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Development of Semolina Starch/Agar-Based Intelligent Films by Incorporating Butterfly Pea Flower Anthocyanins to Monitor the Freshness of Prawns

(Pembangunan Filem Pintar Berasaskan Kanji Semolina/Agar dengan Menggabungkan Antosianin Bunga Telang untuk Memantau Kesegaran Udang)

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ABSTRACT

The natural plant-derived pH indicator film which monitors food freshness during storage has gained attention among researchers due to consumer awareness of food quality, freshness, and food safety. This study aims to develop a semolina/agar-based film (S/A) incorporated with anthocyanins from butterfly pea flower extract (BPE) as a freshness indicator for tiger prawns and freshwater prawns during 4 days of storage at 4±1 °C. The semolina/agar-based butterfly pea extract (S/A/BPE) films were developed with different concentrations of BPE (3, 6, and 9%) by casting method. Increasing BPE up to 9% in the S/A film increased the thickness and moisture content, lowering the films' water permeability. The film also had a smoother surface structure with a darker colour. Different pH solutions (pH 2.0-12.0) also changed the colour of films, depending on the BPE concentrations. The application of S/A/BPE films on tiger prawns and freshwater prawns caused colour changes from dark blue at day 0 (fresh stage) to intense green at day 2 (beginning of spoilage) indicating the spoilage of samples. The pH values of both prawns were gradually increased. However, no major changes were observed in the texture profile analysis. These results indicate that S/A/BPE film has the potential to be used as a pH indicator film to detect the freshness of prawn samples.

Keywords: Butterfly pea extract; freshwater prawns; pH indicator film; semolina; tiger prawns

ABSTRAK

Filem penunjuk pH daripada tumbuhan semula jadi yang memantau kesegaran makanan semasa penyimpanan telah mendapat perhatian dalam kalangan penyelidik kerana kesedaran pengguna tentang kualiti makanan, kesegaran dan keselamatan makanan. Kajian ini bertujuan untuk menghasilkan filem berasaskan semolina/agar (S/A) yang digabungkan dengan antosianin daripada ekstrak bunga telang (BPE) sebagai penunjuk kesegaran udang harimau dan udang galah selama 4 hari penyimpanan pada suhu 4±1 °C. Filem ekstrak bunga telang berasaskan semolina/agar (S/A/BPE) telah dihasilkan dengan kepekatan BPE (3, 6 dan 9%) yang berbeza dengan kaedah tuangan. Peningkatan BPE sehingga 9% dalam filem S/A meningkatkan ketebalan dan kandungan lembapan, menurunkan kebolehtelapan air bagi filem. Filem ini juga mempunyai struktur permukaan yang lebih halus dengan warna yang lebih gelap. Larutan pH yang berbeza (pH 2.0-12.0) juga mengubah warna filem, bergantung pada kepekatan BPE. Penggunaan filem S/A/BPE pada udang harimau dan udang galah menyebabkan perubahan warna daripada biru tua pada hari 0 (segar) kepada hijau pekat pada hari ke-2 (permulaan kerosakan) yang menunjukkan kerosakan sampel. Nilai pH kedua-dua udang telah meningkat secara beransur-ansur. Walau bagaimanapun, tiada perubahan besar diperhatikan dalam analisis profil tekstur. Keputusan ini menunjukkan bahawa filem S/A/BPE berpotensi untuk digunakan sebagai filem penunjuk pH untuk mengesan kesegaran sampel udang.

Kata kunci: Ekstrak bunga telang; penunjuk pH; semolina; udang galah; udang harimau

Introduction

In recent years, consumers are aware of fresh food, food quality, and food safety in preventing the occurrence of foodborne illness. This is where intelligent packaging emerges as a captivating approach that has garnered significant attention from researchers. Intelligent packaging, also known as smart packaging, can monitor food's freshness during storage, food deterioration, and microbial contamination, and improve food quality (Kim, Roy & Rhim 2022; Yan et al. 2021). Generally, a pH indicator film comprises a pH-sensitive dye, which can either be artificially synthesized or derived from natural sources. Synthetic dyes such as methyl red or chlorophenol have been widely used in colourimetric indicator film to detect the pH, however, it is toxic and harmful when in contact with food products (Bhargava et al. 2020). Thus, recent research focuses on natural dyes extracted from plants that are rich in pigments containing anthocyanin, chlorophyll, carotenoids, curcumin, betalains, and tannins (Rahim et al. 2020). The natural dyes are environmentally friendly and a safe source to detect pH changes from food spoilage (Hasanah et al. 2023; Hashim et al. 2022).

Anthocyanins, the largest water-soluble pigments, belong to flavonoids that contribute to the purple, orange, violet, and red colours of flowers, fruits, and vegetables. These pigments not only serve as pH indicators but also play crucial roles in processes like pollination and protection against UV radiation in flowers (Vidana Gamage et al. 2021). Their chemical structure undergoes variations in response to pH changes, resulting in a spectrum of colours. This property makes them highly valuable as natural pH indicators in various applications, including intelligent packaging. (Boonsiriwit et al. 2021). Butterfly pea flower (*Clitoria ternatea* L.) is rich in polyacrylate anthocyanins, which is responsible for blue colour anthocyanin extract that changes colour in different pH conditions (Vidana Gamage et al. 2021). The anthocyanins can be extracted using organic solvents like ethanol or water combined with conventional and non-conventional extraction methods such as maceration and ultrasound-assisted extraction (UAE), respectively, to maximize the yield of the extract.

A solid matrix is important to immobilize with the natural dye, whereas starch can act as a biopolymer matrix in a film. Most biopolymers are non-toxic, have low water sensitivity, have good film-forming properties, and can capture pH-responsive pigments (Chawla, Sivakumar & Kaur 2021; Halász & Csóka 2018). Semolina starch is a polysaccharide and a type of wheat that has a higher amount of gluten content made up of complex carbohydrates and proteins that allow it to produce a cohesive film (Jafarzadeh et al. 2018). Semolina grain is translucent, extra hard, and light-coloured. However, the semolina-based film has a poor water barrier and low physical strength (Jafarzadeh et al. 2017). The addition of agar into starch-based films could help to immobilize the starch and create a strong inter-molecular interaction that enhances the physical and chemical linking. Agar is a heterogeneous complex mixture composed of linear polysaccharides of gelling fraction agarose with non-gelling charged polysaccharides, called agaropectin (Mostafavi & Zaeim 2020). It also has good gelling and film-making properties (Choi et al. 2017; Roy, Rhim & Jaiswal 2019). A specific ratio of starch to agar demonstrates enhanced mechanical properties and increased hydrophilicity in the films (Guo et al. 2021).

The food products produced metabolites such as volatile amines and microbes due to deterioration at different storage conditions (Bhargava et al. 2020). Usually, the spoilage of seafood is mainly caused by volatile chemical release during seafood deterioration. Prawn generates total volatile basic nitrogen (TVB-N) such as ammonia, dimethylammonium and trimethylamine, as well as microbial spoilage during deterioration (Kang et al. 2020). The volatile compounds can be detected by pH-sensing colourimetric film through visible colour changes.

Semolina starch/agar-based film has the potential to be used as a biopolymer film, but the properties of the film as a pH indicator have not yet been studied. Hence, it is our interest to study the potential of semolina-based film as a pH indicator film. Moreover, this study also focuses on tiger prawns and freshwater prawns that may have different resistance against spoilage. This study also aims to incorporate anthocyanins from BPE that can be immobilized in semolina/agar-based film (S/A/BPE) as a natural dye. Subsequently, the S/A/BPE film will be employed for assessing the freshness of prawns during storage at 4 °C. The development of intelligent films for monitoring prawn freshness could potentially improve food safety, reduce waste, and enhance product quality for the food industry while offering researchers an exciting avenue for exploration and innovation in food packaging and biotechnology.

Materials and Methods

Materials

The fresh butterfly pea flower was collected from the farm in Mantin (Negeri Sembilan, Malaysia). Semolina flour and agar were obtained from Choconis Bakery Ingredients Home (Mantin, Negeri Sembilan, Malaysia) while freshwater and tiger prawns were purchased from Seri Kembangan wet market, Selangor, Malaysia. The potassium dihydrogen phosphate anhydrous (KH_2PO_4), potassium hydrogen phthalate ($C_8H_5KO_4$), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Chemiz (Selangor, Malaysia). Other chemicals and solvents used were of analytical grade bought from Sigma-Aldrich (UK).

Preparation of Butterfly Pea Extract (BPE)

Ultrasonic-assisted extraction was carried out to extract the anthocyanin from BPE according to Zahari et al. (2020). The extraction process involved macerating approximately 15 flower petals in a 200 mL solution of 50% ethanol. The ultrasonic probe (Q500 QSonica Sonicator, USA) was immersed directly into the solvent containing the BPE and was sonicated for 40 min at 40 °C with 160W. The solution was vacuum-filtered using a Buchner funnel and Whatman No. 1 filter paper (Whatman, Buckinghamshire, UK), and the extract was freeze-dried in a freeze-dryer (Labconco, Kansas City, Missouri) for 48 h. The obtained freeze-dried BPE was stored in a desiccator until further use.

UV-Vis Spectroscopy of BPE

The UV-vis spectroscopy was carried out by following the methods by Chen et al. (2020) and Choi et al. (2017) with slight modifications and using Thermo Genesys IOS UV-Vis (Thermo Fisher, USA). Four mL of the pH buffers ranging from pH 2 to 12 were reacted with the 1 mL BPE solution and were measured in the range of 400-700 nm.

Film Preparation

The semolina/agar-based colour indicator film was fabricated using the casting method. Two grams of semolina and agar were weighed separately and dissolved in 200 mL of distilled water. Then, 30% glycerol (w/w film base) was added and the film-forming solution was stirred continuously at 90 °C for 1 h to allow gelatinization. The solution was cooled to 50 °C before incorporating the BPE (3, 6, and 9% w/w film base) and was stirred for 20 min (Kim, Roy & Rhim 2022). The mixed solution was cast onto a petri dish of approximately 30 mL and dried in air dried oven (Dynamic Oven Binder, Tuttlingen, Germany) for about 55 °C for 6 h (Koshy et al. 2022). The same procedure was repeated for control without the addition of BPE.

Thickness of the Film

The thickness of the film was measured by using a digital micrometre (547-401, Mitutoyo, Japan). Five random positions on the film were measured and the results were expressed as the average of films in mm.

Moisture Content

The moisture content of the films was determined using a moisture analyser (SHS and MX-50, Japan) by cutting a square shape (2 cm × 2 cm) and drying the film until constant weight. The analyses were carried out in triplicate and results were expressed as the average % of the moisture content.

Water Vapour Permeability (WVP)

The WVP of films was carried out with some modifications according to Mary et al. (2020) and Zhang et al. (2020). Each crucible was filled with 6 mL of distilled water and the openings of the crucible were covered by the films. Then, the crucibles were placed in a desiccator with a relative humidity of 50 ± 5 % at 25 ± 5 °C. The weight of the crucible was measured and recorded every 1 h for 8 h. The WVP was measured with three replicates and calculated according to the following equation:

$$\mathrm{WVP} = \frac{\Delta W \times L}{t \times A \times \Delta P}$$

where ΔW is the weight change (g) of the crucible after 8 h; time taken (s); L is the film thickness (m); A is the permeation area (m^2); and ΔP is the partial water vapour pressure difference (Pa) across the film.

Colour Measurement of pH Indicator Film

Each film was evaluated using a CR-410 chromameter (Konica Minolta, Inc., Japan). The results were expressed as L^* (lightness), a^* (redness), and b^* (yellowness) parameters to evaluate the colour changes of the film at different pH buffers (2.0–12.0). The total colour

difference (ΔE) was calculated according to the following equation (Yan et al. 2021):

$$\Delta E = \sqrt{(L* - L)^2 + (a* - a)^2 + (b* - b)^2}$$

where $L*$, $a*$, and $b*$ are the colour values of the standard white plate.

COLOUR RESPONSE OF THE FILMS TO DIFFERENT pH

The films were cut into a square shape (2 cm × 2 cm) and then immersed in 7 mL of each buffer solution between pH 2.0 and 12.0 for 15 min. The films were removed from the buffer and were photographed.

MICROSTRUCTURE OF THE FILM

The scanning electron microscope (SEM) (JSM 6400, Jeol, Tokyo, Japan) was used to observe the surface microstructure of the films. The films were mounted onto a stub and were sputtered with gold using a Sputter Coater SCD 005 (BAL-TEC AG, Balzers, Liechtenstein) before being visualized at 500× magnification.

APPLICATION OF S/A/BPE FILMS TO MONITOR THE FRESHNESS OF THE PRAWNS

The S/A/BPE 3%, 6%, and 9% films were used to monitor the freshness of both tiger prawns and freshwater prawns. The films were cut into a square (2 cm × 2 cm) and attached to the lid of the container (10 cm × 10 cm × 4.5 cm). Each container contains 1 sample (approximately 30 g) of whole fresh prawn. The samples were stored in a chiller (4 ± 1 °C) for 4 days where all samples were analysed every 24 h. The prawn samples were evaluated for their texture, pH values and appearance by the naked eye. For pH measurements, the prawn was homogenized with distilled water completely for about 5 min using a homogenizer (Heidolph Silent Crusher, Schwabach, Germany). Whereas the colour changes of the pH indicator film were evaluated using a chromameter (Husin et al. 2020).

TEXTURE PROFILE ANALYSIS (TPA) OF PRAWN

The texture profile analysis was carried out using texture analyzer XT plus (Stable Micro System Ltd., Godalming, Surrey, UK) with some modifications according to Wu, Sun and He (2014). The prawn samples were cut into small pieces from different locations and were compressed about 70% from their original shape at 115 mm/min speed with a 120 N compression load by using a cylindrical-shaped probe. The results were expressed as hardness (kg), cohesiveness, springiness (mm), gumminess (kg), and chewiness (kg) of the prawn samples. This analysis was carried out throughout the 4 days of storage.

STATISTICAL ANALYSIS

The results from multiple samples were reported using one-way analysis of variance (ANOVA) from Minitab version 21 using the average values ± standard deviation. The analysis was carried out in triplicate. The significance of the values was according to the Tukey test defined at ($p<0.05$).

RESULTS AND DISCUSSION

COLOUR RESPONSE OF THE BUTTERFLY PEA EXTRACT (BPE)

Colour variations of BPE in different buffer solutions (pH 2-12) were measured, as presented in Figure 1. Colour changes in the BPE were observed when it was tested under acidic, neutral, and alkaline conditions. The colour of the BPE exhibited a slight pink hue at low acidity (pH 2). As the pH increased to pH 3, pH 5, and pH 7-8, the colour shifted progressively to violet, blue, and bluish-green, respectively. The colour changes from purple to blue were due to the structural transformation of anthocyanins from a purple quinoidal anhydrous base to a deep blue ionized anhydrous base (Yan et al. 2021). At high alkalinity, the BPE was greenish blue (pH 9) and green in colour (pH 10-12). In this study, the colour response of BPE in buffer solutions was comparable to the research reported by Boonsiriwit et al. (2021), Hashim et al. (2022), and Mary et al. (2020).

CHARACTERIZATION OF SEMOLINA/AGAR-BASED (S/A/BPE) FILMS

The thickness, moisture content (MC) and water vapour permeability (WVP) of the film were measured (Table 1). Results showed that there was no significant ($p < 0.05$) difference observed when added with different concentrations of anthocyanin. This was due to the BPE extract that was well distributed in the semolina/agar matrix (Yong & Liu 2020). As we can see the trend, the thickness gradually increased when the percentage of the extract was increased up to 9%. An increase in film

thickness resulted in a larger free-volume network, which was influenced by the disruption and restructuring of the intermolecular polymer chain networks. This effect was primarily caused by the plasticizing action of glycerol and the presence of anthocyanin (Sanyang et al. 2015).

The moisture content (MC) of a film represents the total free volume occupied by water molecules in the film network. The MC % was recorded lowest for control films (S/A 0%) which was significantly ($p > 0.05$) lower than films with different concentrations of BPE. Notably, increasing BPE content to 9% increases the moisture content of the S/A film due to the availability of hydroxyl groups from the interaction between water molecules within the S/A film matrix (Lam et al. 2020), resulting in increased moisture content of the fabricated films.

Water vapour permeability (WVP) is an important barrier to measuring the moisture transfer of polymeric materials. It was also able to determine the quality of packaging film to protect the food products that can enhance the food's shelf life (Hashim et al. 2022). There was no significant ($p < 0.05$) difference between all films, but it was observed that S/A film showed a decreasing trend in WVP values after the incorporation of BPE compared to the control film. Limited availability of the free hydroxyl group to interact with the free water molecules was caused by the interaction between hydrogen and covalent bond of the semolina starch-agar as well as the anthocyanin pigment, which led to a decrease in hydrophilicity of the films (Mary et al. 2020). Similar findings were reported by Sohany et al. (2021) whereby the WVP of the sweet potato starch films decreased gradually when increasing the commercial anthocyanin loads from 1 to 2%.

COLOUR PROPERTIES OF FILMS

Colour is an important parameter for a pH indicator film to monitor the freshness of a food product. Table 2 shows the L^*, a^*, b^* and ΔE values for the S/A films. The L^* value (lightness) of the film significantly ($p< 0.05$) decreased due to the addition of BPE extract, making the films appear darker compared to the control film. The a^* (red/green) value significantly ($p<0.05$) increased from 1.39 to 14.9, leaning towards red. The b^* (yellow/blue) value significantly ($p<0.05$) decreased from -0.659 to -36.897, indicating darker bluish films. The ΔE of semolina/agar/BPE starch film increased with the increase in BPE content. According to Mohammadalinejhad, Almasi and Moradi (2020), the ΔE value must be greater than 5 to see a visible colour by the naked eye.

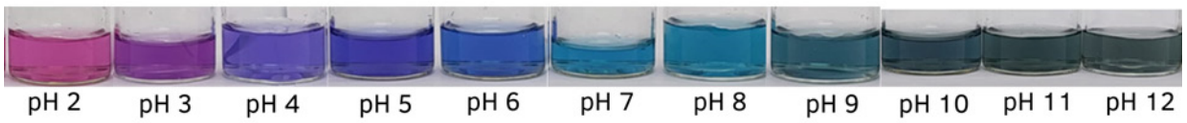


FIGURE 1. BPE in different buffer solutions

TABLE 1. The thickness, moisture content and water vapour permeability of the films

Film	Thickness (mm)	Moisture content (%)	WVP ($\times 10^{-8}$ g.m.P^{-1}. s^{-1})
S/A 0%	0.082 ± 0.084^a	15.30 ± 1.05^b	8.23 ± 1.44^a
S/A/BPE 3%	0.076 ± 0.008^a	14.53 ± 2.91^b	6.94 ± 0.87^a
S/A/BPE 6%	0.091 ± 0.019^a	20.83 ± 0.52^a	7.48 ± 0.50^a
S/A/BPE 9%	0.094 ± 0.007^a	23.60 ± 1.46^a	6.51 ± 1.18^a

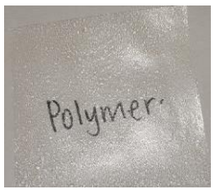
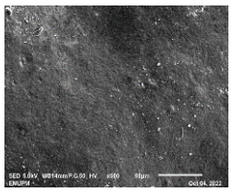
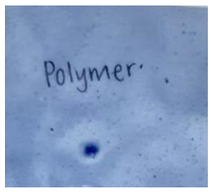
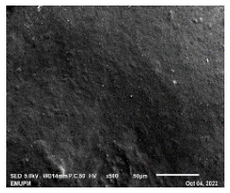
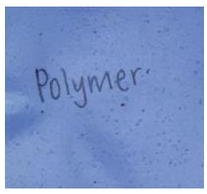
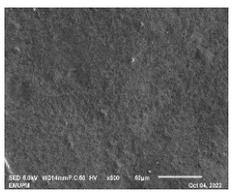
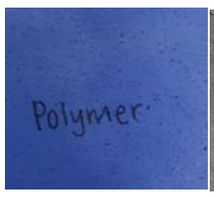
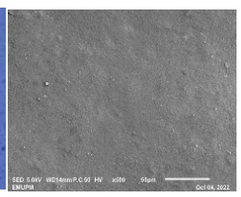
Values are given as mean ± standard deviation. Different superscript letters indicate that the means are significantly ($p<0.05$) different between films. S/A 0% = control, S/A/BPE 3% = film with 3% extract, S/A/BPE 6% = film with 6% extract; S/A/BPE 9% = film with 9% extract

The blue intensity of the film increases with increased concentration of the BPE, where the S/A film (control) is transparent and colourless. The colour parameters were also influenced by the incorporation of phenolic compounds in the BPE extract (Qin et al. 2019). Similar findings were reported by Mary et al. (2020), who observed the blue colour of BPE films gradually deepened as extract content increased.

THE STRUCTURAL SURFACE OF THE S/A FILMS

The microstructure of control, S/A/BPE 3%, S/A/BPE 6 %, and S/A/BPE 9 % films were examined via SEM (Table 2). As shown in the surface image, the S/A film without the extract has a rough surface compared to the film with the extract. The semolina starch granules were still visible at S/A/BPE 3% film which might be caused by the undissolved starch granules. Increasing the concentration of the BPE extract results in a smoother surface, suggesting a more homogeneous blending of the polymer with the anthocyanin extract. Furthermore, as the concentration of anthocyanin increases, the microstructure becomes progressively smoother (Yong & Liu 2020). This was due to the formation of hydrogen bonds of the hydroxyl group from the anthocyanin with the starch and protein molecules within the S/A matrix (Zhai et al. 2018).

TABLE 2. The colour properties, appearance, and microstructure of the surfaces (magnification: 500×) of the films

Film	*L**	*a**	*b**	*ΔE*	Appearance	Microstructure (surface)
S/A 0%	88.19±0.012^{a}	1.39±0.01^{d}	-0.653±0.01^{a}	0		
S/A/ BPE 3%	68.11±0.12^{b}	5.53±0.02^{c}	-22.66±0.08^{b}	30.06		
S/A/ BPE 6%	58.16±0.06^{c}	10.59±0.02^{b}	-31.83±0.06^{c}	44.25		
S/A/ BPE 9%	51.93±0.24^{d}	14.91±0.02^{a}	-36.897±0.08^{d}	53.02		

Values are given as mean ± standard deviation. Different superscript letters indicate that the means are significantly ($p<0.05$) different between each film. S/A 0% = control, S/A/BPE 3% = film with 3% extract, S/A/BPE 6% = film with 6% extract; S/A/BPE 9% = film with 9% extract

COLOUR RESPONSE OF S/A FILMS IN DIFFERENT pH BUFFERS

The colour variation of S/A/BPE films was important to evaluate the sensitivity of the changes of film in different pH. The change of film in different buffer solutions can be used to indicate the spoilage of the food and to design smart packaging as well as colourimetric sensors (Alizadeh-Sani et al. 2020). The colour response of S/A-BPE films containing 3%, 6%, and 9% BPE after being immersed in different buffer solutions (pH 2 -12) is shown in Figure 2. The colour intensity of S/A/BPE films increases with concentration and the colour change of S/A/BPE 9% was darker compared to others.

In some cases, the low content of anthocyanin was insufficient to produce visible colour changes (Yan et al. 2021). The SA/BPE films were dark pink at pH 2. The colour changes to violet at pH 3, then to blue at pH 5-6, and bluish-green at pH 7-8. The films changed towards greenish blue at pH 9 and green at pH 10-12. The colour of the indicator films was in a similar colour range as the BPE extracts in Figure 1. The typical colour variations of the films from pink, violet, and blue to green were comparable to Boonsiriwit et al. (2021) and Kim, Roy and Rhim (2022). However, the colour of the film is slightly different from Mary et al. (2020) due to the extraction method, the solvent used and the properties of semolina and biopolymer that influenced the colour of the film. Overall, the films can be used as a visual indication of food freshness since they can be changed under various pH conditions (Hashim et al. 2022).

APPLICATION OF S/A/BPE AS A FRESHNESS INDICATOR FILM FOR TWO TYPES OF PRAWN DURING STORAGE

Based on Figure 3, the L^*, a^*, b^*, and ΔE of S/A/BPE film for tiger prawn (S/A/BPE-TP) and freshwater prawn (S/A/BPE-FP) had significant changes ($p<0.05$) during 4 days of storage. The L^* and a^* values of S/A/BPE-TP and S/A/BPE-FP films significantly ($p<0.05$) reduced at the end of storage time. Decreasing positive a^* values indicates the film leaning towards green in colour. However, an increase ($p<0.05$) in b^* values on day 4 for all S/A/BPE films containing tiger prawn and freshwater prawn showed a shift towards green and pale blue. Initially, on day 0, the indicator film was blue. Then, the film started to change colour from day 1 onwards which was greenish blue (S/A/BPE-TP) and dark green (S/A/BPE-FP) on the 4th day of storage (Figure 4). The S/A/BPE 9 % film showed distinct colour change due to the high concentration of pigment.

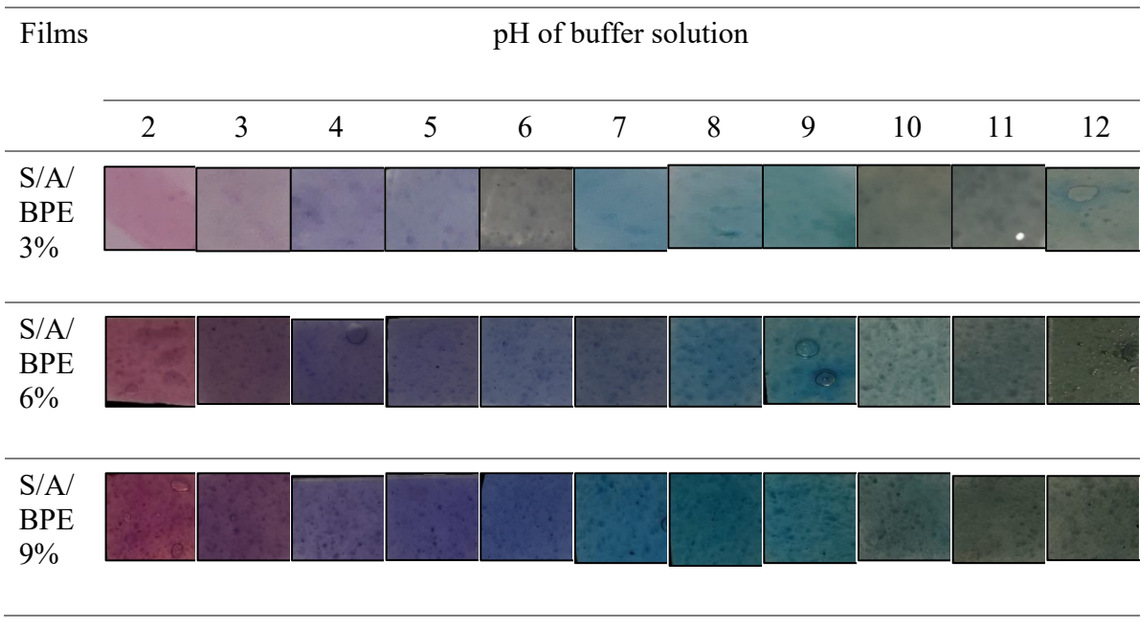


FIGURE 2. Colour response of films in different buffer solutions (pH 2 – 12). S/A/BPE 3% = film with 3% extract, S/A/BPE 6% = film with 6% extract; S/A/BPE 9% = film with 9% extract

The trend of total colour difference (*ΔE*) for all films S/A/BPE decreased after day 3. This is because the film colour changed from dark blue at day 0 (fresh stage) towards dark green (day 4), indicating spoilage for both prawns during storage (Figure 4). The colour changes of the indicator films were stimulated by the volatile compounds formed from spoilage of the prawn (Mary et al. 2020). However, the *ΔE* value trend of S/A/BPE films was inconsistence on day 1, this might be due to the intensity of the dye slightly leaching, where the anthocyanin decreased and then became watery on the surface of the packaging due to water droplets that formed during storage. Overall, the colour changes of the indicator films were stimulated by the volatile compounds formed from spoilage of the prawn (Mary et al. 2020). Similar research was also observed by Hashim et al. (2022), where the colour changes from blue (0 h) to green (24 h) of an indicator film during the application of shrimp. This shows that semolina/agar incorporated with natural anthocyanin extract from BPE can be used to monitor the freshness of food products.

TEXTURE PROFILE ANALYSIS FOR TIGER PRAWN AND FRESHWATER PRAWN DURING STORAGE

The texture profile analysis of tiger prawn and freshwater prawn conducted for 4 days is shown in Table 3. The quality of the prawn was determined by its hardness (maximum force required to compress a sample), cohesiveness (the extent of sample deformation before rupture), springiness (the ability to recover after being compressed), gumminess (the force needed to disintegrate semisolid sample to a ready state before swallowing), chewiness (energy needed to chew a sample to a ready state for swallowing) and resilience (ability of sample to regain its original state from deformation) (Gokoglu et al. 2022).

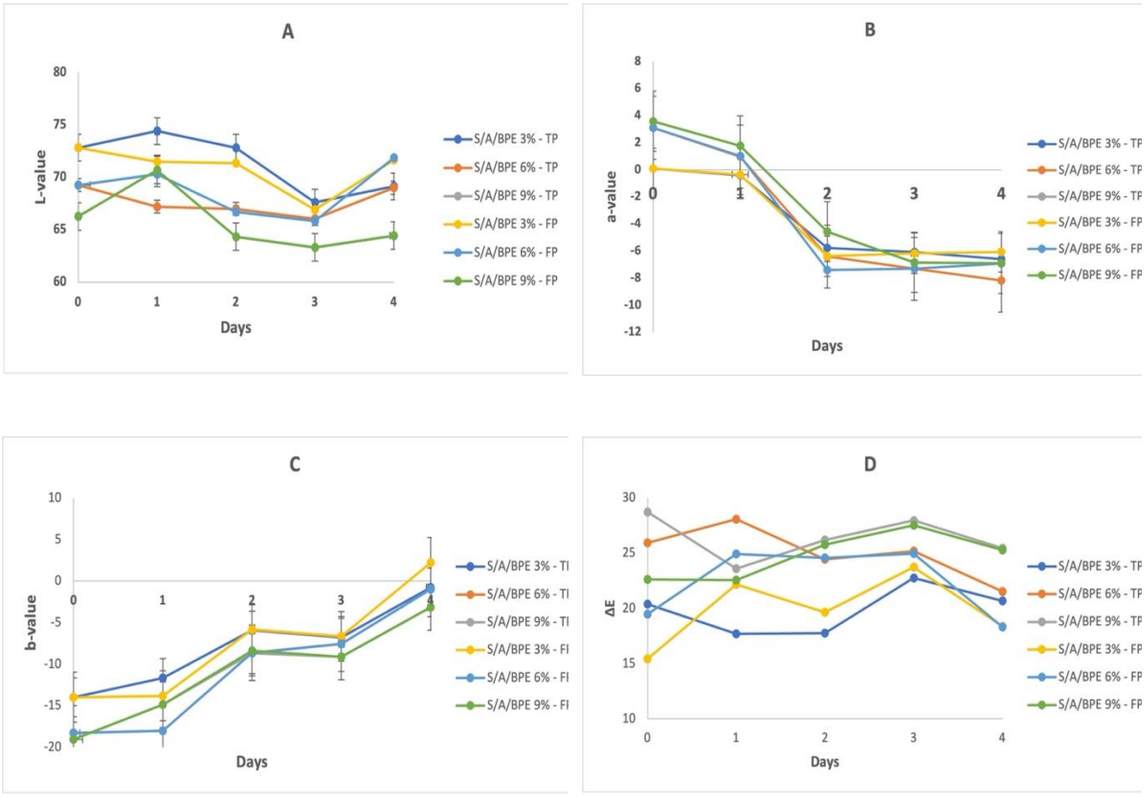


FIGURE 3. (A) *L**, (B) *a**, (C) *b**, and (D) *ΔE* for S/A/BPE film as a freshness indicator of prawn during storage. TP: Tiger prawn; FP: Fresh prawn

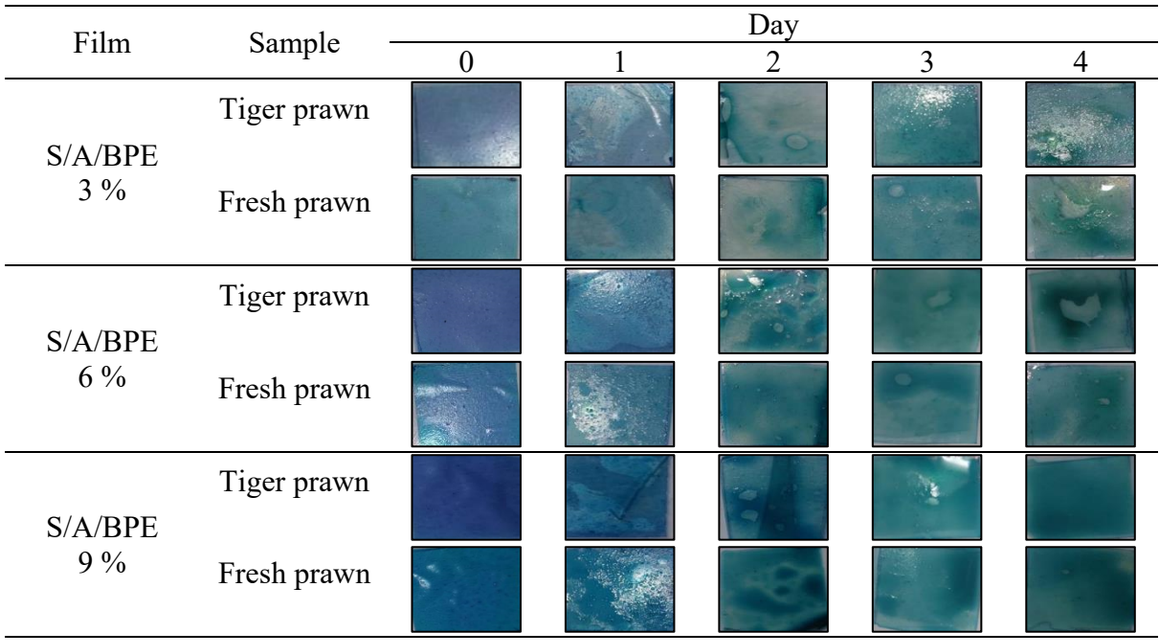


FIGURE 4. The films' colour appearance during 4 days of storage for tiger prawns and freshwater prawns. S/A/BPE 3% = film with 3% extract, S/A/BPE 6% = film with 6% extract; S/A/BPE 9% = film with 9% extract

TABLE 3. Texture Profile Analysis (TPA) for tiger prawn and freshwater prawn during storage

Type	Day	Texture profile analysis					
		Hardness (kg)	Springiness (mm)	Cohesiveness	Gumminess (kg)	Chewiness (kg)	Resilience
Tiger prawn	0	7955±620^{a}	0.225±0.018^{ab}	0.124±0.009^{a}	1080±144^{a}	244±51^{a}	0.064±0.006^{b}
	1	7388±266^{a}	0.260±0.008^{a}	0.149±0.002^{a}	1099±23^{a}	268±38^{a}	0.078±0.003^{ab}
	2	8443±1913^{a}	0.216±0.006^{bc}	0.127±0.003^{a}	1073±219^{a}	216±30^{a}	0.067±0.001^{ab}
	3	9655±1450^{a}	0.239±0.006^{ab}	0.142±0.009^{a}	1565±560^{a}	196±62^{a}	0.073±0.005^{ab}
	4	12740±1780^{a}	0.184±0.003^{c}	0.145±0.006^{a}	2207±142^{a}	351±47^{a}	0.079±0.001^{a}
Freshwater prawn	0	3470±397^{b}	0.176±0.018^{a}	0.164±0.013^{a}	567±21^{ab}	100±7^{ab}	0.090±0.004^{ab}
	1	3915±914^{b}	0.183±0.002^{a}	0.150±0.047^{a}	447±119^{b}	76±14^{b}	0.078±0.003^{b}
	2	6013±504^{ab}	0.177±0.004^{a}	0.125±0.008^{a}	882±77^{ab}	114±43^{ab}	0.067±0.008^{b}
	3	3755±149^{b}	0.184±0.004^{a}	0.133±0.006^{a}	570±106^{ab}	101±13^{ab}	0.066±0.005^{b}
	4	7701±862^{a}	0.194±0.004^{a}	0.199±0.014^{a}	1045±235^{a}	351±47^{a}	0.124±0.009^{a}

Values are given as mean ± standard deviation. Different superscript letters indicate that the means are significantly ($p<0.05$) different between each day for the same type of prawn

Both tiger prawn and freshwater prawn had 37% and 55% increase in hardness at the end of storage. Besides, the overall texture of both tiger prawn and freshwater prawn did not have a significant change although increasing trends in terms of cohesiveness, gumminess, chewiness, and resilience were observed. However, the springiness for tiger prawns had a 22% reduction ($p<0.05$) compared to a 9% ($p>0.05$) increment for freshwater prawns during the last day of storage. The deterioration rate of prawn during storage was influenced by microbial activity, melanosis, proteinase enzyme, lipid and protein oxidation that might affect the textural attributes of the prawn (Lin et al. 2022).

THE pH VALUES FOR TIGER PRAWN AND FRESHWATER PRAWN DURING STORAGE

The pH is an indication of acidity that was correlated with the growth of microbes in seafood. The pH level for both tiger prawns and freshwater prawns increased ($p<0.05$) with prolonged storage days (Figure 5). The initial pH of the tiger prawn is 6.80 and 7.38 for the freshwater prawn. On the last day of storage, the pH increased by 10% and 7%, respectively, for tiger prawn and freshwater prawn. Suratsawadee et al. (2022) and Xu et al. (2019) observed an increase in pH during the storage of shrimp which was influenced by both enzymatic and microbiological activities. The deterioration of the prawn released volatile compounds causing changes in the pH of the sealed headspace. The alkaline volatile nitrogenous compounds caused a structural change of the anthocyanin to the carbinol base resulting in a colour response of the indicator film (Merz et al. 2020).

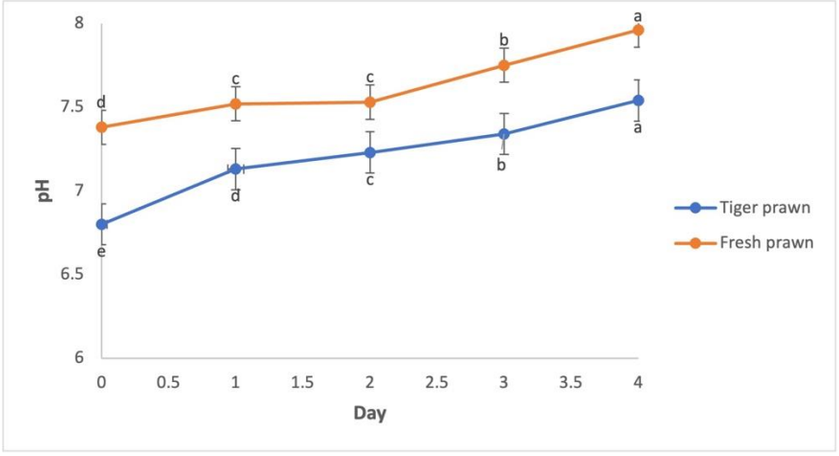


FIGURE 5. The pH values for tiger prawn and freshwater prawn during 4 days of storage at 4 °C

CONCLUSION

In this study, semolina/agar-based (S/A) intelligent films incorporated with the BPE extract were successfully developed. The colour of the BPE extract ranged from red to blue then green at various pH from pH 2.0-12.0. The addition of extract from 3% to 9% increased the thickness and moisture content of the film where a decrease in WVP was observed. The 9% BPE extract and film had a bolder and darker colour when immersed in buffer solution (pH 2-12) compared to 3% and 6%. The appearance of S/A/BPE 9% film was intensely blue and darker compared to the lowest concentration of the extract. The S/A/BPE indicator film changed from blue (fresh stage) to green (spoiled) upon the deterioration of both tiger prawn and freshwater prawn during storage. The relationship between pH and visible changes in the appearance of the prawn was observed. The freshness assessment of both prawns concluded that the spoilage

of prawns started on day 1 according to the slight colour changes of the indicator. Therefore, it can be concluded that the semolina/agar-based BPE films have the potential to serve as environmentally friendly pH indicator films for monitoring the freshness of tiger and freshwater prawns.

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