



## RESEARCH ARTICLE

# Use of freshness indicators containing red beetroot and red cabbage extracts in monitoring shrimp freshness

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### ABSTRACT

This study aimed to develop natural extract-based freshness indicators for real-time monitoring of shrimp spoilage during refrigerated storage (4°C) for up to nine days. The indicators were composed of 1% (w/v) red cabbage or red beetroot extract, 2% (w/v) sodium alginate, and 10% (v/v) glycerol, and were incorporated into packaging materials. To assess shrimp quality and spoilage, pH, total volatile basic nitrogen (TVB-N), total mesophilic aerobic bacteria (TMAB), and total psychrophilic aerobic bacteria (TPAB) counts were monitored. The results showed significant increases in pH (from 7.52 to 7.88), TVB-N (from 20.3 mg/100 g to 44.80 mg/100 g), TMAB (from 3.72 log CFU/g to 7.29 log CFU/g), and TPAB (from 6.30 log CFU/g to 8.21 log CFU/g) confirming microbial spoilage. The spoilage threshold for TVB-N (35 mg/100 g) was exceeded on the fifth day, indicating the end of shrimp shelf life. Accordingly, the color change ( $\Delta E$ ) values of the indicators changed markedly over time: the  $\Delta E$  value increased from 0 to 11.34 for the red cabbage-based indicator, while it decreased from 8.53 to 3.51 for the red beetroot-based indicator. The red cabbage-based indicator exhibited strong and statistically significant correlations with pH ( $r = 0.86$ ,  $p = 0.029$ ) and TVB-N ( $r = 0.83$ ,  $p = 0.040$ ), indicating high potential for freshness detection. In contrast, the red beetroot-based indicator showed weaker and statistically insignificant correlations. These findings suggest that natural extract-based freshness indicators, particularly those containing red cabbage extract, offer a promising and non-invasive alternative to conventional methods for monitoring shrimp spoilage and enhancing food packaging systems.

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## Introduction

In recent years, consumer interest in seafood has been steadily increasing due to its role as a primary source of long chain polyunsaturated fatty acids, essential vitamins, and minerals, all of which significantly contribute to human health (Mohammadalinejad et al., 2020). Among seafood products, shrimp stands out as a valuable source of high-quality fatty acids and proteins, while containing minimal carbohydrates. Additionally, shrimp provides essential micronutrients such as vitamin B3, folate, calcium, magnesium, phosphorus, and potassium, making it an important component of a well-balanced diet. Due to its high protein content and low caloric value, shrimp is particularly recommended for individuals seeking a nutrient dense diet (Fan et al., 2022). However, despite its nutritional benefits, fresh shrimp has a limited shelf life (typically 4-6 days under refrigeration) and is highly susceptible to quality deterioration due to its elevated moisture content, free amino acids, and other soluble non nitrogenous compounds (Mohammadalinejad et al., 2020). During storage, enzymatic reactions and microbial contamination accelerate the deterioration of seafood products, significantly affecting their quality and safety. Consequently, monitoring the freshness of seafood has become a critical concern for consumers, retailers, and the food industry (Wen et al., 2023; Kılınç et al., 2023; Ho et al., 2024). Traditional methods for evaluating the freshness of seafood typically rely on chemical, microbiological, or sensory analyses. While these methods provide reliable results, they often require extensive processing time, skilled personnel, and labor-intensive procedures, making them impractical for rapid assessments (Wu et al., 2019; Kuswandi et al., 2022). To overcome these limitations, various advanced techniques, including electrochemical sensors, Raman spectroscopy, electronic noses, electronic tongues, and hyperspectral imaging, have been developed for seafood freshness evaluation. While these approaches offer high sensitivity and ease of use, their widespread application remains limited due to the need for costly instrumentation and the lack of real time monitoring capabilities. Hence, there is an urgent need to develop cost effective, non-destructive, and real time assessment techniques for monitoring the freshness of seafood products within the industrial food chain (Wu et al., 2019; Wen et al., 2023). Conventional food packaging systems, on the other hand, do not provide real time information about the condition of the packaged food or the internal environment, making it difficult for consumers to assess product freshness accurately (Ho et al., 2024).

Intelligent packaging films represent an advanced packaging technology designed to monitor the condition of food products during storage and transportation, thereby enhancing food safety and quality. These systems often incorporate colorimetric indicator films, which respond to environmental changes such as variations in pH. When the pH level of the packaged food fluctuates, the colorimetric indicator undergoes a visible color change, allowing consumers to assess food freshness without opening the package. This approach has the potential to enhance food quality while simultaneously reducing food waste (Ho et al., 2024). Given their efficiency, cost effectiveness, and ease of application, colorimetric pH sensitive indicator tags could serve as real time freshness monitoring tools for food products (Yan et al., 2021). A typical visual pH indicator consists of two fundamental components: a pH responsive dye and a solid support matrix that immobilizes the dye. Natural dyes are generally preferred over synthetic alternatives due to their lower toxicity, environmental compatibility, renewability, and non-polluting nature (Mohammadalinejad et al., 2020). These natural pH indicators can be derived from various plant-based sources, including flowers, fruits, vegetables, and food industry by products. Several groups of natural pigments, such as anthocyanins, betalains, curcumin, chlorophyll, and shikonin, exhibit pH responsive behavior and possess additional functional properties such as antimicrobial and antioxidant activities, making them highly suitable for intelligent food packaging applications (Abedi-Firoozjah et al., 2022; Zheng et al., 2022; Chaari et al., 2024; Kaewprachu et al., 2024; Tavana et al., 2024).

Red cabbage (*Brassica oleracea* L.) is recognized as a rich source of natural anthocyanins, which are valued for their nutritional benefits and bioactive properties. This vegetable contains high levels of micronutrients, oligosaccharides, minerals, vitamins, and bioactive compounds such as flavanols and glucosinolates, which contribute to human health. Due to its low cost, widespread availability, and strong halochromic properties, red cabbage anthocyanins have garnered significant attention for use in pH responsive indicator films compared to anthocyanins from other natural sources (Abedi-Firoozjah et al., 2022; Cheng et al., 2022).

Similarly, red beetroot (*Beta vulgaris* L.), a biennial herbaceous plant belonging to the Amaranthaceae family, serves as an abundant source of betalains and phenolic compounds, making it a potent antioxidant (Aykın-Dinçer et al., 2021). Red beetroot contains two primary pigment groups: red betacyanins and yellow betaxanthins, collectively referred to as betalains (Eshaghi et al., 2020). In addition to their strong

biological and antioxidant properties, betacyanins exhibit notable pH sensitivity, particularly under alkaline conditions, making them promising candidates for freshness monitoring in protein rich food products such as meat, fish, pork, and poultry (Chaari et al., 2024).

This study aims to develop a cost effective, eco-friendly, and pH sensitive freshness indicator by incorporating red beetroot and red cabbage extracts, with the goal of enabling real-time and non-destructive monitoring of shrimp freshness and spoilage. By doing so, it seeks to advance the development of intelligent food packaging systems that enhance consumer safety and contribute to the reduction of food waste.

## Material and Methods

### Materials

For the purposes of this study, 1 kg of fresh red beetroot (*Beta vulgaris* L.) and red cabbage (*Brassica oleracea*), along with 2 kg of fresh shrimp (*Parapenaeus longirostris*), were sourced from local markets in Izmir.

### Methods

#### *Preparation of Extracts from Red Beetroot and Red Cabbage*

Fresh red cabbage and red beetroot were washed with tap water to remove dust and other impurities. They were then dried in a drying oven at 50°C and ground into powder. A total of 100 g of each dried plant material was used for extraction. Specifically, 20 g of dried red beetroot and 20 g of red cabbage powder were extracted using 400 mL of ethanol (80% v/v). The solvent was removed using a rotary evaporator, and the extracts were lyophilized in a freeze dryer. The resulting extracts were stored at +4°C in a refrigerator until further use (Alizadeh-Sani et al., 2021).

#### *Preparation of Freshness Indicators*

A 2% (w/v) sodium alginate solution was prepared by dissolving 2 g of sodium alginate in 100 mL of distilled water, followed by stirring and maintaining the mixture in a shaking water bath at 60°C for 40 min to ensure complete dissolution. Then, 10% (v/v) glycerol was added as a plasticizer and homogenized using a magnetic stirrer for 30 min. Subsequently, 1% (w/v) red cabbage and red beetroot extracts were incorporated into the mixture, followed by continuous stirring for an additional 30 min.

The final mixture was cast into 20 cm diameter metal trays and dried in an incubator at 30°C for 48 hours under controlled conditions. The dried films were then cut into approximately 3 × 3 cm<sup>2</sup> squares and stored in a desiccator until use. As a result, two distinct types of freshness indicator films (red cabbage based and red beetroot based) were obtained (Chen et al., 2023; Ranjbar et al., 2023; Wu et al., 2024).

### *Packaging and Storage of Shrimp Samples*

Shrimp samples (100 g per container) were placed into lidded plastic containers. The developed freshness indicators were affixed to the inner upper surface of the containers to prevent direct contact with the shrimp. The packaged shrimp samples were stored at +4°C for nine days, and chemical, microbiological, and color analyses were performed at regular intervals.

### *Chemical Analyses*

To assess the chemical changes during storage, pH and Total Volatile Basic Nitrogen (TVB-N) levels were analyzed. For pH measurement, a 10 g shrimp sample was homogenized in 100 mL of distilled water, and the mixture was filtered. The pH was then determined using a digital pH meter (Hanna, HI11312, UK) (Guran et al., 2015). TVB-N analysis were performed as described by (Goulas & Kontominas, 2005), and results were expressed in mg/100 g of shrimp sample.

### *Microbiological Analyses*

Microbiological analyses were conducted to determine the total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB). For this purpose, 10 g of shrimp sample was transferred into 90 mL of sterilized Maximum Recovery Diluent solution under aseptic conditions, followed by homogenization. Serial dilutions were prepared up to a 10<sup>6</sup> dilution level using a 1:10 dilution ratio. From the appropriate dilutions, 0.1 mL aliquots were plated on Petri dishes containing Plate Count Agar (PCA, Merck). The inoculated plates for TMAB enumeration were incubated at 30°C for 24 to 48 hours, while the plates for TPAB were incubated at 6.5°C for 10 days. Following incubation, colony counts were performed to determine the bacterial load (Halkman, 2005).

### *Color Analysis of Freshness Indicators*

The color properties of the freshness indicators were evaluated using a portable colorimeter (Color Muse, Variable Inc., Tennessee, USA). The *L* (lightness), *a* (redness), and *b*

(yellowness) values were recorded for each indicator type. To assess the overall color variation during storage, the total color difference ( $\Delta E$ ) was calculated using the following formula (1) (Chen et al., 2023):

$$\Delta E = \sqrt{[(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2]} \quad (1)$$

where  $L_0$ ,  $a_0$ , and  $b_0$  represent the initial color values measured on Day 0, and  $L_1$ ,  $a_1$ , and  $b_1$  correspond to the values obtained on subsequent storage days. In the results table, “Day 0” values are explicitly designated as the reference (initial) values used in  $\Delta E$  calculations.

### Statistical Analysis

The data obtained were analyzed using one-way ANOVA (analysis of variance), and group differences were evaluated using Duncan’s Multiple Comparison Test. Prior to ANOVA, the underlying assumptions of normal distribution and homogeneity of variances were verified and found to be satisfied. Additionally, Pearson correlation analysis was performed to assess the relationships between  $\Delta E$  values and pH, TVB-N, TMAB, and TPAB counts. The strength and direction of these associations were determined by calculating correlation coefficients ( $r$ ), with statistical significance set at  $p < 0.05$ . All statistical analyses were conducted using IBM SPSS Statistics 2012.

## Results and Discussion

### Changes in the pH and TVB-N Values of Shrimp

Table 1 presents the pH values of packaged shrimp over a nine-day storage period. Due to microbial growth and the decomposition of protein substances in shrimp, pH levels increased significantly ( $p < 0.05$ ), rising from 7.52 on day 0 to 7.82 on day 9. Oner et al. (2018) reported that the pH value of shrimp varied between 6.81 and 8.67 during storage. Similarly, Uçak (2019) observed that the initial pH values of 7.26 and 7.13 in shrimp increased to 7.82-8.30 at the end of eight days of storage at 4°C. Additionally, Bilgin et al. (2006) reported that pH values of brown shrimps stored under refrigerator conditions (4°C) increased from 6.83 at the beginning to 7.95 by day 5. Çolakoğlu et al. (2006) found that the pH value rose from 7.01 to 7.91 after six days of storage at 7°C. The increase in pH is primarily attributed to microbial metabolic activities, notably the deamination and decarboxylation of amino acids, which result in the accumulation of basic compounds such as

ammonia, trimethylamine, and dimethylamine (Balamatsia et al., 2007; Kim et al., 2022).

**Table 1.** The changes in pH and TVB-N values of the shrimps

Day	pH	TVBN (mg/100 g)
0	7.52±0.07 <sup>b</sup>	20.30±0.70 <sup>c</sup>
1	7.84±0.06 <sup>a</sup>	21.35±0.35 <sup>c</sup>
3	7.76±0.03 <sup>a</sup>	22.05±1.75 <sup>c</sup>
5	7.85±0.07 <sup>a</sup>	33.60±5.60 <sup>b</sup>
7	7.88±0.01 <sup>a</sup>	42.00±1.40 <sup>ab</sup>
9	7.82±0.06 <sup>a</sup>	44.80±2.80 <sup>a</sup>

**Note:** Mean(n=2) ± standard error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column ( $p > 0.05$ )

TVB-N, a widely accepted freshness index, quantifies the sum of these volatile nitrogenous compounds (Balamatsia et al., 2007; Kim et al., 2022). In our study, a statistically significant increase in TVB-N levels was observed throughout the storage period ( $p < 0.05$ ). As refrigeration time increased, microbial and enzymatic activity led to protein decomposition, resulting in the formation of amines. These amines reacted with organic acids formed by protein degradation, causing the TVB-N content to rise to 44.80 mg/100 g by day 9. Bilgin et al. (2006) reported a similar trend, with TVB-N levels reaching 42.53 mg/100 g in shrimp stored at 4°C by day 5. A TVB-N concentration exceeding 35 mg/100 g is considered unacceptable for fish and shrimp (Varlık et al., 2000). In the present study, the TVB-N value surpassed this threshold on day 7 of refrigeration at 4°C (Table 1), indicating the end of the shrimp’s shelf life.

### Microbial Quality Assessment of Shrimp

The microbial quality of the shrimp was evaluated based on total mesophilic aerobic bacteria (TMAB) counts and total psychrophilic aerobic bacteria (TPAB) counts, as presented in Table 2. The initial TMAB count was 3.72 log CFU/g on day 0 and remained relatively stable until day 3. However, a statistically significant increase ( $p < 0.05$ ) was observed after day 5, reaching 4.90 log CFU/g. By day 7, TMAB levels had increased to 7.19 log CFU/g, and by day 9, the bacterial count had further increased to 7.29 log CFU/g ( $p < 0.05$ ).

The rapid microbial growth during the later storage days is likely due to favorable conditions for bacterial proliferation, including nutrient availability and enzymatic activity. Similar trends have been reported in previous studies. For instance, Bilgin et al. (2006) found that TMAB levels in refrigerated shrimp exceeded 5 log CFU/g after five days of storage,



indicating the end of shelf life. According to international seafood safety standards, a microbial load above 7 log CFU/g is considered unacceptable for human consumption (ICMSF, 1986). In the present study, the TMAB count surpassed this threshold on day 7, suggesting that the shrimp were no longer suitable for consumption beyond this point.

**Table 2.** Total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB) counts of the shrimps (log CFU/g)

Day	TMAB (log CFU/g)	TPAB (log CFU/g)
0	3.72±0.27 <sup>c</sup>	6.30±0.13 <sup>c</sup>
1	3.54±0.11 <sup>c</sup>	6.15±0.03 <sup>cd</sup>
3	4.12±0.08 <sup>c</sup>	5.96±0.01 <sup>d</sup>
5	4.90±0.28 <sup>b</sup>	7.39±0.03 <sup>b</sup>
7	7.19±0.01 <sup>a</sup>	8.29±0.06 <sup>a</sup>
9	7.29±0.12 <sup>a</sup>	8.21±0.07 <sup>a</sup>

**Note:** Mean(n=2) ± standard error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column (p> 0.05)

The TPAB counts of shrimp samples significantly increased (p<0.05) during storage, with the initial value of 6.30 log CFU/g reaching 8.21 log CFU/g on the ninth day. The rapid increase in TPAB observed, especially after the fifth day, indicates the onset of microbial spoilage. This increase highlights the tendency of psychrophilic bacteria to proliferate under cold storage conditions and their significance as a microbial indicator of product spoilage. Similarly, in a study by Yang et al. (2017), it was reported that in shrimp stored at 4°C, psychrophilic bacteria count exceeded those of mesophilic bacteria by the sixth day, indicating the beginning of spoilage. This suggests that psychrophilic bacteria become dominant during cold storage, limiting the shelf life of the product. Furthermore, in a study by Canizales-Rodríguez et al. (2015), it was reported that

in blue shrimp stored on ice, the initial psychrophilic bacteria count of 2.61 log CFU/g increased to 7.14 log CFU/g after 18 days, and this increase led to significant quality losses. These findings support the idea that psychrophilic bacteria are a reliable indicator of spoilage in shrimp and other seafood, and that TPAB counts are an important parameter for monitoring product freshness. The microbial spoilage correlated with pH and TVB-N increases, indicating a close relationship between microbial activity and the biochemical degradation of shrimp proteins.

### Color Change of Freshness Indicators

The colors of freshness indicators during the storage period are given in Tables 3 and 4. The present study demonstrated a continuous increase in the ΔE value of freshness indicators containing red cabbage and red beetroot extracts during storage. Specifically, the ΔE value increased from 0 to 11.34 in the freshness indicator containing red cabbage extract, whereas it decreased from 8.53 to 3.51 in the freshness indicator containing red beetroot extract.

The visual progression of color changes in freshness indicators over the storage period is presented in Figure 1. As illustrated, the indicator containing red cabbage extract exhibited a noticeable color shift, whereas the indicator containing red beetroot extract showed a gradual darkening. These visual observations correspond well with the numerical ΔE values reported in Tables 3 and 4, thereby reinforcing the reliability of the colorimetric response to pH changes induced by spoilage.

The accumulation of volatile basic amines, resulting from microbial activity in packaged shrimp within the food package cavity, led to an increase in pH and a shift in the color of the freshness indicator (Kim et al., 2017). These changes align with the chemical and microbiological spoilage parameters discussed previously.

**Table 3.** The colors of freshness indicators containing red cabbage extract during the storage period

Day	L	a	b	ΔE
0	73.11±1.44 <sup>a</sup>	4.77±1.01 <sup>a</sup>	14.03±0.31 <sup>a</sup>	0.00±0.00 <sup>c</sup>
1	70.94±1.94 <sup>ab</sup>	2.47±1.34 <sup>b</sup>	10.63±0.72 <sup>b</sup>	5.21±0.57 <sup>b</sup>
3	67.33±2.12 <sup>bc</sup>	2.27±0.39 <sup>b</sup>	10.01±0.20 <sup>b</sup>	7.58±0.24 <sup>b</sup>
5	65.72±3.81 <sup>cd</sup>	1.93±0.62 <sup>b</sup>	14.43±2.54 <sup>a</sup>	10.56±2.25 <sup>a</sup>
7	62.53±1.46 <sup>d</sup>	1.30±1.31 <sup>b</sup>	14.61±1.47 <sup>a</sup>	11.25±1.99 <sup>a</sup>
9	62.86±0.21 <sup>d</sup>	1.46±0.52 <sup>b</sup>	15.80±2.3 <sup>a</sup>	11.34±0.06 <sup>a</sup>

**Note:** Mean(n=2) ± standard error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column (p> 0.05). In the ΔE calculation, Day 0 (L<sub>0</sub>, a<sub>0</sub>, b<sub>0</sub>) used as the initial values.



**Figure 1.** Color evolution of freshness indicators containing red cabbage and red beetroot extracts during the storage period. The top labeled samples correspond to red beetroot extract-based indicators, while the bottom labeled samples correspond to red cabbage extract-based indicators

An examination of the relationship between total color change values ( $\Delta E$ ) and the results of chemical and microbiological analyses revealed a strong correlation between increasing  $\Delta E$  values and rising TVB-N, pH, TMAB, and TPAB counts. Both freshness indicators exhibited significant color shifts over time ( $p < 0.001$ ), demonstrating their potential effectiveness in indicating shrimp spoilage. However, statistical analysis (independent samples t-test,  $p = 0.471$ ) showed no significant difference between the color changes in red cabbage and red beetroot-based indicators, suggesting that both films performed similarly in detecting spoilage.

A strong positive correlation was observed between  $\Delta E$  values of the red cabbage indicator both pH ( $r = 0.86$ ,  $p = 0.029$ ) and TVB-N ( $r = 0.83$ ,  $p = 0.040$ ) as shown in Table 5. In contrast, the correlation between pH and TVB-N values and the red beetroot-based freshness indicator was lower and statistically insignificant ( $r = 0.69$ ,  $p = 0.129$  and  $r = 0.43$ ,  $p = 0.398$ , respectively). The total mesophilic aerobic bacteria count showed a moderate positive correlation with  $\Delta E$  red cabbage ( $r = 0.75$ ,  $p = 0.087$ ), whereas its correlation with  $\Delta E$  red beetroot was weaker ( $r = 0.55$ ,  $p = 0.243$ ). Similarly, the total psychrophilic aerobic bacteria count exhibited a moderate correlation with  $\Delta E$  red cabbage ( $r = 0.78$ ,  $p = 0.068$ ) but was lower for  $\Delta E$  Red beetroot ( $r = 0.49$ ,  $p = 0.312$ ). These findings suggest that the red cabbage extract-based indicator is more sensitive to changes in shrimp spoilage parameters compared to the red beetroot-based indicator.

The observed color changes in freshness indicators are mechanistically linked to biochemical spoilage processes in shrimp. As microbial activity progresses, proteins undergo enzymatic degradation, leading to the accumulation of volatile basic compounds such as ammonia, trimethylamine, and dimethylamine. These compounds elevate the pH of the packaging environment, altering the chemical structure of pH-sensitive pigments in the indicators. Specifically, increased alkalinity promotes structural transitions in anthocyanins, thereby inducing visible color changes that correlate with the degree of spoilage. Thus, the real-time colorimetric response of the indicators is not merely a statistical observation but reflects underlying biochemical transformations in the shrimp matrix (Balamatsia et al., 2007; Kim et al., 2022; Hu et al., 2024).

**Table 5.** Pearson correlation coefficients between shrimp quality parameters and freshness indicators containing red cabbage and red beetroot extracts

Parameters	$\Delta E$ Red Cabbage (r; p)	$\Delta E$ Red Beetroot (r; p)
pH	0.86; 0.029	0.69; 0.129
TVB-N	0.83; 0.040	0.43; 0.398
TMAB	0.75; 0.087	0.55; 0.243
TPAB	0.78; 0.068	0.49; 0.312

**Note:** Bold values indicate statistically significant correlations at  $p < 0.05$

These findings indicate that pH and TVB-N levels are strongly correlated with the red cabbage extract-based freshness indicator, reinforcing its potential as an effective tool for real time freshness assessment in seafood products. The weaker and statistically insignificant correlations observed for the red beetroot-based indicator suggest that this extract might be less responsive to pH and TVB-N fluctuations during storage, potentially limiting its reliability in seafood freshness monitoring. Similarly, the moderate correlation between TMAB and TPAB counts and  $\Delta E$  red cabbage suggests that color changes may indirectly reflect microbial activity. However, the lack of statistical significance in these correlations highlights the need for further investigation.

In a study conducted by Fang et al. (2024), colorimetric labels incorporating red cabbage anthocyanins were utilized to monitor the freshness of shrimp, and the results demonstrated a strong positive correlation between TVB-N values and  $\Delta E$  values. Similarly, in another study where blueberry anthocyanins were employed as a freshness indicator, a comparable correlation between TVB-N and  $\Delta E$  values was

observed, indicating that  $\Delta E$  values are closely associated with the degree of spoilage (Hu et al., 2024). A similar correlation was also identified in a study conducted by Chen et al. (2025) using purple sweet potato anthocyanins. In a similar study conducted by Ezati et al. (2019) it was reported that indicators containing Alizarin, used for detecting fish freshness, exhibited a positive correlation between changes in color values and TVB-N levels.

The stronger correlation between the red cabbage extract-based indicator ( $\Delta E$  red cabbage) and shrimp spoilage parameters compared to the red beetroot-based indicator ( $\Delta E$  Red beetroot) can be attributed to differences in anthocyanin composition, stability, and pH sensitivity (Chigurupati et al., 2002; Khoo et al., 2017). In contrast, red beetroot pigments mainly consist of betalains, including betacyanins and betaxanthins, which, while also pH responsive, are generally more stable in mildly acidic to neutral conditions and exhibit a narrower color transition range (Giuliani et al., 2016).

Additionally, anthocyanins from red cabbage undergo significant structural modifications in response to pH, transitioning from red (acidic) to purple (neutral) and eventually green/yellow (alkaline) due to changes in their molecular charge distribution (Khoo et al., 2017; Chen et al., 2025). This results in a more pronounced  $\Delta E$  value that accurately reflects spoilage related pH changes. In contrast, betalains degrade more gradually and are less sensitive to small pH variations, potentially explaining the weaker correlation with TVB-N and microbial spoilage indicators.

Another factor influencing the performance of the freshness indicators is their oxidative stability. Betalains are known to be more susceptible to oxidation and thermal degradation than anthocyanins, which may have contributed to inconsistencies in the colorimetric response of the red beetroot-based indicator (Yang et al., 2021).

These findings suggest that the red cabbage extract-based freshness indicator provides a more reliable and sensitive real time assessment of shrimp spoilage due to its superior pH responsiveness, oxidative stability, and ability to interact with spoilage related volatile compounds. Future studies should further investigate the structural stability of different natural pigments in smart packaging applications to optimize their performance.

## Conclusion

The results demonstrated a strong correlation between the  $\Delta E$  values of the red cabbage extract-based indicator and key

spoilage parameters, including pH and TVB-N, reinforcing its reliability as a freshness marker. Although TMAB and TPAB counts also showed moderate correlations with  $\Delta E$  red cabbage, these were not statistically significant. In contrast, the red beetroot-based indicator exhibited weaker and statistically insignificant correlations with all tested parameters, limiting its potential for effective freshness monitoring.

Based on the chemical and microbiological quality criteria, the shrimp meat exhibited clear signs of deterioration by the fifth day, which aligns with the observed changes in  $\Delta E$  values. Therefore, these findings suggest that freshness indicators containing red cabbage and red beetroot extracts can serve as reliable, visual tools for monitoring shrimp freshness without requiring complex laboratory analyses.

These natural, biodegradable, and visually detectable indicators provide a practical, cost effective, and consumer friendly solution for monitoring seafood freshness. Their integration into food packaging could enhance food safety, extend shelf life, and reduce food waste, making them a promising tool for the food industry. Further studies should focus on testing these freshness indicators with other protein-rich and highly perishable foods, such as poultry and fish. Additionally, efforts should be made to optimize the formulation for enhanced sensitivity and to evaluate their performance under real-life supply chain conditions through pilot-scale packaging applications.

## Compliance With Ethical Standards

### Authors' Contributions

FÖ: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing

HG: Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing,

All authors read and approved the final manuscript.

### Conflict of Interest

The authors declare that there is no conflict of interest.

### Ethical Approval

For this type of study, formal consent is not required.

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Not applicable.

## Data Availability

All data generated or analyzed during this study are included in this published article.

## AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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