Potential of Anthocyanin from Young Fruit Skin of *Elaeis Guineensis* as a pH Sensor

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Abstract. The increased consumer demand for the freshness and safety of food products would contribute to providing new technologies, which could function intelligently and could monitor the freshness of packaged foods. Chemical indicators are often used to monitor the freshness of food, but they have toxic properties and potential carcinogens. Therefore, an alternative method was needed to monitor the freshness of food. This work was aimed to explore the potential of young oil palm fruit skin (*Elaeis guineensis*), which was carefully designed on filter paper and exactly used as a pH indicator. The pH sensor was developed by simple immersion of filter paper into a solution of *E. guineensis* young fruit skin extract. Color change of the pH indicator was caused by the addition of various pH buffer solutions and was directly analyzed by colorimetry to obtain the color parameter values (L*, a*, b*, and ΔE). The results obtained that the total anthocyanin content was 0.512 ± 0.019 mg/100 g of fresh anthocyanin in *E. guineensis* sample. Meanwhile, values of total color change (ΔE) were found in a linear range at pH (9–11), with a linear regression value at $y = 3.7083x + 5.6983$, and the value of regression coefficient at $R^2 = 0.92021$. Meanwhile, the repeatability value of the pH sensor was measured at OD 530 nm and OD 620 nm, at pH (4–12), and found a relative standard deviation value (RSD, $n = 5$) of (6.35–7.85) %. The developed pH sensor has a wide total color change and has a high potential to be used as a pH indicator. Furthermore, this research will be applied to freshness monitoring in packaging.

Keywords: Anthocyanin · pH sensor · Elaeis guineensis · Oil palm fruit skin

1 Introduction

Oil palm (*Elaeis gueineensis*) is a very important monocotyledonous plant for many countries such as Indonesia, Malaysia, Africa, and Colombia. This plant is exactly classified in a family of Aceraceae. This plant is found to originate from Africa and has a genetic advantage with high productivity and is widely adopted in Asia [1]. This plant has become an important commodity in Indonesia. This is well evidenced by the increasing number of oil palm plantations in Indonesia. In 2012, the total number of Indonesian oil palm plantations was 10 million hectares [2] and these plantations were found to be 14.6 million hectares in 2021 [3]. This large number of oil palm plantations has made

E. Susanti et al. (Eds.): ICGT 2022, AER 221, pp. 331–338, 2023.
https://doi.org/10.2991/978-94-6463-148-7_33

Indonesia the largest producer of crude palm oil (CPO) in the world. The realization of Indonesian CPO exports and other palm oil reached 20.57 million tons with a value of US$ 19.35 billion in 2012 [4]. The world market demand for Indonesian palm oil is increasing every year.

Meanwhile, the chemical content of oil palm fruit and leaves were found to contain qualitatively carotenoids, tocopherols, flavonoids and anthocyanins [5, 6]. Compounds of the flavonoid and anthocyanins were grouped in phenolic compounds. They were used as an anti-oxidant and plant defense system against biotic and abiotic threats [5]. Oil palm fruit ripening is started with enlargement of fruit and formation of mesocarp, kernel and oil synthesis [7]. The oil synthesis is carried out in the mesocarp of the oil palm fruit and is followed by the formation of chlorophyll compounds, carotenoids, tocopherols and tocotrienols [8]. Along with increasing fruit maturity, the content of flavonoid and anthocyanins compounds are found to decrease during the ripening process. These compounds are regularly decreased starting from raw, unripe, and ripe fruit [6]. The oil palm fruit maturity is characterized by a change in color from black/purple to reddish-orange or green to yellow-orange [7, 9].

Anthocyanins are a family of flavonoid compounds and are grouped in polyphenol compounds. This compound has a natural color and is found in various plant species. The natural color of anthocyanin will be well dissolved in water and has properties such as the ability to remove free radicals and good antioxidants [10]. This anthocyanin is very interesting to study because it has an anthocyanidin glycosylated structure [11], can change color over a wide pH range, is abundantly available in nature, antimicrobial and antioxidant properties [12]. Currently, there are six types of anthocyanins found in nature such as malvidin, peonidin, petunidin, delphinidin, pelargonidin, and cyanidin [13], and more than 600 anthocyanins have been found in various plant species [14]. Various colors such as magenta, purple, pink, red, reddish hue, blue, and so on have been found from various flowers, fruits, leaves, and roots contributed by anthocyanins. The color change of the anthocyanins is highly dependent on changes in pH [13]. Anthocyanins are found to function as biologically active ingredients, which are used as anti-cancer, free radical scavengers, anti-diabetic, antimicrobial, anti-aging, blood lipid-lowering agents, and food additives [15, 16]. Meanwhile, studies on anthocyanins are increasing rapidly, this is contributed by anthocyanins as non-toxic natural dyes, having a wide range of color changes, being able to monitor the quality of packaged foods, monitor food shelf life, and as color indicators in packaged foods [11].

Several recent publications on anthocyanin compounds have been extensively used for the development of pH sensors. For example, red cabbage (*Brassica oleracea),* red dragon fruit (*Hylocereus undatus*), sweet potatoes (*Ipomoea batatas*) (husks and peels), roselle (*Hibiscus sabdariffa*), butterfly pea (*Clitoria ternatea*), and mangosteen (*Garcinia mangostana*) (husks and peels) were carefully immobilized into gelatin film [17], rice berry was embedded on chitosan matrix [18], black carrot anthocyanin was inserted into starch matrix [19], *Ocimum sanctum* L., was absorbed on filter paper [10], red cabbage extract was embedded on cellulose acetate film [20], *Echium amoenum* extract was immobilized on cellulose film [21], black rice extract was encapsulated in chitosan [22], red cabbage was immobilized on chitosan [23], *Clitoria sp* and Brassica sp

extracts were immobilized on l-carrageenan film [24]. The reported studies have successfully utilized to monitor the freshness of packaged foodstuffs. Meanwhile, exploration of oil palm fruit skin used as a pH sensor has not to need reporting.

This work was focused on exploring the use of young fruit skin of *E. guineenses* as a pH sensor, which was designed by using filter paper. The color changes of anthocyanins were exposed to various pH buffer solutions and analyzed by utilizing colorimetry to obtain values of total color difference.

2 Experimental

2.1 Materials and Reagents

The young fruit of *Elaeis guineensis* was obtained from a farmer (Riau, Indonesia), the filter paper was purchased from Whatman, Hydrochloric acid was obtained from Sigma Chemical Co (MO. USA), Natrium acetate was purchased from Darmstadt, ethanol (96%) was purchased from Merck. pH meter was purchased from Hanna Instrument, Colorimeter was obtained from Colorimeter Lab Tools, Version 1.6.6.5, Samsung SM-A305F. Spectrophotometer was obtained from Shimadzu (Japan).

2.2 Extraction of E. guineensis Anthocyanins

Samples of *E. guineensis* were carefully cut into small pieces using a knife and mashed using a blender for 15 min. Then, the samples were simply soaked using 95% ethanol in a ratio of (2: 3) w/v and left overnight at 4 °C. The samples were filtered using a cotton cloth to remove coarse particles and continued by centrifugation at 5000 rpm at room temperature for 10 min. The extracted anthocyanins were directly stored in dark bottles at 4 °C for further use.

2.3 Determination of Total Anthocyanin Content

The total anthocyanin content (TAC) of *E. guineensis* was determined by different pH conditions [21]. Briefly, 3.6 mL of KCl solution (0.025 M, pH = 1) and CH3COONa solution (0.4 M, pH = 4.5) were added separately to 0.4 ml of anthocyanin extraction. The mixture was measured using a UV-Vis spectrophotometer at wavelengths 510 and 700 nm. The total anthocyanin content was determined as mg cyaniding-3-glucoside/100 mL anthocyanin using Eqs. (1) and (2).

$$A = (A_{510} - A_{700})_{pH=1} - (A_{510} - A_{700})_{pH=4.5} \tag{1}$$

$$TAC = \frac{A \times MW \times DF \times 100}{MA} \tag{2}$$

Where, A is absorbance (nm), MW is the molecular mass of cyaniding-3 glucoside (449.2 g/mol), DF is dilution factor and MA is molar absorptivity (26900). All experiments were conducted three times.

2.4 Fabrication of pH Sensor

The fabrication of the pH sensor was designed using Whatman paper following the procedure proposed by Suthar and Saran, [20] with slight modifications. The Whatman paper was prepared by immersing it directly into the solution of anthocyanin and allowed to stand overnight at 4 °C. Furthermore, the developed pH sensor was directly dried by leaving it at 4 °C for 30 min. The master Whatman paper was cut to ±400 mm^2 and used for the assessment of the pH of the solution.

2.5 Determination of Color Change

The designed pH sensor tested for its performance against various pH buffer solutions. The test was conducted by adding a buffer solution of sodium acetate in pH range of 1–13 and left for 2 min to obtain a complete color change. The buffer solution at alkaline pH was adjusted using 0.1 M NaOH solution, and the acidic condition was adjusted using 0.1 M HCl. Experiments were carried out in a triplet manner at room temperature.

2.6 Color Measurements

The color change of pH indicator was regularly evaluated at different pH values. The buffer solution was directly prepared in the pH range of (1–13). Color parameters were detected with a colorimetry tool. The color parameters were carefully analyzed at coordinates (L*, a*, b*), where ((L*) is brightness, (a*) is the level of redness or greenness, and (b*) is the level of yellowness or bluish. The obtained color was used in estimating the total color difference (ΔE*) using Eq. (3).

$$\Delta E^* = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 \right]^{1/2} \tag{3}$$

Where, $\Delta L^* = L^* - L_0^*$; $\Delta a^* = a^* - a_0^*$; $\Delta b^* = b^* - b_0^*$. L_0^*, a_0^*, and b_0^* were the color values of the pH sensor before testing. Values of L*, a*, and b* were taken after testing against various pH buffer solutions.

2.7 Repeatability

Repeatability analysis was conducted according to Ahmad et al. [24] with several modifications. The repeatability response to pH sensor was carried out 5 times and using a buffer solution with a different pH. The repeatability response to the pH sensor was measured 5 times at different pH conditions. The buffer solution was utilized in the pH range of 4–12. Furthermore, the pH sensor was measured at absorbance wavelengths of 530 nm and 620 nm.

3 Results and Discussion

3.1 Total Anthocyanin Content from E. guineensis

The total anthocyanin content (TAC) of *E. guineensis* was directly analyzed using a spectrophotometer at absorbance of 510 nm and 700 nm, at pH 1 and pH 4.5 [21]. The results showed that the TAC in the skin of *E. guineensis* was obtained at 0.512 ± 0.019 mg/100 g of fresh anthocyanin samples from skin of young oil palm fruit. The color of oil palm fruit was caused by the presence of anthocyanins, flavonoids, and carotenoids in the fruit. An anthocyanin content was decreased with increasing fruit maturity. Where young fruit of oil palm found anthocyanin concentration of 5.25% [6]. Meanwhile, secondary metabolic content in the skin of young oil palm fruit was mostly phenolic compounds such as anthocyanins, tannins, flavonols, hydraxinal acids, and cafrine [25]. Other polyphenolic compounds such as vitamin E and other vitamins were also found in the young fruit of *E. guinensis* and were used as antioxidants [26].

3.2 Response of the pH Sensor to Change in pH and Analysis of Total Color Difference

The color change of the designed pH indicator on filter paper was analyzed by adding distilled water (as a control) and buffer solution at pH 1–13 (Fig. 1B). The pH indicator did not change color with the addition of distilled water. Furthermore, anthocyanin extract was added with a buffer solution (CH_3COONa) at pH 1–7. It was found that the pH indicator also did not change color and was found to be stable at a pale yellow-green color. Meanwhile, the addition of a buffer solution at pH 9–13, the indicator pH was found to be dark green-yellow. This means that the anthocyanin extract from *E. guineensis* as a natural indicator has good sensitivity to pH under alkaline conditions (>pH 9). The change in color of the pH indicator was contributed by alkaline conditions (pH > 9), where the pH indicator in this condition was degraded in its functional group. Changes in the chemical structure of the immobilized anthocyanin compound contributed to the color change of the pH indicator [27, 28].

Color difference (ΔE) is an indicator used to evaluate the ability of the human eye to distinguish colors, without using a sensory analysis panel. The total color difference (ΔE) of anthocyanins immobilized by filter paper toward changes in pH was demonstrated in Fig. 1B. The color difference is stable at low pH (1 – 7). This shows that the immobilized anthocyanin color changes did not show a significant change. If the addition of pH was increased from pH (9–13), there was a trend of increasing the value of the color difference and was obtained a linear regression equation of $y = 3.7083x + 5.6983$ and $R^2 = 0.98673$. This means that there is a significant change in color from light green to light green–yellow. Meanwhile, the difference in color between two samples being compared could be easily distinguished at value of $\Delta E > 3.0$, while values above 6.0 indicate a very large difference. This means that the value of ΔE obtained in this study indicates that consumers could distinguish color changes at pH > 9.

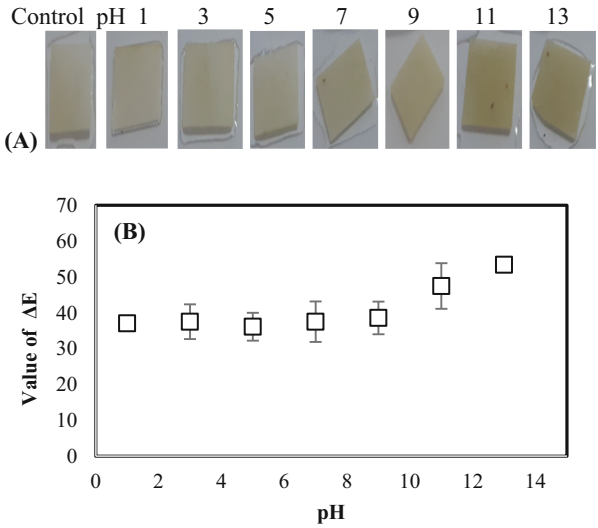

Fig. 1. The color change of pH indicator (A) and total color difference (B) analyzed against change in pH of buffer solution at pH 1–13.

3.3 Repeatability Study

The repeatability performance of pH sensor was tested. The relative standard deviation (RSD) values for repeatability (OD 530 nm) at pH 4, pH 7, and pH 12 were found at 6.65, 7.15, and 5.74%, respectively. Meanwhile, the RSD values for OD 620 nm at pH 4, pH 7, and pH 12 were found to be 5.53, 6.38, and 7.62%, respectively. This RSD value indicates that the absorption is evenly distributed on the filter paper. This RSD value was classified as low and could be accepted as a good pH sensor.

4 Conclusions

In this work, the pH sensor based on anthocyanin from the skin of young fruit *E. guineensis* immobilized on filter paper has been successfully developed. The pH sensor showed promising performance for evaluating the pH value of a solution. This study has a work performance in a wide pH range and good repeatability response. In the future, the developed pH sensor will be applied to monitoring the freshness of packaged foods.

Acknowledgments. This research was conducted with the assistance of the LPPM Universitas Riau (UNRI) DIPA Fund in 2022, through a research grant No. 1495/UN19.5.1.1.3/PT.01.03/2022. Thus, we would also like to thank all those who assisted in this research.

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