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Development of pH Indicator Film Composed of Corn Starch-Glycerol and Anthocyanin from *Hibiscus Sabdariffa*†

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Food packaging featuring colorimetric pH indicators is among the most reliable and intelligent packaging system. In the present study, the pH indicator film was made up of corn starch and glycerol, with the addition of a natural dye extracted from Roselle calyxes, *Hibiscus sabdariffa*. This work emphasized on the development of a pH indicator film made of corn starch-glycerol and anthocyanin to detect food spoilage. Corn starch and glycerol acted as polymer and plasticizer, respectively, thus creating a solid matrix to immobilize anthocyanin within the film. Fourier transform infrared (FTIR) and ultraviolet-visible (UV-Vis) spectra confirmed the effective incorporation of anthocyanin in the corn starch-glycerol mixture. The film color variation was measured using a chromameter after immersing the film in acidic and basic solutions of different pH values. In order to evaluate the applicability and reliability of the proposed pH indicator film, a test on real samples, i.e. milk and meat, was conducted. The indicator film turned pink and green when in contact with spoiled milk and meat, respectively, which proved the acidic and basic features of spoiled food. Therefore, it shows that this pH indicator film can be used as a sensor in order to notify the quality of food.

Key words: Anthocyanin; Roselle; plasticizer; film; sensor; food spoilage

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In this modern era, many food packaging techniques have been developed to meet the new concept where it becomes more convenient for the consumers and provides efficient information about the safety of the food [1]. In order to satisfy consumer demands, the development of intelligent food packaging has become a major business for the developers in order to find the ideal food packaging that can serve the purpose properly. Nowadays, many manufacturers often use natural products as a component in the manufacturing processes due to its eco-friendly properties, which do not bring harm to the environment. Anthocyanin is a water-soluble natural product that contains a pigment and it is very responsive towards pH changes [2] due to the chemically unstable cation in its flavium core structure [3]. Anthocyanin can be extracted from fruits and flowers of plants, such as *Hibiscus sabdariffa* (*H. sabdariffa)* as the calyxes have a very high amount of anthocyanin [4]. Anthocyanin is categorized as a flavonoid, which is very effective in protecting body cells from free radicals in the body [5]. Although anthocyanin can be found in any plants, it is reportedly confirmed that anthocyanin from different types of plants will give varying processing and storage properties (e.g. storage temperature, pH, and light) [6]. In the film production process, corn starch is much preferred due to its convenience, economical price, and higher stability compared to other plant materials. Besides, starch is known to have a high quantity of polysaccharides such as amylose and amylopectin, as well as properties of biodegradability and mechanical strength [7]. Although the bioplastic production of corn starch is considered as a substitute for synthetic plastic, it still has several weaknesses, such as brittleness and hydrophilic nature [8]. To overcome these problems, the addition of plasticizers is required to prolong lifespan and improve strength [9]. In this study, pH indicator films were made up by the combination of corn starch, glycerol, and anthocyanin. Solvent extraction of anthocyanin was applied and the films were made by using the casting technique. The films were analyzed using FTIR and UV-Vis spectroscopies. The color changes of the films upon food spoilage were measured using a chromameter.

MATERIALS AND METHODS

Materials

H. sabdariffa calyxes were obtained from Pasar Besar Melaka, Bandar Melaka. Commercial corn starch was purchased from Gedung Sri Minang, Kuala Pilah. Meanwhile, 99% glycerol, 30% of 1 M sodium hydroxide (NaOH), 40% ethanol, and 37% of 1 M

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hydrochloric acid (HCl) were supplied by Sigma Aldrich Sdn. Bhd., Shah Alam.

Extraction of Anthocyanin

H. sabdariffa calyxes were washed and the seeds were removed, which was followed by the drying process under the sun for three days until fully dried. The dried *H. sabdariffa* calyxes were blended into a fine powder and placed in a container to dry at ambient temperature. Then, 3 g of *H. sabdariffa* powder was mixed in 30 mL of acidified ethanol with 1.5 M HCL (85:15). The extraction process was conducted at room temperature (25°C) for three hours. Next, the extracted sample was heated up to 100°C for 5 minutes at pH 1.5. The sample extract was tested for its absorbance at the maximum wavelength (λ_{max}) using a UV-Vis spectrophotometer (PG instrument T80/ T80+) in the range of 190-900 nm and a FTIR spectrophotometer (Perkin Elmer model FTIR spectrum 100) in the range of 600-4000 cm^{-1}.

Preparation of pH Indicator Film

3.0 g of commercial corn starch powder was mixed with 80 mL of distilled water. Then, 1.5 g of glycerol was dissolved in distilled water and added into the corn starch solution. The solution was heated at 250°C and stirred at 800 rpm for two hours. After 2 hours, the solution was cooled until it reached 40°C. Following this, 5 ml of anthocyanin extract from the Roselle powder was aliquoted into a 250 ml volumetric flask and diluted with distilled water. The diluted extract was added into the corn starch-glycerol solution and stirred for 30 minutes at 100°C. The pH of the mixture was adjusted to pH 5 by using 1 M sodium hydroxide. The mixture was homogenized at 8000 rpm for 20 minutes. The mixture was poured on a clean mould and dried for 48 hours. Then, the dried films were cut into rectangular shaped pieces with the dimension of 10 mm × 40 mm, having an average thickness of 100 μm. The dried films with and without anthocyanin were analyzed using UV-Vis and FTIR spectrophotometers in the range of 190-900 nm and 600-4000 cm^{-1}, respectively [10].

Color Responses Analysis

A pH indicator film was cut into eight pieces of rectangular shaped films. The films were immersed in 1 M HCl (pH 3.0, 4.0, 5.0, and 6.0) and 1 M NaOH (pH 7.0, 8.0, 9.0, and 10.0) solutions, each in different pH values [11]. Then, the pH indicator films were kept at room temperature for 10 minutes prior to analysis using a chromameter (MiniScan EZ Hunterlab).

Application of pH Indicator Film on Milk

The test was conducted at room temperature and the pH of the milk was checked using a digital pH meter every day throughout the test period. A pH indicator film was submerged in 10 mL of milk for 10 minutes in each test. After 10 minutes, the colour change which occurred in the pH indicator film was analyzed using a chromameter. The measurements were taken at three random locations from different surfaces of the film [11].

Application of pH Indicator Film on Meat

10 g of minced meat was kept at room temperature for 48 hours in a fume cabinet. The pH of the meat was analyzed using a digital pH meter throughout the test period. A pH indicator film was placed on the minced meat for 10 minutes in each test. After 10 minutes, the colour change which occurred in the pH indicator film was analyzed using a chromameter. The measurements were taken at three different locations from different surfaces of the film [1].

RESULTS AND DISCUSSION

FTIR Analysis

Figure 1 shows the FTIR spectra of: (a) anthocyanin, (b) corn starch, (c) glycerol, (d) pH indicator film without anthocyanin, and (e) pH indicator film with anthocyanin. The spectra are summarised in Table 1. Anthocyanin (Figure 1(a)) exhibited a broad peak that corresponded to a hydroxyl group (O-H) at 3386.64 cm^{-1} and other significant peaks at 1635.27 cm^{-1} and 1223.02 cm^{-1} due to the stretching of C=C bond that arise from the pyran ring and aromatic $C=CH_2$ of the anthocyanin, respectively [11]. For the corn starch (Figure 1(b)), the broadband at 3357.39 cm^{-1} and a peak at 1339.06 cm^{-1} showed the stretching and bending vibration of the O-H bond, respectively. The presence of O-H bending in the starch indicated that tightly bound water was present in the starch and become the main reason for it to dissolve in the mixture of water and glycerol during the film preparation [4]. Glycerol (Figure 1(c)) consisted of C-H stretching vibration at 2936.83 cm^{-1} and $CH-CH_2$ bending vibration at 1420.71 cm^{-1} [12]. Figures 1(d) and 1(e) are of the pH indicator films without and with anthocyanin, respectively. The films without and with anthocyanin exhibited peaks at 3382.68 cm^{-1} and 3378.11 cm^{-1}, respectively, which were stretching vibrations of O-H bands. It is possible that both films inherited this broadband from the corn starch due to it being the major component in the film. Moreover, the peaks at 1654.27 cm^{-1} and 1648.33 cm^{-1} were attributed to C=O vibrations that appeared from the corn starch. It is believed that C=O from the amylose and amylopectin helps the film to withstand force and support the structure of the film [13]. The addition of anthocyanin in the film mixture induced chemical and physical interactions with corn starch and glycerol, which involved the addition of pyran ring detected at 1239.30 cm^{-1} and shifted to a higher wavenumber. This phenomenon makes the film much stronger as an intelligent food packaging [14].

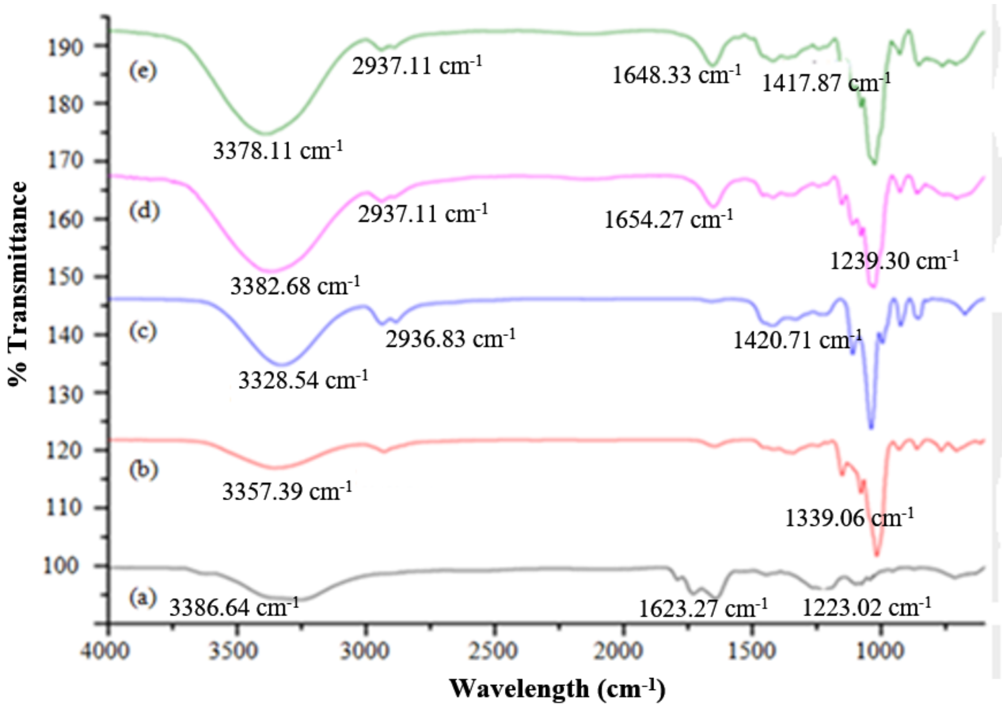

Figure 1. FTIR spectra of (a) anthocyanin, (b) corn starch, (c) glycerol, (d) pH indicator film without anthocyanin, and (e) pH indicator film with anthocyanin.

Table 1. Functional group assignments of anthocyanin, corn starch, glycerol, and pH indicator films with and without anthocyanin.

Compound	Wavenumber (cm^{-1})	Description
(a) Anthocyanin	3386.64	Hydroxyl group
	1623.27	C=C stretching
	1223.02	Aromatic $C=CH_2$
(b) Corn starch	3357.39	O-H stretching
	1339.06	O-H bending
(c) Glycerol	2936.83	C-H stretching vibration
	1420.71	$CH-CH_2$ bending vibration
pH indicator film		
(d) With anthocyanin	3382.68	O-H stretching
	1654.27	C=O vibrations that appear from corn starch
	1239.30	Pyran ring
(e) Without anthocyanin	3378.11	O-H stretching
	1648.33	C=O vibrations that appear from corn starch

UV-Vis Analysis

Figure 2 shows the UV-Vis spectra of: (a) anthocyanin and pH indicator films (b) with and (c) without anthocyanin. This analysis was conducted to identify the λ_{max} of anthocyanin and study the compatibility between anthocyanin and corn starch with glycerol. Based on Figure 2(a), anthocyanin showed the maximum absorbance at 520 nm, which described the presence of C=C conjugation in the anthocyanin structure. The film without anthocyanin (Figure 2(c)) showed no absorption peak in the visible region, while the film with anthocyanin (Figure 2(b)) showed an absorption peak at 515.00 nm [1]. The presence of anthocyanin in the pH indicator film enabled the film to change color in different pH values.

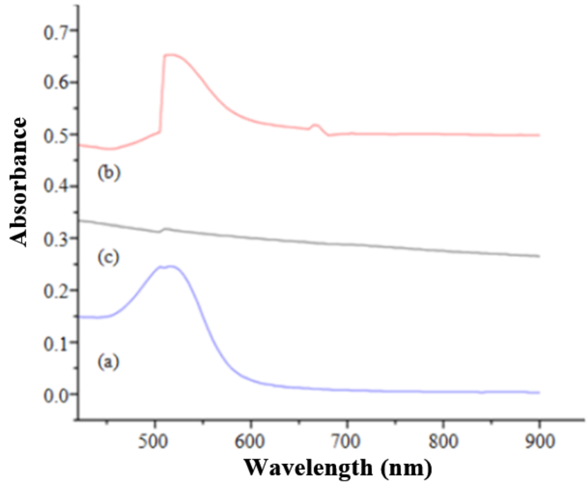

Figure 2. UV-Vis spectra of (a) anthocyanin and pH indicator films (b) with and (c) without anthocyanin.

Color Response Analysis

Figure 3 shows the color response analysis of the pH indicator films with anthocyanin. The films were placed in contact with different pH valued solutions (pH 3.17 to 10.22) and the color varied from bright pink to green [1]. Typically, the film showed bright pink colour at pH 3; as the pH value increased, the coloration was light pink at pH 4 and turned to slightly colourless at pH 5 and 6. Meanwhile, in basic solutions (pH ≥ 7), the colour of the pH indicator films was green. As the pH value of the solutions increased, the intensity of the green colour became stronger and turned into a brighter green colour.

Color Parameter Analysis

The colour parameters were presented in the value of L* (lightness), a* (red-green), and b* (yellow-blue). The L* parameter showed the value of luminosity, where if the value approached 100.00, the film experienced transparency that could lead to a transparent film. On the other hand, if the value approached 0.00, then the film was in the opaque state. For parameters a* and b*, it indicated the intensity of red & green and yellow & blue, respectively.

Table 2 shows that the value for L* dramatically increased from 43.21 to 51.78 when the films got in contact with acidic media from pH 3 to 6. Then, the value decreased from 51.78 to 37.93 when the pH increased from 6 to 10. It indicated that as L* lost its intensity, the films became clearer, which were almost transparent [11]. This can be observed in Figure 3, where the brightness of the films decreased at pH 6 and pH 7 compared to pH 3 and pH 10. The value of a* was the highest at pH 3.17, where the film was bright pink in colour. Gradually, the value started to depreciate as the films were in contact with the basic media, thus changing into green color. Typically, the higher the value of a*, the brighter the red color detected on the films. For the lower a* values, green color was detected. In Table 2, the value of b* shows the highest amount in both the lowest and highest pH values. Based on these values, it can be deduced that yellow color helps in increasing the intensity and brightness of the pink and green colour [10]. In addition, high b* value at pH 7.21 was probably due

Table 2. Color parameter of pH indicator film in different pH medium.

pH	Color parameter		
	L* (lightness)	a* (red – green)	b* (yellow – blue)
3.17	43.21	12.07	21.95
4.25	46.76	10.18	20.80
5.13	50.70	7.41	19.42
6.15	51.78	4.79	18.73
7.21	42.10	4.80	22.90
8.16	45.89	2.32	18.04
9.27	39.80	1.95	29.31
10.22	37.93	0.11	29.81

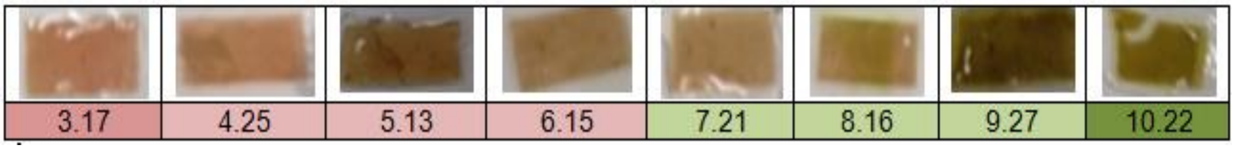

Figure 3. pH values and color of the films after having contact with acid and base solutions.

to the brighter film intensity, as shown in Figure 3.

pH Indicator Film Response on Milk

Spoiled milk is acidic due to lactic acid accumulation from microbial contamination [11]. At the beginning of the test, the fresh milk recorded a pH value of 6.21, which was considered slightly acidic. During the test period, the pH of the milk decreased to 5.98, signalling the formation of lactic acid. The colour changes of the films were difficult to analyze through naked eye observation, but the color parameter analysis reported significant figures. Based on the reported values in Table 3, the a* value started to increase as the pH value dropped, which indicated the spoilage of milk through microbial contamination. Thus, it can be deduced that the a* value may serve as a significant value to indicate milk spoilage.

pH Indicator Film Response on Meat

Initially, the pH of the fresh meat was 5.72. Throughout the test period, the pH of the meat increased to 6.47 as reported in Table 4. The main factor that increased the pH value was the decomposition of several substances within the meat, such as protein, lipid, and fiber. The microbial metabolism that occurred in the meat led to the decomposition process, thus producing ammonia and several amino sugars. This caused the contamination of the meat [1]. Ammonia is a base that increases the pH value, as meat spoils. The value of a* that decreased from 4.26 to 2.16 was shown by the film becoming green in color due to the pH of the meat that increased to 6.47 on the last day of the analysis. The film color changes were visible to the naked eye, thus it could detect the quality of the meat.

CONCLUSION

In conclusion, the presence of common functional groups in anthocyanin was supported by FTIR and UV-Vis analyses. The pH indicator film was successfully prepared using the casting technique and comprised of corn starch, glycerol, and anthocyanin from *H. sabdariffa.* The film was very responsive towards colour change and it could be seen during the test conducted. The film turned pink when in contact with acidic solution and green with basic solution. In the milk and meat test, the pH indicator film showed slight colour changes according to the pH of the food itself. The anthocyanin-based pH indicator film developed has a great potential as a pH sensor for food spoilage detection based on its color-responsive property.

Table 3. pH and color parameters L*, a*, and b* of the films tested on milk.

Day	pH value	Colour parameter		
		L*	a*	b*
1	6.03	71.16	6.98	18.03
2	6.01	67.71	7.05	21.06
3	5.98	62.08	8.59	21.41

Table 4. pH and color parameters L*, a*, and b* of the films tested on meat.

Day	pH value	Colour parameter		
		L*	a*	b*
1	6.12	80.77	4.26	12.39
2	6.35	74.71	3.95	15.29
3	6.47	72.21	2.16	16.55

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