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Preparation of an Intelligent pH Film Based on Biodegradable Polymers and Roselle Anthocyanins for Monitoring Pork Freshness

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Abstract

This study aims to develop an intelligent indicating film based on biodegradable polymers incorporated with roselle anthocyanins to monitor pork freshness. Three different films were prepared by using two substances of starch, polyvinyl alcohol and chitosan. The UV-vis spectra and color of anthocyanins changed at pH 2-12. SEM photographs showed that the compatibility of films were improved with the addition of anthocyanins. Furthermore, the polyvinyl alcohol/ chitosan/ roselle anthocyanins film had the highest tensile strength (98.28MPa). The starch/polyvinyl alcohol/roselle

anthocyanins film had the highest antioxidant activity (524.07%) and the best color stability. The starch/polyvinyl alcohol/roselle anthocyanins film showed visible changes from red to green when employed to monitor the freshness of pork stored at 25°C, before the TVB-N value of the pork gradually increased to the rejection limit (15 mg/100 g) at 36 h. Therefore, the indicator film can be used to monitor pork freshness for intelligent packaging.

Keywords: Intelligent packaging; Pork; Freshness; Roselle anthocyanins; film

1. Introduction

Intelligent food packaging can indicate the changes in the environment of the food packing to provide consumers with information on food quality during storage and transportation (Fang, Zhao, Warner, & Johnson, 2017). Particularly, colorimetric pH indicators have been applied in food intelligent packaging because of its convenience to use and low cost (Choi, Lee, Lacroix, & Han, 2017).

Proteins decomposition of meat could produce a large number of basic volatile organic amines, such as trimethylamine resulting in pH increase of the headspace of the packaging with meat. Therefore, various pH indicators have been used to detect meat freshness. For example, a pH indicator based on a mixture of bromothymol blue and bromocresol green and polyaniline were used to monitor the freshness of skinless chicken and fish freshness, respectively (Kuswandia, Restyana, Abdullah, Heng, & Ahmad, 2012; Rukchon, Nopwinyuwong, Trevanich, Jinkarn, & Suppakul, 2014). However, the synthetic chemical dye with potential harmful influence to human health were not ideal materials for food packaging (Dainelli, Gontard, Spyropoulos,

Zondervan-van den Beuken, & Tobback, 2008). Hence, non-toxic pH sensitive dyes or pigments are high in demand.

Anthocyanins are natural and non-toxic pigments and they can show visible color changes towards pH changes in wide ranges. Nowadays, there was an increasing studies focusing on fabricating pH indicators by using anthocyanins. For example, pH indicator films with purple sweet potato anthocyanins was developed to monitor the freshness of pork (Choi, Lee, Lacroix, & Han, 2017). Roselle is an herb, whose calyx contains a large number of anthocyanins (Sinela, Rawat, Mertz, Achir, Fulcrand, & Dornier, 2017) . Roselle anthocyanins have been used as a natural colorant in a many foods (Rodriguez-Amaya, 2016). Therefore, in this study, we aimed to use roselle anthocyanins as the pH sensitive pigment.

In order to immobilize anthocyanins, some biodegradable polymer have been reported including polyvinyl alcohol, starch, chitosan and other different polymers (Yoshida, Maciel, Mendonça, & Franco, 2014). Chitosan has advantages of safety, good film-forming property (Bilas, Sriram, Maheswari, & Sheriffa Begum, 2017). However, poor mechanical property have been described as one of the disadvantages chitosan films, while it could be improved by incorporating chitosan with other polymers. For example, Pereira Jr VA developed a composite film resulting that films from blending chitosan and PVA have modified physical properties when compared to films prepared only from chitosan (Silva-Pereira, Teixeira, Pereira-Júnior, & Stefani, 2015). Starch is a natural polysaccharides polymer formed by dehydration with condensation of α-D-glucose and mainly found in plants (Collado-González,

Montalbán, Peña-García, Pérez-Sánchez, Víllora, & Baños, 2016). It is the earliest and most widely used in packaging. Moreover, it has good biocompatibility and biodegradability. Polyvinyl alcohol (PVA) is a vinyl-polymer that non-toxic and good mechanical properties (Mallakpour & Motirasoul, 2017). Some researchers have shown that blending with these film-forming substrate materials can overcome shortcomings of single component (Bof, Bordagaray, Locaso, & García, 2015; A. Cano, Cháfer, Chiralt, & González-Martínez, 2016; Liu, Xu, Zhao, Liu, Zhao, & Li, 2017).

In this study, we aimed to develop three composite films by using roselle anthocyanins as the pH sensitive pigment, and two substances of starch, polyvinyl alcohol and chitosan as the film-forming matrix. The microstructures, mechanical properties, color stabilities and antioxidant activities of these three films have been investigated and compared. The roselle anthocyanins/starch/polyvinyl alcohol compo--site film was used for pork freshness detection.

2. Materials and methods

2.1 Materials

Roselle calyx and fresh pork were obtained from the local supermarket. Chitosan, starch, anhydrous and ethylene glycol (EG, 99.8%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Glycerin, polyvinyl alcohol (MW: 1750 ± 50), ammonia hydroxide ($NH3 \cdot H_2O$, 25%~28%), hydrochloric acid solution and acetic acid solution were acquired from Shanghai Natural Wild-insect Kingdom Co. Ltd. Buffers with range of 2.0-12.0 were prepared with citric acid/disodium

hydrogen phosphate and their pH values were measured by using a digital pH meter. Disodium citrate was obtained from Jiangsu Thorpe Group Co. Ltd. Plastic Petri dishes were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Extraction of Roselle anthocyanins

Roselle extract that was rich in anthocyanins was prepared according to the literature with a slight modification (M. Monica Giusti, Luis E. RodríguezSaona, Donald Griffin, & Ronald E. Wrolstad, 1999). The roselle calyx was dried and powdered. Subsequently, 15 mL of 80% ethanol was added to 1 g of roselle powder and the pH value was adjusted to 2.0 with HCl (1 M). Then, the sample was heated at 50 °C for 1 h and centrifuged at 3000 r/min for 6 min to get the extract. After that the solvent was removed with a rotary evaporator (RE-200A, Zheng Zhou HEB Biotechnology, China) at 50°C in dark. Finally the RS extract was frozen and dried to get RS powders.

2.3 UV-Vis spectroscopy of anthocyanins

UV-Vis spectroscopy of anthocyanins was measured using an UV-visible spectrophotometer (Agilent CARY 100, Varian Corporation, USA). The spectra of solutions in pH values of 2.0–12.0 were measured in the range of 400–800 nm.

2.4 Preparation of composite films

The composite films were prepared by a two-step process, including mixing and drying (Ma & Wang, 2016). The starch hydrogel and PVA hydrogel were prepared by dissolving 2 g of starch and polyvinyl alcohol in 100 mL of distilled water under a magnetic stirrer (F-101S,YUHUA,China) at 100°C for 30 minutes respectively. The

chitosan hydrogel was prepared by 2 g chitosan in 100 mL of 1% acetic acid solution (v:v) under a magnetic stirrer for 12 h at 37°C .The final solution was prepared by mixing the starch and PVA hydrogel (SP), the starch and chitosan hydrogel (SC), the PVA and chitosan hydrogel (PC) at a ratio of 1:1(v/v), with incorporation of RS respectively. The anthocyanins concentration used in the films was established as 2.5% of the total mixture of hydrogels. The mixed solutions were stirred until they dissolved and homogenized in an ultrasonic machine (Ultra Turrax IKA T25 digital, Germany) at 25°C for 5 min. Then, 18 mL of the mixed solutions were spread on a plastic petri dish (8 cm in diameter) and dried in an incubator at 25 °C with 50% RH for 18 h. Finally, three different films were formed for further use.

2.5 Characterization of films

2.5.1 Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra were acquired by a Nicoletis50 infrared spectrometer (Perkine Elmer 16 PC spectrometer, Boston, USA). The spectra were operated with attenuated total reflection (ATR) mode. FT-IR spectra were measured at a resolution of 2 cm^{-1} between 4000 and 650 cm^{-1} wave range, with three 3 scans.

2.5.2 Scanning electron microscopy (SEM)

The cross-section of the films was captured by a scanning electron microscopy (S-4800, Hitachi High-Technologies Corporation, Japan) with an accelerating voltage of 5 kV. The films were coated with a thin gold layer prior to observation.

2.5.3 Thickness, mechanical properties and moisture content

The thickness for each film was measured with a digital micrometer (Sanfeng

Group Co., Ltd., Taiwan, China) with a precision of 0.1 μm. The tensile strength and elongation at break of the films were measured using a universal texturometer (TA-XT2i, Instron Corporation, USA) according to GB 13022-1991. Before test, the films were cut into rectangular strips (60 mm ×20 mm). The initial grip separation was set to 40 mm and the traction speed was set to 0.06 mm/s, with 150 kgf load cell. Each sample was analyzed for three times at the room temperature.

The moisture content (MC) of films was determined by thermally drying to an equipoise weight at 105°C in an oven. MC was calculated on the basis of the following equation:

$$MC(\%) = 100 \times (M_i - M_f) / M_i \quad (1)$$

Where M_i was the initial weight of films stored in 75% RH to moisture equilibrium (g) and M_f was the final weight of films dried at 105°C (g).

2.5.4 Color stability

The stability of the films was identified according to the color change. The films were stored in a constant temperature and humidity chamber at 4°C and 25°C with a humidity of 75% respectively. The images of the composite films were determined every two days of two weeks in a Hunterlab spectrophotometer (PSC-30,EVERFINE, China). The values of L^* were lightness, a^* was the red to green and b^* was the yellow to blue. And the total color difference ΔE was calculated according to the equation (Vbv, Cmp, & Franco, 2012):

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

Where $\Delta L^* = L^* - L_0^*$; $\Delta L^* = a^* - a$; $\Delta b^* = b^* - b_0^*$; L_0^*, a_0^* and b_0^* were the initial gray

values of the films, L^*, a^* and b^* were the color after storage.

2.5.5. Antioxidant activity of films

The antioxidant activities of the film samples were measured using the method reported by Peng Y(Peng, Wu, & Li, 2013). Firstly, the films were cut into 20×20 mm, then placed in test tube containing 4 mL methyl alcohol. The mixture was stirred for two hours at 25°C. After those periods, 3 mL of the sample supernatant and 1 mL 150 μM DPPH methanol solution was mixed and the absorbance of the solution was measured at 517 nm (A_1). At the same time, 3 mL of the sample supernatant and 1 mL 150 μM methanol solution was mixed and the absorbance of the solution was measured at 517 nm (A_0) (Dou, Li, Zhang, Chu, & Hou, 2018).DPPH scavenging rate was measured according to the following equation:

$$DPPH\ scavenging\ rate(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

2.6 Reaction of the indicating film to ammonia

Response of composite films to volatile ammonia was tested according to the method of Kuswandi B with some modifications(Kuswandia, Restyana, Abdullah, Heng, & Ahmad, 2012). A 500 mL Erlenmeyer flask was charged with 80 mL ammonia solution (8 mM), and the films (20×20 mm) were suspended at 1 cm above the solution for 24 min at 25℃. The films were test by using scanner every 4 minutes, and the R, G, and B of indicating films were obtained. Then the response sensitivity (S_{RGB}) of films to ammonia was calculated according to the following equations:

$$\Delta R = \left| R_a - R_b \right|$$

$$\Delta G = \left| G_a - G_b \right|$$

$$\Delta B = \left| B_a - B_b \right| \quad (4)$$

$$S_{RGB} = \frac{\Delta R + \Delta G + \Delta B}{R_a + G_a + B_a} \times 100\%$$

where R_a, G_a, B_a were the initial values of the red, green and blue, R_b, G_b, B_b were the gray values after storage.

2.7 Application of films for the freshness of pork

2.7.1 Pork spoilage trial

To achieve the application test on pork, the films (2×2 cm) were fixed on the headspace of a packing box (20×10×6 cm) containing 250 g of pork sample, as shown in Fig. S1. Then the packing box was placed in an incubator at 25°C. The images of the film were collected with a camera (ImSpector, V10E, Specim, Finland) in the top of the light box, and fluorescent lights on the each sides. When pictures were collected at each time, the position, incident angle and intensity of the fluorescent lights kept constant.

2.7.2 Determination of TVB-N

The TVB-N level of the pork sample was measured by a Kjeldahl method (Li, Liu, Zou, He, Xu, Zhou, 2017). 10 g of pork was blended with 50 mL of water, and this mixture was homogenized by using a homogenizer. Then the homogenate was filtered by using filter paper. 5 mL of the filtrate and 5 mL of magnesium oxide suspension (10 g/L) were added into the reaction chamber. In the blank experiment, distilled water was used in place of the filtrate of pork. The condensate tube was inserted into 10 mL of 2% boric acid solution. After the reaction was terminated, the

197 boric acid solution was titrated with 0.01 M hydrochloric acid solution until the
198 solution became a blue-violet color. The TVB-N content was calculated as the
199 following equation:

200 $$X = \frac{(V_1 - V_2) \times c \times 14}{m \times 5/100} \times 100 \quad (5)$$

201 Where X is the TVB-N of the pork sample, mg/100g; V_1 is the volume of
202 hydrochloric acid consumed by the sample, mL; V_2 is the volume of hydrochloric acid
203 consumed in the blank, mL; c is the concentration of hydrochloric acid, M; m is the
204 sample quality, g.

205 2.8 Statistical analysis

206 Data and mean differences were analyzed using a statistical analysis of Statistic
207 Package for Social Science (SPSS) with Duncan's multiple range tests. Differences
208 were defined to be statistically significant at a P value less than 5%.

209 ## 3. Results and discussion

210 3.1 UV-vis spectra of RS anthocyanins in different pH

211 The colors of RS anthocyanins in pH 2-12 buffer solutions were measured. As
212 shown in Fig.1a, the RS anthocyanins changed from red to blue then to yellow when
213 the pH increased from 2.0 to 12.0. RS became red when the pH was no more than 5.0,
214 pink at pH 5.0-6.0, purple at pH 8.0-9.0, and yellow at pH 11.0-12.0. Those changes
215 might due to chemical structure transformation of anthocyanins. (Castañedaovando,
216 Galánvidal, Pachecohernández, Rodríguez, & Páezhernández, 2009). Fig.1b shows
217 the corresponding absorption spectra change of the RS anthocyanins with the
218 increasing pH. The maximum absorption peak appeared at around 527 nm at pH 2,

and the absorbance decreased with the increase of pH in the range of 2-4. When the pH was higher than 5, the maximum absorption peak appeared at 548 nm in the range of 5-6, and the corresponding absorbance increased. Furthermore, the absorbance at 597 nm decreased in the range of 7-12. The color variation and the corresponding peak shift assign to the reason that RS has different structures (C Grajeda-Iglesias, Figueroa-Espinoza, Barouh, Baréa, Fernandes, De, et al., 2016). At pH 2.0-3.0, anthocyanins mainly present in the form of yellow salt ions, the solution appears red; and at pH 4.0-6.0 their structures gradually transform into quinoid so that the red decreased; at pH 7.0-9.0 their structures transform to colorless pseudo-base and the color gradually turns to blue; Finally when the pH value is greater than 9.0, anthocyanins degrade in the strongly alkaline environment and the color changes into yellow-green (Claudia Grajeda-Iglesias, Salas, Barouh, Baréa, & Figueroa-Espinoza, 2017).

Fig.1

3.2 Fourier transform infrared spectroscopy (FT-IR) analysis

Fig. 2 shows the FT-IR spectra of RS, chitosan, starch, PVA, SP, SPR, SC, SCR, PC, and PCR. The peak at 3270 cm^{-1} was ascribed to free vibration of O-H stretch as all the materials contain hydroxyl group (Hong, Chen, Zeng, & Han, 2016). Bands at 2932 cm^{-1} and 2946 cm^{-1} were attributed to the -CH2 and –CH stretching for methyl groups respectively (Taokaew, Seetabhawang, Siripong, & Phisalaphong, 2013). With the addition of RS anthocyanin, it can be facile to observe the spectra of SPR film having a weak band at 1779 cm^{-1} attributing to pyran ring stretches of flavonoid

241 compounds. Furthermore, the SPR spectra shows a clear absorption band at 1646 cm^{-1}
242 and 1556 cm^{-1} corresponding to stretching vibrations of C=C aromatic rings, which
243 was also observed in the other two composite films (Silva-Pereira, Teixeira,
244 Pereira-Júnior, & Stefani, 2015). The spectrum of chitosan at 1392 cm^{-1} was a
245 characteristic peak attributing to the N-H vibration in amide band of chitosan
246 (Staroszczyk, Sztuka, Wolska, Wojtaszpająk, & Kołodziejska, 2014), while this
247 shifted to around 1409 cm^{-1} in the SCR and PCR film spectra. The reason possibly
248 was the inter-molecular interactions between the amide functionality of chitosan and
249 the anthocyanins (Maciel, Yoshida, & Franco, 2015).From spectral region of starch,
250 SP and SC, the bands presented at 1018,1025 and 1149 cm^{-1} were assigned to bending
251 and asymmetric stretching of C-O, C-C and O-H in alcoholic COH moieties (Capron,
252 Robert, Colonna, Brogly, & Planchot, 2007). And the PVA and PC spectrum showed
253 1235 cm-1 corresponding to the C-O group (Silva-Pereira, Teixeira, Pereira-Júnior, &
254 Stefani, 2015). However, with the addition of anthocyanins, the intensity of these
255 absorption peaks changed partially which was attributed to the changes of the
256 chemical and physical interaction between the aromatic rings of anthocyanins and
257 starch (Maciel, Yoshida, & Franco, 2015). These above results indicate that the RS
258 was successfully immobilized in the composite films matrix.

259 **Fig. 2**

260 3.3 Morphology observation by SEM

261 The SEM images obtained from the cross-sections of composite films are shown
262 in Fig. 3. As shown in SC and PC film, the surface were smooth and homogeneous

263 structure, indicating a good compatibility between chitosan and PVA/starch (Talón,
264 Trifkovic, Nedovic, Bugarski, Vargas, Chiralt, 2016). However SP film presented a
265 heterogeneously-fractured layer of the cross section, which points to the ordered
266 arrangement of some segments of the polymer chains (A. I. Cano, Cháfer, Chiralt, &
267 González-Martínez, 2015). But the results have changed with the addition of
268 anthocyanins. SCR film showed an inferior homogeneous structure the results
269 indicate that films structure become rougher in the polymer network. Roselle
270 anthocyanins may destroy the orderly arrangement between the chitosan and starch
271 and increased the brittleness (Talón, et al. 2016). The PCR film and SPR film have
272 smooth and regular cross-section structure and corresponding to good mechanical
273 properties in the Table S1. The phenolic hydroxyl groups in roselle anthocyanins can
274 bond with the hydroxyl groups in the PVA that reduce the chain molecules in the
275 polymer and improve the mechanical properties (Jiang, Jiang, Gan, Zhang, Dai, &
276 Zhang, 2012).

277 **Fig. 3**

278 3.4 Mechanical properties

279 The thickness, tensile strength, elongation at break, and water content of films
280 are shown in Table S1. As it can be seen, the thickness of indicator films did not
281 obviously change while the water content has evidently distinguished. The results
282 showed the water content of SPR, SCR and PCR film was 17.95%, 29.81% and
283 21.07% respectively. The water content SPR film have the most obvious decreased
284 compared the other two film (43.02%). This was possibly because the interaction

285 between SP and RS anthocyanins could reduce the availability of hydroxyl groups of
286 SP, which would cut water interactions (Wang, Dong, Men, Tong, & Zhou, 2013). SC
287 and SCR film showed the lowest elongation at break were corresponding to SEM
288 results. The tensile strength of SCR and PCR film increased 40.87% and 47.97%, in
289 contrast, the elongation at break decreased 14.06%and 20.13%, respectively. These
290 results associated to addition of RS anthocyanins into chitosan-films increasing the
291 hydrogen bond interactions with chitosan-films matrix, as chitosan chains act more
292 regular and the tensile strength of chitosan-film increased. Furthermore, RS
293 anthocyanins reducing the water molecules interactions with chitosan-films matrix,
294 that the elongation at break of chitosan-film decreased (Yoshida, Maciel, Mendonça,
295 & Franco, 2014). SPR film showed high elongation at break (88.16%), the behavior
296 revealed that the hard interaction between starch and hydroxyl groups of PVA chains.
297 And the results also showed that tensile strength of SPR film was lower than that of
298 PCR film while higher than that of SCR film. This may be due to their different
299 crystal structures and intense of intramolecular hydrogen bonding (Picone & Cunha,
300 2011).

301 3.5 Color stability

302 As the use of the composite film for the detection of meat products freshness is
303 based on the color change of the composite film, while the stability of the composite
304 film itself directly affects the color, so it is necessary to characterize the stability of
305 the films. The color variations of three different indicator films at two level
306 temperatures (4 and 25°C) are shown in Fig. S2. From which can be seen apparent

307 difference, then these results of films stability: SPR > PCR > SCR were be found.
308 Otherwise the results clearly showed that the indicator films stored at 4° C had small
309 relative color changes. But the indicator films stored at 25 °C had higher color
310 changes relatively within 16 days. The color change of SCR film and PCR film were
311 obviously yellowish at 25 °C. Similar color instability of anthocyanins-chitosan film
312 was also found in a previous study (Vbv, Cmp, & Franco, 2012). In the previous study,
313 it told that the color variation was associated with anthocyanins structure changes.
314 With the temperature rising, pyrylium ring of anthocyanins was opened and formed a
315 chalcone structure which producing a yellow pigmentation (Islam, Jalaluddin, Garner,
316 Yoshimoto, & Yamakawa, 2005). Moreover, it can be seen that the SPR film stored at
317 4°C and 25°C both had small ΔE values which were lower than 5% within 16 days,
318 indicating that it had excellent color stability.

319 3.6 Antioxidant activity of films

320 DPPH radical is a nitrogen-centered and very stable free radical. DPPH radical
321 scavenging rate is also widely used as a standard method for the determination of
322 antioxidant activity of composite films (Ubonrat & Brucer, 2010). Fig. 4 shows that
323 the antioxidant capacity of SC, PC and SP films were very weak. After the addition of
324 roselle anthocyanins, the antioxidant activity of the composite films increased
325 significantly ($P < 0.05$), and SCR, PCR and SPR film increased by 335.19%, 387.24%
326 and 524.07%, respectively. Anthocyanins are polyphenols, which contain a large
327 number of phenolic hydroxyl groups. Phenolic hydroxyl groups eliminate free
328 radicals by forming phenoxy groups. (Fogarasi, Kun, Tankó, Stefanovits-Bányai, &

329 Hegyesné-Vecseri, 2015). However, the antioxidant activity of SCR, PCR and SPR
330 films were significantly different ($P < 0.05$), where DPPH radical scavenging rate of
331 SPR film was 95.79%, followed by SCR film (60.60%) and PCR film (40.20%). The
332 SCR and PCR films had lower antioxidant activity, probably because chitosan made
333 the film in an alkaline environment and thus reduced the total phenolic content of
334 films (Kanatt, Rao, Chawla, & Sharma, 2012). The antioxidant activity of composite
335 films was mainly dependent on the release of active components and other influence
336 of factors, such as the compound interaction between active components and
337 polymers, and microstructure of films. (Piñeros-Hernandez, Medina-Jaramillo,
338 López-Córdoba, & Goyanes, 2016).

339 **Fig. 4**

340 3.7 Sensitivity of films to ammonia

341 Fig. 5 shows the sensitivity of the composite films to ammonia that can be used
342 to simulate the release of volatile nitrogen compounds in the process of meat spoilage.
343 The mechanism of the results was that ammonia firstly diffused into the film and then
344 hydrolyzed to produce hydroxyl ions which made an alkaline environment of the film.
345 As a result, the structure of anthocyanins converted to chalcone making the composite
346 films color changes. In comparison, the SPR film presented the highest sensitivity to
347 ammonia. The S_{RGB} of the SPR film gradually increased to a plateau within 16 min,
348 when the S_{RGB} was 30.77% and then remained stable without significant changes.
349 SCR film obtained maximum value up to 9.54% at 12 min, and the sensitivity of the
350 PCR film was the lowest, reaching a maximum to 7.66% at 16 min. An alkaline
351 environment made the pH fluctuations in anthocyanins environment of composite film.
352 However the difference in sensitivity between the composite films may be related to
353 the different pH of the film-forming substrate. It was generally expected that indicator
354 film could have fast response .Therefore SPR film exhibited the highest color

355 variation rate would contribute to its application .

356 **Fig. 5**

357 3.8 Application of films for the freshness of pork

358 Proteins in meat ingredients are very susceptible to bacteria and mildew. During
359 spoilage, various volatile nitrogenous compounds, such as ammonia and amines were
360 produced (Xiaobo, 2008). In this study, SPR film was used to monitor the pork
361 freshness due to its highest color stability and highest color variation rate. The SPR
362 indicator films showed different color with the changes of pork freshness at
363 25 °C .The TVB-N of pork and the parameter ΔE of the films were shown in Fig. 6. It
364 can be seen that the amount of TVB-N and the value of ΔE continuously increased
365 during the storage periods. In the first 24 h, the TVB-N slightly increased from 7.52 to
366 11.28 mg/100 g, and then the value of TVB-N increased to15.69 mg/100 g at 36 h.
367 According to Chinese Standard (GB 2707-2016), the rejection limit of TVB-N level
368 for pork is 15 mg/100 g. This result indicated that the pork sample was not fresh at 36
369 h. However, the value of TVB-N increased to 23.79 mg/100 g showed that proteins in
370 pork start to decompose and produce organic amines at 48 h. The value of TVB-N
371 was 30.78 mg/100 g at 60 h, indicating that the meat was already in the decay period.

372 The SPR film presented a purple color at the beginning, then brown color at 48 h
373 and finally yellow color after 72 h. The color of the indicator films was purple
374 indicating that pork was in fresh period at 24h, at the same time, the ΔE value was
375 8.08. The color of the film turned brown while ΔE value was 25.42 at 48 h. The
376 results meant that the meat began to spoil, the color change of film was seen by naked
377 eyes. And the value of ΔE increased to 29.61with the films consistently turned yellow

at 60 h. These results showed that the SPR film had good ability to indicate the freshness of pork.

Fig. 6

4. Conclusion

In this study, three different intelligent indicator films were prepared by natural Roselle anthocyanins combined with biodegradable materials. Fourier transform infrared spectroscopy showed that roselle anthocyanins can be well compatible with different film-forming substrates. The results of SEM showed that the anthocyanins extract of roselle had a significant effect on the microstructure of the composite film and had good biocompatibility with polyvinyl alcohol / starch matrix. The roselle anthocyanins / starch / polyvinyl alcohol composite film possessed the highest DPPH scavenging rate and best color stability. Therefore, the SPR film was applied to the pork freshness research. When the storage time of meat reaches 36 h, the TVB-N value was 15.69 mg/100 g, and otherwise the color of film changes from red to blue and the value of ΔE was 15.06, the pork sample was beginning to spoil. Therefore, the results show that the roselle anthocyanins is an ideal raw material for smart indicator films and it can be used to develop smart packages which indicate the freshness of meat products.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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