

Article

The Potential Use of Anthocyanin in Butterfly Pea (*Clinotertia ternatea*) Petals as Colorimetric Indicator in Intelligent Food Packaging Article

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Article Info

Article history:

Received: 31 August 2021

Accepted: 11 January 2022

Published: 17 March 2022

Academic Editor:

Nur Huda Faujan

Malaysian Journal of Science,
Health & Technology

MJoSHT2022, Volume 8, Issue No. 1

eISSN: 2601-0003

<https://doi.org/10.33102/2022243>

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Abstract— Butterfly pea is a perennial leguminous twiner that lives within the tropical belt and certain warm areas. Butterfly pea contains anthocyanin that are highly sensitive towards any change of pH, making them suitable to be used as pH indicator. The mechanism of the pH sensing capabilities of anthocyanin from butterfly pea involves four reversible structure of anthocyanin that are present in different range of pH: flavylium cation, hemiketal, quinonoid base and chalcone. Colorimetric indicator is a tool that are used in an intelligent food packaging. Its purpose in intelligent food packaging is to provide information of the food contained inside through colour changes that are visible to naked eyes. This colorimetric indicator utilizes pH indicator to assess the freshness of the food by measuring the change of pH that the product undergoes. Anthocyanin from butterfly pea provides an option of natural and organic pH indicator as opposes to synthetic pH indicators that can cause health issues. Anthocyanin from butterfly pea is capable to react towards any change of pH both acidic and basic, stable at least up to 60 days and are organic, perfect to be used in intelligent food packaging and other food related purposes.

Keywords— Anthocyanin, Butterfly pea extract, Acid-base indicator, Colorimetric indicator, Intelligent food packaging.

I. INTRODUCTION

Intelligent food packaging is an extension and an improved version of traditional food packaging. Traditional food packaging are materials that are used with purpose of preventing contaminants from contacting the packaged goods. It works on the principles of preserving the condition and quality of the goods and protection against stresses and contaminants [1]. Intelligent food packaging basically is a traditional food packaging with more added functions. The principal behind intelligent food packaging are protection,

communication, convenience, and containment. Protection means the packaging can provide protection against mechanical and chemical stresses like traditional food packaging but with more durability and more effective. Communication means that it can provide information of the product as well as real-time information such as the freshness and the condition of the food. Convenience is referring to the convenience for the user to transport, use, and dispose of the packaging. There are also some other factors that can be integrated to the packaging such as the reusability and the

recyclability of the packaging. Containment refers to the ability of the packaging to properly contain the product and avoiding the product to escape or leaked out and avoiding entry of contaminants and foreign objects. For these purposes, there were technologies that were integrated into the design of the intelligent food packaging to achieve the desired goal. These technologies include the use of sensors, indicators, and radio frequency identification (RFID) system [2]. Sensors and indicators are tools that are used in the making of an intelligent food packaging. Its main purpose is to use different principles to provide information about the current condition of the food. This includes colorimetric indicator. Colorimetric indicator is an indicator that provide real-time information such as the freshness of the food through colour. It uses pH indicators to detect the change of pH caused by the release of chemicals from the breakdown of the product. The proposed research is to use the capability of *Clitoria ternatea* which is highly sensitive towards any change of pH to be used as the organic chemical in the colorimetric indicator.

Butterfly pea or *Clitoria ternatea* is a flowering plant of Fabaceae family and of Papilionaceae subfamily. It is a perpetual leguminous twiner that winds itself around supports. Currently There are 60 species of butterfly pea that are distributed within the tropical belt and some area with warm temperatures [3]. The etymology of *Clitoria ternatea* can be traces back to an island that is in an Indonesian archipelago named Ternate. This is where it was recorded that Linnaeus has produced the specific description of the flower. There is an ambiguity of the native range of the species since the island Ternate is grown in the Molucca Sea in eastern Indonesia and not in the Indian Ocean. Thus, it is inferred that *Clitoria* arose around the Indian Ocean from the center of diversity for this subgenus [4]. According to the researcher, the habitat of *Clitoria ternatea* is in open forest with moderate or well-balanced supply of moisture or shrubs. Moreover, in Australia, *Clitoria ternatea* could be found in open areas of tropical regions with plenty of sunlight allowed by sparse canopy and in area where fresh water gathers such as wetlands, small gullies, or at the base of rocky hillsides. It thrives in temperature of 24°C to 32°C but it can survive well in frost conditions and retain it leaves for 7 days. The researcher also stated that the woody part of the plant can typically recover from the conditions. *Clitoria ternatea* is most productive in fertile and warm temperature where the rainfall of the area is 650-1250 mm (26-50 inches). It is influenced by its growing season which is in summer and spring where moisture is in high amount with high temperature and are limited in sparse moisture and low temperature [5]. It grows well in different ranges of soil that has pH of 5.5-8.9 like calcareous soil. Its main propagation method is by seed. It produces large number of seed and reseed mainly because of seed shattering when it reaches maturity [6]. It has a very low germination rate. For culturing purposes, it is advised that the seed are stored for 6 months as well as promoting the uses of chemical scarification can help in increases the seed germination rate.

II. ANTHOCYANIN BUTTERFLY PEA EXTRACT AS COLORIMETRIC INDICATOR

A. pH Detection Mechanism in Anthocyanin from Butterfly Pea Extract

Anthocyanin is a colour pigment that are responsible for many colours in flowers. It is widely distributed among plants and are responsible for wide range of colours including red, pink mauve, orange, magenta and purple. It is also the one that gives the blue and white colour for *Clitoria ternatea* depending on the species. Anthocyanins are water soluble and are stable in mild acid conditions. The colour of anthocyanin can also change which ranges from deep blue to magenta when contacting different type of pH [7]. Most found anthocyanins are glycosides which contains one of many aglycone cores. In Butterfly pea the major aglycone cores are kaempferol [7]. Anthocyanins exist naturally in the form of glycosides of flavylium (2-phenylbenzopyrylium) salts. A study stated that there have been more than 635 structures of anthocyanin and up to 95% of it are derivations of 6 anthocyanidins which are cyanidin, delphinidin, malvidin, peonidin, pelagornidin and petunidin [8]. The mechanism that anthocyanin exhibit in the detection of pH is as follows (Fig.1). In pH of 3.5, red Flavylium cation is hydrated to form colourless hemiketal. When the pH increases, hemiketal will undergoes tautomerization and changes to cis-chalcone and trans-chalcone. The blue and purple colour comes from quinoidal base that was drained from the flavylium cation, hemiketal and both chalcone [9].

The present colour of anthocyanin is based on the number of structures that are formed the most in the pH its contained in. In neutral conditions, the blue quinoidal base and colorless hemiketal are high in number thus projecting the colour that leans toward blue or purple. As contented by [10], when the solution it contained in starts to become more acidic, the red flavylium cation was produced as response to the reaction thus increasing the concentration of red colour and reducing the concentration of blue colour. Additionally, when the solution become more basic red flavylium cation change into hemiketal and later the pair of cis-chalcone and trans-chalcone which is light yellow in colour. The changes of colour can be referred to the colour range in Fig. 2.

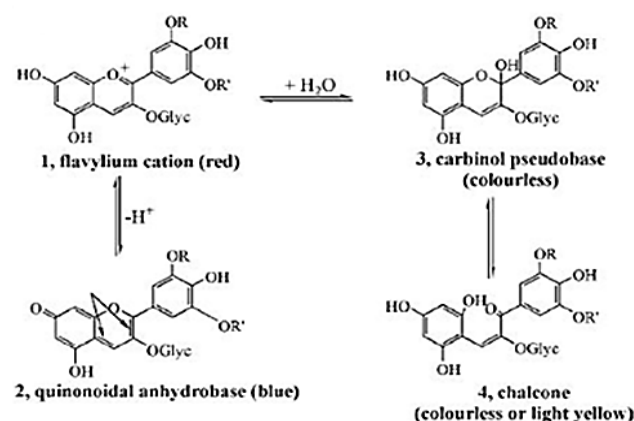


Fig. 1 Structural changes of anthocyanins in different pH conditions

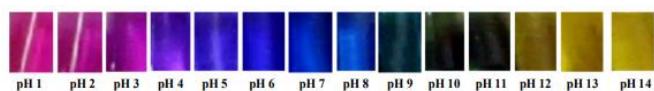


Fig. 2. The colour changes of *Clitoria ternatea* Anthocyanin extract towards different pH

B. *Clitoria Ternatea* Anthocyanin Extract as pH Indicator

pH indicator or acid-base indicator is substances that shows reaction normally in the form of change of colour when in contact of different pH. It is mainly used to mark and signals the completion of an acid-base titration. pH indicators that are widely used in experiments includes phenolphthalein, methyl orange, bromothyl blue and alizarin yellow. These indicators share one common feature which is the ability to show visible, clear, and instantaneous result. Anthocyanin in *Clitoria ternatea* makes a great pH indicator since it is also able to produce clear, visible and instantaneous result [11]. It is highly reactant towards any change of pH both in acidic and basic conditions its contained in. The colour changes in different pH can be seen as in Fig. 3.

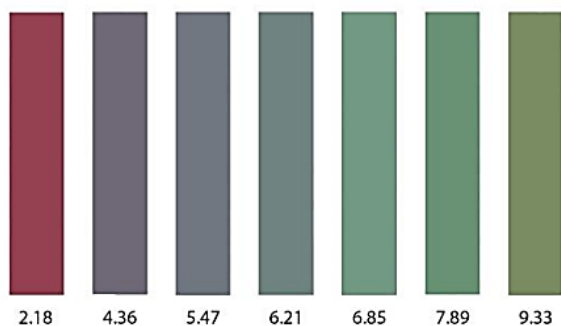


Fig. 3. Butterfly Pea Extract pH Indicator Solution Colour Scale.

As depicted in the figure, the colour changes are clear and very distinguishable from one another. These criteria make it a very good pH indicator. Pair up with its ability to react almost instantaneously, butterfly pea extract makes for a good option. Butterfly pea extract has great thermal stability on top of having great colour stability [12]. The result can be seen as provided in Table 1. It is noted that the *Clitoria ternatea* Anthocyanin extract for pH 1.5, 3.0 and 6.0 are more stable at 7 °C. the intensity of the colour remained around 80-90% on day 60 of the original colour and continues for about a year. This makes it a viable pH indicator. Plus, it is important to note that *Clitoria ternatea* extract are fully organic which is better than most standard pH indicators which are synthetic. This is because synthetic chemicals prone to cause problems and can be hazardous especially if it is to be used for food purposes [13] and has given the advantage to *Clitoria ternatea* especially to be used as colorimetric indicator in intelligent food packaging, as its water-soluble property making it able to produce high yield.

TABLE I
REMAINING ANTHOCYANIN'S PERCENTAGE (%) IN
ANTHOCYANIN EXTRACT OF CLINOTERIA TERNATEA ON
60 DAYS PERIOD AT DIFFERENT PH VALUE WITH
TEMPERATURE OF 7, 27 AND 37 °C

pH	Percentage of residual anthocyanin on day 60 (%)		
	7°C	27 °C	37°C
1.5	91	79	78
3.0	90	71*	59*
6.0	79	33	32
7.4	nd	22	21
8.5	nd	32	29
9.0	nd	18	18

* Estimated by extrapolation, the study was conducted for 30 days only. nd: not determined

C. *Clitoria Ternatea* Anthocyanin Extract as Colorimetric Indicator

Colorimetric indicator is one of the tools used in intelligent food packaging. It is a tool that provides information through mainly colour changes. This makes it a good tool for intelligent food packaging since it does not require further processes and can be observed directly with naked eyes. There are many types of colorimetric indicators including pH-sensitive indicators, biogenic amines indicator and carbon dioxide indicators. One of the examples for colorimetric indicator are a colorimetric hydrogen sulfide indicator. A study has been done inspecting the synthesis of hydrogen sulfide (H₂S) colorimetric sensor using silver nanoparticles (AgNPs) and Gellan gum (GG). Hydrogen sulfide is a volatile gas that were released in the breakdown of meat during spoilage. Gellan Gum (GG) does not exhibit any colour and appear colourless. When coupled with silver nanoparticles (AgNs) it will produce gellan gum-silver nanoparticles (GG-AgNPs) that exhibit yellow colour to naked eyes. Silver nanoparticles (AgNPs) are highly reactive to H₂S which will produce Ag₂S. Thus, in the presence the GG-AgNPs will react to H₂S producing Ag₂S eroding the yellow colour of GG-AgNPs. As more H₂S are present, more Ag₂S are produced decreasing the intensity of the yellow colour of the solution [14]. This can be seen as provided in Fig. 4. From Fig. 4, the GG-AgNPs colorimetric indicator possesses high colour stability and had only a slight change after storage of 30 days at 25 °C. The GGAgNPs solution also showed good stability in high ionic strength environments. It shows little to no changes when comparing between freshly prepared sample with a sample prepared after 30 days. Also, it appears stable in pH higher than 5. This concept is the one that are used in the making of Anthocyanin of Butterfly Pea extract colorimetric indicator.

From the previous studies, some things are to be noted for the making of a colorimetric indicator. The colorimetric indicator is made using materials and solution that possess colour changing properties such as pH sensitive material. The biochemical and microbiological reactions that the food undergoes produces carbon dioxide and ammonia gases which can also lead to a different of pH reading. The change of pH will react with the pH sensitive indicator causing it to change colour which can be used to gauge the freshness as well as the condition of the food [15]. As the researcher contended, this intelligent food packaging with colorimetric indicator could help in reducing wastage. Food spoilage can leave a huge impact on security, environment, quality and food safety. It also can have great impact on cost whereas food that looks bad

when it is still in safe condition could be thrown away and cause wastage. These losses can cause an impact towards environmental as well as economic damage to the retailers. For example, misjudgment on the quality of the food causes still in safe condition to be thrown together with spoiled food causing the retailer to throw away the food in bulk which can attract pests and possibly spreading diseases. Thus, it is beneficial to invest and focuses more efforts on intelligent food packaging with colorimetric indicator, stressed by the researcher.

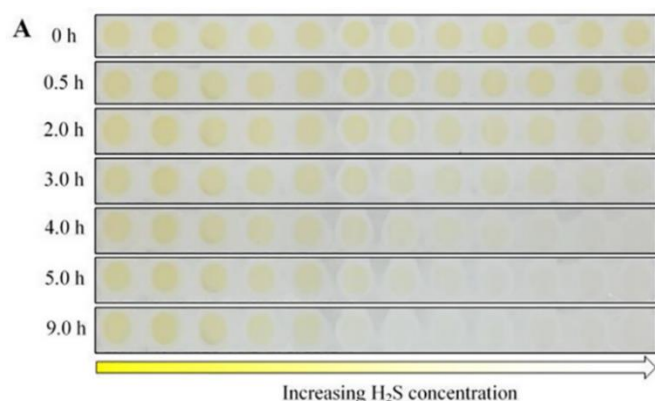


Figure 4. The color evolutions of sensory hydrogels on exposure to H₂S vapor produced in an enclosed microcentrifuge tube (10 mL) containing 5 mL H₂S solution at 25 °C. The concentration of H₂S solution (from left to right): 0, 1.8, 3, 4.8, 7.2, 12, 15, 18, 21.6, 25.2, 30 and 36 μ M.

It is important for this colorimetric indicator to be safe for consumption since it is vulnerable and exposed to the food itself. Existing intelligent packaging that utilizes pH indicator uses bromophenol blue and chlorophenol red which both are highly sensitive to the change of pH. However, both chemicals are synthetic and there are concerns as well as studies that proven the danger of using synthetic material. The concerns stem out from the possibility of the material to migrate into the food and causes harmful effects. Thus, researchers have focuses studies as well as experimentation to search for natural sources for pH indicator that are safe for consumption if accidental migration of the chemical does happen. Anthocyanin in butterfly pea can change its colour depending on the pH its contained in. Butterfly pea extract in general itself has been proven to be safe for consumption making it a perfect candidate to be used in the colorimetric indicator. The other option for plant sourced pH indicators includes *Arnebia euchroma* root, red cabbage, purple grapes, purple cabbages, and purple sweet potato extracts which the principle behind the pH indication of these materials is the utilizing of anthocyanin though each material exhibits a different type of anthocyanin. The anthocyanin pigment in *clitoria ternatea* has been proven to be better in term of quality. Anthocyanin in butterfly pea exhibit the same colour changes to other pH indicator while colour changes of anthocyanin from mangosteen and sweet potatoes only shows three colour changes while anthocyanin from red cabbages exhibit great range of colour changes but appear colourless at pH 5-7 [16] This proven that anthocyanin from butterfly pea is the better option for plan sourced pH indicator.

A study has been done on developing a colorimetric pH sensor base on *Clitoria sp* and *Brassica sp* for monitoring of food spoilage using chromametry [17]. The objective of this study is to develop a colorimetric sensor that utilizes the pH sensing capabilities of anthocyanin from *Clitoria Ternatea* as well as the anthocyanin from *Brassica sp* (red cabbage). Anthocyanin from butterfly pea is dissolved in distilled water and then the liquid is run through a blender followed by filtration using two layers of muslin cloth and run through centrifugation to remove any particles resulting in a pure butterfly pea extract. As for the red cabbage, the red cabbage is grinded and soaked in ethanol. It was stirred continuously for 12 hours. After that, it was put through the same processes which are blend, filtration, and centrifugation. The solutions are then mix with a 1:1 ratio to make the anthocyanin solution used for this experiment. Carrageenan films are used as medium to hold the solution together. The anthocyanin mixture solution and the anthocyanin colorimetric solution are run through several testing including testing the response of the anthocyanin mixture solution and the anthocyanin colorimetric film towards different pH and testing the response with the spoiling of food sample. The anthocyanin mixture solution and anthocyanin colorimetric film shows great response when tested with different pH and the resulted colour variation are dependent on pH changes and its colour changes were easily distinguishable as provided in the Table II.

TABLE II. THE COLOURS OF SOLUTIONS OF MIXED NATURAL DYE, BRASSICA SP, CLITORIA SP EXTRACT AND COLORIMETRIC pH SENSOR FILM IN DIFFERENT pH BUFFERS SOLUTIONS

Type/ pH	1	2	3	4	5	6	7	8	9	10	11	12	13
A	Red	Orange	Yellow	Green	Blue	Dark Blue	Teal	Light Green	Dark Green	Light Yellow	Dark Yellow	Orange	Red
B	Red	Orange	Yellow	Green	Blue	Dark Blue	Teal	Light Green	Dark Green	Light Yellow	Dark Yellow	Orange	Red
C	Red	Orange	Yellow	Green	Blue	Dark Blue	Teal	Light Green	Dark Green	Light Yellow	Dark Yellow	Orange	Red
D	Red	Orange	Yellow	Green	Blue	Dark Blue	Teal	Light Green	Dark Green	Light Yellow	Dark Yellow	Orange	Red

Table II: The color of solutions of (A) mixed natural dye (Brassica sp + Clitoria sp); (B) Brassica sp; (C) Clitoria sp extract) and (D) Colorimetric pH sensor film in different pH buffers solution (pH 1–13) (Ahmad et al., 2019).

As for the testing with food samples goes, the result can be referred to the Table III. The result of the change of colour are recorded and measured with the colour parameter (L^* , a^* , b^* , C^* and ΔE^*). For prawn sample, as the storage time increases, there is a decrease in L^* value, a^* value drops to the negative section and b^* value increase. The decrease of L^* value means the film has become darker. The drops of a^* value means that it becomes less red and subsequently turn green while increase of b^* value means that it is changing from blue to yellow. Conclusively, for the spoilage of prawn sample, the film turned darker, greener, and slowly diminishing of blue colour. Means that it is slowly turning dark green. When compared to the colour spectrum of pH changes of anthocyanin this signals an increase of pH in the food sample. This correlates with the nature of spoilage for meat and seafood where the product will have an increase of pH as time pass by. For durian sample, there is a decrease in L^* value, decrease in a^* value and increase in negative b^* value. This can be translated to the change of the colour of the film to be darker, decrease in red colour intensity

and increase in blue colour intensity. In short, it is turning from blue to dark purple. This correlates to the nature of durian spoilage to be more acidic upon time [17].

TABLE III. PARAMETERS CHANGES (L*, A*, B*, C* AND ΔE^*) OF SENSING FILM FOR PACKAGED SHRIMP AND DURIAN STORED AT AMBIENT TEMPERATURE ($28 \pm 1^\circ\text{C}$)

Storage time (h/day)	L*	a*	b*	C*	ΔE^*
Prawn sample					
1.0 h	38.61 $\pm$ 0.61	2.24 $\pm$ 0.20	-5.06 $\pm$ 0.19	5.53 $\pm$ 0.18	56.88 $\pm$ 0.65
2.0 h	30.45 $\pm$ 0.67	0.06 $\pm$ 0.10	-5.11 $\pm$ 0.24	5.11 $\pm$ 0.24	64.90 $\pm$ 0.74
2.5 h	24.93 $\pm$ 0.67	-0.11 $\pm$ 0.20	-4.69 $\pm$ 0.40	4.69 $\pm$ 0.47	70.33 $\pm$ 0.55
3.0 h	23.35 $\pm$ 0.71	-0.67 $\pm$ 0.12	-4.36 $\pm$ 0.39	4.41 $\pm$ 0.38	71.86 $\pm$ 0.68
4.0 h	21.75 $\pm$ 0.22	-1.14 $\pm$ 0.08	-3.43 $\pm$ 0.81	3.61 $\pm$ 0.76	73.37 $\pm$ 0.29
Durian Samples					
0 day	37.15 $\pm$ 0.75	3.59 $\pm$ 0.24	-4.16 $\pm$ 0.29	5.49 $\pm$ 0.42	58.28 $\pm$ 0.87
2 days	27.62 $\pm$ 0.59	3.49 $\pm$ 0.12	-3.94 $\pm$ 0.18	5.26 $\pm$ 0.27	67.69 $\pm$ 0.65
4 days	26.38 $\pm$ 0.30	3.14 $\pm$ 0.06	-2.86 $\pm$ 0.06	4.25 $\pm$ 0.12	68.79 $\pm$ 0.31
6 days	25.67 $\pm$ 0.09	3.06 $\pm$ 0.06	-2.73 $\pm$ 0.16	4.10 $\pm$ 0.17	69.48 $\pm$ 0.15

IV. CONCLUSIONS

Anthocyanin from butterfly pea holds great potential in term of uses and advances especially in the food and health industry. Aside from its other benefits that lean more towards health sector, the ability of anthocyanin to change its colour to different pH can benefit the food sector greatly. This ability allows butterfly pea extract to be used as a universal pH indicator generally that is safe and does not cause health complication. Deriving from its pH sensing capabilities it can be used as colorimetric indicators in intelligent food packaging as spoilage and freshness indicator. The mechanism of its pH sensing capabilities traces back to the 4 reversible structures of anthocyanin that changes based on the surrounding pH. With great stability in term of colour stability and thermal stability on top of being organic and less risky toward health of consumer, this has butterfly pea extract a perfect pH indicator for food purpose. According to the study it works well as colorimetric indicator and shows great reaction towards the spoilage of shrimp and durian sample.

ACKNOWLEDGMENT

This review paper would not have been made possible without assistance of my co-authors and support from Faculty of Science Technology, Universiti Sains Islam Malaysia. Therefore, I would like to say many thanks to those parties. Last but not least, I am very grateful for the beneficial insights and comments from anonymous peer reviewers at Malaysian Journal of Science, Health and Technology.

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