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Characterization and Properties of Chitosan/PVA Bio-based Film Incorporated with *Clitoria ternatea* L. (Butterfly pea) Extract and Its Application in Foods

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Abstract

Natural anthocyanin pigments gained awareness to use to improve the function of food packaging based on dye sensitivities when chemical composition changes. In this study *Clitoria ternatea* L. (Butterfly pea) extract (10-30%) was incorporated into chitosan/poly-vinyl alcohol (PVA) based film to produce pH-sensing elements. Physical, mechanical and barrier properties of film, e.g., swelling index, thickness, water vapor permeability coefficient (WVPC) and tensile strength were evaluated. Film incorporated with 30% *Clitoria ternatea* L. extract showed an increase in film thickness and decreased in tensile strength and swelling index. No significant difference ($p>0.05$) was observed in WVPC between treatments. Films were immersed in six levels of pH buffer (1, 3, 5, 7, 9 and 12) and exhibited color response ranging from brownish-red (acidic), bluish-green (neutral) and yellow (basic), respectively, within 15 min which was confirmed by color parameters (L*, a*, b*, chroma and hue angle). Total color different (ΔE) greatly rose under extreme pH conditions (pH 1 and 12). Films were also performed on a model of low (pasteurized milk and chicken meat) and high (fresh-cut pineapple) acid foods. The visual color of film responded when pH shifted, which changed into pale pink (pasteurized milk), green (chicken meat) and red (fresh-cut pineapple) compared to an initial color (bluish-green). This study revealed that an embedding of *Clitoria ternatea* L. extract into chitosan/PVA based films had a pH-sensing potential material for application in smart packaging.

Introduction

In the current consumer environment, quality and safety of food are important factors that consumers are concerned about. The modern consumer demand is for convenience products due to changes in modern lifestyles, however, food safety must go hand in hand with good nutrition. Therefore, fresh food and minimally processed products are now gaining greater attention, especially among the healthy-lifestyle consumer because of the high nutritional content. However, shelf-life of fresh food is short and must be stored in a temperature controlled condition throughout the storage periods. Likewise, foods that have been on the shelf for a long

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period result in a reduction of food quality which increases with storage times. Intelligent or smart packaging has been developed for solving this problem and guaranteeing quality, food safety, and traceability (Vanderroost et al., 2014). The system comprises a smart function such as sensing, detecting, tracing, recording, communicating and sending information to the external environment (Schaefer & Cheung, 2018). Examples of intelligent packaging are radio frequency identification (RFID) sensors (Vanderroost et al., 2014), time-temperature indicators (Lu et al., 2013b; Pereira et al., 2015; Wu et al., 2015), gas sensing devices (Vu & Won, 2013), freshness and ripeness indicators (Kuswandi et al., 2013), and a microbial indicator (Yousefi et al., 2018).

Freshness sensor offers quality data resulting from microbial metabolism or chemical change of the food product, based on a color shift (Vanderroost et al., 2014) which is made by embedding a pigment in various kinds of material such as natural polymers and paper. Currently, natural pigments are noticeable used instead of artificial dyes such as phenol red and bromothymol blue (Hidayat et al., 2019), since its plays an important role in non-poisoning and widely response to pH ranges (Kungsuwan et al., 2014). Anthocyanin, a water-soluble natural pigment, has a great potential as a pH sensor which is exhibited in red, purple, or blue based on the pH of the environment. Moreover, the expression of anthocyanin color is markedly influenced by its structure, temperature, pH, ultraviolet light, and oxygen (Kungsuwan et al., 2014). Extraction of anthocyanin from red cabbage (Pereira et al., 2015), grape peel (Golasz et al., 2013), blakeana flower (Zhang et al., 2014), rose flower (Shukla et al., 2016) and purple sweet potato (Liu et al., 2017) have been noted for utilizing in a smart packaging system.

Clitoria ternatea L. flowers (Butterfly pea) are a perennial herbaceous plant with a distinctive deep blue color that grows in Tropical Asia, and are found in Africa, Australia and America (Sinha et al., 2012). In Asia, a blue color of *Clitoria ternatea* L. is widely used as a natural coloring for foods (Kungsuwan et al., 2014), textiles and other industries (Sinha et al., 2012). The color of anthocyanin extracted from *Clitoria ternatea* L. can shift to various shades which show a red color in acid, blue in neutral and green in base condition (Kungsuwan et al., 2014). The embedding *Clitoria ternatea* L. extracted in polyvinyl alcohol (PVA) film showed positive response to NH_3 vapor as a model volatile amine. However, the limitation of PVA based film has shown poor performance and appearance because of a swelling problem in the excess moisture environment which should be modified by blending with other polymers (Sukprasong, 2013). Based on the results of the above study, there is a possibility to use anthocyanin from *Clitoria ternatea* L. in an intelligent packaging system. However, studies on the blend of PVA with other polymers, a composite film, are needed to reduce their limitations accordingly. The ratio between chitosan and PVA embedded with fresh Butterfly pea extract was noted by Hidayati et al. (2021). However, the optimum anthocyanin concentration to produce the colorimetric pH indicator and its application in various kinds of food have not been reported.

Currently, the demand for modern packaging in the global food industry is growing continuously in order to provide food safety and shelf stability. Unfortunately, it also brings a pollution crisis on the resources and the environment. From this perspective, increase interest is occurring in environment-friendly packaging materials, a natural source, such as polysaccharide (gum, starch, chitosan, cellulose, and their derivatives), protein (whey, soy, corn, zein, gelatin and wheat gluten, etc.) and lipid (Mangaraj et al., 2019). Chitosan is a cationic amino polysaccharide derived from chitin (N-deacetylated derivative) which has been widely used as edible coating and film for preserving food qualities. The properties of chitosan film depends on the source (molecular weight and degree of methylation), solvent, drying condition, temperature and storage time (Park et al., 2002). However, low mechanical strength is a limit of chitosan film, so other polymers are usually incorporated, e.g., PVA (Pereira et al., 2015) which is non-toxic, biodegradable and a water-soluble. PVA is often used by mixing with other types of polymers for reducing the water solubility and improving mechanical properties (Jayasekara et al., 2004). Higher molecular weight of chitosan improved the strength of the film but did not affect water vapor permeability (Park et al., 2002). Moreover, the antimicrobial effect of chitosan has been noted (Zivanovic et al., 2005). There have been reports that a blend of chitosan and PVA cross-linked with formaldehyde using glycerol as a plasticizer enhanced thermal stability by reducing the percentage of weight loss (Abraham et al., 2016). Liu et al. (2018) found that chitosan/PVA blended film (75:25) prepared by electrospray technique improved the permeability of water vapor and showed a higher antimicrobial effect. Thus, the physical, mechanical, and barrier properties of film are an important factor for food packaging in order to be durable to use in various storage

conditions. These factors also has an effect on the quality and shelf life of food. The potential of chitosan/PVA solution or film for application in contact food has been reported in minimally processed tomato (Tripathi et al., 2009), sliced fresh *Channa argus* (Lu et al., 2013a) and chicken sausage (Nwabor et al., 2020).

The objective of this study was to evaluate the performance of a chitosan/PVA based film incorporated with an anthocyanin extract from *Clitoria ternatea* L. as pH-sensing elements. The efficacies of the sensing film were tested in various pH-solutions and both low (pH > 4.6) and high acid food (pH < 4.6) samples. Mechanical, physical and barrier properties of films were also investigated.

Materials and methods

1. Raw material and reagents

Dried *Clitoria ternatea* L. was bought from domestic markets in Sathorn District, Bangkok, Thailand. Chitosan medium molecular weight with 75-85% deacetylate was obtained from Sigma-Aldrich, Germany. Sodium tripolyphosphate and polyvinyl alcohol were used as food grade, which was obtained from Chemipan, Thailand. Sodium dihydrogen phosphate (Ajex Finechem, Australia), disodium hydrogen phosphate (Loba Chemie Pvt., Ltd, India), lactic acid (Loba Chemie Pvt., Ltd, India), acetic acid (food grade; QP, Thailand) and sodium hydroxide (NaOH; Sigma-Aldrich, Germany).

2. *Clitoria ternatea* L. extraction

Clitoria ternatea L. was extracted according to the method of Pereira et al. (2015) with minor modifications. Fifty grams of dried *Clitoria ternatea* L. was ground in 150 mL of the mix solutions of water and ethanol (7:3) followed by filtering using cotton cheesecloth. The extract was adjusted to pH 2.0 using 1 mol/L HCl and kept at 5°C in the refrigerator for 24 h. After that, the extract solution was filtered again through Whatman filter paper No. 1. The pH of supernatant was adjusted to 7.0 using 2.5 mol/L NaOH and kept in an amber bottle at 5°C until the experiment. Total anthocyanin content was analyzed by spectroscopic method at 535 nm and calculated using extinction coefficient (ε) of 98.2 given by Ranganna (1986) as shown in equation (1) and (2).

$$\text{Total optical density} = \frac{\text{OD at 535 nm} \times \text{Volumn made}}{\text{Weight of sample}} \times 100 \quad (1)$$

$$\text{Total anthocyanin content} = \frac{\text{Total optical density}}{98.2} \quad (2)$$

3. pH-sensing film preparation

The biodegradable base film was prepared by two types of polymer, chitosan and PVA, as a composite film. Chitosan/PVA film preparation was described by Pereira et al. (2015) using a casting technique with some adjustments. One percent of PVA was prepared in distilled water at 70±2°C. One percent of chitosan solution was prepared in 1% (w/v) acetic acid and heated up to 70±2°C while stirring continuously using a magnetic stirrer for 24 h. The composite film was done by mixed PVA and chitosan in the ratio of 3:7 (v/v). Then, *Clitoria ternatea* L. extract (10, 20 and 30%) was added in the chitosan/PVA solution. Sodium tripolyphosphate (1.5%) was used as a plasticizer by adding the volume of 0.1% (w/v) into the mixed solution. The final pH of the mixed solution was adjusted to 6.1 using 0.1 mol/L NaOH (shade changed from purple to blue) and then poured onto a petri dish (90 mm of diameter) with 42 mL per dish. To eliminate the solvent in the composite film, the petri dishes were placed in a hot air oven at 35°C for 5 h followed by placing at room temperature (25°C) for 48 h. Dried film was kept in an aluminum foil bag and placed in a desiccator filled inside with silica gel until experiment.

4. Swelling index (%Si)

Dried film was tested for swelling index which was described by Cavalcanti et al. (2002). The method was done by cutting the film into a size of 2 cm^2. Weight of initial film was measured before immersion in a plastic cup containing 200 mL of distilled water. Then, the film was weighed after immersion for 0.5, 1, 3, 5, 7, 10, 15 and 20 min, respectively, using two decimals digital scale. The experiment was done at 25°C using three samples per treatment. Swelling index was calculated according to the following equation (3).

$$\text{Si (\%)} = \frac{\text{(Final weight – Initial weight)}}{\text{Initial weight}} \times 100 \quad (3)$$

5. Film thickness

Film thickness was measured using a manual micrometer (Mitutoyo, Japan) at room temperature (25°C). Film was cut into a size of 2 cm^2 and then randomly measured in 3 points with three replications of each treatment.

6. Tensile strength

Mechanical property of film was reported as tensile strength using Texture analyzer (Ta.xt.plus, Stable Micro Systems, Surrey, UK) according to Zhang et al. (2019) with some modifications. Film was cut into a size of 20

mm x 75 mm. Tensile strength was measured using A/SPR probe at 25°C. Texture analyzer was set to a test speed of 1 mm/sec, pre-test speed of 1mm/sec, post-test speed of 10 mm/sec and distance of 10 mm. Three samples were analyzed in each test and tensile strength was calculated by the following equation (4)

$$\text{Tensile strength (MPa)} = \frac{\text{Maximum force (N)}}{\text{Film width (mm)} \times \text{Flim thickness (mm)}} \quad (4)$$

7. Water vapor permeability

Water vapor transmission (WVTR) of film was measured by the cup method using ASTM E96-87 (1989), which was utilized in accordance with the recommendation in Caner et al. (1998) with some modifications. This method was based on water vapor transmitted out of the cup. The non corroding aluminum cup was filled with distilled water. The film without visible scratches or leaks was mounted on the cup and tightly fixed with paraffin wax and then placed in a desiccator with 11% RH monitored at room temperature (26±2°C). The weight loss of the test cup was recorded using a four-digit digital scale (ATX224; Shimadzu Corporation, Japan) as a function of time for 48 h. WVTR ($g/h.m^2$) was calculated using equation (5) given by ASTM E97-87 (1989). To calculate the water vapor permeability coefficient (WVPC), film thickness was used to multiply with WVTR and the obtained value was then divided by the pressure difference as expressed in equation (6).

$$\text{WVTR} = \text{Slope/ Film Area} \quad (5)$$

$$\text{WVPC} = \text{WVTR} \times \text{thickness}/\Delta\text{Vapor pressure} \quad (6)$$

8. Film sensitivity in standard pH solutions

The color response of pH-indicator film was studied in six levels of standard pH solution (pH 1.0, 3.0, 5.0, 7.0, 9.0 and 12.0). Phosphate buffer was prepared by mixing of 0.1% sodium dihydrogen phosphate (acid) and 0.1% disodium hydrogen phosphate (base) into pH 7.0 and 9.0. Lactic acid (0.2 M) was used to adjust the pH of the phosphate buffer into an acidic condition (pH 1.0, 3.0 and 5.0). For basic conditions, NaOH (0.1 M) was used to adjust the pH of phosphate buffer to 12.0. Films were cut into a size of 2 cm^2 and then immersed into pH solution for 15 min at 25°C to allow a development of color. The changes in color of indicator films were measured by colorimeter (MiniScan XE Plus, USA) and reported in both of the CIE L* C* h and CIE L*, a*, b* scale. L* value indicated lightness which ranged from black (0) to white (100). Chroma (C*) represented the distance from the lightness axis (L*) and ranged from 0-100. Hue angle (h) expressed in degrees, which ranged from 0° (red) to 270° (blue). The values of a* represented red-green and b* was yellow-blue. Total color difference (ΔE) between each pH level (L, a and b) and the film element before experiment (L_0, a_0 and b_0) was calculated using equation (7). Tests were performed in triplicate at 25°C.

$$\Delta E = \sqrt{(L - L_0)^2 (a - a_0)^2 (b - b_0)^2} \quad (7)$$

9. Film sensitivity in food samples

The efficacy of pH-sensing film was tested on low (pH > 4.6, a_w > 0.85) and high acid food (pH <4.6, a_w > 0.85). Low acid foods were studied in pasteurized milk (pH 5.50) and chicken meat (pH 6.80), while pineapple cv. Pattawia was considered as a high acid food sample (pH 3.36). Cow pasteurized milk was purchased from a convenience store and transferred to the laboratory within 15 min. Skinless chicken breast and pineapple were bought from a wholesale hypermarket store in Sathorn District, Bangkok, Thailand, and then brought for preparation within 1 hour.

Pineapple was washed with tap water and then drained to remove excess water. Fruit was peeled and prepared into a cube of 3x4 cm (6 g per piece) using a sharp stainless knife. Skinless chicken breast was cut into a size of 3x3 cm with weights of 6 g per piece. Each piece of both food samples were placed in a plastic cup and had a pH-sensing element on the top followed by a covering with PVC film (Aro, Thailand) and then stored at 5±1°C for 4 days. The study in pasteurized milk was conducted by the procedures according to Pereira et al. (2015) which a film was maintained in contact with food (40 mL) in a sterile petri dish (diameter 9 cm) covered with a glass lid and then stored at a controlling temperature of 25±2°C for 4 days. Changes in pH of food samples and visual color of films were observed during storage periods. pH was measured using pH meter (Model 7011; Ezdo, Taiwan) which was calibrated with standard buffer 4.0, 7.0 and 10.0 before each measurement.

10. Statistical analysis

All measurements were done with three replications and represent all data as mean ± standard deviation. Statistical program was used (SPSS V. 26; An IBM Company, Ontario, Canada) to analyze data. Mean values were compared by Duncan's multiple range test to determine the difference between treatments.

Results and discussion

1. Physical, mechanical and barrier properties of pH-sensing film

Physical, mechanical and barrier properties of pH-indicator film were reported by%Si, WVPC, film tensile strength and thickness. Percent swelling index of pH-indicator film incorporated in 10-30% *Clitoria ternatea* L. extract is shown in Table 1. Overall, it can be seen that that higher percentage of butterfly pea extract (30%) results in a lower%Si compared to other treatments. Percent swelling index of all treatments increased gradually from an initial at 0.5 min (36-46%) reaching the peak at around 5 to 7 min and then falling, with table 1 showing 59.22 and 46.03% for 10 and 20% film, and 44.99% for 30% film, respectively. The results showed that the composite film between chitosan and PVA demonstrated swelling ability in the water as a hydrogel due to the high hydrophilic properties of PVA. It was noticeable that, a higher content of *Clitoria ternatea* L.extract, a phenolic compound, retarded a rise of swelling rate which was observed in 30% treatment at 7 min. Liu et al. (2019) reported the%Si of PVA film decreased by adding the tea polyphenol extract because of the hydrophobic properties of phenyl rings resulting in an increase of the hydrophobicity of the matrix systems leading to reduce swelling capacity. Similar observation was noted in the incorporation of lignin nanoparticles in PVA/chitosan hydrogel film (Yang et al., 2018).

Table 1 Swelling index (%Si) of pH-indicator film incorporated with 3 levels of *Clitoria ternatea* L. extract

Time (min)	*Clitoria ternatea* L. extract (%)		
	10	20	30
0.5	46.37 ± 2.84^{bA}	41.92 ± 2.69^{abcAB}	36.17 ± 0.21^{bB}
1	50.99 ± 2.24^{abA}	38.75 ± 1.91^{cB}	37.93 ± 1.24^{bB}
3	51.20 ± 3.14^{abA}	37.41 ± 0.34^{cB}	39.84 ± 1.45^{bB}
5	59.22 ± 6.59^{aA}	46.03 ± 0.67^{aB}	38.98 ± 1.71^{bB}
7	54.10 ± 1.08^{abA}	44.51 ± 1.09^{abB}	44.99 ± 0.91^{aB}
10	45.81 ± 0.30^{bA}	39.96 ± 5.02^{abA}	36.81 ± 4.26^{bA}
15	52.90 ± 2.12^{abA}	40.36 ± 1.73^{bcB}	36.32 ± 0.10^{bB}
20	49.69 ± 4.68^{bA}	42.61 ± 0.54^{abcA}	25.57 ± 2.08^{cB}

Remark: Data represented mean ± standard deviation
Mean value in each column of each immersion time followed by distinct lower letter cases indicates significant differences ($p \leq 0.05$).
Mean value in each row of each film treatment followed by distinct upper letter cases indicates significant differences ($p \leq 0.05$)

Film thickness, tensile strength and WVPC properties of pH-sensing film incorporated with 10-30% *Clitoria ternatea* L. extract is shown in Table 2. Addition of the highest percentage of *Clitoria ternatea* L. extract (30%) into composite resulted in the elevated thickness of the film which was 0.094 mm. Tensile strength was considered to reduce as the amount of *Clitoria ternatea* L. extract increased from 10 to 30%, with Table 2 showing 1.310, 1.112 and 0.874 MPa, respectively. This could be due to the supplementation of natural extract causing a change in proportion and density between base polymers chitosan/PVA and sodium tripolyphosphate as a plasticizer that resulted in a weakening of internal interaction bonding. This result was in accordance with Pereira et al. (2015) who revealed that the chitosan/PVA blend film embedded with red cabbage extract presented a lower tensile strength compared to pure chitosan or PVA film. However, the film form from pure chitosan with glycerol as a plasticizers incorporated with green tea extract showed an increase in tensile strength (Siripatrawan & Harte, 2010) which was the effect of hydroxyl groups (-OH) of polyphenols and NH_3^+ reactive groups of the chitosan backbone.

Table 2 Thickness, tensile strength and water vapor permeability coefficient (WVPC) of pH-sensing film incorporated with 3 levels of *Clitoria ternatea* L. extract

Clitoria ternatea L. extract (%)	Thickness (mm.)	Tensile strength (MPa)	WVPC^{ns} (10^{-8} g/ m.h.atm)
10	0.062 ± 0.006^{c}	1.310 ± 0.117^{a}	2.81 ± 0.10
20	0.075 ± 0.012^{b}	1.112 ± 0.220^{ab}	2.63 ± 0.10
30	0.094 ± 0.007^{a}	0.874 ± 0.112^{b}	2.46 ± 0.22

Remark: Data represents mean ± standard deviation. Mean value in each column of each film property followed by distinct lower letter cases indicates significant differences ($p<0.05$). ns = not significant

The capabilities of water vapor molecules moved through the film was slightly limited when the amount of *Clitoria ternatea* L. extract increased. However, no significant difference ($p>0.05$) was observed as compared to other treatments. From this result, the thickness of the film may be an important factor that affected WVPC apart from the nature of molecules incorporated (Miranda et al., 2004). WVPC is an important factor for high moisture food, e.g., meat and poultry when kept in a low WVPC packaging generally presents a fog inside which could enhance microbial growth and spoilage. Fruit and vegetables still remain respiration and transpiration which enhanced a deterioration rapidly because of a condensation problem (Turan, 2021).

2. Film sensitivity in standard pH solutions

Efficacy of pH-sensing films incorporated with 10-30% *Clitoria ternatea* L. extract were tested in a standard pH solution ranging from 1.0-12.0. The visible shade and color parameters are shown in Fig. 1 and Table 3. The visual color in all film treatments ranged from brownish-red (pH 1.0) to yellow (pH 12.0), which had

stronger color intensity when anthocyanin content increased. An indicator film observed at pH 1.0 and 3.0 represented in a brownish-red color which was evidenced by hue angle, with table 3 showing 31.96° and 69.46°, respectively. Increasing *Clitoria ternatea* L. extract (30%) was noted in a more vivid shade of the same color which was confirmed by positive a* value approximately 13, while the 10 and 20% treatments were 2 and 6, respectively (Table 3). The pH-sensing film showed a dark blue color at pH 5.0 and the shade was changed into green when the pH increased to 7.0 and 9.0, respectively, which was approved by negative a* value when pH increased (Table 3). At pH 12.0, a yellowish-green color was observed and showed the highest b* value (40.94-59.90) compared to pH 7.0 (2.30-25.18) and 9.0 (12.86-35.63) (Table 3). The 30% film showed a more intense yellowish-green sight by eye while the vivid color was faded in 10% treatment (Fig. 1). The hue angle of film incorporated with 10, 20 and 30% *Clitoria ternatea* L. extract were 102.82°, 95.31° and 79.65°, respectively, which indicated that the color appearance ranged from yellow (60°) to green (120°). The degree of color brilliance was explained by C* value which was greater in treatment with 30% *Clitoria ternatea* L. extract leads to a decline in lightness (L* value) (Table 3). Total color change (ΔE) approved that all film treatments had responsibility for pH shift (Table 3). A little change of ΔE for all film samples investigated at pH between 5 to 7 due to its revert to a color close to the beginning (pH 6.1). The color of the film from this result was more intense than that of the Hidayati et al. (2021) which was probably due to the higher amount of anthocyanin content incorporated. However, the color shade of the film at different pH levels were similar.

Similar observation of the color pattern of anthocyanin extract from *Clitoria ternatea* L. was reported by Kungsuwan et al. (2014) and Ahmad et al. (2020) which exhibited a red in acidic and green in basic condition. Total anthocyanin content in *Clitoria ternatea* L. extract in this study was 12.22 mg/100 g, demonstrating that there is a potential for utilization as a source of anthocyanin for an indicator in pH measurement. The results were roughly similar to the experiment made by Pham et al. (2019), the maximum amount of anthocyanin content in *Clitoria ternatea* L. was 13.28 mg/100g. For color shift sensitivity, anthocyanin structure can form as the predominant flavylium cation under acidic conditions which could absorb photons in higher wavelengths of visible spectra such as red and magenta. In basic conditions, the degree of disruption in the conjugated double bond in the central ring controlled an absorption of photons in a higher wavelength such as yellow, blue and purple (Chen & Gu, 2013; Kungsuwan et al., 2014). However, numerous factors are affected on anthocyanin color which depend on the main type of anthocyanin raw materials, structure and the test conditions, such as pH ranges, type of solutions and temperatures. The performance of anthocyanin pigment on pH sensitivities were recognized in red cabbage (Pereira et al., 2015; Silva-Pereira et al., 2015), purple sweet potato (Choi et al., 2017; Liu et al., 2017) and black bean seed coat mixed with red cabbage (Prietto et al., 2017). The embedding

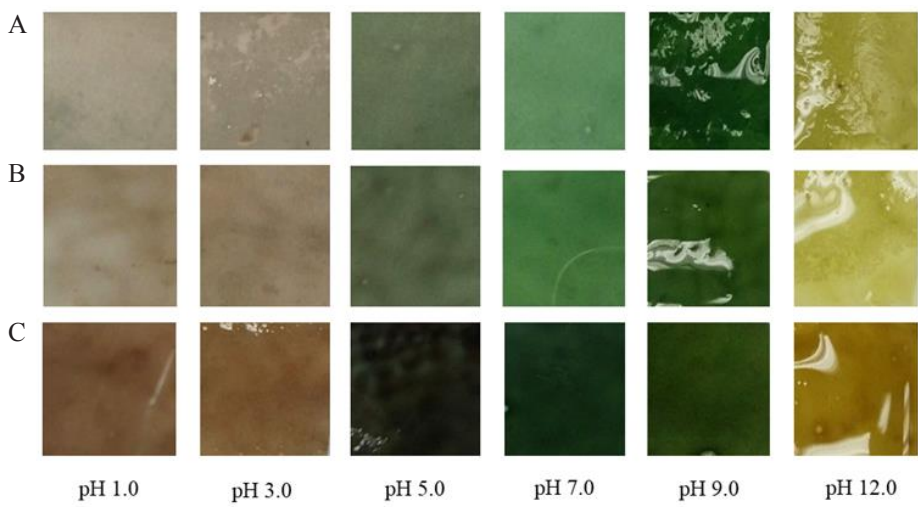

Fig. 1 Changes in color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extract in a variety of pH levels

red cabbage extract in chitosan/PVA composite film showed a wide color spectrum between pH 1-12, shifted from red to pink, purple, light blue, blue and green, respectively (Pereira et al., 2015).

3. Film sensitivity in food samples

The efficacies of pH-sensing films were tested in chicken meat, pasteurized milk and pineapple, as the model for low and high acid food. Changes in pH samples and visual color of film incorporated with 10, 20 and 30% *Clitoria ternatea* L. extract are shown in Fig. 2-4. Tested in pasteurized milk, the color of film changed from an initial of blue-green to light red color at the end of the storage. An initial pH of pasteurized milk was 6.78-6.80 (Fig. 2 A-C) which showed a similar shade of color as observed in standard pH solution at pH 7.0 (Fig. 1). During storage at 25°C (accelerated condition), pH of pasteurized milk continuously decreased to 3.48-3.51 on day 4 appearing as a light red color, corresponding to the color observed in standard pH solution at pH 3.0 (Fig. 1). However, no visual difference between all treatments after pH dropped below 4.2 on day 2 (Fig. 2 A-C) clearly indicating milk spoilage due to an accumulation of lactic acid by microbial metabolism can cause the declining pH value (Pereira et al., 2015).

The test of pH-sensing film in chicken meat is shown in Fig. 3. During storage at 5°C for 4 days, pH of chicken meat rose from an initial of 5.42-5.61 to 6.05-6.49 at the end of the storage, which was detected as off-flavor (Fig. 3 A-C). The blue color of pH-sensing film on an initial storage was shifted to green after storage for 4 days as clearly observed in 10% treatment which was expressed in a similar pattern with 7.0 pH solution. On the contrary, the change in color of 20-30% treatments were not noticeable when viewed with eyes. A change in film color studied in chicken meat was in accordance to Ahmad et al. (2020) in which the sago film incorporated with Butterfly pea extract turned to a green color after 48 h storage at exposed condition. The deterioration of chicken meat is caused by microorganisms and activity of protease enzymes. Proteolytic bacteria is able to hydrolyze protein into short chain peptide or amino acid and also produce ammonia as a volatile compound resulting in an increase of pH value (Zhang et al., 2016). The response of film embedded with anthocyanin extract from butterfly pea on volatile amine (NH_3 vapor) was reported by Sukprasong (2013).

An initial pH of fresh-cut pineapple was acidic ranging between 3.34 to 3.39 (Fig. 4 A-C). The remarkable changes in color was obviously found on day 1 while the pH values slightly dropped to around 3.17-3.18. After 2 days of storage, the color of 10-30% treatments shifted from red to more vividness when the pH dramatically fell to 2.45-2.60 on day 4. It is noticeable that a red color of *Clitoria ternatea* L. extract

Table 3 L value (L*), a*, b*, Chroma (C*), Hue angle (h) and ΔE of indicator film incorporated with 10-30% *Clitoria ternatea* L. extract immersed in various pH solutions

Treatments	pH	L*	a*	b*	C*	h	ΔE
10%	control*	39.55±0.67^{dA}	-28.48±0.57^{eB}	7.66±0.27^{cB}	29.76±0.71^{bB}	164.55±0.78^{cA}	-
	1	57.83±0.40b^{cA}	1.80±0.19^{aC}	1.20±0.52^{eC}	2.20±0.31^{fC}	31.96±1.22^{gC}	35.96
	3	76.73±0.40^{aB}	1.90±0.13^{aB}	5.27±0.22^{dB}	5.47±0.11^{eB}	69.46±0.75^{fB}	48.07
	5	39.77±0.20^{dA}	-15.80±0.20^{eA}	-2.51±0.16^{fC}	15.95±0.10^{dA}	188.81±0.45^{aA}	16.26
	7	56.42±01.21^{cA}	-14.97±0.37^{cA}	2.30±0.30^{eC}	15.35±0.31^{dB}	171.07±2.17^{bA}	22.27
	9	27.43±0.91^{eA}	-19.90±0.39^{dB}	12.86±0.41^{bC}	21.85±0.77^{cB}	149.42±0.44^{dA}	15.73
	12	60.70±0.23^{bA}	-9.29±0.06^{bC}	40.94±0.26^{aC}	40.98±0.24^{aC}	102.82±0.59^{eA}	43.85
20%	control	40.75±0.67^{eA}	-30.29±0.57^{fB}	13.99±0.27^{dA}	32.94±0.71^{cA}	155.72±0.78^{aB}	-
	1	58.84±0.40^{bA}	6.07±0.19^{aB}	10.39±0.52^{eB}	12.02±0.31^{eB}	57.83±1.22^{dA}	40.77
	3	87.22±0.40^{aA}	-0.31±0.13^{bC}	2.82±0.22^{gC}	2.54±0.11^{gC}	96.50±0.75^{cA}	56.42
	5	37.05±0.21^{fB}	-7.28±0.20^{cB}	7.11±0.16^{fB}	9.69±0.10^{fC}	137.06±0.45^{bB}	24.30
	7	49.29±1.21^{dB}	-23.60±0.37^{dC}	17.46±0.30^{cB}	28.79±0.31^{dA}	140.64±2.18^{bB}	11.39
	9	39.94±0.91^{eB}	-26.79±0.39^{eC}	25.14±0.41^{bB}	36.44±0.77^{bA}	137.21±0.44^{bB}	11.71
	12	57.32±0.23^{cB}	-6.94±0.06^{cB}	54.47±0.26^{aB}	54.60±0.24^{aB}	95.31±0.58^{cB}	49.58
30%	control	19.22±0.67^{fB}	-10.09±0.57^{eA}	4.98±0.27^{gC}	10.27±0.71^{gC}	155.97±0.78^{aB}	-
	1	47.13±0.40^{aB}	13.25±0.19^{bA}	18.20±0.52^{eA}	22.02±0.31^{eA}	53.47±1.22^{gB}	38.71
	3	42.63±0.40^{cC}	14.56±0.13^{aA}	22.34±0.22^{dA}	26.23±0.11^{dA}	56.50±0.75^{fC}	38.17
	5	26.49±0.20^{eC}	3.10±0.20^{dC}	14.14±0.16^{fA}	14.24±0.10^{fB}	78.30±0.45^{dC}	17.73
	7	26.33±1.20^{eC}	-17.26±0.37^{gB}	25.18±0.30^{cA}	29.75±0.31^{cA}	124.85±2.17^{bC}	22.58
	9	29.26±0.91^{dA}	-10.80±0.39^{fA}	35.63±0.41^{bA}	36.29±0.77^{bA}	107.00±0.44^{cC}	32.26
	12	44.13±0.23^{bC}	10.96±0.06^{cA}	59.90±0.26^{aA}	60.85±0.24^{aA}	79.65±0.58^{dC}	63.87

Remark: * Control represents an indicator film before examination. Data represents mean ± standard deviation
Mean followed by the distinct lower letter case represents the significantly different results between pH levels ($p \leq 0.05$)
Mean followed by the distinct upper letter case represents the significantly different results between film treatments ($p \leq 0.05$)

film at pH ranging from 2-3 was different which showed a brownish-red color observed in standard pH solution (pH 2.0-3.0) since it used lactic acid for adjusting pH. These results indicated that the type of acid in raw material along with other components may be affected by color expression. In this case, citric acid was the main feature acid found in pineapple. However, yeasts and lactic acid bacteria can metabolize sugar (glucose, sucrose and fructose) as a nutrient resulting in a production of lactic acid that leads to deterioration in fresh-cut pineapple (Zhang et al., 2013). The decline in pH was shown in the same pattern which was reported by Antoniolli et al. (2012) in minimally-processed pineapple dipped in ascorbic and citric acid as a browning agent. Therefore, changes in flavor quality or spoilage of fresh-cut pineapple can be detected and monitored through a pH-sensing film in this work.

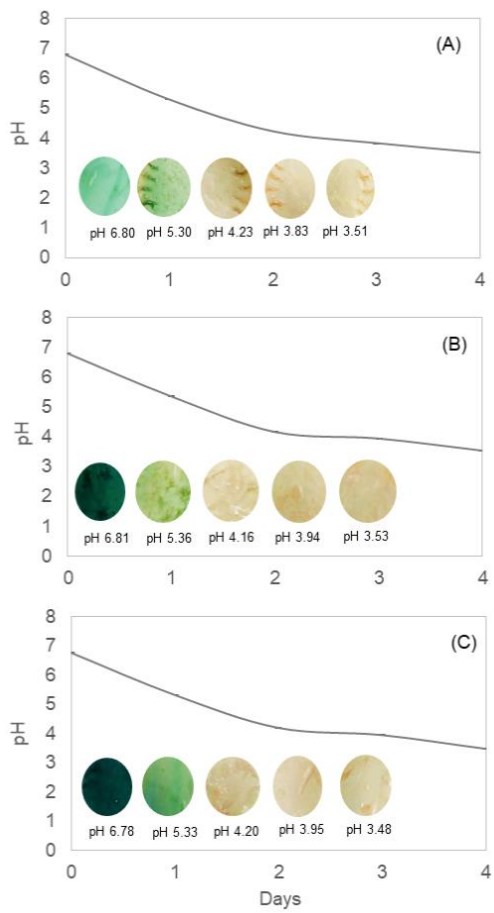

Fig. 2 Changes in pH and visual color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extract observed in pasteurized milk during storage at 25°C for 4 days

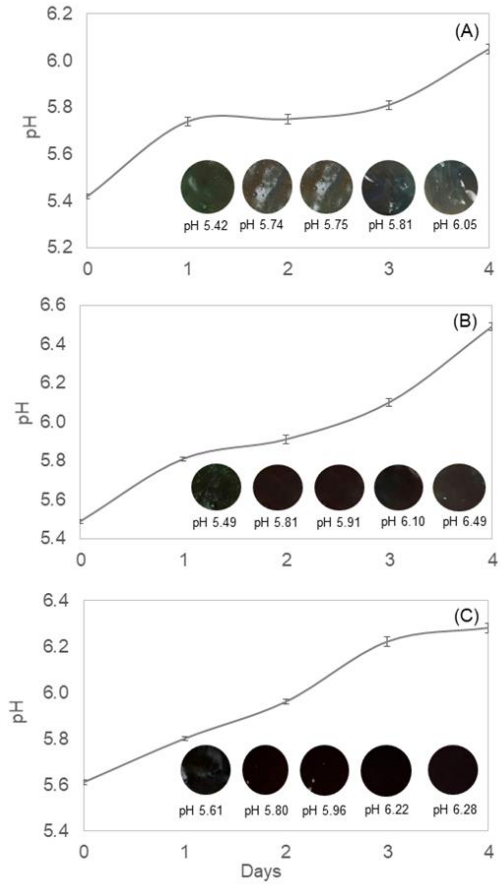

Fig. 3 Changes in pH and visual color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extract observed on chicken meat during storage at 5±1°C for 4 days

Conclusion

Ten and/or twenty percent of *Clitoria ternatea* L. extract showed the optimum concentration for producing pH-sensing elements depending on the type of food applied. Both treatments showed no difference of visual color shift (pH buffers and food samples) when compared to 30% corresponding to the total color difference represented by ΔE value. The 20% films contained good physical properties with neither too much nor too little in terms of swelling index, tensile strength and WVPC. For description, the swelling was minimal and had the ablity to maintain its shape when exposed to water or humidity. Film physical properties were flexible, not hard and brittle, and water vapor was allowed to pass more than 30% treatment. Shelf-life and film performance in food samples by noncontact method should be studied in the future for the detection of volatile chemical changes upon food spoilage.

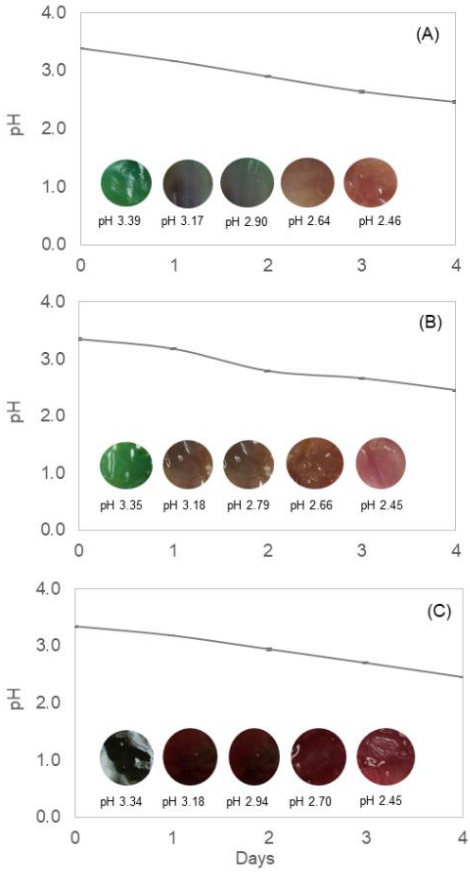

Fig. 4 Changes in pH and visual color of pH-sensing film incorporated with 10 (A), 20 (B) and 30% (C) *Clitoria ternatea* L. extracted observed on pineapple cv. Pattavia during storage at 5±1°C for 4 days.

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