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Utilisation of black sticky rice (*Oryza sativa* L.) extract in chitosan-methylcellulose film

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Abstract

Botanical extracts have potential for application in active food packaging systems in terms of antioxidant and antimicrobial activities, including other smart functions. In the present work, black sticky rice (*Oryza sativa* L.) extract (BE; 10 - 30%) was incorporated into a composite film of chitosan (1.0%) and methylcellulose (0.5, 1.0, and 1.5%), and prepared by a casting technique using polyethylene glycol (PEG 400) as a plasticiser. Application of 1.5% methylcellulose in combination with chitosan slightly increased film solubility and the water vapour permeability coefficient (WVPC), and improved physical properties as compared to other treatments. Film with a high BE content (20 - 30%) was thicker, and had a decreased WVPC. The tensile strength of the film increased in contrast to a statistically significant decrease ($p \leq 0.05$) in the percentage of elongation at break. The surface morphology of the film was flat and smooth, and the cross section was more rigid when viewed under a scanning electron microscope. The colour of the film containing 30% BE changed clearly from red (pH 1.0) to pinkish brown (pH 6.0) and finally yellow (pH 12.0), indicated by decreased a* value and increased b* value. ΔE presented the total colour difference that changed from an initial before immersing. The film was responsive when tested in food systems by mounting it on the lid of the box, showing a visible orange-brown colour in torpedo scad, greyish brown in chicken tenderloin, and light orange in minimally processed pineapple when foods spoiled. The present work revealed the potential of anthocyanin extract derived from purple sticky rice for use as a natural pH indicator in chitosan-methylcellulose-based films for intelligent packaging.

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Introduction

Nowadays, packaging technology is constantly evolving and growing, and attention is paid to the choice of biodegradable materials such as starch, cellulose, and chitosan due to the environmental problems non-biodegradable materials will cause (Siracusa *et al.*, 2008). Packaging not only protects food and retards its deterioration, but also, by using current technology, can be made with special features, for example antimicrobial and antioxidant properties, including a smart function which is also known as "smart packaging" or "intelligent packaging". Smart packaging refers to a type of packaging that can assess the quality and safety of food, and then transmit the data to the external environment (Luchese *et al.*, 2017).

Many natural extracts from plants or agricultural waste have been reported in food packaging to increase the usability and added value of raw materials, such as by incorporation into a film matrix or used as a coating solution for preserving foods. Apart from having antimicrobial and antioxidant properties, plant pigments, especially anthocyanins, can be utilised as food spoilage indicators based on pH change. The colour of anthocyanins ranges from red to blue, and their stability depends on pH, light, and temperature (Khoo *et al.*, 2017). An advantage of natural indicators is their non-toxicity as compared to synthetic dyes such

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as phenol red and bromothymol blue (Hidayat *et al.*, 2019), and ability to be in contact with food. A pH indicator is commonly used as an element in smart packaging because the colour shift can be easily noticed by the eye. There have been reports of the application of anthocyanin pigments extracted from grape skin (Golasz *et al.*, 2013), purple bauhinia flower (Zhang *et al.*, 2014), purple cabbage (Pereira *et al.*, 2015; Silva-Pereira *et al.*, 2015; Zia *et al.*, 2021), rose (Shukla *et al.*, 2016), jambolan fruit (Merz *et al.*, 2020), red radish (Zhai *et al.*, 2018; Chayavanich *et al.*, 2020), blueberry peel (Shi *et al.*, 2021), and mulberry fruit (Yang *et al.*, 2021) for use as pH indicators by incorporating them into various kinds of polymer. Therefore, the study of other kinds of anthocyanin-source plants is a challenge. Moreover, reports of the utilisation of pigments extracted from black or purple sticky rice in chitosan and methylcellulose film are limited, although this type of rice seems to be a potential source of anthocyanins, including cyanidin-3-glucoside, peonidin-3-glucoside, malvidin, pelargonidin-3,5-diglucoside, cyanidin-3,5-diglucoside, and pelargonidin-3-glucoside (Rerkasem *et al.*, 2015).

Chitosan is a derivative of chitin, a by-product obtained from the aquaculture processing industry, and has been employed in a variety of food manufacturing applications such as edible film and coating. Chitosan is a high molecular weight polymer consisting of units N-acetyl-D-glucosamine connected by β (1–4) glycosidic bonds. For film production, the properties depend on the source of chitosan (molecular weight and degree of deacetylation), solvent, drying conditions, storage time, and temperature (Park *et al.*, 2002). However, the limitation of pure chitosan film is its brittleness. In order to solve this problem, blending with methylcellulose helps to improve the tensility of chitosan film (Rachtanapun and Wongchaiya, 2012). Methylcellulose is a water-soluble hydrocolloid derived from cellulose, where methyl groups ($–CH_3$) substitute the hydroxyls of anhydro-D-glucose units by etherification (Nasatto *et al.*, 2015). The utilisation of methylcellulose in a blended film enhances the film's strength, toughness, flexibility, and water solubility. Moreover, film obtained from methylcellulose is translucent, oil resistant, and acts as a barrier to oxygen, carbon dioxide, and aromatic compounds (Park and Chinnan, 1995).

Therefore, the objective of the present work was to determine the optimum ratio of chitosan and methylcellulose for preparing a film incorporated with black sticky rice extract for observing the pH-sensing potential. Physical, mechanical, and barrier properties of the film were also evaluated.

Materials and methods

Extraction of anthocyanins from black sticky rice

Black sticky rice (*Oryza sativa* Linn.) grown in Nan province, Thailand, during August-December 2018, was purchased from a local supplier. The rice materials were dried and rid of insects by heating in a hot air oven at 70°C for 2 h. To extract anthocyanins, the procedure described by Pedro *et al.* (2016) was followed; rice was mashed in a blender and then extracted with a mixed solution of ethanol and 1.0 mol/L citric acid (80:20) at a ratio of 1:10 for 2 h by shaking continuously using an automatic shaker (HS-260 basic, IKA, Germany). The extraction process was performed in a light-protected environment at 25 ± 1°C using aluminium foil to cover the extraction device. The residue was separated with a strainer, and then the supernatant was filtered through No. 1 filter paper. Anthocyanin extract was kept in an amber glass bottle, and stored at 4 ± 1°C in a refrigerator for no more than 3 h until film preparation.

Study of the optimum ratio for chitosan-methylcellulose film preparation

The preparation of film from chitosan (1.0%) in combination with methylcellulose (0.5, 1.0, and 1.5%) was studied. The film-forming procedure was conducted according to Sungsuwan *et al.* (2008) with some modifications, using a casting technique and polyethylene glycol (PEG 400) as a plasticiser. To prepare the film, medium molecular weight chitosan (75 - 85% deacetylated; Sigma-Aldrich, Germany) was dissolved in 1% acetic acid, and mixed with methylcellulose (viscosity 4,000 cP; Sigma-Aldrich, Germany) which was prepared in 50% ethanol at a ratio of 1:1 using a magnetic stirrer at 70°C. Next, 1 mL of PEG 400 was added to 100 mL of mixed solution, and stirred continuously at 70°C for 20 min. The mixture was kept at room temperature for about 1.5 h to remove the gas bubbles in the film solution before pouring into a Petri dish (diameter 13.5 cm; 30 mL per dish). The plate was allowed to dry at room temperature for 48 h. After that, the dried film was manually peeled from the dish using stainless steel sharp-tipped pliers. Physical (film thickness and film solubility) and barrier properties (water vapour

transmission rate) were analysed for each film formulation.

Preparation of chitosan-methylcellulose film incorporated with black sticky rice extract

The optimum ratio of chitosan and methylcellulose was used to prepare film in combination with anthocyanin extract from black sticky rice (BE). After the blend of chitosan and methylcellulose was dissolved completely, it was allowed to cool down to around 40°C, and then BE was added to the mixed solution to give a final volume of 10, 20, or 30% of anthocyanins using PEG 400 as a plasticiser. Film formation and drying were done using the method for film preparation as earlier described.

Film thickness

Film thickness was measured using a micrometre (Winton, Japan). Three pieces of film for each treatment were used in the experiment; five different points were measured, and the mean film thickness was reported in mm.

Film solubility

Film solubility in water was determined by placing the cut film (3 × 3 cm) in a Petri dish filled with 10 mL of distilled water for 6 h; the film was allowed to dry at room temperature for 24 h. The weight of film before and after immersion in water was noted using a 4-digit digital scale (ATX224, Shimadzu Corporation, Japan). The percentage of film solubility was calculated using Eq. 1 (Brychcy *et al.*, 2015):

$$\text{Film solubility (\%)} = \frac{\text{Initial dry weight - Final dry weight}}{\text{Initial dry weight}} \times 100 \quad \text{(Eq. 1)}$$

Water vapour transmission rate (WVTR)

The WVTR of film was determined by the cup method using ASTM E96-87 as recommended by Caner *et al.* (1998) with minor modifications. The experiment was done by placing the film sample in an aluminium cup containing distilled water. Then, the cup was sealed by covering it tightly with paraffin film without any leaks. The initial weight of cup samples was measured using a 4-digit digital scale (ATX224, Shimadzu Corporation, Japan), and then the cup was placed in a desiccator at 11 ± 2% RH and 26 ± 2°C. The sample cup was weighed at 6 h intervals for 48 h. WVTR (g/h·m^2) was calculated using Eq. 2 given by ASTM E97-87, where the slope obtained from linear regression (R^2 = 0.97 - 0.99) of the plot between the amount of water lost against time was divided by the tested film area (3.0175×10^{-3} m^2). The film thickness (mm) and the difference in water vapour partial pressure (atm) between the inside (p_1) and outside (p_2) of the cup were used to calculate water vapour permeability coefficient (WVPC) using Eq. 3.

$$\text{WVTR} = \text{Slope/Film area} \quad \text{(Eq. 2)}$$

$$\text{WVPC} = (\text{WVTR} \times \text{Thickness}) / \Delta\text{Vapour pressure} \quad \text{(Eq. 3)}$$

Tensile strength and elongation at break

The tensile strength and elongation at break of film were analysed following the standard method ASTM D882-12 using a universal testing machine (Instron model 1123, USA). The film sample was cut into pieces of 25.4 mm wide; five pieces being used per test. The machine was set up with spacing between the grip of 50 mm, and crosshead speed of 25 mm/min. The conditions of the test were 27 ± 1°C and 65 ± 2% relative humidity. Tensile strength was reported in megapascal (MPa) and elongation at break in percentage (%).

Film morphology

The surface morphology and cross section of chitosan-methylcellulose film incorporated with BE was observed by scanning electron microscope (JSM-5800LV, JEOL, Japan). Film was prepared by cutting with a razor blade into 0.8 × 80 mm pieces for the cross-sectional study, and 0.8 × 0.8 mm pieces for surface study. For the cross-sectional study, the film sample was immersed in liquid nitrogen, and broken horizontally using a blade. After film preparation, the samples were coated with gold using an ion sputter coater (SC 7620, Polaron Range, UK) then adhered to an aluminium stub with carbon tape, analysed under an acceleration voltage of 10 kV, and viewed at 200 and 1,500× magnifications.

Colour responsivity of film in pH buffer

The method for preparing pH buffers was described by Pereira *et al.* (2015). Phosphate buffer (1% disodium hydrogen phosphate mixed with 1% sodium dihydrogen phosphate) was adjusted with lactic acid (0.2 M) or sodium hydroxide (0.1 N) to

reach a pH of 1 - 12, which was measured by a pH meter (F20, Mettler Toledo, Switzerland). Film was cut into 2 × 2 cm pieces and immersed in pH buffer for 10 min. The colour response of film was analysed using a colorimeter (Color Quest XE; Hunter Lab, USA), and reported as L*, a*, b*, chroma, and hue values. The total colour difference (ΔE) was calculated using Eq. 4, using film before the experiment as a standard ($L_0, a_0,$ and b_0).

$$\Delta E = \sqrt{(L - L_0)^2 (a - a_0)^2 (b - b_0)^2} \qquad \text{(Eq. 4)}$$

L* was lightness which ranged from black (0) to white (100); chroma (C*) was distance from the lightness axis (L*), and ranged from 0 to 100; hue angle (h) ranged from 0° (red) to 270° (blue); a* was red-green; and b* was yellow-blue.

Film colour responsivity for detecting food freshness

Minimally processed pineapple (*Ananas comosus*), chicken tenderloin, and torpedo scad (*Megalaspis cordyla*) were used as samples for simulating food deterioration under accelerated conditions of 36 ± 2°C. Pineapple (cv. Pattavia) was washed with tap water and peeled; half of the fruit was cut into six parts, and then sliced to a thickness of 2 cm. Each sample (88 - 98 g) was packed in a plastic box. Chicken tenderloin (38 ± 3 g/piece) and torpedo scad (162 ± 5 g/fish) were kept in plastic boxes (15 × 17 cm; one piece per box).

Film performance was studied by a non-contact method. The cut film (2 × 2 cm) was attached to the lid of the box with masking tape at three positions (left, middle, and right). The visual colour response of the film in correlation with pH change was considered until the end of food shelf-life.

Statistical analysis

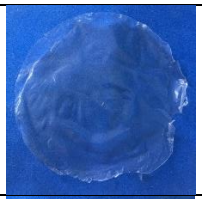
The mean values between treatments for each experiment were compared by Duncan's multiple range test using a statistical software program (SPSS V. 26, IBM Company, Ontario, Canada).

Results and discussion

Optimum ratio for chitosan-methylcellulose film preparation

The physical and barrier properties of a basic formula film which was prepared from 1% chitosan in combination with 0.5, 1.0, and 1.5% methylcellulose are shown in Table 1. Results showed that on application of methylcellulose at the lowest concentration of 0.5%, a dried film could not be formed. The film could be easily peeled off from the Petri dish when the concentration of methylcellulose was increased to 1.0 and 1.5%; it was thicker and had increased WVPC. Moreover, the physical characteristics of 1.5% methylcellulose film were the most complete; no film damage was observed, as seen in Table 1. Methylcellulose is hydrophilic because it contains hydroxyl groups; therefore, it has a lower resistance for water vapour diffusion (Nasatto *et al.*, 2015). For this reason, the film prepared with 1.5% methylcellulose had a

Table 1. Physical and barrier properties of chitosan (CH)-methylcellulose (MC) film.

Treatment	Film thickness (mm)	WVPC (10^{-4} g/m·h·atm)	Film solubility (%)	Film appearance
1.0% CH + 0.5% MC	- *	- *	-*	-*
1.0% CH + 1.0% MC	0.058 ± 0.003^{b}	3.85 ± 0.003^{b}	46.49 ± 1.17^{ns}	
1.0% CH + 1.5% MC	0.060 ± 0.003^{a}	3.93 ± 0.021^{a}	47.25 ± 2.15^{ns}	

Asterisks (*) indicate that a film could not be formed. Values are mean ± standard deviation. Means followed by different lowercase superscripts in a column are significantly different at $p \leq 0.05$. ^{ns}Not significant.

significantly higher WVPC ($p \leq 0.05$) than 1.0% methylcellulose film: 3.93 and 3.85×10^{-4} g/m·h·atm, respectively (Table 1). However, the advantage was it could prevent the condensation of water vapour inside the package, an important factor supporting microbial growth (Park and Chinnan, 1995). This result is in agreement with the observation by Chambi and Grosso (2011) that methylcellulose films created by blending one or two kinds of polysaccharides (glucomannan and/or pectin) had significantly improved WVPC as compared to that of pure polymer films.

The percentage solubility of films containing the two levels of methylcellulose (1.0 and 1.5%) were not significantly different ($p > 0.05$), with values between 46 and 47%. A similar trend was observed in a chitosan (2%) and methylcellulose (1%) blended film, in which, increasing the proportion of methylcellulose to more than 80% of the total components resulted in a significant increase ($p \leq 0.05$) in film solubility, while application at a lower ratio of methylcellulose had a slight effect (Mura *et al.*, 2011). Film solubility is an important factor when the film is used under conditions of a high moisture content and relative humidity associated with application in foods with high amounts of water such as fruits, vegetables, and meats; so that it must be insoluble (Sothornvit and Krochta, 2000). From these results, the optimum ratio for preparing a basic film formula was determined as 1% chitosan in combination with 1.5% methyl cellulose.

Characteristics of chitosan-methylcellulose film incorporating black sticky rice extract

Physical, mechanical, and barrier properties

The physical, mechanical, and barrier properties of chitosan-methylcellulose film incorporated with BE in terms of film thickness, tensile strength, elongation at break, and WVPC are shown in Figure 1. The addition of a higher percentage of BE resulted in a significant increase ($p \leq 0.05$) in film thickness as compared to pure blended film. Film incorporating 10% BE was 0.062 mm thick, and the thickness increased to 0.065 and 0.072 mm, respectively, when 20 and 30% BE were added. WVPC represents the mobility of water vapour molecules through the film matrix. Results showed that incorporating BE at all concentration levels decreased WVPC ($2.84 - 3.34 \times 10^{-4}$ g/m·h·atm) as compared to pure blended film (3.93×10^{-4} g/m·h·atm). This might have been due to particles of anthocyanin molecules (polyphenol compounds) which can bind with the polymer matrix, thus preventing the movement of water vapour through the film. This result is in agreement with Pastor *et al.* (2012) who observed that the addition of resveratrol to the matrix of a chitosan and methylcellulose-based film led to a decrease in WVPC, and the film experienced a loss of strength and became more brittle. It can be clearly seen from the SEM image in Figure 2 that the cross section of film containing 30% BE was noticeably rough (Figure 2 A-3) when compared with pure blended film (0% BE) that showed a homogenous layer throughout the matrix (Figure 2 A-1). The 30% BE film was visibly thicker in the SEM image (Figure 2 A-3). A flat smooth surface with no pores was observed for both film formulas (Figure 2 A-2 and A-4), thus indicating continuous hydrogen bonding throughout the film matrix. The results are similar to those for the morphology of the same type of polymer blend reported by Rachtanapun and Wingchaiya (2012).

The tensile strength of film was greater when the percentage of BE increased from 10 to 30%, while elongation at break decreased when compared with pure blended film. The chitosan-methylcellulose film incorporating 30% BE had the highest tensile strength at 20.3 MPa, and the lowest elongation at break at 63.2% (Figure 1C and 1D). The incorporation of BE changed the proportions between the two polymers and plasticiser, thus leading to the production of a compact and rigid structure, which in turn indicated a reduction of the workload required to cause film breakage. The results are in agreement with those found for the same composite film incorporated with silica nanoparticles, inducing film strength (Mura *et al.*, 2011). Jimtaisong and Saewan (2018) also confirmed the functionality of the crosslinking potential of polyphenol compounds, *i.e.* gallic and ferulic acids in chitosan-methylcellulose composite film. A similar trend has also been observed in other types of polymers, including a gelatine film incorporating a polyphenol from mango peel extract, in which tensile strength increased and elongation at break decreased (Adilah *et al.*, 2018).

Film sensitivity in pH buffer

In this experiment, the film incorporated with 30% BE was used to investigate the film's responsivity to pH shift, since it contained a strong

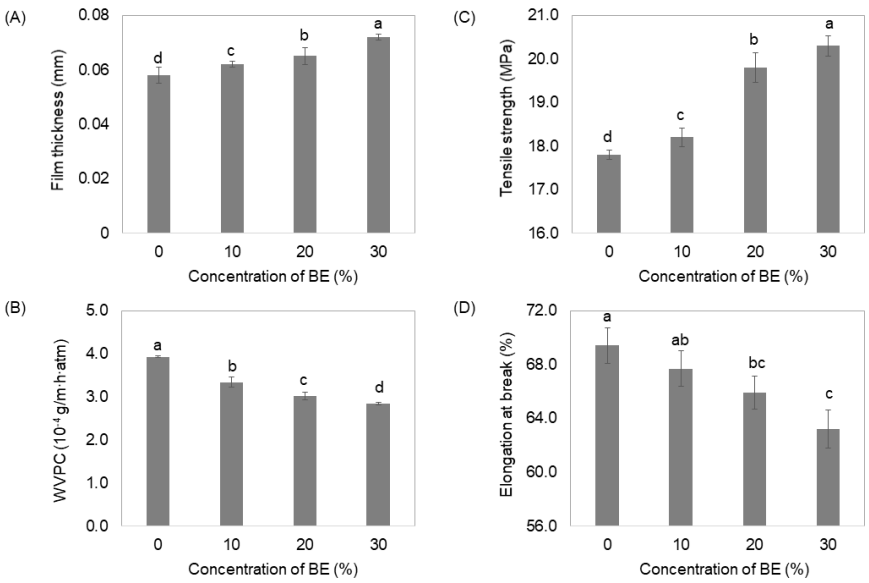


Figure 1. Physical [film thickness **(A)**, tensile strength **(C)**, and elongation at break **(D)**] and barrier properties [WVPC **(B)**] of chitosan-methylcellulose film incorporated with 10 - 30% of black sticky rice extract (BE). Bars represent the standard deviation of the mean at $p \leq 0.05$.

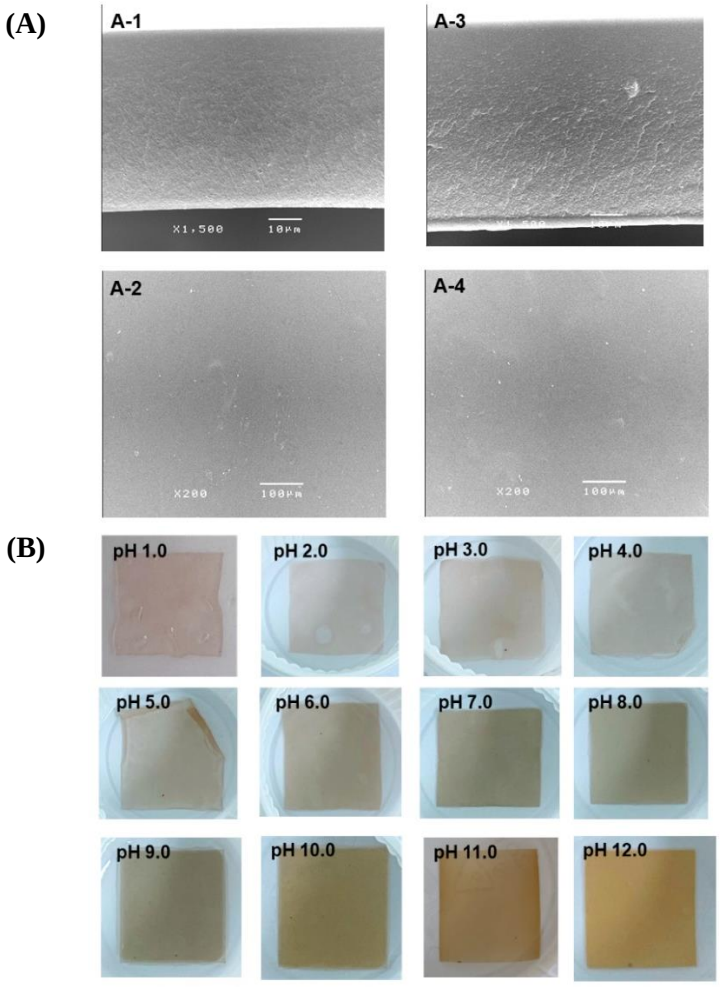


Figure 2. Cross section and surface of chitosan-methylcellulose film (A-1 and A-2) and chitosan-methylcellulose film incorporated with 30% black sticky rice extract (A-3 and A-4) at 200× and 1,500× magnification **(A)**, and film sensitivity tested in buffers of different pH levels **(B)**.

anthocyanin pigment and the colour change could be observed visually. The results are shown in Figure 2B. The film responded to both acidic and basic conditions. At pH 1.0, the film was reddish pink and the colour changed to light pink at pH 2.0 - 4.0. At pH 5.0 - 6.0, the film was pinkish brown, which was similar to its initial colour. At pH 7.0 - 9.0, the film was yellowish brown, and at pH 10.0, it was a brownish yellow colour. At basic conditions of pH 11.0 - 12.0, the film presented an intensive yellow colour. It was noticeable that the film was outstandingly responsive to pH shifts, so it could be applied to measure pH as a result of changes in food chemical composition.

The colour values (L*, a*, b*, chroma, and hue angle) of film at different pH levels (1.0 - 12.0) are shown in Table 2. The hue angle of film at pH 1.0 - 3.0 was in the range of 21.78 - 24.12°, and expressed a red shade in accordance with a higher a* value, and the visual colour presented in Figure 2B. Hue angle significantly increased ($p \leq 0.05$) to 42.37 - 44.66° when the pH increased to 4.0 - 5.0 (Table 2), thus indicating that the colour shifted to an orange-brown tone, and the colour intensity declined as observed by the low chroma value (2.88 - 3.29). Hue angle continuously increased to a more orange and yellow tone at pH 6.0 - 7.0, 8.0 - 10.0, and 11.0 - 12.0, being 59.77 - 60.85°, 63.39 - 65.62°, and 69.21 - 75.68°, respectively (Table 2). The greater value of total colour difference (ΔE) at each pH level indicated that the colour of the film was noticeably changed when compared with that before measurement.

Other works have used similar raw material, purple or black rice and its bran, incorporated with other kind of polymer matrix such as chitosan (Yong *et al.*, 2019), chitosan/oxidised chitin nanocrystals (Wu *et al.*, 2019), and filter paper. The pH-sensing elements changed from red to blue (Yong *et al.*, 2019), pink-red to purple brown (Wu *et al.*, 2019), and red to yellow when the pH shifted from acidic to basic. This might be dependent on the type and concentration of anthocyanins which differ by cultivar (Sivamaruthi *et al.*, 2018), the type of polymer on which the film is based (Kurek *et al.*, 2019), and the interaction between the film matrix and anthocyanin molecules (Halász and Csóka, 2018). A low or high level of anthocyanin extract results in a distinct colour in each pH range (Wu *et al.*, 2019; Merz *et al.*, 2020).

Table 2. Colour values (L*, a*, b*, chroma, and hue angle) and total colour difference (ΔE) of chitosan-methylcellulose film incorporated with 30% black sticky rice extract at different pH levels.

pH	L*	a*	b*	Chroma	Hue angle	ΔE
1	76.48 ± 0.16^{d}	7.88 ± 0.99^{a}	0.40 ± 0.04^{l}	7.42 ± 0.22^{e}	19.78 ± 0.17^{k}	5.18
2	77.92 ± 0.04^{a}	4.11 ± 0.01^{b}	0.69 ± 0.02^{k}	6.86 ± 0.00^{f}	21.78 ± 0.48^{j}	4.10
3	71.00 ± 0.02^{f}	3.31 ± 0.10^{e}	0.74 ± 0.01^{j}	6.38 ± 0.01^{h}	24.12 ± 0.04^{i}	3.64
4	76.81 ± 0.01^{b}	2.81 ± 0.00^{g}	2.03 ± 0.01^{i}	2.88 ± 0.01^{k}	44.66 ± 0.21^{g}	2.75
5	76.75 ± 0.01^{c}	3.78 ± 0.01^{d}	2.22 ± 0.02^{h}	3.29 ± 0.01^{j}	42.37 ± 0.25^{h}	2.54
6	72.79 ± 0.01^{e}	3.23 ± 0.01^{f}	5.79 ± 0.02^{f}	6.63 ± 0.01^{g}	60.85 ± 0.13^{e}	3.70
7	69.52 ± 0.01^{i}	4.00 ± 0.00^{c}	6.87 ± 0.02^{d}	7.95 ± 0.02^{b}	59.77 ± 0.10^{f}	6.50
8	70.81 ± 0.01^{g}	2.58 ± 0.02^{i}	5.28 ± 0.04^{g}	5.88 ± 0.03^{i}	63.96 ± 0.23^{d}	4.59
9	69.22 ± 0.05^{j}	3.20 ± 0.02^{f}	6.98 ± 0.01^{c}	7.69 ± 0.01^{c}	65.37 ± 0.18^{c}	6.80
10	69.69 ± 0.01^{h}	2.75 ± 0.01^{gh}	6.06 ± 0.01^{e}	6.65 ± 0.01^{g}	65.62 ± 0.09^{c}	5.89
11	68.63 ± 0.01^{k}	2.70 ± 0.02^{h}	7.11 ± 0.03^{b}	7.61 ± 0.02^{d}	69.21 ± 0.22^{b}	7.37
12	64.42 ± 0.01^{l}	3.60 ± 0.01^{d}	14.07 ± 0.04^{a}	14.53 ± 0.03^{a}	75.68 ± 0.07^{a}	15.24

Values are mean ± standard deviation. Means followed by different lowercase superscripts in a column are significantly different at $p \leq 0.05$.

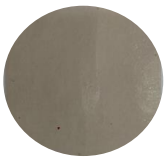
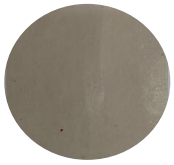
Film sensitivity in food samples

The performance of the film for indicating the pH changes in food which are involved in their deterioration during storage was evaluated under accelerated conditions (36 ± 2°C). The changes in pH and film colour during 2-day storage are shown in Table 3. Increased temperature induced a decrease in the pH of minimally processed pineapple from an initial 4.35 ± 0.07 to 3.88 ± 0.01, and the colour of the film changed to light orange at the end of the storage

period (Table 3). The changes in pH of chicken tenderloin and torpedo scad were similar, increasing continuously throughout the storage period. An increase in pH value is associated with meat deterioration when the total volatile base nitrogen content in aquatic muscle increases during the spoilage process due to enzymatic or microbial activity (Dudnyk *et al.*, 2017; Kanatt, 2020; Yang *et al.*, 2021). Moreover, proteolytic bacteria can break down protein with their protease enzyme to smaller protein molecules and amino acids, which will be further decomposed to volatile substances such as ammonia, thus resulting in an increase in pH (Nychas and Tassou, 1997). For non-fresh foods, the film turned an opaque orange-brown shade in torpedo scad, and dark greyish brown in chicken tenderloin at the end of the storage period (Table 3).

Other works have also reported the responsivity of indicator film to monitor the freshness of fish, *e.g.*, spade nose shark fillets (Kanatt, 2020), salmon fillets (Lan *et al.*, 2021), and crucian fish (Yang *et al.*, 2021), using intelligent film made from *Amaranthus* leaf (Kanatt, 2020), red apple pomace (Lan *et al.*, 2021), and mulberry extract (Yang *et al.*, 2021). The source of anthocyanins (Rodrigues *et al.*, 2021) and type of polymer on which the film is based (Kurek *et al.*, 2019) are important factors that affect its colour. In other research on chicken meat, a red cabbage sensor film changed from purple-pink to green-blue (Dudnyk *et al.*, 2017), and red radish film changed from red to grey-purple (Chayavanich *et al.*, 2020), while a foam indicator with red cabbage turned from purple to greenish-grey (Zia *et al.*, 2021) when the food spoiled.

Table 3. Film sensitivity to pH change tested in food samples under accelerated storage conditions (36 ± 2°C).

Food sample	Day	pH	Initial storage	End of storage
Minimally processed pineapple	0	4.35 ± 0.07[a]		
	1	4.07 ± 0.07[b]		
	2	3.88 ± 0.01[c]		
Chicken tenderloin	0	5.19 ± 0.01[c]		
	1	5.45 ± 0.01[b]		
	2	5.76 ± 0.01[a]		
Torpedo scad	0	5.46 ± 0.01[c]		
	1	5.55 ± 0.01[b]		
	2	5.92 ± 0.02[a]		

Values are mean ± standard deviation. Means followed by different lowercase superscripts in a column are significantly different at $p \leq 0.05$.

Conclusion

The composite chitosan-methylcellulose film (1% chitosan and 1.5% methylcellulose) incorporating 30% BE had good physical and mechanical properties. The film responded in a wide range of pH environments (acidic to basic), and was able to detect food deterioration which appeared as a change in film colour. The film did not disintegrate during storage under experimental conditions. Therefore, BE appears to be an interesting new source of a natural pH indicator for application in food packaging. Future studies should investigate changes in the chemistry of foods that are associated with food spoilage, and their relation to the film's response.

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