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Research article

Colorimetric indicator films based on carboxymethyl cellulose and anthocyanins as a visual indicator for shrimp freshness tracking

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ABSTRACT

This study aimed to evaluate the response efficiency of colorimetric indicator films based on carboxymethyl cellulose (CMC) incorporated with different anthocyanins [Karanda alone (CMC/AK), butterfly pea alone (CMC/AB), and a mixture of anthocyanins from Karanda and butterfly pea (CMC/AK75/AB25)] for tracking shrimp freshness during storage at different temperatures and times (4 °C for 8 days and 25 °C for 30 h). The mathematical models were also applied to predict their freshness and shelf life. The CMC/AK75/AB25 indicator film was the most sensitive and clearly changed color, which could be distinguished by the naked eye. Color changes indicated the shrimp deterioration processes: dark purple (fresh), purplish gray or gray (semi-fresh), and olive green or brown (spoilage). During shrimp storage at temperatures of 4 and 25 °C, the pH reached 7.52 and 8.14, TVB-N 35.98 and 72.72 mg/100 g, and TVC 5.75 and 7.88 log CFU/g, respectively, indicating shrimp had completely deteriorated. Furthermore, there was a positive correlation between the ΔE value of the indicator film and both TVB-N and TVC. These findings suggest that the CMC/AK75/AB25 indicator film could serve as a real-time visual indicator for tracking shrimp freshness and could enhance the guarantee of shrimp safety.

1. Introduction

Shrimp is a highly perishable food and has a short shelf life due to its high protein content and moisture level. It usually stores for 4–6 days in the refrigerator after slaughter [1]. However, the chemical composition of shrimp varies depending on species, feeding diets, and growing environment [2]. Over time, the freshness of shrimp consistently decreases. The loss of freshness means chemical, physical, microbial, and sensory changes have occurred. The volatile basic compounds such as trimethylamine (TMA), dimethylamine (DMA), and ammonia (NH_3), commonly known as a group of total volatile basic nitrogen (TVB-N), are produced in spoiled shrimp by microbial action [3]. Shrimp spoilage can be detected by monitoring the change in TVB-N values, which are frequently used as markers for seafood quality. Therefore, a non-destructive tool is required to track shrimp quality changes and inform consumers about shrimp deterioration in real-time without opening the packaging during distribution and retail sale.

In recent years, there has been a growing interest in smart packaging systems, particularly in the form of indicators, in order to

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guarantee quality and safety. Indicators are a simple tool that is important in terms of ensuring food quality and safety. They can monitor food quality, safety, microbial contamination, and spoilage degree during storage and distribution in real-time [4]. Indicators typically consist of two parts: a solid base and a sensitive dye. Anthocyanin is a natural water-soluble dye and mostly contributes color (red, purple, and blue) to fruits and vegetables. Recently, natural anthocyanin dyes, such as anthocyanins in butterfly pea flowers, *Carissa carandas* fruit, purple sweet potato, and red poppy, have revealed great potential for use as a dye and the development of smart colorimetric indicator films due to their ability to change color in response to pH change [5–8]. Among the indicators, freshness or spoilage indicators have garnered significant attention due to their ability to communicate quality changes resulting from reactions between microbial metabolites produced and indicators through visible information via color changes during storage [4,9].

Various colorimetric indicator films have been successfully developed for tracking food freshness. Some include bacterial cellulose films containing anthocyanin from *Echium amoenum* [3], sodium alginate film incorporated with butterfly pea extract [5], chitosan-poly(vinyl alcohol) films containing anthocyanins isolated from *Clitoria ternatea* and *Carissa carandas* [6], gelatin/agar films integrated with anthocyanin from *Clitoria ternatea* flower [7], gelatin/pectin film containing anthocyanin from butterfly pea flower [8], riclin/polyvinyl alcohol films incorporated with purple sweet potato anthocyanin [10], chitosan films incorporated with anthocyanins of red poppy (*Papaver rhoeas* L.) [11], polyvinyl alcohol films containing purple tomato anthocyanin [12], and cassava starch/polyvinyl alcohol incorporated with anthocyanin from *Aronia melanocarpa* [13].

A previous experiment [14] showed that smart colorimetric sensing films based on carboxymethyl cellulose (CMC) incorporated with a combination of anthocyanins from Karanda fruit pomace (AK) and butterfly pea flower (AB) at a ratio of 75:25 had excellent performance in terms of physicochemical properties and responsive sensibility to pH changes and volatile ammonia. However, information regarding the use of CMC/AK75/AB25 indicator film in real food systems is lacking. Therefore, the objective of this study was to evaluate the response efficiency of the colorimetric indicator films based on CMC incorporated with different anthocyanins [Karanda alone (CMC/AK), butterfly pea alone (CMC/AB), and a mixture of AK and AB (CMC/AK75/AB25)] for monitoring shrimp freshness during storage at 4 °C for 8 days (normal storage condition) and 25 °C for 30 h (accelerated storage condition). The shrimp quality attributes, such as pH, total volatile basic nitrogen (TVB-N), and total viable count (TVC), and the color changes of colorimetric indicator films were investigated. Furthermore, the mathematical models were also applied to predict their freshness and shelf life.

2. Materials and methods

2.1. Materials

Karanda fruit pomace (*Carissa carandas* L.) was supplied by Sirisompong Farm (Samut Songkhram, Thailand). Dried butterfly pea (*Clitoria ternatea*) was purchased from a local market (Samut Sakhon, Thailand). Fresh shrimp was purchased from Mahachai seafood market (Samut Sakhon, Thailand). Commercial carboxymethyl cellulose (CMC) powder, trichloroacetic acid (TCA), potassium carbonate, boric acid, and 3M™ Petrifilm™ Aerobic Count Plate were purchased from Union Science Co., Ltd. (Chiang Mai, Thailand). Other chemical reagents were of analytical grade.

2.2. Preparation of anthocyanins from Karanda fruit pomace and butterfly pea flower

Anthocyanins from Karanda fruit pomace and butterfly pea flowers were extracted as described in Kaewprachu et al. [14]. Briefly, plant samples were added to distilled water (1:10, w/v), sonicated (Misonix, Inc., Farmingdale, NY, USA) for 15 min, and then filtered through Whatman filter paper No. 4 (Schleicher & Schuell, Maidstone, England). The filtrate was freeze-dried, kept in polypropylene bags, and stored at −18 °C for further study. he obtained powders were subsequently referred to as "anthocyanin from Karanda fruit pomace (AK)" and "butterfly pea flower anthocyanin (AB)".

2.3. Preparation of colorimetric indicator films

The colorimetric indicator films were prepared as described in Kaewprachu et al. [14]. The formulation for the preparation of the colorimetric indicator film is presented in Table 1. Two g of CMC were mixed with the distilled water. The 30 % (w/w, based on CMC content) glycerol was added to the mixture and heated at 80 °C for 30 min vigorously stirred to obtain CMC film-forming solution (FFS). A natural anthocyanin indicator (1 %, w/v) (Karanda; AK, butterfly pea; AB, and a mixture of Karanda and butterfly pea at a ratio of 75:25; AK75/AB25) was added into FFS and stirred at a temperature of 45–50 °C for 15 min. After that, the bubble in FFS was removed using sonication for 5 min. Finally, the FFS (6 ± 0.01 g) was cast into a 50 × 50 mm rimmed silicone resin plate in an oven at 30 °C for 24 h to evaporate water. The colorimetric color film was dried in an oven at 30 °C for 24 h and further in a ventilated oven

Table 1
Formulations to obtain colorimetric indicator films (g/100 mL).

Films	CMC	Gly	AK	AB
CMC/AK	2	0.6	1	–
CMC/AB	2	0.6	–	1
CMC/AK75/AB25	2	0.6	0.75	0.25

CMC: carboxymethyl cellulose; Gly: glycerol; AK: Karanda; AB: butterfly pea.

environmental chamber at 50 ± 5 % relative humidity (RH) and 25 ± 0.5 °C for 24 h. The colorimetric indicator films prepared by adding AK alone, AB alone, and AK75/AB25 were denoted as CMC/AK, CMC/AB, and CMC/AK75/AB25, respectively.

2.4. Application of colorimetric anthocyanin indicator films for monitoring shrimp freshness

A colorimetric indicator film was cut into 2 × 3 cm and attached inside the polyethylene terephthalate (PET) box lid (7.5 cm × 11.5 cm × 4.3 cm) like a label. Two shrimp (~25 g) of similar size were put into the plastic box and stored at two conditions, including at 4 °C for 8 days (normal storage condition) and at 25 °C for 30 h (accelerated condition). The pH, total volatile basic nitrogen (TVB-N), and total viable count (TVC) were analyzed at days 0, 2, 4, 6, and 8 after storage at normal conditions and examined at 0, 6, 12, 24, and 30 h after storage at accelerated conditions, respectively.

2.5. Determination of color changes of colorimetric indicator films

The visual aspect of the colorimetric indicator films was photographed by a smartphone camera (iPhone 14 Pro Max, Apple Inc., Cupertino, CA, USA). Color changes of colorimetric indicator films were measured in L^* (lightness), a^* (redness), and b^* (yellowness). The average L*, a^*, and b^* values were used in order to calculate the total color difference (ΔE).

2.6. Determination of quality attributes of shrimp

2.6.1. pH measurement

A digital pH meter (Starter 3100, Ohaus Corporation, Parsippany, NJ, USA) was used to measure the pH of the mixture of shrimp sample (~2 g) and chilled distilled water (10 mL) that was already homogenized and placed at room temperature for 10 min.

2.6.2. Total volatile basic nitrogen (TVB-N) analysis

TVB-N was determined according to the method described in Kaewprachu et al. [15]. The experiments were done in triplication. The amount of TVB-N was calculated and expressed as mg N/100 g sample.

2.6.3. Determination of total viable count (TVC)

Briefly, shrimp samples (10 g) and 0.85 % (w/v) saline solution (90 mL) were blended and homogenized. A series of decimal dilutions was carried out, and 1 mL of each appropriate dilution was deposited onto a 3M™ Petrifilm™ Aerobic Count Plate (3 M Petrifilm, St. Paul, MN). The plate was incubated for 24–48 h at 37 °C. TVC was counted and reported as log CFU/g.

2.7. Mathematical model to describe microbial growth of shrimp

Mathematical model for predicting freshness of shrimp was modified from Arrhenius equation by Yang et al. [16] and adopted for describing the change of TVC as follows Equation:

$$B_{TVC} = B_{TVC0} + 1.00 \times 10^6 t exp(-33555.30 / RT) \tag{1}$$

where B_{TVC} is bacteria counts (log CFU/g), B_{TVC0} is bacteria counts (log CFU/g), t is time (day), R is the gas constant (8.314 J mol^{-1}. K^{-1}), and T is absolute temperature (K)

The data at 4 °C was used to validate the models. Both the bias factor (BF) and accuracy factor (AF) calculated, respectively, by Equations (2) and (3), were used to check the degree of estimation.

$$BF = exp\left(\frac{\sum ln(B_p - B_e)}{n}\right) \tag{2}$$

$$AF = exp\left(\frac{\sum |ln(B_p - B_e)|}{n}\right) \tag{3}$$

where B_p is the predicted bacteria counts (log CFU/g), B_e is the actual bacteria counts (log CFU/g) and n is number of data.

Mean Square Error (MSE) value was also determined to see the quality of estimation by model:

$$MSE = \frac{1}{n}\sum_{l}^{n}(B_p - B_e)^2 \tag{4}$$

2.8. Statistical analysis

All of the data was subjected to one-way analysis of variance (ANOVA) using SPSS software. Duncan's multiple range tests were used to analyze the differences between means ($p < 0.05$). The mathematical models for prediction of freshness and shelf life were constructed using linear regression (Microsoft 365, Excel, version 16.83 and MathWorks MATLAB R2023b v23.2.0.2515942).

3. Results and discussion

3.1. Changes in color of colorimetric indicator films

Fig. 1 presents the visual color changes of the CMC/AK, CMC/AB, and CMC/AK75/AB25 indicator films in response to the quality changes of shrimp. The package box containing the colorimetric indicator films as on-package label and shrimp was then stored at two different storage temperatures and times: 4 °C for 8 days and 25 °C for 30 h, to evaluate the response efficiency of the colorimetric indicator films related to the quality attributes of shrimp. As shown in Fig. 1A, the initial color of the CMC/AK indicator film was plum, changed to red (days 2–6), and finally to brown on days 8 of storage. The CMC/AB indicator film had only two colors, including dark blue (days 0–2) and turned dark teal after days 8 of storage. Furthermore, no remarkable color change was observed after 4 days of storage. For the CMC/AK75/AB25 indicator film, it had various colors during storage; the initial color was dark purple, purple (day 2), purplish gray (day 4), gray (day 6), and olive green (day 8), which were easy to distinguish using the naked eye. Notably, the CMC/AK75/AB25 indicator film possessed the most obvious color change among the three films. Narayanan et al. [8] reported the same pattern of color changes in gelatin/pectin film containing anthocyanin from butterfly pea flowers when monitoring the freshness of Tilapia fish during storage at 4 °C for 7 days. The film changed color from purple-blue on day 0 to bluish gray on day 4, and finally to olive/dark green on day 7.

The color changes of indicator films at a temperature of 25 °C for 30 h after applied to shrimp, as shown in Fig. 1B, showed that the CMC/AK indicator film was plum, changed to red (6–12 h), brown (24 h), and finally to orange–brown after 30 h of storage. The CMC/AB indicator films exhibited the same trends in color as the films stored at 4 °C. While the CMC/AK75/AB25 indicator film was dark purple, purple (6 h), gray (12 h), olive green (24 h), and brown (30 h), changes in the color of indicator films during shrimp storage are due to an increase in volatile compound secretions from the shrimp samples. Cold storage conditions can slow down the shrimp deterioration process, resulting in less volatile compounds being generated by the shrimp and less drastic color changes in the indicator

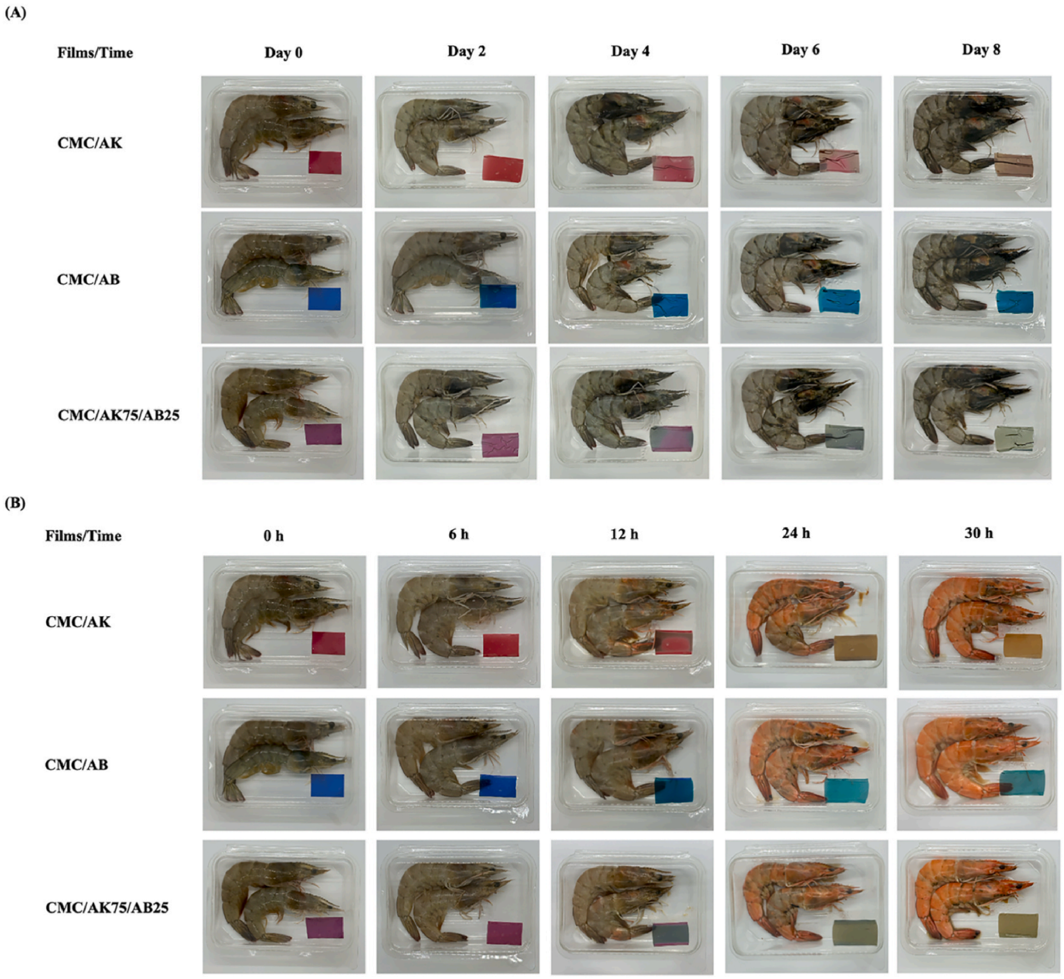

Fig. 1. Visual color changes of the colorimetric indicator films for monitoring shrimp freshness during storage at different temperatures. (A) temperature of 4 °C for 8 days and (B) temperature of 25 °C for 30 h.

films. While high storage temperatures cause the shrimp to break down more quickly, increasing the content of volatile compounds emitted and accelerating production [17], this phenomenon causes the colorimetric indicator films to change color significantly. A similar observation was reported in a study by Listyarini et al. [17] using cellulose paper labels incorporated with *Ruellia simplex* flowers to assess shrimp freshness during storage at temperatures of 13, 25, and 40 °C for 24 h. The color of the label was changed from purplish pink (fresh), purplish blue (began spoilage), greenish gray, and yellowish gray (spoilage). They also concluded that the rate of color change of the label increased as the storage temperature increased. Mohseni-Shahri and Moeinpour [18] also reported that a mixture of roselle anthocyanin and curcumin in a ratio of 4:1 (v/v) provided two different colors that related to shrimp freshness when stored at 4 °C for 10 days. Liang et al. [19] also observed that the funoran films incorporated with *Ophiopogon japonicus* seed anthocyanins exhibited high sensitivity for assessing the freshness of shrimp when stored at 25 °C for 7 h. The color of the films changed from light purple to yellowish-gray to light gray and finally turned green when shrimp spoiled. In this study, it can be revealed that the CMC/AK75/AB25 indicator film changed color most clearly and was visible to the naked eye when compared to the CMC/AK and CMC/AB indicator films. When the shrimp is in fresh condition, the CMC/AK75/AB25 indicator film was dark purple and changed to purplish gray or gray when the shrimp can still be consumed even though the quality has decreased. Finally, when the shrimp is very rotten or cannot be consumed, the CMC/AK75/AB25 indicator film was turned to olive green or brown. This color explanation of the CMC/AK75/AB25 indicator film can help the consumer quickly decide the freshness level of the shrimp and whether it is still fresh or spoiled, which is easily visible to the unaided eye without the need for opening the packaging.

The total color difference (ΔE) values of the CMC/AK, the CMC/AB, and the CMC/AK75/AB25 indicator films after being applied to shrimp during storage at different temperatures and times (4 °C for 8 days and 25 °C for 30 h) are presented in Table 2. The results showed that the ΔE values of the three colorimetric indicator films significantly increased as storage times increased ($p < 0.05$). At refrigerator (4 °C), the ΔE values of the CMC/AK indicator film were 11.40 (days 2), 13.46 (days 4), 25.16 (days 6), and 35.47 at the end of storage (days 8). The ΔE values of the CMC/AB indicator film were 24.70 (days 2), 44.41 (days 4), 46.56 (days 6), and 49.20 at the end of storage (days 8). The ΔE values of the CMC/AK75/AB25 indicator film were 13.75 (days 2), 26.26 (days 4), 38.30 (days 6), and 45.47 at the end of storage (days 8). In general, the calculated ΔE values greater than 5 can be considered visually perceptible to distinguish color difference changes by the human eye [20,21]. However, the ΔE value of the CMC/AB indicator film changed slightly after 4 days of storage, and the color change cannot be recognized by the naked eye due to its ΔE below 5.

At room temperature (25 °C), the ΔE values of the CMC/AK indicator film were 11.46 (6 h), 13.22 (12 h), 42.19 (24 h), and 45.51 at the end of storage (30 h). The ΔE values of the CMC/AB indicator film were 11.87 (6 h), 47.88 (12 h), 54.66 (24 h), and 54.25 at the end of storage (30 h). The ΔE values of the CMC/AK75/AB25 indicator film were 8.95 (6 h), 32.51 (12 h), 52.24 (24 h), and 56.80 at the end of storage (30 h). All colorimetric indicator films showed a slight color difference after 24 h of storage. A similar result was reported by Shi et al. [22], who found that the ΔE value of the freshness indicator film based on starch and polyvinyl alcohol incorporated with blueberry peel anthocyanin after monitoring the tilapia fillets freshness showed negligible change after storage at 25 °C for 24 h. Hashim et al. [23] also reported that sugarcane wax/agar films incorporated with butterfly pea flower extract had a slight change in the ΔE value after tracking shrimp freshness for 18 h (at 25 °C for 24 h).

The ΔE values for the CMC/AK75/AB25 indicator film at 4 and 25 °C were all above 5 throughout the entire storage period, indicating that the CMC/AK75/AB25 indicator film was the most sensitive for monitoring shrimp freshness. Notably, the greatest color changes of the colorimetric indicator films occurred at 25 °C. The changes in ΔE values of the colorimetric indicator films were related to the changes in pH, TVB-N, and TVC values (Table 3) of the shrimp during storage. During shrimp storage, the volatile basic compounds were continuously released from shrimp and became denser in the headspace of the package box. These compounds were further absorbed by the CMC/AK, the CMC/AB, and the CMC/AK75/AB25 indicator films, which were attached to the inner surface of the package lid. Noticeably, the increase of hydroxyl ions in the film by these compounds causes the deprotonation of hydroxyl groups [20]. As a result, anthocyanin's structure altered and shifted in color when compared with its initial color. Liang et al. [19], Eze et al. [24], and Hashim et al. [25] reported similar observations for tracking shrimp freshness using chitosan film incorporated with broken riceberry anthocyanin, an agar and methylcellulose film containing purple cauliflower anthocyanin, and funoran films incorporated

Table 2

Changes in total color difference (ΔE) of colorimetric indicator films in response to volatile compounds according to shrimp spoilage during storage at temperatures of 4* and 25** °C.

Films	Storage time (days)*				
	0	2	4	6	8
CMC/AK	0.00^{e}	11.40 ± 0.33^{d}	13.46 ± 1.02^{c}	25.16 ± 1.06^{b}	35.47 ± 2.16^{a}
CMC/AB	0.00^{e}	24.70 ± 2.28^{d}	44.41 ± 0.58^{c}	46.56 ± 0.98^{b}	49.20 ± 0.81^{a}
CMC/AK75/AB25	0.00^{e}	13.75 ± 0.86^{d}	26.26 ± 4.87^{c}	38.30 ± 0.62^{b}	45.47 ± 0.65^{a}
Films	**Storage time (h)****				
	0	6	12	24	30
CMC/AK	0.00^{d}	11.46 ± 0.79^{c}	13.22 ± 3.03^{c}	42.19 ± 1.20^{b}	45.51 ± 0.90^{a}
CMC/AB	0.00^{d}	11.87 ± 2.17^{c}	47.88 ± 1.56^{b}	53.66 ± 0.63^{a}	54.25 ± 1.71^{a}
CMC/AK75/AB25	0.00^{e}	8.95 ± 0.51^{d}	32.51 ± 3.18^{c}	52.24 ± 0.17^{b}	56.80 ± 0.83^{a}

Values are given as mean ± SD (n = 5).

Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 3
Changes in pH, TVB-N, and TVC analysis of shrimp during storage at temperatures of 4* and 25** °C.

Parameters	Storage time (days)*				
	0	2	4	6	8
pH	6.61 ± 0.03^{e}	6.90 ± 0.03^{d}	7.23 ± 0.10^{c}	7.36 ± 0.07^{b}	7.52 ± 0.07^{a}
TVB-N (mg/100 g)	1.66 ± 0.43^{d}	11.36 ± 0.86^{c}	19.29 ± 0.48^{b}	22.72 ± 1.02^{b}	35.98 ± 5.26^{a}
TVC (log CFU/g)	3.80 ± 0.03^{b}	3.74 ± 0.17^{b}	4.18 ± 0.48^{b}	4.48 ± 0.28^{b}	5.75 ± 0.76^{a}
Parameters	Storage time (h)**				
	0	6	12	24	30
pH	6.61 ± 0.03^{e}	6.91 ± 0.02^{d}	7.02 ± 0.03^{c}	7.27 ± 0.02^{b}	8.14 ± 0.08^{a}
TVB-N (mg/100 g)	1.66 ± 0.43^{e}	6.55 ± 1.21^{de}	14.23 ± 1.86^{c}	57.49 ± 6.96^{b}	72.72 ± 7.32^{a}
TVC (log CFU/g)	3.80 ± 0.03^{d}	5.57 ± 0.19^{c}	7.37 ± 0.13^{b}	7.79 ± 0.14^{a}	7.88 ± 0.12^{a}

Values are given as mean ± SD of triplications.
Different superscripts in the same row indicate significant differences ($p < 0.05$).

with *Ophiopogon japonicus* seed anthocyanins, respectively. This study suggests that among the three films, the CMC/AK75/AB25 indicator film has potential for use as a visual indicator to track shrimp freshness.

3.2. pH changes in shrimp

Table 3 presents the pH value of the shrimp samples during storage at different temperatures and times (4 °C for 8 days and 25 °C for 30 h). The pH of shrimp samples significantly increased during storage ($p < 0.05$). At the beginning of the storage period, the shrimp samples initially had a pH of 6.61 (for days 0 and 0 h). The pH value continuously increased to 7.52 on day 8 at a temperature of 4 °C and to 8.14 after storage at a temperature of 25 °C for 30 h. The rise in pH over time indicates the level of spoilage in shrimp. This is because basic compounds like TMA, DMA, and NH_3 are produced due to autolytic and microbial reactions [26]. According to Mohammadalinejhad et al. [3], shrimp that had a pH higher than 7.6 were not acceptable. Despite other values exceeding the standard, the pH value of shrimp stored at 4 °C for 8 days was acceptable in this study. However, in the shrimp sample that was stored at 25 °C, the pH value exceeded 7.6 at 30 h. This is consistent with Romruen et al. [5], who found that after 24 h of storage (25 °C), the pH of shrimp changed from 6.74 to 7.65. Tavassoli et al. [11] and Mohseni-Shahri and Moeinpour [18] found that the pH values of shrimp were in the range of 7.26 (0 h)–7.90 (48 h) and 6.96 (day 0)–8.50 (day 10) when held at 25 °C for 48 h and 4 °C for 10 days, respectively.

3.3. TVB-N of shrimp during storage

Total volatile basic nitrogen (TVB-N) refers to low molecular weight substances and alkaline compounds, particularly TMA, DMA, and NH_3, which produce off-flavors and sensory rejection during the deterioration process. The TVB-N level is a typical index to assess the freshness or spoilage of shrimp during storage. Table 3 presents the TVB-N content of shrimp at two different storage temperatures and times. The initial TVB-N level of fresh shrimp was 1.66 mg/100 g, which indicated that the shrimp were fresh. On day 8 of storage in the refrigerator (4 °C), the TVB-N level gradually increased from 1.66 to 42.05 mg/100 g. The TVB-N level gradually increased from 1.66 to 78.13 mg/100 g for 30 h of storage at room temperature (25 °C). Rukchon et al. [27] found that as the temperature rose, the TVB-N's vapor pressure increased more quickly. The increases in the TVB-N levels were coincidental with increases in the TVC count due to microbial growth (Table 3). At the index of spoilage, shrimp were deemed unsuitable for consumption due to the presence of an off-flavor and the TVB-N level being above the acceptable limit on day 8 at 4 °C (35.98 mg/100 g) and at 25 °C for 24 h (57.49 mg/100 g). According to Lannelongue et al. [28], the TVB-N acceptability ratings for raw shrimp are as follows: fresh (less than 12 mg N/100 g), edible but somewhat decomposed (between 12 and 20 mg N/100 g), borderline (between 20 and 25 mg N/100 g), and decomposed and inedible (more than 25 mg N/100 g).

3.4. TVC changes in shrimp

Table 3 presents the TVC changes in shrimp at two different storage temperatures and times. During storage, microorganisms produce the metabolites responsible for off-flavors and off-odors, which ultimately cause sensory rejection. At the beginning of the storage period, the initial TVC of the shrimp was 3.80 log CFU/g. As storage time increased, the TVC of shrimp during storage at 4 °C reached 5.75 log CFU/g on day 8, which did not exceed the limit of acceptability, and 7.88 log CFU/g at 25 °C for 30 h. In general, the acceptable limit on bacterial count in shrimp is 7 log CFU/g [2]. TVC subsequently increased, accompanied by TVB-N, during storage for both conditions. This is consistent with Bao et al. [29], who reported that the TVC value of fresh shrimp was in the range of 3.16 (0 h)–6.33 (36 h) log CFU/g at 4 °C, which correlated with the TVB-N value (39.20 mg/100 g) at the storage time of 36 h. They suggested that the shrimp had completely spoiled.

3.5. Correlation between ΔE values of the colorimetric indicator films and shrimp freshness

The correlation analysis between ΔE values of the CMC/AK, CMC/AB, and CMC/AK75/AB25 indicator films and shrimp freshness at temperatures of 4 and 25 °C is shown in Figs. 2 and 3, respectively. In both storage conditions, it showed a positive correlation between ΔE values and TVB-N/TVC. At refrigerator (4 °C), the correlation between ΔE values of the CMC/AK, CMC/AB, and CMC/AK75/AB25 indicator films and TVB-N/TVC was $R^2 = 0.9513$ and 0.8184, $R^2 = 0.8146$ and 0.4495, and $R^2 = 0.9450$ and 0.7152, respectively. At room temperature (25 °C), the correlation between ΔE values of the CMC/AK, CMC/AB, and CMC/AK75/AB25 indicator films and TVB-N/TVC was $R^2 = 0.9662$ and 0.7147, $R^2 = 0.6475$ and 0.9604, and $R^2 = 0.8743$ and 0.8904, respectively. From these results, all indicator films provided a good indication of the shrimp deterioration process. However, when considering the color variations in the colorimetric indicator film, the CMC/AK75/AB25 indicator film was visible and could be detected by the human naked eye, which is correlated with the shrimp freshness. Similar observations for freshness monitoring of shrimp have been reported

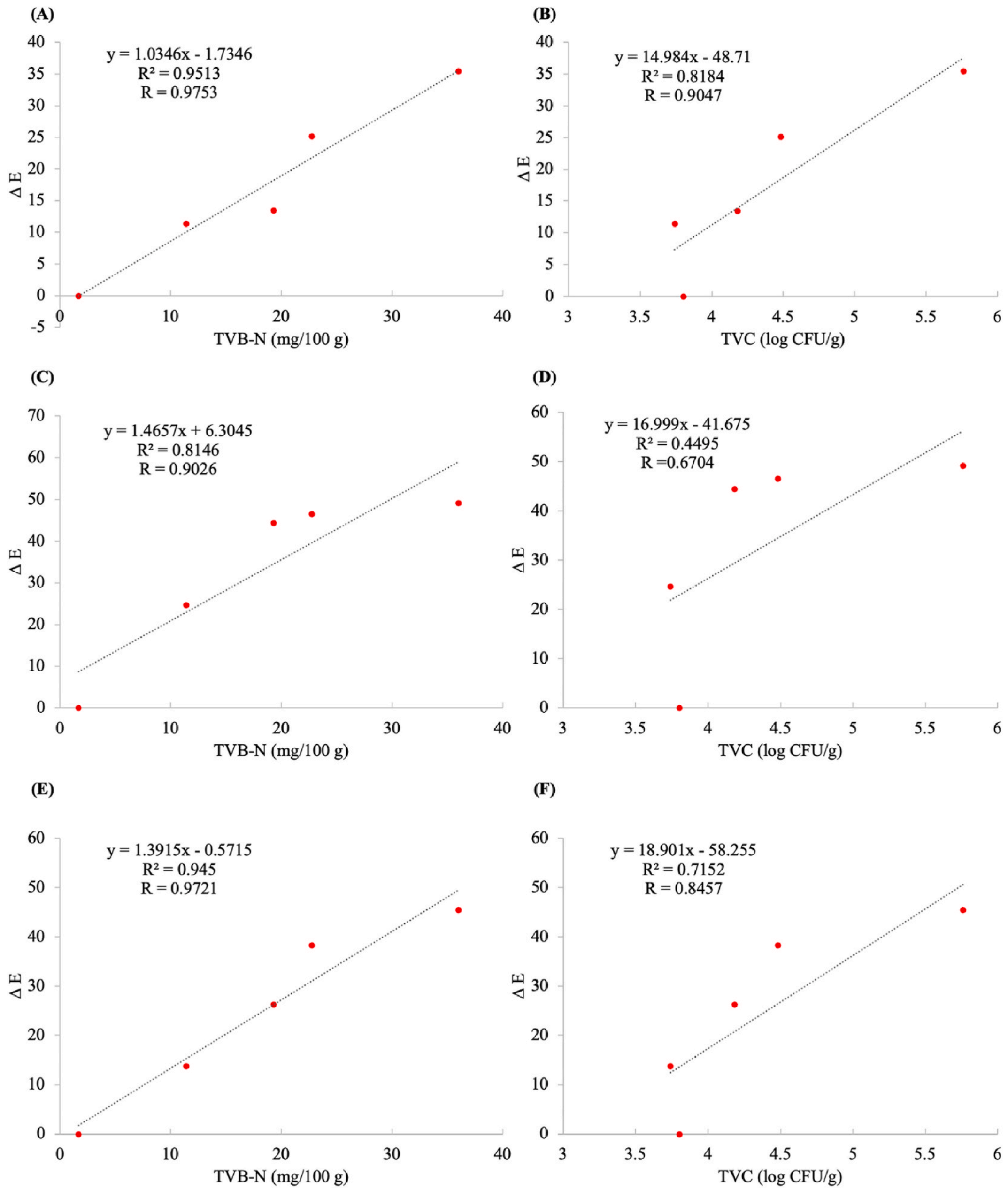

Fig. 2. The correlation between TVB-N, TVC, and ΔE values of the colorimetric indicator films at 4 °C: (A–B) CMC/AK film (C–D) CMC/AB film (E–F) CMC/AK75/AB25. TVB-N: total volatile basic nitrogen; TVC: total viable count; ΔE: total color difference.

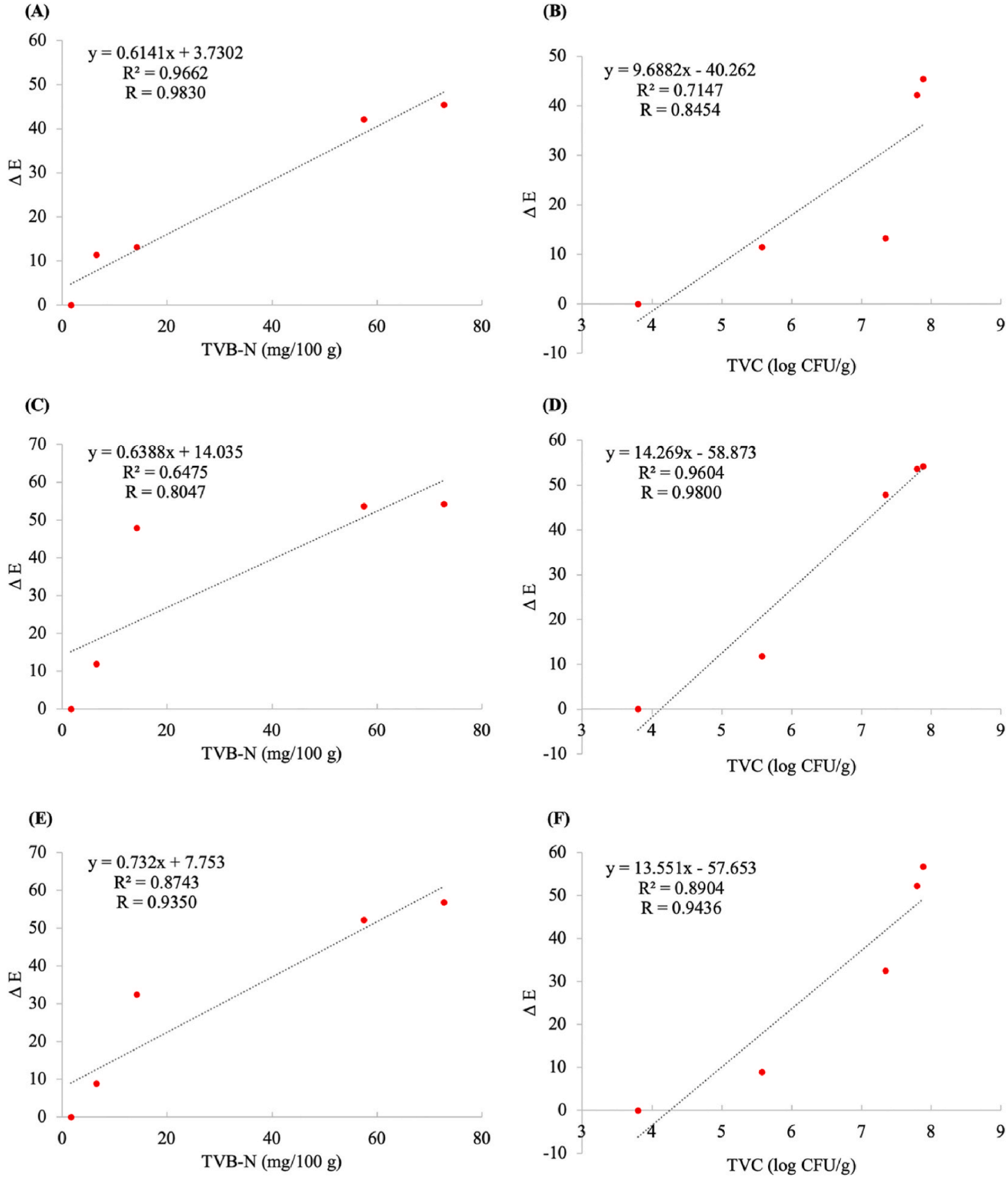

Fig. 3. The correlation between TVB-N, TVC, and ΔE values of the colorimetric indicator films at 25 °C: (A–B) CMC/AK film (C–D) CMC/AB film (E–F) CMC/AK75/AB25. TVB-N: total volatile basic nitrogen; TVC: total viable count; ΔE: total color difference.

for the bacterial cellulose films containing anthocyanin from *Echium amoenum* (correlation between ΔE and TVB-N/TVC; $R^2 = 0.8077$ and 0.9892, respectively) [3], the starch/blueberry anthocyanin/chondroitin sulfate films (correlation between ΔE and TVB-N/TVC; $R^2 = 0.9728$ and 0.8707, respectively) [29], and the carrageenan film mixed natural dyes from edible plants *Clitoria* sp. and *Brassica* sp. (correlation between ΔE and TVB-N/TVC; $R^2 = 0.5271$ and 0.8856, respectively) [30].

3.6. Validation of the prediction model

The solution of Equation (1) by Yang et al. [16] under constant temperature was compared to actual data as shown in Fig. 4. There was fairly good agreement between the prediction and reported data, with a slight underprediction of microbial. Both the bias factor (BF) and accuracy factor (AF) were slightly close to 1.0 (Table 4), showing the adequateness of the model [31]. The mean square error (MSE) value was 0.15, indicating a pretty good prediction by model [32] (Table 4). As a result, our work extends the prediction model to the microbial changes in fresh seafood. However, in the commercialized application of the fresh shrimp prediction model, factors

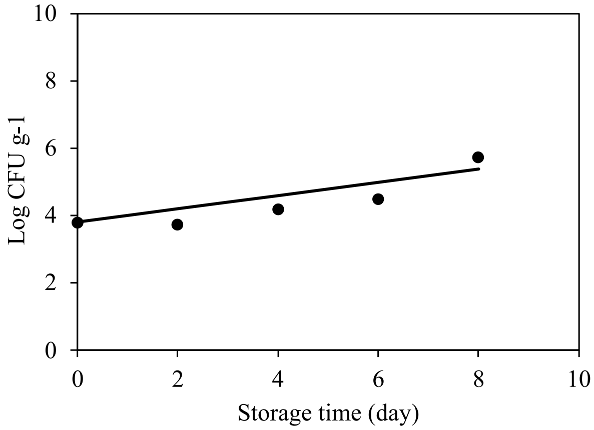

Fig. 4. Comparison of predicted microbial counts and actual microbial counts under 4 °C. Circular filled dots are actual data and solid lines are predicted ones.

Table 4
Evaluation of the microbial change prediction of fresh shrimp under constant temperature conditions.

BF	AF	MSE
1.05	0.92	0.15

such as maturity, species, seasonality, harvesting, etc., which are known to significantly affect the freshness kinetic of shrimp, need to be considered along with the uncertainty problem [33–36].

4. Conclusion

In this study, the CMC/AK, CMC/AB, and CMC/AK75/AB25 indicator films have been used to track shrimp freshness based on volatile alkaline compounds, particularly ammonia and trimethylamine, in the headspace of shrimp packages during storage at different temperatures and times (4 °C for 8 days and 30 °C for 30 h). During the storage of shrimp, the pH values, TVB-N levels, and TVC counts significantly increased throughout the storage time. The quality of shrimp samples that were stored at 30 °C rapidly changed compared to those stored at 4 °C. Furthermore, the color response of the colorimetric indicator films is temperature–dependent. The CMC/AK75/AB25 indicator film was the one with the most sensitive detection and clearly changed color after reacting with metabolites. It can be indicated the three different shrimp deterioration processes as color changes: dark purple in the shrimp is fresh, purplish gray or gray in the shrimp can still be consumed even though the quality has decreased (semi-fresh), and olive green or brown at the spoilage. Of these, the CMC/AK75/AB25 indicator film is regarded as the most applicable shrimp freshness indicator because it showed a distinct color change, and the response of the indicator film had a positive correlation with the shrimp deterioration process.

Data availability statement

The data will be made available on request.

CRediT authorship contribution statement

Pimonpan Kaewprachu: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chalalai Jaisan:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Saroat Rawdkuen:** Writing – review & editing, Supervision, Resources. **Kazufumi Osako:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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