Effect of pH on the color thermal degradation of *Clitoria ternatea* anthocyanin-impregnated Whatman paper: a study on its potential as high-temperature colorimetric indicator

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Abstract. *Clitoria ternatea* flowers are a readily extractable source of anthocyanin. This study aimed to understand the effect of pH (5, 7, and 8) on the thermal stability of anthocyanin-impregnated Whatman paper (AIP) at elevated temperatures (80, 90, and 98 °C). Color degradation, expressed in terms of Chroma, was monitored using the CIE Lab color model (a*, b*, and C*). The results showed that the degradation rate increased with pH and temperature, with degradation rate constants at 80, 90, and 98°C for AIP5, AIP7, and AIP8 being 0.0004, 0.0010, 0.0018; 0.0015, 0.0041, 0.0059; and 0.0031, 0.0047, 0.0065 min^{-1}, respectively. This indicates that pH 8 is the most unstable, so it causes the highest amount of color change. Activation energies were highest for AIP5 (89.35 kJ/mol), indicating that pH 5 is the most responsive to change in reaction rate with temperature fluctuations compared to AIP7 (82.26 kJ/mol) and AIP5 (43.70 kJ/mol). These findings suggest that AIP8's rapid degradation at high temperatures makes it a potential candidate for high-temperature indication, while AIP5's greater temperature sensitivity could be exploited for applications requiring the detection of smaller temperature variations.

1 Introduction

Clitoria ternatea L. (CT), or the butterfly pea, produces an intense, dark blue color. This color indicates a high content of anthocyanin pigments. The flower survives in areas with prolonged rainfall and dry seasons and can grow throughout the world's tropics. This flower will serve as an accessible source of anthocyanin blue coloring pigment [1]. CT has been a natural colorant in various food products, including beverages, bread, noodles, and pizza. BP is a cost-effective source of natural anthocyanin extracts [2].

Anthocyanins constitute a prominent group of natural food colorants. Anthocyanins are secondary metabolites found in plants and represent the most extensive water-soluble pigments in the kingdom Plantae. They are part of the flavonoid category, which provides many health advantages. The displayed colors fluctuate, including red, orange, purple, and blue, contingent upon the pH level [3]. Numerous factors, including chemical structure, temperature, pH, relative humidity, oxygen, light, sulphur dioxide, sulfites, enzymes, co-pigments, and metal ions, influence the durability of these natural pigments [4–6]. The structural alteration of anthocyanins through hydroxyl, acyl, methoxyl, and glycosyl groups influences their stability. Acylated anthocyanins exhibit more excellent stability than unacylated anthocyanins [7]. Anthocyanins with complex glycosylation and acylation structures have good stability against pH changes and heat treatments [8]. Sources of anthocyanins with that structure can be found in CT [9]. Ternatins, the primary polyacylated anthocyanins in CT flowers, are delphinidin 3,3',5'-triglucoside derivatives, demonstrating significant stability in low acidic and neutral environments [10].

The unique characteristics of CT anthocyanin stability have captivated researchers. Marpaung et al. [11] investigated the degradation of anthocyanins from CT extract flower at pH 7 at different temperatures (7, 30, 45, 60, 75, and 90°C) in bottles with 0% and 50% volume of headspace. The result of the research is that the color and chemical degradation were satisfactorily characterized by first-order reaction kinetics. In addition to CT flowers, other anthocyanin stability studies have also been conducted. Gamage & Choo [12] investigated the stability of anthocyanin-based dyes from other sources such as black goji berry to find the effect of pH treatment (3, 4, 5, and 6) combined with thermal treatment (60, 80, and 100°C) for 10 and 30 minutes on the stability of dye preparations from black goji berry and purple sweet potato. The dye derived from black goji berry exhibited superior color stability compared to purple sweet potato after thermal treatment, although it demonstrated reduced stability during storage at 4 and 26°C. The colorant formulations derived from black goji berry exhibited significant stability at 60 °C for durations of 10 and 30 minutes, as well as substantial storage stability at pH 3 when stored at 4 °C for 30 days. Despite these studies, a comprehensive evaluation of the thermal stability of CT

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anthocyanin across different pH levels and temperatures remains underexplored [13]. Therefore, additional research should evaluate the thermal stability of anthocyanin CT flowers.

Investigations on the application of anthocyanins for the production of temperature colorimetric indicators have been conducted, focusing on the influence of pH and temperature but using anthocyanins from red cabbage. Halász conducted a study on TTI labels utilizing red cabbage extract with cellulose matrices prepared at varying pH levels (pH 2, pH 7, and pH 9) to assess how pH influences the label's response to temporal and thermal variations while elucidating the correlation between color change and time across different temperatures (4 °C, 23 °C, 40 °C, 80 °C, and 100 °C) following incremental time intervals. The outcome indicates that labels produced with red cabbage extract exhibit varying behaviors at different pH levels. The acidic pink TTI label exhibited lower color stability. The label utilizing acidic red cabbage extract is the most appropriate TTI label for indicating long-term temperature storage, whereas the label utilizing neutral red cabbage extract is the most suitable TTI for indicating short-term storage through color change [14].

This study aims to understand the effect of pH 5, 7, and 8 on the color stability of *Clitoria ternatea* (CT) anthocyanin-impregnated Whatman paper at high temperatures (80, 90, and 98 °C) over 6 hours. Color stability was assessed using standard colorimetric techniques. This research could provide an optimal pH for CT anthocyanin extracts for the effective potential of high-temperature colorimetric indicators.

2 Materials and methods

2.1 Materials

Clitoria ternatea flowers (CT) were harvested from a garden in Dramaga, Bogor, Indonesia. The blue part of the blooming flower was detached from the white part. Then, it is dried by freeze-drying. The dried flowers were ground. The powder was packed in a tight container and stored in a freezer until it was used. Buffer solutions pH 5 (citric acid monohydrate- sodium citrate dihydrate), pH 7, and 8 (disodium hydrogen phosphate dihydrate- sodium dihydrogen phosphate monohydrate) were procured from Merck®. All reagents utilized were of analytical quality and required no additional purification.

2.2 Preparation of extract

The modified Marpaung method was utilized to extract anthocyanin from the CT flower [15]. One gram of powder was extracted from 40 ml of deionized water at 60°C for 30 minutes in darkness with constant agitation. The suspension was filtered through a filter paper [11].

2.3 Impregnated extract to Whatman paper

The extract was diluted with buffer solutions at pH 5, 7, and 8, with a dilution factor of 5, and allowed to equilibrate for one hour. Whatman paper (4 cm x 4 cm) was then soaked in each diluted solution for one hour. This process impregnated the Whatman paper with anthocyanin extract, resulting in colored paper at different pH levels: pH 5 (AIP5), pH 7 (AIP7), and pH 8 (AIP8). The impregnated paper was dried for 24 hours and subsequently laminated with retortable plastic.

2.4 Color stability test

To assess color stability, the impregnated paper was exposed to temperatures of 80, 90, and 98 ° C. The CIE (Commission Internationale d'Eclairage) Lab method was used to quantify color changes. For each temperature and pH condition, three individual paper samples were tested.

The initial color coordinates (redness or greenness: a*, yellowness or blueness: b*) were measured at ten points of each paper. After heating at a specified temperature for a predetermined duration, the paper was photographed, and its color coordinates were analyzed using digital colorimetric analysis with Adobe Photoshop 2022 software (Adobe Systems Incorporated, America) [16]. Based on the color coordinates, other color parameters, Chroma (C*), can be calculated as follows:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

2.5 Kinetics of Color Degradation

The degradation kinetics of the color of anthocyanin-impregnated paper were modeled utilizing both zero and first-order reaction kinetics (Eq. 2 and 3).

$$C = Co - k.t \text{ (zero order)} \quad (2)$$
$$C = Co.e^{-k.t} \text{ (first order)} \quad (3)$$

C represents the chroma value; Co denotes the initial chroma value, and k is the rate constant of the reaction. The activation energy (Ea) of the degradation reaction was determined using Eq. 4.

$$k = k_0 e^{\frac{-Ea}{RT}} \quad (4)$$

Where ko is the pre-exponential factor, R is the ideal gas constant (8.314 J/mol·K), and T is the absolute temperature in Kelvin. By fitting the experimental data to these kinetic models, the rate constants and activation energy were determined.

3 Results and discussion

Change of Color in the *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) at Various pH Levels and Elevated TemperaturesTo analyze the change of color, parameters a a* and b* were measured for AIPs at pH 5, 7, and 8. These parameters indicate

color changes such as greenness (negative a*), redness (positive a*), blueness (negative b*), and yellowness (positive b*). AIP5 samples exposed to all temperatures (80, 90, and 98ºC) initially showed an increase in *a-value* up to 85 minutes, indicating a shift towards a redder color. However, after this initial period, the a-value decreased, indicating a gradual changes towards a greener.

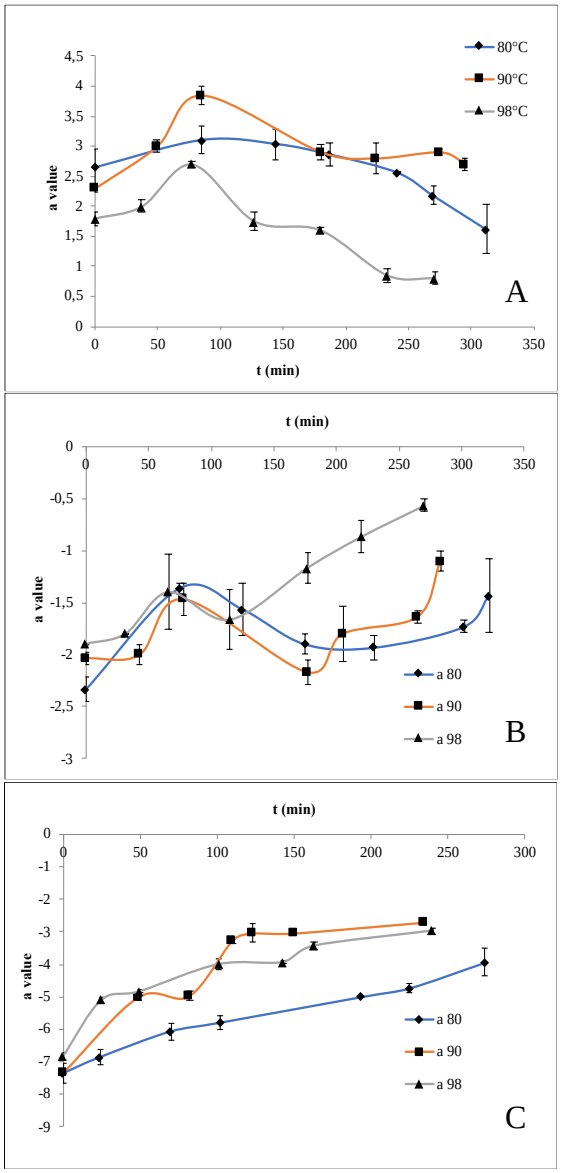

Fig. 1. Degradation of a* value in *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) during heating at 80, 90, and 98 °C and pH 5 (A), pH 7 (B), and pH 8 (C).

AIP7 at all temperatures tended to experience fluctuations of decline and increase until about 300 minutes. An initial decreasing and growing trend continues, indicating that the green color is more dominant at the beginning of the heating process. Still, then, there is a recovery toward a redder color. The values remain in the negative range (-2 to -0.6), meaning the pigment remains more green during heating. At the highest temperatures, there is a more drastic change in the values, where there is an increase toward more positive values, indicating a more robust red color tendency. AIP8 at all temperatures showed an increasing pattern with heating time. The color tended to be slightly redder but remained in the green range. Thus, when comparing the three AIPs at the three temperatures, the anthocyanins showed a strong tendency to change towards red in AIP5 or anthocyanin extract at acidic pH, while in AIP7 and AIP8, the color tended to be more green (Figure 1).

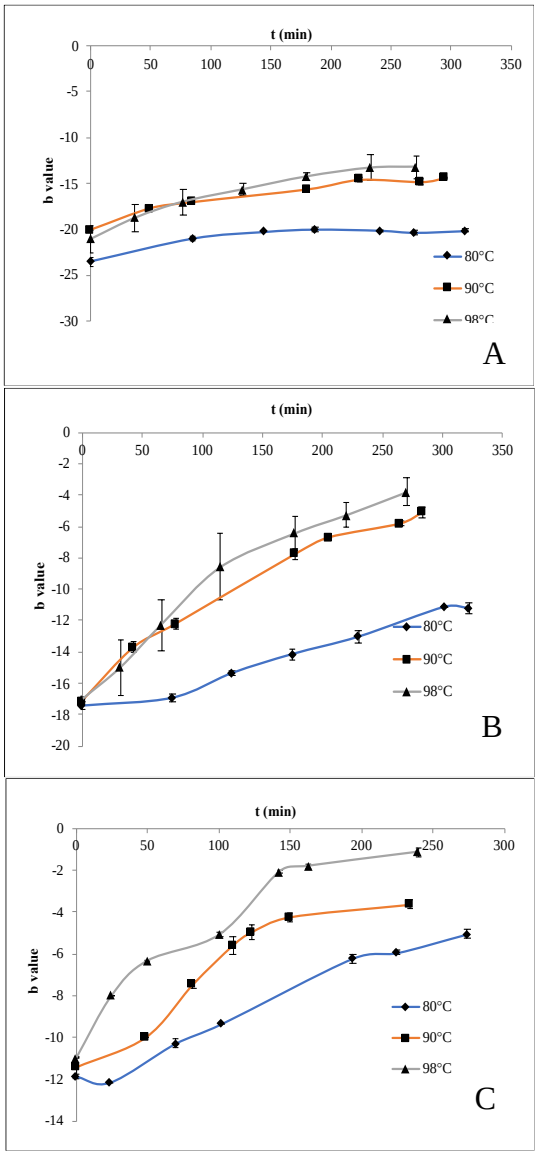

Fig. 2. Degradation of b* value in *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) during heating at 80, 90, and 98 °C and pH 5 (A), pH 7 (B), and pH 8 (C).

The b* values at all pH levels for each temperature increased, although b* remained negative (Figure 2). In AIP5 at 80°C, the *b* value remained close to -23 until the end of the heating time and did not change much towards yellow. AIP5 at 90°C had a greater increase in *b* value from about -20 to close to -14, and AIP5 at 98°C also increased with an initial value of about -21 and increased to -13. In AIP7 at 80°C, the *b* value starts at around -17 and increases slowly to around -11. For 90°C, the b-value increased from -17 and increased to close to -5; at 98°C, the initial *b-value* was around -17 and increased to close to -4 after 270 minutes. AIP8 at 80°C b-value starts at about -11 and increases gradually to about -5. At 90°C, it increases from -11 to close to -3, indicating that the

yellow color is more dominant, and at 98°C, the *b-value* increases from about -11 to almost -1 after 240 minutes. Therefore, it can be said that an increase in the b* value indicates a shift from the blue color range towards yellow.

These changed a* and b* values express the relationship between the parameters a* and b* as chroma (C*), representing the intensity/brightness [17]. Changes in the a* and b* values affect the C* value. Figure 3 shows that all AIPs at 80, 90, and 98°C temperatures showed a decreasing trend in the percentage chroma value, indicating a C* decrease in intensity or color saturation. This decrease indicates a change in the chemical structure of anthocyanins that shifts the color balance due to the influence of temperature. Increased temperature causes anthocyanin

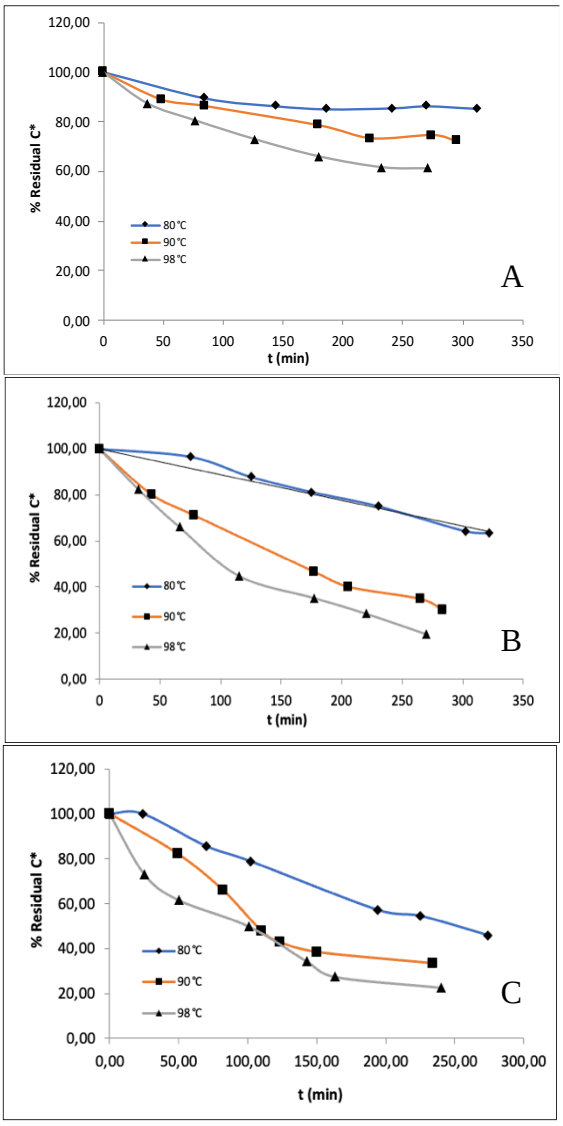

Fig. 3. Degradation of C* value in *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) during heating at 80, 90, and 98 °C and pH 5 (A), pH 7 (B), and pH 8 (C).

degradation due to color degradation [18]. The process involves the cleavage of the pyrillium ring and the resulting formation of chalcones as the first degradation step for anthocyanins [19]. In addition, the figure also illustrates that the three AIPs experience a decreasing rate and still have residual values of color intensity. This indicates that the AIPs can still be heated at higher temperatures.

Adams proposed the hydrolysis of sugar moieties and the subsequent synthesis of aglycones as an preliminary degradation stage potentially induced by the formation of cyclic additives. The author additionally states that anthocyanins decompose upon heating into chalcone structures and sugar [20]. This chalcone compound is expected to cause the yellow color formation due to the formation of this yellow chalcone compound [21]. Furthermore, because AIP is based on CT anthocyanins, the type of anthocyanins is delphinidin, so the degradation continues with the change of delphinidin into phloroglucinaldehyde and gallic acid [22].

A representative schematic of the color change of AIP presented after heating at 80, 90, and 98°C is depicted in Fig. 4, where it can be observed that there is color degradation at both temperature and pH. At pH 5, the color intensity changes from purplish blue to purplish-blue to more yellow. At pH 7, the color intensity changed from blue to yellowish green; at pH 8, the intensity also changed to yellowish green, originally green.

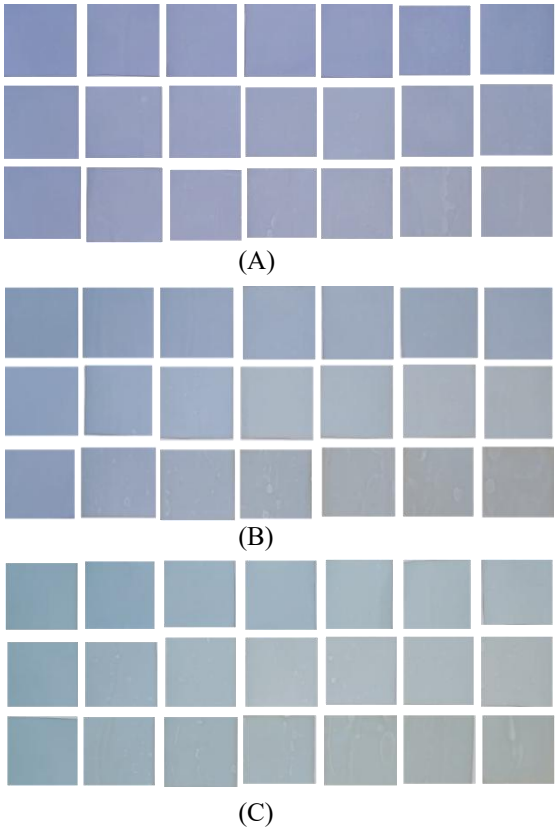

Fig. 4. Color response in *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) during heating at 80, 90, and 98 °C and pH 5 (A), pH 7 (B), and pH 8 (C).

3.1 Color degradation kinetics of the *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) with different high-temperature and pH.

High temperature stability of the *Clitoria ternatea* anthocyanin extracts in Whatman Paper (AIP) was analyzed at various heating temperatures (80, 90, and 98 °C), heating times (0 – 300 minutes), and pH values (5, 7, and 8). The findings indicated that all thermal degradation data of AIP can be accurately modelled using a first-order reaction kinetic model, exhibiting a good regression coefficient R^2 [11,22,23] (Table 1), with a 0.69–0.99 R^2 range. The thermal degradation of anthocyanins from the AIP at pH 5, 7, and 8 is depicted in Figures 5, 6, and 7.

Table 1. Rate constants for the color thermal degradation of *Clitoria ternatea* anthocyanin-impregnated Whatman paper (AIP) at various temperatures and pH values..

pH	T (ºC)	Zero Order		First Order	
		R^2	k (min^{-1})	R^2	k (min^{-1})
5	80	0,6839	0,0095	0,6917	0,0004
	90	0,9173	0,0171	0,9342	0,0010
	98	0,9281	0,0295	0,957	0,0018
7	80	0,9855	0,0216	0,9765	0,0015
	90	0,9659	0,0403	0,9945	0,0041
	98	0,9399	0,0503	0,9876	0,0059
8	80	0,991	0,0296	0,992	0,0031
	90	0,863	0,0425	0,918	0,0047
	98	0,864	0,0389	0,945	0,0065

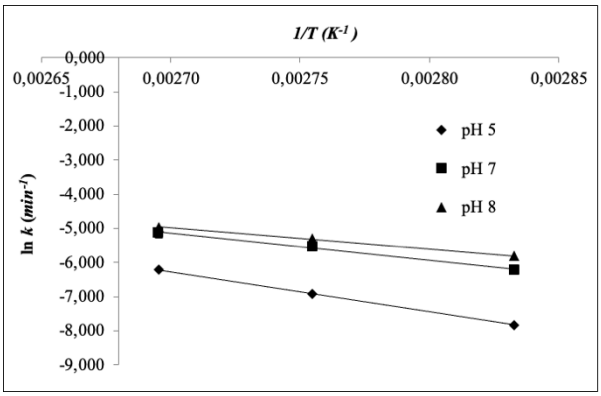

Fig. 5. Arrhenius plot of rate constants for the color thermal degradation of *Clitoria ternatea* anthocyanin-impregnated Whatman paper (AIP) at different pH values

This study (Figure 5) examined the kinetic parameters for the color thermal degradation of *Clitoria ternatea* anthocyanins-impregnated Whatman paper (AIP) at different heating temperatures and pH values. The color degradation rate constant (k-value) of the AIPs generally increased with elevated heating temperatures and pH values. The k-values at 80, 90 and 98°C of AIP5 were 0.0004, 0.0010, and 0.0018 min^{-1}, AIP7 were 0.0015, 0.0041 and 0.0059 min^{-1}, and AIP8 were 0.0031, 0.0047 and 0.0065 min^{-1} (Figure 5). This signified an interaction effect between pH and heating temperature. This result aligns with prior research indicating that anthocyanin degradation increases at higher temperatures and pH levels [24].

The temperature dependency of the AIP color thermal degradation rate constant was analyzed using the Arrhenius relationship (Figure 5). This analysis yielded the activation energy. The activation energies of the degradation reaction for AIP5, AIP7, and AIP8 were 89.35, 82.26, and 43.70 $kJ.mol^{-1}$, respectively. These results are consistent with Shiau's findings, which showed an inverse relationship between pH and activation energy. Shiau reported that activation energies of the degradation for anthocyanins extract at pH 2.5, 3.5, 4.5, and 5.5 were 45.23, 32.43, 22.36, and 19.52 $kJ \cdot mol^{-1}$, respectively [23].

Visually, it was observed that the slope of the line at pH 5 is the steepest. This indicates that pH 5 exhibits the highest activation energy among the tested pH values. A higher activation energy signifies a greater dependence of the reaction rate on temperature. Therefore, the degradation of AIP color at pH 5 is the most sensitive to temperature changes. Relatively small changes in temperature at pH 5 lead to larger changes in the degradation rate (k-value) compared to pH 7 or 8.

4 Conclusion

This study suggests that the color thermal degradation of *Clitoria ternatea* anthocyanin-impregnated Whatman paper (AIP) followed first-order reaction kinetics, with higher temperatures and pH generally leading to increased degradation rates. Arrhenius plot analysis further demonstrated the influence of pH on activation energy (Ea), with AIP5 exhibiting the highest Ea (89.35 kJ/mol) and thus the greatest temperature sensitivity. This inverse relationship between pH and Ea aligns with previous research on anthocyanin extracts, confirming that more acidic conditions enhance thermal stability but also increase the sensitivity of color degradation to temperature fluctuations.

These findings provide a basis for tailoring AIP as a temperature indicator by adjusting the pH during preparation. While AIP8 exhibits rapid degradation at high temperatures, making it potentially useful for high-temperature indication, the greater temperature sensitivity of AIP5 suggests its suitability for applications requiring the detection of subtle temperature changes or operating within a different temperature range. The kinetic data presented here are essential for predicting AIP color stability over time, regardless of the specific application.

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