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Research Article

Development of pH Indicator Film Containing Butterfly Pea Flower (*Clitoria ternatea* L.) Extract for Monitoring Sardines and Catfish Freshness During Chilled Storage

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ABSTRACT

An application of pH indicator film has been utilised in food packaging to ensure quality and safety, as well as monitor the real-time freshness of perishable products; however, previous works have focused on synthetic dyes. This research aimed to develop and determine the physicochemical characteristics of a pH indicator film from potato starch incorporated with butterfly pea anthocyanin extracts (PS-BPE) at different concentrations of BPE (4%, 8% & 12%), and evaluate its potential for monitoring the freshness of sardines and catfish. The films were characterised by microstructure, thickness, moisture content (MC), water vapour permeability (WVP), and colour responses at different pH values (2 - 12). Then, the PS-BPE films were applied to fish to monitor the freshness at 4°C for 6 days of storage. The colour changes, pH, texture profile analysis (TPA), and Quality Index Method (QIM) were analysed every two days of storage. The increase in BPE concentration in the film results in an increased thickness and a decreased MC, with no significant difference in WVP of the film. The colour of PS-BPE films also became significantly darker and bolder with an increase in concentration, and colour changes from purple to blue-green were observed during storage. TPA show no difference between the two fish samples. However, both fish increased in pH and QI scores over time, indicating a decline in the quality and freshness of the fish as the storage period extended. These results suggest that PS-BPE films have the potential to serve as a freshness indicator for fish samples.

Key words: Anthocyanins, butterfly pea extract, fish freshness, fish processing, pH indicator film

INTRODUCTION

Nowadays, food quality and safety have become a significant concern for both consumers and food manufacturers, as they strive to avoid product recalls and food poisoning crises. Biochemical degradation, enzymatic activities, and microbial growth in perishable foods, such as seafood (Amit *et al*., 2017), have been identified as factors contributing to reduced shelf life and overall product quality. Therefore, there is a pressing need for packaging systems that can actively monitor and ensure the real-time freshness of these products. Therefore, researchers have made various efforts to develop intelligent packaging films that potentially satisfy this requirement and improve the functionality of food packaging (Zeng *et al*., 2019).

According to Yam (2012), intelligent packaging is defined as a system that utilises a communication function to enhance decision-making by monitoring and improving storage conditions, thereby enabling the application of proper actions to ensure the quality and safety of food. The pH indicator films are one of the intelligent packaging solutions that incorporate active compounds, such as anthocyanin, which can detect changes in food pH through a colour response due to reactions with volatile amines produced by bacteria (Wei *et al*., 2017). Other than that, pH indicator film is a convenient and accurate method for delivering qualitative information that can be visually indicated by consumers through the colour changes of the films (Wu *et al*., 2021).

However, synthetic colours or dyes such as bromophenol blue, methyl red, and bromocresol green are harmful to human life because they can cause lung disease and skin infection (Husin *et al*., 2020; Shi *et al*., 2021). Alternatively, anthocyanin is one of the water-soluble natural pigments widely used in pH indicator films, as it shows potential and the ability to visibly change colour when the pH of the surrounding environment changes. Butterfly pea flower (*Clitoria ternatea* L.) consists of delphinidin, which is responsible for the anthocyanin for the deep blue to purple colour (Husin *et al*., 2020; Mary *et al*., 2020). Previous research showed that butterfly pea flowers had the potential to act as naturally-derived pH dyes for the colourimetric indicator in monitoring fish freshness, since they provide significant changes in the colour spectra with the changes of pH and the application of butterfly

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pea flowers is perceived as a lower risk to the consumer (Ahmad *et al*., 2019).

However, limited studies have been carried out on the application of pH indicator films to monitor and compare the freshness of saltwater fish (sardines) and freshwater fish (catfish). Both types of fish may react differently to the pH indicator film due to their varying rates of deterioration. In addition to the pH changes, the Quality Index Method (QIM) could further confirm the deterioration of the stored fish. Hence, the objectives of this study were 1) to develop and evaluate pH indicator potato starch matrix films integrated with various concentrations of butterfly pea anthocyanin extracts and 2) to investigate the effectiveness of the developed films in monitoring the freshness of sardine and catfish during storage at 4°C. It is expected that the films developed could function well as indicators of fish freshness.

MATERIALS AND METHODS

Materials

The dried butterfly pea flowers and fish gelatine were purchased from an online shop, and potato starch was obtained from Lotus's IOI City Mall, Putrajaya, 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 and bought from Sigma-Aldrich (UK).

Preparation of butterfly pea flower

To achieve a constant particle size of the sample, the dried flower was ground using a grinder (MX-898 M, Panasonic, Malaysia) and then passed through a 0.2970 mm (50 mesh) sieve, as described by Husin *et al*. (2020), with some modifications. The powdered butterfly pea flower was kept in an air-tight container and placed in desiccators for further extraction and analysis.

Extraction of anthocyanins from butterfly pea flower

Butterfly pea flower extract (BPE) was prepared according to Husin *et al*. (2020) with some modifications. One hundred mL of water was added to 15 g of powdered butterfly pea flower and placed in a 250 mL beaker. The beaker was placed in an ultrasonic bath (8510E-MTH, Bransonic, USA) and sonicated for 30 min at 44 kHz and 230 V. After sonication, the solution in the beaker was filtered through a Whatman filter paper No. 1 to separate and remove solid particles. Then, the filtered extract was centrifuged at 2,000 rpm for 10 min at 4°C. The extracted solution was lyophilised for two days, and the freeze-dried powder was wrapped with aluminium foil and stored in a freezer at -18°C for further analysis.

Colour response of the butterfly pea extract (BPE)

Colour response analysis of the extract solutions was conducted using the method described by Singh *et al*. (2021) and Wu *et al*. (2021), with some modifications. To prepare the extract, 1 g of freeze-dried powder was dissolved in 100 mL of distilled water. This extract (2 mL) was then mixed with 10 mL of different pH solutions ranging from 2.0 to 12.0.

Preparation of pH indicator films

Potato starch films (PS) and potato starch-butterfly pea extract films (PS-BPE) were developed using the casting method, as described by Mary *et al*. (2020), with some modifications. For PS-BPE films, 100 mL of an aqueous dispersion containing 4 g of potato starch and a BPE solution with a volume corresponding to the starch weight (4%, 8% & 12% w/w) was then added. Additionally, 30% sorbitol was added as a plasticiser. The control film (PS) was produced without the addition of BPE. The solution was mixed well using a magnetic stirrer (MS-H280-Pro, DLAB Scientific Inc., Malaysia) and heated to 85°C for 10 min. The solution was subjected to an ultrasonic treatment of 10 min for the removal of air bubbles (Liu *et al*., 2018). Then, the film-forming solution was immediately poured into a Petri plate. The films were dried in an oven (30-750, Memmert, Germany) at 55°C for 18 hr. The dried films were peeled from a Petri plate and kept in a desiccator to prevent them from absorbing moisture from the air and humidity until the evaluation. The films were labelled as PS (control), PS-BPE 4% film, PS-BPE 8% film, and PS-BPE 12%.

Colour determination

The colours of PS and PS-BPE films were determined by using a colourimeter (CR-410, Chroma Meter, Japan) adjusted with the standard D65 light source (Yan *et al*., 2021). The films were recorded for *L** (lightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness) colour coordinates. Measurements were performed in triplicate for each film sample and were calculated according to the formula:

$$\Delta E = \left[(L^*-L)^2 + (a^*-a)^2 + (b^*-b)^2\right]^{\frac{1}{2}}$$

Where *L*, *a*, and *b* are colour parameters of PS (control) and *L**, *a**, and *b** are colour parameters of the PS-BPE films.

Film thickness

The thickness of the films was determined using a digital micrometre (C112XBS, Mitutoyo, Japan), with five different positions randomly selected on each film, to a precision of 0.001 mm (Mary *et al*., 2020; Yan *et al*., 2021).

Moisture content

A moisture analyser (AND MX-50, US) was used at 120°C until the films reached a constant weight (Mary *et al*., 2020). The analysis was carried out in triplicate**.**

Water vapour permeability (WVP)

The WVP of the films were determined gravimetrically using a WVP cup (2.5 cm depth & 4.8 cm diameter) according to the ASTM E96-95 (Ezati & Rhim, 2020). Each film sample was cut into a (7.5 cm × 7.5 cm) and mounted on the top of the WVP cup, which contained 18 mL of distilled water. Then, the cups were tightly sealed using parafilm. The assembled cups were stored in a humidity chamber controlled at 25°C and 55% RH. The weight of the cup was measured at a 1 hr interval for a 6 hr period, and the changes were recorded. The WVP ($g \times m.m^2 \times Pa \times s^{-1}$) of the film was calculated as follows:

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

pH analysis

Ten grams of fish fillet samples were homogenised in 50 mL distilled water and evaluated using a digital pH meter (Eutech pH 700, Thermo Fisher Inc., USA) (Ming-Min & Ismail-Fitry, 2023). The pH meter was calibrated before use to ensure accurate and reliable measurements.

Texture Profile Analysis (TPA)

The texture analysis of each fish was measured using a texture analyser (TA-XT2i, Stable Micro Systems Ltd., Surrey, UK), following the method of Wang *et al*. (2019) with some modifications. Each fish's flesh was placed between parallel, flat plate fixtures equipped with a cylindrical probe-type p/2. The settings were as follows: pre, 1.0 $mm.s^{-1}$; test, 2.0 $mm.s^{-1}$; post, 5.0 $mm.s^{-1}$; time before second compression, 5 sec; trigger force, 5 g and strain 50 %. The parameters were hardness, cohesiveness, springiness, chewiness, and gumminess. The analysis was done in triplicate on days 0, 2, 4 and 6 of storage.

Quality Index Method (QIM) analysis

The freshness assessment of each fish was conducted randomly and evaluated for analysis every 2 days using the QIM scheme (Triqui & Bouchriti, 2003), with the score measured on average.

Statistical analysis

The results were analysed using one-way analysis of variance (ANOVA) from Minitab version 21.1.0, with average values reported as mean ± standard deviation. The significance of the values was defined at $p<0.05$.

RESULTS

Quality of the pH indicator films

Colour change of BPE anthocyanin and film in buffer solution

Figure 2 shows the colour responses of anthocyanins in BPE in different buffer solutions ranging from pH 2-12, while Figure 3 shows the visible colour changes of P/S-BPE films in response to different pH levels after immersing in buffer solution (pH 2 - 12). Both the anthocyanins in buffer solutions and PS-BPE films in buffer solutions showed similar ranges of colour responses. They were red-pinkish at pH 2, purple-violet at pH 3, purple at pH 4-5, deep blue at pH 6, blue-green at pH 7-8, green at pH 9-10 and brownish-green at pH 11-12. Thus, the excellent colour stability and the response of the pH indicator films can make them practical and effective for determining the freshness of food products.

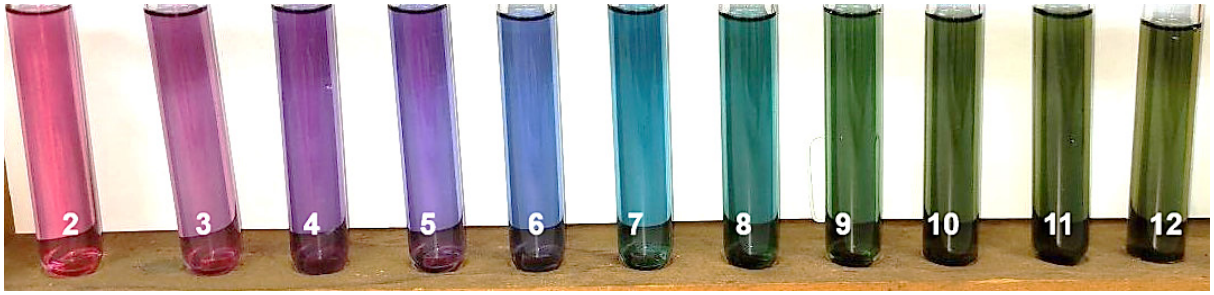


Fig. 2. Colour responses of Butterfly Pea Extract (BPE) in different buffer solutions (pH 2-12)

Fig. 3. Visual appearance of PS-BPE films in different buffer pH solutions (pH 2-12)

Colour of PS, PS-BPE 4%, PS-BPE 8%, and PS-BPE 12% films

Figure 4 shows the surface appearance of the films. The PS (control) film was colourless and transparent, whereas the colour of the PS-BPE films darkened and became purplish as the concentration of BPE increased. Table 1 shows that the *L** (lightness) value of the film significantly ($p<0.05$) decreased after BPE was incorporated into the starch matrix. Other than that, the *a** (red/green) value was significantly ($p<0.05$) increased, whereas the *b** (yellow/blue) value significantly ($p<0.05$) decreased due to the increase in BPE content. The value of the colour difference (ΔE) of PS-BPE films gradually increased with the increase in BPE concentration. The ΔE values were above 3.00 except for the PS film (control). The lowest ΔE value was PS-BPE 4%, which was 22.97, while the highest was PS-BPE 12% with 48.46 (Table 1). This indicates that the colour of every PS-BPE film exhibited visually perceptible differences.

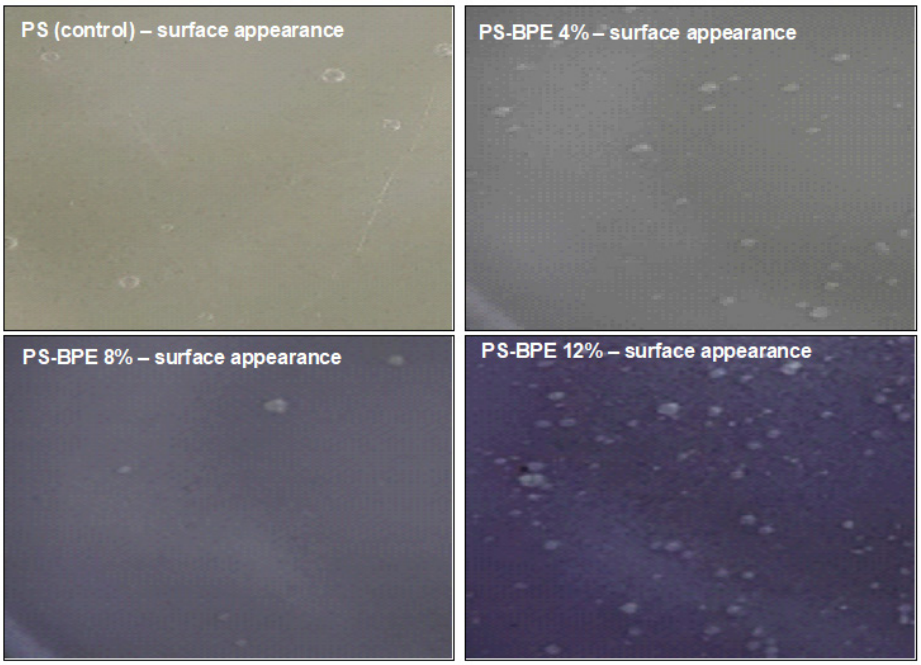


Fig. 4. Surface appearance of the PS-BPE film at different concentrations

Thickness, moisture content and water vapour permeability (WVP) of films

Table 1 shows that the thickness of the PS-BPE film increased significantly ($p<0.05$) from 0.0951 mm to 0.1202 mm with the increase in BPE concentration. Apart from that, the thickness of the PS-BPE 8% and PS-BPE 12% films was relatively higher than that of the S-BPE 4% film. The moisture content of PS-BPE was 12% significantly lower ($p<0.05$) than that of the PS film (control). Table 1 also shows that the WVP value of PS-BPE 12% was the highest among the other films; however, the differences in WVP values among the films were not statistically significant ($p>0.05$).

Table 1. Colour, thickness, moisture content and water vapour permeability (WVP) of films

Films	Colour				Thickness (mm)	Moisture content (%)	WVP ($\times 10^{-10}$ g./m. Pa.s)
	*L**	*a**	*b**	ΔE			
PS (control)	90.70 ± 0.09^{a}	1.25 ± 0.01^{d}	-4.81 ± 0.03^{a}	0.00	0.10 ± 0.01^{ab}	3.43 ± 0.42^{a}	2.00 ± 0.17^{a}
PS-BPE 4%	70.85 ± 0.81^{b}	6.45 ± 0.29^{c}	-15.14 ± 0.39^{b}	22.97	0.09 ± 0.01^{b}	2.32 ± 0.08^{ab}	1.70 ± 0.21^{a}
PS-BPE 8%	59.10 ± 0.76^{c}	11.77 ± 0.19^{b}	-19.51 ± 0.10^{c}	36.41	0.11 ± 0.01^{a}	2.71 ± 0.93^{ab}	2.14 ± 0.41^{a}
PS-BPE 12%	47.73 ± 1.08^{d}	17.08 ± 0.57^{a}	-20.66 ± 0.07^{d}	48.46	0.12 ± 0.01^{a}	1.67 ± 0.02^{b}	2.41 ± 0.67^{a}

All values are mean ± standard deviation of three replicates. Means that do not share the same letter are significantly different (p<0.05) in the same column.

Microstructure of films

The surface microstructure of PS film and PS-BPE films was observed using SEM and presented in Figure 5. The PS film without BPE showed a smooth and homogenous surface. After the addition of BPE in the potato starch matrix, the rough surface of the film was observed. The surface of the PS-BPE 8% film was much rougher than that of the PS-BPE 4% and other films. However, the PS-BPE 12% film had a smooth and dense surface.

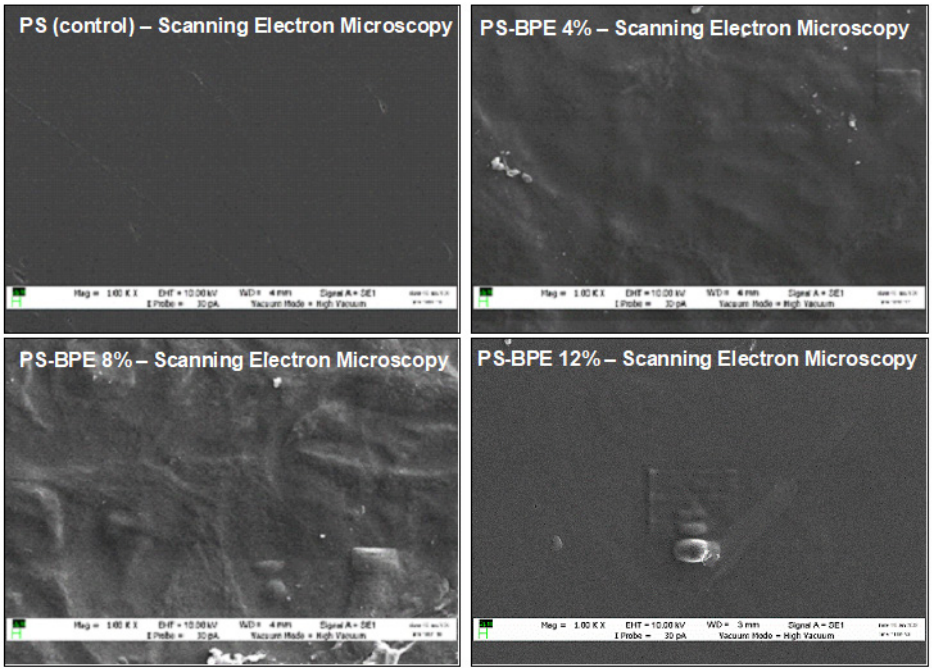


Fig. 5. The Scanning Electron Microscopy (SEM) images of the surface of films (magnification: 1000×)

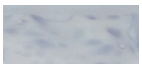
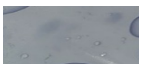
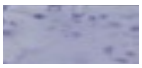
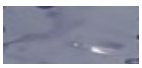
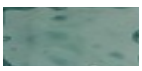
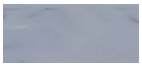
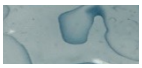
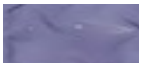
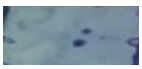
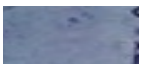
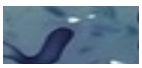
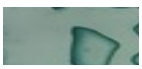
Application of S-BPE films to monitor fish freshness

Colour determination

The PS-BPE films were used to monitor the freshness of sardine fish and catfish during chilled storage (4 ± 1°C) for 6 days. As shown in Table 2, the *L** values of the PS-BPE 4% and S-BPE 12% films significantly increased from 51.78 to 66.65 and from 49.85 to 58.03, respectively, between day 0 and day 6. The *L** value increased due to the anthocyanins' pH sensitivity, resulting in the dye changing from a dark purple-blue to a green colour during storage. Anthocyanins are highly sensitive to pH due to their complex molecular structure, which undergoes protonation or deprotonation as the pH of their environment shifts Khoo *et al*., 2017). Thus, it will affect the film's lightness. The ionic nature of their anthocyanins leads to colour changes at different pH levels, increasing lightness over time during storage. Meanwhile, the *a** and *b** values of the S-BPE 4%, S-BPE 8%, and S-BPE 12% films significantly (p<0.05) decreased from day 0 to day 6. The visual appearance of S-BPE films changes from purple (day 0) to blue-green (day 6). The blue-green colour indicated that the sardine fish had begun to spoil, and the spoilage odour became apparent towards the end of storage. Other than that, the ΔE value of the PS-BPE 4%, PS-BPE 8%, and PS-BPE 12% films also showed an increment from 6.91 to 15.92, 1.73 to 13.04, and 2.52 to 12.29, respectively, from the 0-day evaluation to the last evaluation day. This indicates that the colour changes of S-BPE films at the end of storage can be detected by the human eye when ΔE is greater than 3.00 (Mary *et al*., 2020).

The significant (p<0.05) changes of *L**, *a**, *b** values for PS-BPE 4%, PS-BPE 8% and PS-BPE 12% in catfish samples are also listed in Table 2. The trend for L* and a* showed a similar result with PS-BPE films in the sardine sample, where the *L** value was significantly increased (p<0.05), while the a* value was significantly decreased (p<0.05). However, the *b** value of PS-BPE films showed a significant (p<0.05) increase between day 2 and day 6. The visual appearance of PS-BPE films was purple at day 0, indicating that the catfish was in a fresh condition, and it turned to blue-green on day 6, indicating that the catfish had begun to spoil. The ΔE value of PS-BPE films gradually increased from 9.06 to 15.37, 8.39 to 18.66 and 5.86 to 16.34.

Table 2. The colour response of PS-BPE film at different time intervals (0,2,4,6 days) for monitoring sardine freshness

Day	Films	*L**	*a**	*b**	ΔE	Film appearance
			Sardine Fish Sample			
0	PS-BPE 4%	51.78 ± 5.00^{b}	2.29 ± 0.29^{a}	-5.59 ± 0.93^{b}	0.00	
2		58.65 ± 1.48^{ab}	2.32 ± 0.25^{a}	-6.08 ± 0.53^{b}	6.91	
4		64.90 ± 2.51^{a}	2.21 ± 0.52^{a}	-6.84 ± 0.97^{b}	13.15	
6		66.65 ± 2.13^{a}	-2.97 ± 0.18^{b}	-3.47 ± 0.44^{a}	15.92	
0	PS-BPE 8%	52.93 ± 4.14^{a}	3.36 ± 0.64^{a}	-7.04 ± 1.53^{b}	0.00	
2		54.58 ± 1.30^{a}	3.58 ± 0.08^{a}	-7.52 ± 0.43^{b}	1.73	
4		63.41 ± 6.66^{a}	4.30 ± 0.59^{a}	-8.90 ± 0.43^{b}	10.69	
6		63.30 ± 5.44^{a}	-3.94 ± 0.56^{b}	-4.02 ± 1.01^{a}	13.04	
0	PS-BPE 12%	49.85 ± 2.90^{b}	3.81 ± 0.60^{b}	-7.29 ± 0.68^{b}	0.00	
2		50.99 ± 1.59^{b}	5.05 ± 0.55^{ab}	-8.77 ± 0.84^{b}	2.52	
4		56.77 ± 1.89^{a}	5.62 ± 0.59^{a}	-9.21 ± 1.12^{b}	7.40	
6		58.03 ± 0.49^{a}	-4.87 ± 0.38^{c}	-4.31 ± 0.82^{a}	12.29	
			Catfish Sample			
0	PS-BPE 4%	53.25 ± 2.02^{c}	2.51 ± 0.20^{a}	-5.61 ± 0.75^{ab}	0.00	
2		62.23 ± 1.09^{b}	1.39 ± 0.26^{a}	-6.08 ± 0.20^{b}	9.06	
4		62.34 ± 0.12^{b}	-0.42 ± 0.59^{b}	-5.13 ± 0.39^{ab}	9.56	
6		67.45 ± 1.52^{a}	-3.07 ± 0.74^{c}	-3.76 ± 1.53^{a}	15.37	
0	PS-BPE 8%	50.01 ± 0.83^{c}	3.79 ± 0.23^{a}	-7.10 ± 0.74^{ab}	0.00	
2		58.32 ± 1.11^{b}	3.13 ± 0.18^{a}	-8.04 ± 0.89^{b}	8.39	
4		56.35 ± 2.58^{b}	0.02 ± 0.89^{b}	-7.25 ± 0.82^{ab}	7.38	
6		66.98 ± 1.85^{a}	-3.51 ± 0.95^{c}	-4.48 ± 2.16^{a}	18.66	
0	PS-BPE 12%	46.35 ± 2.24^{c}	4.10 ± 0.28^{a}	-6.94 ± 0.28^{ab}	0	
2		51.73 ± 2.18^{bc}	3.90 ± 1.66^{a}	-8.74 ± 0.933^{b}	5.68	
4		55.11 ± 0.25^{ab}	-0.58 ± 1.59^{b}	-7.63 ± 1.20^{b}	9.96	
6		59.92 ± 3.35^{a}	-4.68 ± 1.44^{c}	-4.56 ± 1.52^{a}	16.34	

All values are mean ± standard deviation of three replicates. Means that do not share the same letter are significantly different (p<0.05) in the same column.

pH determination

The pH values of sardines and catfish are shown in Table 2. During the initial storage period, the pH of both fish was consistently low, and the pH value for the sardine was lower than that of the catfish, which were 6.19 and 6.40, respectively. The pH value of both fish slightly increased after 4 days of storage. At the end of storage, the pH values of sardine and catfish were significantly increased (p<0.05).

Texture profile analysis (TPA)

Table 3 presents the hardness values for both fish, which decreased slightly from day 0 to day 6 but were not statistically different (p>0.05). Nevertheless, the hardness values for sardines were negative due to the softness of the samples. No significant differences (p>0.05) were observed for other parameters (adhesiveness, gumminess, cohesiveness, and chewiness) in either fish. However, only the springiness of sardine showed a significant (p<0.05) decrease from day 2 to day 4.

Quality Index Method (QIM) of sardine and catfish

The freshness of the fish was determined by the total demerit value of the quality index (QI). The QI point increased linearly with the storage time at the chiller temperature. The QI scores for sardines and catfish during storage are shown in Table 3. The QI scores for both fish showed significant (p<0.05) increases with increasing storage time. For the sardine, the freshness stage remained constant from day 0 to day 4, as the QI score was still under 16. The odour and colour of the gills were still seaweedy and dark red. The flesh was still bright and firm. The freshness and quality of the sardine declined on day 6 when the colour of the gills became brownish-red and the odour slightly rancid compared to before, as shown in Figure 6. Other than that, the flesh's appearance also became dull. The QI score on day 6 was also the highest, which was 21. For the catfish, the fish was fresh on day 0, but declined with the increase in storage period. The QI score was significantly (p<0.05) increased from the first day until the end of storage. The colour of the gill changed from dark red (day 0) to bright red (day 4) and finally to brownish red on day 6. The odour of the gill also changed from fresh to sour. Other than that, the flesh of the catfish became dull on day 6.

Fig. 6. QMI freshness index of sardine and catfish samples.

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Table 3. Texture Profile Analysis (TPA), pH and QI score of sardine and catfish samples during storage

Fish sample	Day	Texture Profile Analysis						pH of fish	Quality Index
		Hardness (g)	Adhesiveness (g.s)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (g.mm)		
Sardine	0	-0.09 ± 0.74^{a}	-25.58 ± 12.33^{a}	0.87 ± 0.04^{a}	0.49 ± 0.07^{a}	-0.36 ± 0.90^{a}	-0.30 ± 0.79^{a}	6.19 ± 0.11^{b}	2.33 ± 0.57^{d}
	2	-0.19 ± 1.08^{a}	-35.19 ± 3.30^{a}	0.89 ± 0.08^{a}	0.39 ± 0.10^{a}	-0.18 ± 0.44^{a}	-0.18 ± 0.39^{a}	6.22 ± 0.03^{b}	12.0 ± 1.00^{c}
	4	-0.24 ± 0.72^{a}	-22.28 ± 4.09^{a}	0.61 ± 0.12^{b}	0.45 ± 0.02^{a}	0.28 ± 0.499^{a}	0.214 ± 0.34^{a}	6.25 ± 0.02^{b}	15.33 ± 0.57^{b}
	6	-0.98 ± 0.35^{a}	-30.96 ± 6.26^{a}	0.89 ± 0.06^{a}	0.39 ± 0.13^{a}	-0.40 ± 0.25^{a}	-0.34 ± 0.19^{a}	6.45 ± 0.06^{a}	21.00 ± 1.00^{a}
Catfish	0	84.90 ± 41.10^{a}	-55.30 ± 56.30^{a}	0.87 ± 0.12^{a}	0.40 ± 0.14^{a}	31.41 ± 5.54^{a}	27.72 ± 8.06^{a}	6.40 ± 0.20^{b}	1.67 ± 0.57^{d}
	2	64.46 ± 2.01^{a}	-55.24 ± 10.14^{a}	0.91 ± 0.02^{a}	0.37 ± 0.06^{a}	22.88 ± 4.01^{a}	20.90 ± 4.14^{a}	6.67 ± 0.15^{b}	8.00 ± 1.00^{c}
	4	63.79 ± 3.07^{a}	-68.46 ± 16.12^{a}	0.76 ± 0.11^{a}	0.45 ± 0.06^{a}	28.28 ± 5.94^{a}	21.86 ± 7.67^{a}	6.74 ± 0.015^{b}	11.00 ± 1.00^{b}
	6	62.27 ± 1.70^{a}	-52.63 ± 17.21^{a}	0.92 ± 0.07^{a}	0.43 ± 0.13^{a}	27.54 ± 8.73^{a}	25.82 ± 9.60^{a}	7.09 ± 0.05^{a}	16.00 ± 1.00^{a}

All values are mean ± standard deviation of three replicates. Means that do not share the same letter are significantly different (p<0.05) in the same column (of the same fish species)

DISCUSSION

BPE anthocyanin changes to a different colour due to the variation of chemical structure in anthocyanins in basic, neutral and acidic forms (Yan *et al*., 2021). Spectra of the BPE shifted from acidic form (pH 2 - 5) to basic form (pH 8 - 11) due to the transformation of flavylium cation (red) to anionic quinoidal (purple to blue) and chalcone (yellow to green) structures (Singh *et al*., 2021), similar to the colour changes of BPE from red-pinkish to green in Figure 2 and Figure 3. Similar results were reported by Wang *et al*. (2022) in the rose anthocyanin extracts. Colour is a crucial indicator that directly reflects the different appearance of the control film (PS) and the prepared film incorporated with BPE. The colourless and transparent PS film (control) measured the highest value of *L**, which was 90.70, indicating its white properties. Apart from that, *a** value increased due to the S-BPE films becoming redder and bluer, coming from the BPE anthocyanin.

The visual aspect in Figure 4 confirmed that S-BPE films tended towards a purple colour. According to Mary *et al*. (2020), the colour change of film can be considered visually perceptible to the human eye when the value of the total colour difference (∆E) is higher than 3.00. A study from Rawdkuen *et al*. (2020) observed significant changes in *L**, *a**, *b** and ∆E values of gelatine films after the butterfly pea anthocyanins were added. In addition, a study by Wang *et al*. (2022) also observed changes in the ΔE value of cellulose/polyvinyl alcohol (C/PVA) film with the increase of rose anthocyanin extracts (RAE). Thus, this confirmed that PS-BPE films could be used as indicator films to detect colour changes with the naked eye.

The thickness of the film is a crucial parameter that directly impacts its opacity, water vapour permeability, mechanical strength, and application as a freshness sensor (Mary *et al*., 2020; Alizadeh *et al*., 2021). The higher BPE amount increased the dry matter content, resulting in a thicker film. In contrast, a low amount of BPE can be distributed evenly throughout the starch matrix (Wang *et al*., 2022). In addition, the excess amount of extract would produce a more complex matrix between starch and anthocyanins (Yan *et al*., 2021), thereby increasing the thickness of the PS-BPE 8% and PS-BPE 12% films. Similar results were reported in Wang *et al*. (2019), Alizadeh *et al*. (2021), and Yan *et al*. (2021), where the film thickness increased after the addition of rose anthocyanin extract, red barberry extract, and butterfly pea flower extract, respectively. It can be concluded that a higher concentration of anthocyanin extract yields a greater effect on the film thickness.

Moisture content is a crucial indicator for evaluating the water resistance and physical integrity of films during exposure to food products (Wang *et al*., 2022). The incorporation of BPE in the potato starch film resulted in a reduction in moisture content compared to the control film. This was possible because the interaction between the hydroxyl group of starch and BPE anthocyanins could lower the intermolecular interaction between water molecules and starch molecules (Mary *et al*., 2020; Wang *et al*., 2022). A similar result was observed by Yan *et al*. (2021), who reported that the addition of BPE promoted a significant ($p<0.05$) decrease in the moisture content of chitosan composite films. To conclude, the incorporation of BPE in the starch film has a significant impact on the film's moisture content. WVP is an important barrier property for prepared films, reflecting the film's ability to resist water vapour. No significant changes were observed in this study as the concentration of BPE increased. The results are similar to those of a study by Saravanan *et al*. (2024), which applied BPE in Semolina Starch/Agar films at concentrations of 3, 6, and 9%. The study by Ahmad *et al*. (2024) also showed no changes in WVP when BPE was incorporated at 5 and 10% in quinoa starch-fish gelatine films. Therefore, it can be concluded that the incorporation of BPE in the potato starch film did not provide a greater impact on the WVP of the film.

The microstructure of the film surface with various compositions was examined by SEM analysis at a magnification of 1000×. The control film exhibited a smooth surface, indicating that starch and sorbitol were well-mixed with good dispersion and compatibility. The surface of the PS-BPE 8% film was much rougher, which might be due to the low interaction between the anthocyanin compound and the starch and sorbitol (Prietto *et al*., 2017). The PS-BPE 12% film exhibited a dense surface, indicating that the higher concentration of BPE formed a continuous polymeric matrix and enhanced compatibility with starch molecules (Xue *et al*., 2020; Sohany *et al*., 2021). A parallel result was reported by Xue *et al*. (2020), who found that sago starch films incorporated with a lower content of torch ginger extract (TGE) possessed a rough surface structure. In contrast, films with an excess of TGE formed a compact and dense surface.

During storage, the freshness of fish declines due to microbial growth and the biochemical reactions that produce many volatile nitrogenous compounds (Hasanah *et al*., 2023). In a sealed package, the volatile nitrogenous compound slowly released from the fish to the headspace of the sealed package. As a consequence, these compounds were retained, and the pH of the environment increased with increasing storage time. Therefore, a pH indicator film was used to detect the fish freshness by the colour changes of the film. The ∆E value of PS-BPE films gradually increased, indicating that the colour changes can be perceived by the human naked eye. To summarise, PS-BPE films in both fish samples exhibited significant colour changes from day 0 to day 6, which were attributed to fish spoilage and the formation of volatile nitrogenous compounds. A study by Yan *et al*. (2021) also showed a similar finding, where the colour of the chitosan-butterfly pea (CH-BP) film changes gradually from purple-blue on day 0 to dark green on day 6 during application to tilapia fillets. The colour differences may be due to the varying extraction methods applied and the base ingredient of the film. However, the PS-BPE films with higher BPE content (S-BPE 8%, S-BPE 12%) exhibited a visible colour change bolder than the PS-BPE 4% film. This result also indicated that starch incorporated with BPE has the potential for monitoring the freshness of food samples.

The pH value is an indicator associated with fish freshness. The increase in pH during the storage period may be due to the accumulation of volatile nitrogenous compounds released by fish (Sardar *et al*., 2015). This trend was also consistent with a study by Yan *et al*. (2021), which observed a significant increase in the pH of tilapia fillets from day 0 to day 6 during chiller storage. Another study also produced the same trend, although the fish was stored in an ice storage (Abbas *et al*., 2008). According to Suvanich *et al*. (2000), the acceptable pH range for freshwater fish was 6.8-7.0. In conclusion, the increase in pH values of both sardine and catfish on day 6 indicated that the freshness and quality of the fish were lower, and spoilage was evident at the end of the storage period.

TPA was used to measure the physical changes of sardine and catfish fillets that may indicate their freshness during storage. The hardness values of the fish were reduced, although not significantly. Theoretically, the texture of fish fillets became softer and less elastic throughout the storage period due to the proteolysis of fish protein (Tavares *et al*., 2021). No significant

difference in cohesiveness was observed, as a similar result was also reported by Wang *et al*. (2019), who found no significant changes in cohesiveness in crucian carp fish after 5 days of refrigerated storage. However, only the springiness of sardine showed a significant (p<0.05) decrease. This indicated that the sardine becomes less elastic due to the breakdown of protein during the storage process (Sun *et al*., 2018). In summary, the texture of both fish during chiller storage did not show a significant change. This indicated that the degradation of protein (proteolysis) by endogenous enzymes during the 6-day storage period was still lower. Therefore, the texture of both fish remains fresh and may become softer with increased storage time.

The Quality Index Method (QIM) is a method used to assess the freshness of fish. The QI scores for sardines and catfish increased during storage, corresponding to the passage of time. This indicated that the freshness of both fish declined until the last day of evaluation. According to Triqui and Bouchriti (2003), fish can be regarded as less fresh when the total QIM score exceeds 16. For sardine fish, the results align with a study by Triqui and Bouchriti (2003), which observed an increase in the QI score of sardines (*Sardina pilchardus*) from day 0 to day 5 of iced storage. The spoilage of sardines is more rapid than that of catfish, which may be due to some factors such as mishandling of fish during onboard and landing (Sardar *et al*., 2015). Apart from that, it can be concluded that the spoilage began on day 6. Overall, these results showed a similar correlation with the colour changes of PS-BPE films, from purple (day 0) to blue-green at day 6, indicating that both fish had spoiled by day 6.

CONCLUSION

In this study, pH indicator films were successfully developed by incorporating butterfly pea extract (BPE) anthocyanins into a potato starch matrix using the casting method. Findings revealed that the PS-BPE 12% film exhibited the best film with a significant increase in thickness, a low moisture content, and no significant change in WVP. The SEM micrograph revealed a more compact and denser surface after adding 12% BPE and showed a significant colour change in buffer solutions (pH 2 - 12) and film, which demonstrated the boldest colour shift compared to the other films. This study also demonstrated that the colour of PS-BPE films changes from purple (fresh stage) on day zero to blue-green (beginning of spoilage) during application on fish, serving as a freshness sensor, and this change is readily observable to the naked eye. The freshness assessment (QIM) of both fish confirmed that spoilage of the fish began on day six, correlating with the colour changes of the PS-BPE films. Overall, the development of pH indicator films benefits the food industry by enhancing product quality, reducing costs, and improving food safety, all while benefiting consumers through improved product quality and reduced food waste.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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