ANALYSIS

All experimental data were analyzed using analysis of variance (ANOVA) in Minitab. Statistical significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

EXTRACTION OF NATURAL COLOUR

The absorbance of the *Clitoria ternatea* anthocyanin extract was measured at a wavelength of 568 nm. This wavelength was selected based on the findings reported by Husin et al. (2020), which was conducted as part of the same research project using the same extraction protocol and source

material. In that study, 568 nm was identified as the wavelength of maximum absorbance ($\lambda_{\max}$) for the extract, corresponding to delphinidin-3-glucoside, the predominant anthocyanin pigment in *Clitoria ternatea*. Therefore, the same wavelength was adopted in the present study to ensure methodological consistency and enable reliable comparison of results.

The standard calibration curve established by Husin et al. (2020) was subsequently used to quantify the anthocyanin concentration of the extract. Based on the linear regression equation, $y = 1.228 + 0.0033x$, the anthocyanin concentration of the sample was determined to be 4 g/mL.

APPLICATION OF BEADS ON SHRIMP SAMPLE

The anthocyanin-loaded beads were sealed in a pouch and suspended inside containers containing shrimp samples, which were stored at ambient temperature (25 °C) for 24 hours. The beads exhibited colour changed from blue to green during spoilage detection. The amine compounds released from the shrimp were detected by the beads during spoilage and disappeared when the beads were subsequently exposed to an environment without amine compounds (Rahim et al. 2020). Therefore, the same pouch was reused in three different cycles—Cycle 1, Cycle 2, and Cycle 3—as shown in Figure 1.

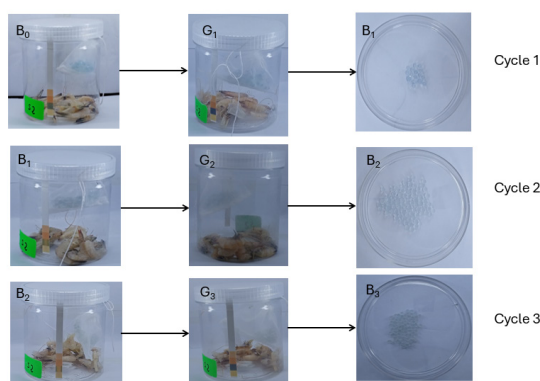


FIGURE 1. Anthocyanin-loaded beads sealed in a pouch were exposed to shrimp samples for 24 hours and subsequently recycled across three consecutive detection cycles. (B₀, – before detection, G₁, G₂, G₃ – after detection, B₁, B₂, B₃ – transformed and recycled beads)

The beads able to detect the spoilage up until Cycle 3. During storage, shrimp spoilage was initiated by the accumulation of non-protein nitrogen (NPN) compounds such as peptides, free amino acids, amines, and other low molecular weight organic molecules. Additionally, limited lactic acid production and increased microbial activity enhanced enzymatic degradation of proteins, leading to

the release of volatile nitrogenous compounds that contributed to pH elevation and spoilage progression (Li et al. 2020).

The successful detection of shrimp spoilage over three consecutive cycles demonstrates that the anthocyanin-loaded beads retained their responsiveness to volatile basic compounds despite repeated use. Anthocyanins are natural pH-responsive pigments that undergo reversible structural transformations when exposed to changes in environmental pH, resulting in distinct colour changes that enable real-time monitoring of food freshness. During seafood spoilage, the production of volatile basic nitrogen compounds increases the local pH, inducing structural changes in anthocyanins that produce the observed blue-to-green colour transition (Yang et al. 2025).

After removal from the shrimp samples, the beads were no longer exposed to freshly generated volatile basic compounds. As the concentration of residual amines surrounding the beads gradually decreased during storage, the local pH returned closer to neutral, enabling the anthocyanins to revert to their original molecular structure and restoring the blue colour. This reversible behaviour is consistent with the well-established pH-responsive nature of anthocyanins, which undergo structural transformations in response to alkaline and neutral environments.

MICROBIAL COUNT AND ANTIBACTERIAL TEST

The progression of spoilage was monitored by quantifying the microbial load present on the shrimp samples. The total bacteria load in fresh shrimp was estimated at 5.3×10^6 CFU/mL. This result follows the recommendation by the International Commission on Microbiological Specifications for Foods (ICMSF), which states that mesophilic microorganism counts should be below 10^7 CFU/mL. In contrast, spoiled shrimp samples exhibited an extremely high microbial load, with all dilution plates categorized as TNTC. This excessive bacteria growth indicates advanced tissue degradation and microbial contamination, likely involving spoilage-associated bacteria such as *Staphylococcus* spp., *Klebsiella* spp., *Aeromonas* spp., and *Shigella* spp. (Samia et al. 2014)

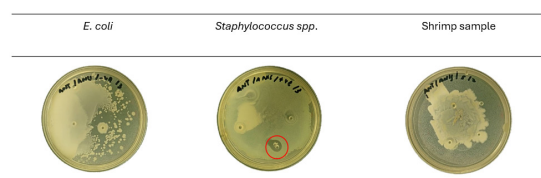


FIGURE 2. Antibacterial test on beads towards *E. coli*, *Staphylococcus* spp and shrimp.

Anthocyanins extracted from *C. ternatea* have been reported to exhibit antibacterial properties against various pathogenic bacteria (Jeyaraj et al. 2022). A distinct inhibition zone (± 10.2 mm) was observed exclusively against *Staphylococcus* spp., as presented in Figure 2. The absence of inhibition zones in the remaining plates could be attributed to limited diffusion or interaction between the bioactive compounds within the beads and the bacteria cells, particularly in the presence of high microbial loads (Hindler & Munro, 2024). The encapsulation matrix may also retard the release of anthocyanins into the agar medium, resulting in lower apparent antibacterial activity than that reported for free *C. ternatea* extracts.

PH MEASUREMENT

Fresh shrimp generally exhibits a pH range between 7.3 and 7.8 (as shown in Figure 3), which indicates acceptable quality (Tripody et al. 2016). As spoilage progresses, microbial activity and enzymatic protein degradation lead to the release of ammonia and other alkaline by-products, causing the pH to exceed 7.8 (Mohseni-Shahri & Moeinpour 2023).

In this study, the anthocyanin-loaded beads successfully detected these changes through visible colour transitions corresponding to the pH shift. The results validate the beads' responsiveness to pH variation, confirming their potential as reliable freshness indicators for shrimp.

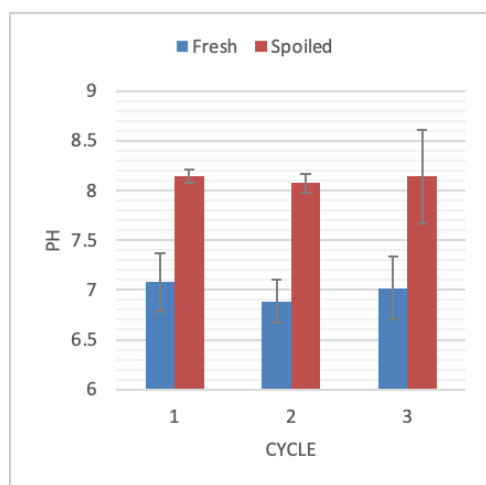


FIGURE 3. pH of fresh and spoiled shrimp for 3 cycles

SPECTRAL ANALYSIS AND FUNCTIONAL PERFORMANCE OF RECYCLED BEDS

FTIR spectral analysis indicated minor structural alterations in the anthocyanin-loaded beads over three reuse cycles, as shown in Figure 4. Initially, prominent absorption peaks

were observed at 3285 cm^{-1} (O–H stretching), 2920 cm^{-1} (C–H stretching), and 1043 cm^{-1} (C–O stretching), corresponding to the characteristic functional groups of the calcium alginate biopolymer matrix (Jain et al. 2016). In Cycle 2, a slight reduction in peak intensities was observed, which may be attributed to moisture loss and minor structural rearrangements within the polymer network during repeated use. By Cycle 3, an additional absorption band emerged at 1414 cm^{-1} , which is characteristic of the symmetric stretching vibration of carboxylate (COO^-) groups in calcium alginate (Pereira et al. 2013; Draget et al. 2005).

The appearance of this band may indicate subtle rearrangement of calcium–alginate interactions or changes in hydrogen bonding between the alginate matrix and anthocyanin molecules during repeated use rather than degradation of the polymer structure. Despite a slight reduction in transparency after successive cycles, the overall FTIR profile remained largely unchanged, suggesting that the chemical integrity and stability of the anthocyanin-loaded beads were maintained throughout the three reuse cycles.

Table 1 presents the colourimetric changes of anthocyanin-loaded beads over three recycling cycles (Cycles 1–3) compared with their initial state (B_0). The RGB (Red, Green, Blue) values quantify the colour intensity of the beads. The images of the beads in the Petri dishes show a progressive loss of colour intensity from Cycle 1 to Cycle 3, indicating gradual pigment degradation during repeated reuse. This trend is consistent with the RGB measurements, where all three colour components decreased with each successive cycle.

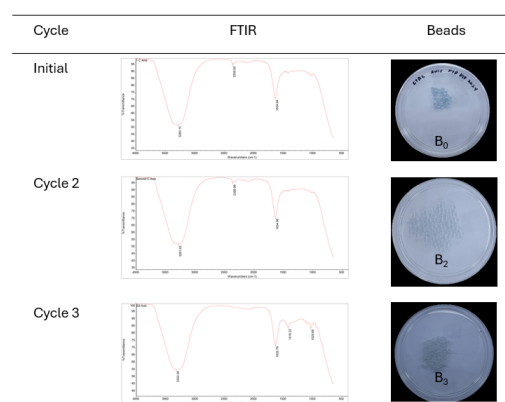


FIGURE 4. FTIR analysis results for Cycle 1, Cycle 2, and Cycle 3

The progressive reduction in colour intensity suggests a decline in the visual clarity and sensitivity of the beads as colourimetric freshness indicators, since anthocyanin degradation reduces the distinctiveness of colour changes

and limits their ability to provide reliable visual responses to spoilage metabolites (Sun et al. 2022; Xue et al. 2024). This behaviour is expected because anthocyanins are susceptible to degradation when repeatedly exposed to environmental factors such as oxygen, light, temperature, and moisture, resulting in reduced colour stability and diminished indicator performance (Oancea, 2021; Xue et al. 2024).

TABLE 1. Colour values for recycled beads. (Different superscripts in the same line indicate significant differences ($p < 0.05$) according to Tukey's test)

Recycle beads				
	Initial	Cycle 1	Cycle 2	Cycle 3
Colour value				
R	156.00 ± 0.60 ^a	154.67 ± 0.62 ^a	148.67 ± 0.41 ^a	119.67 ± 0.59 ^b
G	176.67 ± 0.66 ^a	170.00 ± 0.36 ^{ab}	165.67 ± 0.34 ^b	134.00 ± 0.22 ^c
B	204.67 ± 0.21 ^a	195.33 ± 0.04 ^b	191.67 ± 0.05 ^b	162.00 ± 0.47 ^c

The red (R) component remained relatively stable during the first two reuse cycles, showing only 0.85% and 4.70% reductions in Cycles 1 and 2, respectively, with no significant difference from the initial beads ($P > 0.05$). However, a significant decrease of 23.29% was observed in Cycle 3, as indicated by the different superscript ('b'), demonstrating a substantial loss of red colour intensity after repeated reuse. The green (G) component exhibited a gradual decline throughout the recycling cycles, decreasing by 3.77%, 6.23%, and 24.15% in Cycles 1, 2, and 3, respectively.

The statistically significant differences, particularly between the initial beads (B_0) and Cycle 3, indicate progressive degradation of the green colour component. Similarly, the blue (B) component showed an early reduction, decreasing by 4.56% in Cycle 1 and 6.35% in Cycle 2, with the most pronounced decline of 20.84% occurring in Cycle 3. The significant reduction in the blue component from the first reuse cycle suggests that blue pigments were more susceptible to degradation during repeated use, which may reduce the contrast and visual distinctiveness of the colourimetric response for spoilage indication.

The progressive reduction in RGB values is likely attributable to the gradual degradation of anthocyanins during repeated use. Repeated exposure to oxygen, moisture, light, and fluctuations in pH can promote oxidation, hydrolysis, and structural transformation of anthocyanin molecules, resulting in reduced pigment

stability and colour intensity (Oancea, 2021; Sun et al. 2022; Xue et al. 2024). Furthermore, repeated swelling and drying of the calcium alginate matrix may facilitate the diffusion or leaching of anthocyanins from the beads, accelerating colour loss and consequently reducing the sensitivity and visual clarity of the colourimetric freshness indicator (Yong et al. 2020; Xue et al. 2024).

CONCLUSION

This study successfully demonstrated the potential of anthocyanin-loaded beads as reusable indicators for monitoring food freshness. The beads maintained structural integrity and functional responsiveness for up to two usage cycles, with only minor degradation observed by the third cycle. The RGB values decreased by only 4.7–6.4% during Cycles 1 and 2 compared with the initial beads, indicating that the colourimetric response was largely preserved. In contrast, Cycle 3 exhibited a more pronounced reduction in RGB values of 20.8–24.2%, which may reduce the visual clarity and reliability of spoilage indication.

These results support the feasibility of bead reuse for up to two cycles to ensure reliable visual detection. Importantly, the observed limitations highlight the need for further optimization of anthocyanin encapsulation to improve colour stability and extend usability across additional reuse cycles. Overall, the findings underscore the beads' durability, biodegradability, and strong potential as a sustainable colourimetric system for freshness monitoring in perishable food products.

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DECLARATION OF COMPETING INTEREST

None.

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