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BUTTERFLY PEA FLOWER-INFUSED QUINOA STARCH FILM AS A pH INDICATOR FOR SHELLFISH FRESHNESS ASSESSMENT

(Filem Kanji Kuinoa yang Diserapkan dengan Bunga Telang sebagai pH Indikator untuk Penilaian Kesegaran Kerang-Kerangan)

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Abstract

The pH indicator film has been utilised in food packaging to guarantee quality and safety as well as to track the freshness of perishable food in real-time. However, previous studies have concentrated on synthetic dyes, so this study investigated the use of butterfly pea flower (BPF) which contains anthocyanin, a natural pigment that is sensitive to a wide pH range. Quinoa starch (QS) contains 10-21% amylose with a small starch granule size of around 1 µm that allows for easier dispersion, making it a promising material to produce pH indicator films. BPF extract was incorporated into QS matrix films as a pH indicator to monitor the freshness of different types of shellfish (blood cockles and short-necked clams) stored at 4°C. The quinoa starch-fish gelatine-butterfly pea flower extract (QS/F-BPF) films were developed with different concentrations of BPF (5% and 10%), and then their characteristics including physical appearance, colour, thickness, water vapour permeability (WVP), moisture content, and colour responses were evaluated at different pH values (pH 1-12). The addition of different concentrations of BPF to the quinoa-gelatine-based film did not significantly affect the thickness, moisture content, and WVP. Meanwhile, the micrograph of the films showed good surface morphology. The colour and pH of the QS/F-BPF film were examined every two days for six days of storage of shellfish at 4°C, with the colour changing from blue to green with increasing pH on day 6, indicating spoilage. In conclusion, the QS/F-BPF pH indicator film is effective for monitoring the freshness of different types of shellfish stored in chiller conditions.

Keywords: butterfly pea flower, quinoa starch, pH indicator, anthocyanin, shellfish

Abstrak

Filem penunjuk pH telah digunakan dalam pembungkusan makanan untuk menjamin kualiti dan keselamatan serta untuk mengesan kesegaran makanan mudah rosak dalam masa nyata. Walaubagaimanapun, kajian lepas tertumpu kepada pewarna sintetik, jadi kajian ini menyiasat penggunaan bunga telang (BPF) yang mengandungi antosianin, pigmen semulajadi yang sensitif kepada julat pH yang luas. Kanji kuinoa (QS) mengandungi 10-21% amilosa dengan saiz butiran kanji kecil sekitar 1 µm yang membolehkan penyebaran lebih mudah, menjadikannya bahan yang berpotensi untuk menghasilkan filem penunjuk pH. Ekstrak BPF telah dimasukkan ke dalam matriks filem QS sebagai penunjuk pH untuk memantau kesegaran pelbagai jenis kerrang-kerangan (kerang dan lala kepah) yang disimpan pada suhu 4°C. Filem kanji kuinoa-gelatin ikan-ekstrak bunga telang (QS/F-BPF) telah dihasilkan dengan kepekatan BPF yang berbeza (5% dan 10%), dan kemudian ciri-cirinya termasuk rupa fizikal, warna, ketebalan, kebolehtelapan wap air (WVP), kandungan lembapan dan tindak balas warna dinilai pada nilai pH yang berbeza (pH 1-12). Penambahan kepekatan BPF yang berbeza kepada filem berasaskan kuinoa-gelatin tidak menjejaskan ketebalan, kandungan lembapan dan WVP dengan ketara. Sementara itu, mikrograf filem menunjukkan morfologi permukaan yang baik. Warna dan pH filem QS/F-BPF diperiksa setiap dua hari selama enam hari penyimpanan kerrang-kerangan pada suhu 4°C, dengan warna berubah daripada biru ke hijau dengan peningkatan pH pada hari ke-6, menunjukkan kerosakan. Kesimpulannya, filem penunjuk pH QS/F-BPF berkesan untuk memantau kesegaran pelbagai jenis kerrang-kerangan yang disimpan dalam keadaan suhu dingin.

Kata kunci: bunga telang, kanji kuinoa, pH indikator, antosianin, kerang-kerangan

Introduction

Food spoilage is the deterioration of food caused by microbial and biochemical spoilage, and this will lead to changes in food pH [1]. Food spoilage can be detected through smell, touch or sight and is easily detected on perishable foods such as raw chicken, beef, and seafood products. Thus, to ensure the food is safe for human consumption, intelligent packaging made from polymer immobilized with active compounds such as anthocyanin can help identify the changes in food pH through a colour response due to reactions between the anthocyanin and volatile amines produced by bacteria and the enzymes [2].

Anthocyanins are coloured polar pigments that belong to the phenolic group [3]. Anthocyanins have a colour-sensitive characteristic after being applied to a wide pH range. The changes in different colours occur due to the anthocyanins' chemical structure change along with the different pH values. Thus, Anthocyanin is a natural colour pigment that can act as an environment-friendly pH indicator [4]. *Clitoria ternatea* or butterfly pea is known as a perennial herbaceous plant from the Fabaceae family [5]. The butterfly pea flower is well known for its stability and application as a natural food colourant as well as an antioxidant.

Recently, several studies used various sources of anthocyanins and biodegradable polymers to form the solid matrix pH film including butterfly pea with sago (Metroxylon sagu) [6], blackberry pulp with arrowroot starch [7] and mulberry with k-carrageenan [8]. Anthocyanin and starch combined create a smooth and homogeneous surface with an intermolecular hydrogen bond that extends the wavelength of the biopolymer [9]. In addition, the primary biopolymers used in the creation of biodegradable films are proteins and polysaccharides. Carboxymethyl cellulose, starch, and chitosan are the polysaccharides most frequently used for food-based biodegradable films [10]. Proteins have a higher gas barrier and mechanical properties like fish gelatine. Meanwhile, polysaccharides such as quinoa starch can enhance the functionality of films [11].

Quinoa is a flowering plant that is grown as a crop primarily for its edible seeds which are rich in protein, dietary fibre, B vitamins and dietary minerals in higher amounts than many other grains. Most of the dry matter in quinoa grains which is up to 70% is made up of starch [12]. Araujo-Farro et al. [13] reported that quinoa starch contains about 10-21% amylose with a small starch granule size of around 1μm that allows for easier dispersion which is a promising material to produce pH films. To immobilise the natural dyes from butterfly pea flower extract, a solid matrix made from natural and biodegradable polymers, which is quinoa starch, was used in this research as it has better mechanical properties and barrier properties compared to corn and amaranth starch [12]. Although there were many studies on butterfly pea extract with other starch, quinoa starch has not been used as a pH film indicator. Thus, it is the main interest in researching BPF extract incorporated with quinoa starch as a solid matrix of pH indicator films.

Shellfish is a marine life that has a shell and can be categorised as crustaceans, molluscs or echinoderms. Molluscs are divided into three categories, which are bivalves such as cockle, clam, mussel, oyster or scallop, univalves such as abalone, conch or snail and cephalopods such as octopus or squid. Shellfish that completely seal their shells can be kept for up to seven days except for the storage life of mussels is three to four days [14]. Perishable foods such as shellfish can easily deteriorate and cause biochemical degradation, enzymatic activities, and microbial growth, which will affect the quality characteristics. By developing intelligent film for packaging, where the colour changes from blue to green colour that indicating the spoilage (volatile nitrogenous) of shellfish, the observation of shellfish freshness can be done just using the naked eye in real-time. To the authors' knowledge, limited studies were found to observe the freshness of different types of shellfish using pH indicator films, specifically, films made up of quinoa starch. Therefore, the main objectives of this study were 1) to develop a pH indicator film by incorporating different concentrations of BPF extract into the quinoa starch matrix and determine its physicochemical properties and 2) to evaluate the potential of the developed film for monitoring the freshness and quality characteristics of blood cockles (*Tegillarca granosa*) and short-necked clams (*Paphia undulata*) during storage at 4 °C.

Materials and Methods

Materials

The butterfly pea flower was collected from a local area in Negeri Sembilan. Quinoa flour and fish gelatine were purchased from a bakery supplier in Bangi, Selangor. The blood cockles and short-necked clams were purchased from the Seri Kembangan wet market in Selangor, Malaysia.

Extraction of anthocyanin from butterfly pea flower

The extraction was carried out through ultrasound–assisted extraction of fresh petals. Butterfly pea flower petals were separated from the pods and leaves prior to immersion in 50% ethanol as solvent. A water bath was used to regulate the temperature and was positioned beneath the extraction setup. The solvent containing butterfly pea flowers was submerged completely with the ultrasonic probe and sonicated for 30 mins at 40 °C with 160W [15]. The extracted solution was then gone through a rotary evaporator (Eyela 1L Rotary Evaporator with Water/Oil Bath, N-1001V-WD, China) to remove solvent and will undergo lyophilized for 48 h. Then, the freeze-dried BPF powder was wrapped with aluminium foil and stored in the freezer for further analysis.

Colour responses of anthocyanin in BPF extract to pH change

The colour changes were carried out by adding 0.01g of freeze-dried butterfly pea flower extract to 4 mL of distilled water. The buffer solutions containing different pH of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 were prepared with an aqueous solution of 0.1 M HCl and 0.1 M NaOH and distilled water, then checked by a digital pH meter. The BPF extract (1 mL) was mixed in buffer solution (4 mL) ranging from pH values of 1.0 to 12.0 and the colour changes were photographed using a digital camera [4].

Development of quinoa starch films by immobilizing the anthocyanin pigment

The quinoa starch films were developed through solution casting with slight modification [4]. Solution with 100 mL of an aqueous dispersion containing 2.5g of quinoa starch (Q), and 2.5g of fish gelatine was prepared. Then, 30% glycerol was added as the plasticiser on a starch-dry basis. The solution was mixed well using a magnetic stirrer (MS-H280-Pro, DLAB Scientific Inc, Malaysia) which was then heated to 90 °C for 1 hour. Following that, the BPF extract amount (5% and 10% w/w) that corresponded to the weight of starch was added after the solution cooled down to at least 50 °C. The control film (QF) was made without adding BPF extract. The film-forming solution was immediately poured into a petri plate and film hydrogels were formed. Then, the hydrogels were dried to film at room temperature under a fan for 12 hours. After that, the film was peeled from the Petri plate and dried quinoa starch films were preserved in controlled conditions for further use. The films were labelled as QF, Q/F-BPF 5% and Q/F-BPF 10%.

Appearance and colour of film

The colour of the films was measured by a colourimeter (Chroma Meter CR-410, Japan) with L* (light/dark), a* (red/green), and b* (yellow/blue) values in triplicate and the average values were taken [4]. The total colour difference (ΔE) of the films was calculated according to the following equation (1):

$$\Delta E = \sqrt{(L*-L)^2 + (a*-a)^2 + (b*-b)^2} \qquad (1)$$

Where L*, a* and b* were the colour parameters of the BPF incorporated with quinoa starch films while L, a and b were the colour indices of the control film (QF).

Film thickness

The thickness of the films was determined by a digital micrometre (C112XBS, Mitutoyo, Japan) by randomly selecting five different positions on each film with a precision of 0.001 mm [4].

Moisture content

The moisture content of the film was determined using a moisture analyser (SHS AND MX-50, Japan) until it reached constant weight. The analyses were performed in triplicate and the results were recorded.

Water vapour permeability (WVP)

The WVP for BPF incorporated with composite QF films was performed by cutting the films into (7 cm x 7 cm) and then mounted on the top of the 50 ml crucible that contains 6 ml distilled water. The initial weight was recorded prior to being in a desiccator with 55% RH at 25°C. Then, the crucible weight was measured and recorded at 1-hour intervals for 8 hours [16]. WVP was calculated according to the following equation (2):

$$Water\ vapour\ permeability = \frac{W \times X}{t \times A \times \Delta P} \qquad (2)$$

Where W is the test tube weight (g), X is the film thickness (m), t is the time for the gain in test tube weight, A is the area of film sample for permeation (m2) and ΔP is the water vapour pressure difference between the two sides of the film (Pa).

pH sensitivity

The films were cut into (2 cm x 2 cm) and then immersed in 9 ml of buffer solutions consisting of different pH values ranging from 1.0–12.0 for around 5 min. The colour changes of the films were photographed with a white background [4, 16].

Microstructural characterization of the film

The morphology of the QF composite films was observed through a Scanning Electron Microscope (SEM) at room temperature. The films were cut into (13 mm x 13 mm), then placed on an aluminium base and covered with a gold layer then observed for the upper surface and the cross-section of films operated at 10kV [7].

Application of quinoa starch films on observing shellfish freshness

The pH films were cut into a square shape (1 cm × 1 cm) and then fixed on the inner lid of each polypropylene container's headspace containing different types of shellfish (cockles and clams). The container was stored in a chiller at 4 °C for 2 to 6 days. Six samples were taken randomly for analysis every 2 days. The changes in the colour of pH indicator films were measured by a hand-held colourimeter (Chroma Meter CR-410, Konica Minolta Inc., Tokyo, Japan) and recorded for triplicate until the end of storage [4].

Determination of pH on shellfish sample

The pH values of the ground cockles and clam samples were measured by homogenizing the sample in distilled water and determined with a pH meter (Eutech pH 700, Thermo Fisher Inc., USA). The pH values of the samples were observed on days 0, 2, 4 and 6.

Statistical analysis

The results obtained from the samples were reported using one-way analysis of variance (ANOVA) from Minitab version 19.1.1.0 and reported by the average values ± standard deviation. The significance of the values was defined at $p < 0.05$.

Results and Discussion

Colour change of butterfly pea flower extract and film

The BPF extract displayed various colour changes after exposure to a wide pH range (Figure 1), turning to pinkish-red colour in a buffer with a pH of 1 and 2 or a strong acid, then to purple or purplish-blue at pH 3 until pH 6 which is slightly acidic and displayed in its original blue colour at natural pH 7. The colour of the BPF extract turned bluish-green at pH 8, greenish at pH 9 and deep green at pH 10 until pH 12. These colour changes were similar to the previous studies by Hidayati et al. [17] and Hashim et al. [18].

Anthocyanin colour changes are influenced by increasing pH from acidic to alkaline conditions [2] and involve broad values (pH 1.0 to 12.0) with four different stages (flavylium cation, quinoidal base, carbinol pseudobase, and chalcone). Anthocyanins extracted from BPF consist of glycosides which contain flavylium cations that produce the red colour in acidic pH due to the formation of a double bond [19]. The red colour fades at higher pH due to the hydrolysis of the flavylium cation [20], with a slightly higher pH causing the dominance of carbinol, a purplish colour that was perceptible at pH 3-5. At neutral pH, carbinol does not provide any colour with the high abundance of polyacylated anthocyanins being responsible for the pigment's stable blue colour [21]. As the pH increases (pH >8), the colour changes from green to yellow because of chalcone formation [2]. Taken together, BPF is a promising anthocyanin pigment that can be used to develop pH indicator films.

Figure 1. Colour response of BPF extracted in different pH buffer solutions (pH 1.0-12.0)

Figure 2. Colour response of Q/F-BPF 10% films in different pH buffer solutions (pH 1.0-12.0)

Figure 2 shows that the visible colour of the Q/F-BPF films changed due to the response to different pH and were slightly leached after immersing for 5 min in buffer solution (pH 1.0−12.0). The colour gradually changed from red (pH 1) to pink (pH 2) to violet (pH 3) to purple (pH 4−5) to deep blue (pH 6) to original blue (pH 7) to greenish-blue (pH 8) to greenish (pH 9) and darken green (pH 10−12). A previous study reported similar colour changes from pink to yellow of starch BPF films after immersion in pH 1−14 which indicates that the films have a prominent colour response to pH [4]. The response of the pH indicator films is practical and effective for their application in determining the freshness of food products. In addition, the range (pH 1.0−12.0) in this study was sufficient as the colour changing of the film is usually from neutral to acidic or alkaline involving small shifts of pH values when the food deteriorates.

Thickness of Films

Table 1 presents the thickness of the films showing no significant ($p < 0.05$) difference between different concentrations of anthocyanin. The anthocyanins are evenly distributed throughout the solid mass of the film matrix due to abundant OH groups in BPF, starch and fish gelatine. This mixture of anthocyanin and starch can strongly bond like a bridge with a protein matrix from gelatine through intermolecular interactions [22].

Table 1. Physical properties of the composite films.

Films	Appearance	Colour				Thickness (mm)	Moisture Content (%)	WVP (x 10^{-10} g/m.Pas)
		L*	***a****	***b****	**ΔE**			
QF (control)		71.01 ± 1.46^{a}	1.52 ± 0.15^{a}	6.22 ± 0.18^{a}	0	0.23 ± 0.023^{a}	18.74 ± 5.40^{a}	1.40 ± 0.00^{a}
Q/F-BPF 5%		33.80 ± 5.83^{b}	15.71 ± 0.70^{b}	-29.36 ± 5.93^{b}	53.40	0.23 ± 0.02^{a}	14.22 ± 0.91^{a}	1.41 ± 0.00^{a}
Q/F-BPF 10%		29.06 ± 0.76^{b}	18.51 ± 0.84^{c}	-28.39 ± 0.41^{b}	56.98	0.24 ± 0.01^{a}	16.25 ± 2.17^{a}	1.64 ± 0.00^{a}

Note: 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.

Film moisture content

Moisture content is an important factor for food packaging material because high moisture is likely to encourage microbial growth on the surface of packaged foods [23]. The moisture content of QF and QF composite indicator films are listed in Table 1 showing no significant difference in moisture content because gelatine is highly hydrophilic and the interaction between the hydrophilic BPF anthocyanin results in more polar groups which can absorb moisture from the surroundings. However, the control film exhibits a slightly high moisture content possibly because the interaction between the hydroxyl group of starch and anthocyanins present in BPF could lower the number of free hydroxyl groups of starch to integrate with moisture during the drying process [24].

Film water vapour permeability

WVP is a crucial measure of application performance and indicates the barrier property which reflects the ability to modulate the flow of moisture [25]. The WVP values are tabulated in Table 1 showing no significant (p < 0.05) differences between films. However, the composite film with BPF anthocyanin shows a slightly higher increase than the control film, possibly due to the good solubility of BPF anthocyanin extract. Q/F-BPF 10% had a slightly increased WVP value of 1.64 ×10-10 g/m.Pa.s. because it has a higher concentration of BPF anthocyanin and anthocyanin helped in the transportation of water vapour due to its hydrophilic nature [26]. Therefore, it can be concluded that the quinoa starch and fish gelatine immobilised with BPF had a greater impact on the film WVP

Colour appearance of films

Colour is a crucial parameter for developing pH film indicators. Moreover, colour illustrates the different appearance of the control film and the films immobilised with BPF as shown in Table 1. The QF film was transparent and colourless whereas Q/F-BPF films were opaque and dark blue with the increased BPF concentration. The L* (lightness) of the films was significantly (p <0.05) decreased after immobilised with BPF in the starch matrix. This could be due to the white properties of quinoa starch without any anthocyanin colour. The a* (redness) value significantly (p <0.05) increased from 1.52 to 18.51 while the b* (yellowness) value significantly (p <0.05) decreased along with the increase of BPF concentration. The results indicated that the colour tends to be redder and bluer on Q/F-BPF films.

The colour difference (ΔE) of the films presented in Table 1 increases with the increase in BPF concentration. When the total colour difference (ΔE) was higher than 3, the colour changes are visible to the naked eye [4]. According to Table 2, the (ΔE) values for Q/F-BPF films were above 3 where the lowest was 53.40 and the highest was 56.98, indicating that the colour portrayed by each Q/F-BPF film was visually discernible. A study by Husin et al. [27] distinguished significant changes in L*, a* and b* of delphinidin-based colour-indicator films coalesced with butterfly anthocyanin. Therefore, these results indicate that Q/F-BPF films are potential indicator films and that the colour changes are visible to the naked eye.

Film microstructure

Figure 3 indicates the surface microstructure as well as the cross-section of QF and Q/F-BPF films. The microstructures are crucial to determining the effect of BPF on the surface and cross-section of the starch gelatine matrix. The QF films exhibited smooth and homogeneous surfaces which demonstrate good compatibility as well as dispersion. The two polymers are very compatible with hydrogen bonding between the carboxyl groups (COO) of the protein and the -OH groups of the polysaccharide, especially when used in the same ratio [28].

The Q/F-BPF film surface was a little rough with a homogeneous and dense structure. Starch and BPF significantly interact, and the anthocyanin extract was thoroughly mixed. The development of hydrogen bonds with the starch matrix is facilitated by the addition of phenolic compounds like anthocyanin [4]. According to Kim et al. [28], although they tended to aggregate in some areas of the film, overall, they were evenly dispersed throughout the polymer matrix. The cross-section specifies the compact and dense surface with the dispersion of the polymers.

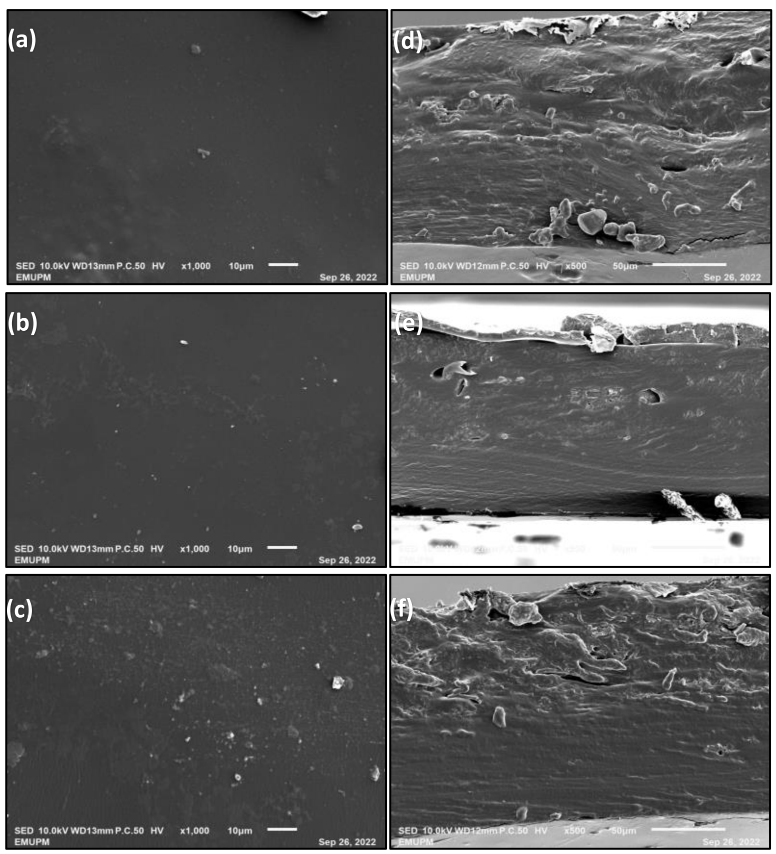


Figure 3. SEM images of the surface of the QF (A), Q/F-BPF 5% (B), and Q/F-BPF 10% (C) indicators and cross sections of the QF (D), Q/F-BPF 5% (E), and Q/F-BP 10% (F) indicators.

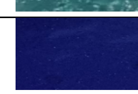
Application of Q/F-BPF films to monitor shellfish freshness

The spoilage of seafood products is initially caused by microbes as well as biochemical amine reactions which lead to freshness decline and the release of volatile nitrogenous compounds. Ammonia, trimethylamine, and dimethylamine are volatile foul-smelling gases released by microbial activity and enzyme degradation of proteins in seafood [25]. The pH rises when these volatile nitrogenous compounds accumulate in the headspace of the air-tight containers during extended storage. Thus, pH indicator films are used to detect the shellfish freshness. The Q/F-BPF films were implemented to observe the shellfish freshness under chiller storage (4 °C for 6 days).

The results showed the Q/F-BPF film colour changes indicated shellfish spoilage, with the odour becoming obvious towards the end of the storage. The initial colour of the Q/F-BFE 5% and Q/F-BPF 10% indicator was used as a reference for ΔE calculation and changes in ΔE during storage are presented in Table 3. The ΔE value after 2 days of storage on Q/F-BPF 5% was 5.57 for blood cockles and 7.79 for short-necked clams, indicating an increase in spoilage parameters. The ΔE value of the Q/F-BPF 5% indicator sharply increased to 24.33 for blood cockles and 16.70 for short-necked clams after 6 days of storage and the indicator colour changed from blue to green. Thus, the colour of the Q/F-BPF 5% indicator changed according to the changes in the quality of blood cockles and short-necked clams. The colour changes of the Q/F-BPF films at the end of the storage were visible to the naked eye when the ΔE was more than 3 [4].

Taken together, Q/F-BPF films showed a significant colour change throughout the 6 days of storage of blood cockles and short-necked clams due to the spoilage and the formation of volatile nitrogenous compounds. A previous study reported that the colour of S-BPF films turned to intense green from pink on the 6th day of shrimp storage [4]. Similarly, the recognisable anthocyanin colour changes during storage showed that the quality of the shrimp had declined [28]. The colour changes were more distinct in the Q/F-BPF 10% film which had a higher concentration of BPF extract. These results depicted that BPF immobilised with quinoa starch and fish gelatine has the potential to monitor shellfish freshness and other food samples.

Table 3. The colour response of Q/F-BPF films at different time intervals (0, 2, 4, 6 days) for shellfish freshness

Day	**Films**	**Colour**				**Films Appearance**
		L***	***a******	***b******	**ΔE**	
		Blood Cockles Sample				
0	Q/F-BPF 5%	53.90 ± 1.54^{a}	3.31 ± 0.39^{a}	-6.28 ± 2.49^{a}	0	
2		57.60 ± 5.03^{a}	3.54 ± 0.34^{a}	-10.43 ± 1.23^{b}	5.57	
4		62.21 ± 4.36^{a}	3.87 ± 0.64^{a}	-11.51 ± 1.24^{a}	9.84	
6		65.62 ± 2.32^{a}	0.91 ± 2.77^{a}	14.90 ± 29.30^{a}	24.33	
0	Q/F-BPF 10%	55.98 ± 2.96^{a}	3.45 ± 0.40^{a}	-6.73 ± 1.72^{a}	0	
2		64.50 ± 4.64^{a}	2.59 ± 0.06^{b}	-6.11 ± 0.84^{a}	8.59	
4		68.20 ± 4.59^{a}	2.63 ± 0.85^{a}	-7.86 ± 3.47^{a}	12.30	

Day	Films	Colour				Films Appearance
		L^*	a^*	b^*	ΔE	
6		63.20 ± 4.74^a	0.547 ± 0.61^a	-3.38 ± 0.21^a	8.47	
Short-necked Clams Sample						
0	Q/F-BPF 5%	51.94 ± 5.25^a	3.81 ± 0.55^a	-8.51 ± 1.46^b	0	
2		59.44 ± 1.95^a	3.71 ± 0.19^a	-10.58 ± 1.58^b	7.79	
4		66.91 ± 2.26^a	0.93 ± 1.14^a	-6.70 ± 1.12^a	15.35	
6		66.93 ± 2.98^a	-1.55 ± 2.18^a	-3.46 ± 0.04^a	16.70	
0	Q/F-BPF 10%	60.94 ± 2.04^a	3.07 ± 0.37^a	-5.41 ± 0.22^a	0	
2		64.07 ± 3.17^a	2.95 ± 0.095^b	-5.66 ± 0.77^a	3.15	
4		69.73 ± 0.38^a	2.95 ± 0.09^a	-5.66 ± 0.77^a	8.87	
6		66.28 ± 1.84^a	0.08 ± 0.99^a	-3.39 ± 0.36^a	6.44	

Note: 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 as an indicator of shellfish freshness

Table 4 presents a summary of the pH values of blood cockles and short-necked clams. The pH for both shellfish initially was low, 5.8 for blood cockles and 5.9 for short-necked clams, then increased significantly ($p < 0.05$), reaching 8.2 for blood cockles and 8.1 for short-necked clams by day 6. This was due to the accumulation of volatile nitrogenous compounds released by those shellfish [29]. This trend was in line with the significant increase in the pH of mackerel (*Scomber scombrus*) from day 0 to day 15 when stored in a chiller [30]. Hence, the increase in pH of the blood cockles and short-necked clams on day 6 depicted that the freshness and quality of the shellfish declined, and that spoilage had begun.

Table 4. pH value of blood cockles and short-necked clams during storage

Fish Sample	Day	pH
	0	5.83 ± 0.15^d
	2	6.60 ± 0.10^c
	4	7.30 ± 0.36^b
Blood cockles	6	8.22 ± 0.25^a
	0	5.97 ± 0.21^d
	2	6.73 ± 0.21^c
	4	7.50 ± 0.20^b
Short-necked clams	6	8.20 ± 0.14^a

Note: 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.

Conclusion

In conclusion, the anthocyanin extract from BPF incorporated into QS solid matrix films produces a good pH film indicator which has good colour intensity, and physical properties, and can be applied to seafood products. The addition of BPF to the quinoa starch and fish gelatine base did not greatly affect the thickness, moisture content or WVP. The film has good surface morphology with an even dispersion of quinoa starch, fish gelatine, BPF and glycerol. The Q/F-BPF film colour changed from blue to green indicating the spoilage (volatile nitrogenous) of shellfish during storage. The freshness assessment confirmed the shellfish spoilage started on day 1 and corresponded to the colour changes of the Q/F-BPF films on day 6, which were visible to the naked eye. Therefore, Q/F-BPF pH indicator films have promising potential to be used as a pH indicator to monitor shellfish freshness.

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