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Effects of different physical parameters on the stability of anthocyanin from coconut (*Cocos nucifera*) husk

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Abstract

Anthocyanin, a bioactive compound present in plants, possesses the characteristic of being an extractable pigment. Due to its bioavailability and wide spectrum of colors, extensive research has been conducted on anthocyanin to explore its medicinal advantages and its potential as a natural coloring agent. However, it is reported to be easily degraded by external environmental factors due to its high reactivity. The husk from coconut (*Cocos nucifera*) has been proven to contain a significant amount of anthocyanin. Therefore, this study aims to evaluate the effects of temperature, autoclaving condition, pH, and light on the stability of anthocyanin from coconut husk. The anthocyanin extracted from the mesocarps of brown coconut husks was tested at different temperatures (50 ℃ to 70℃), pH (1 to 14), autoclaving conditions (15 minutes to 60 minutes), and light exposure (room temperature for 70 days). In extreme temperatures and long durations of autoclaving conditions, anthocyanin extracts were slightly degraded in terms of colour strength and total anthocyanin content. The acidity and alkalinity of the samples affected the stability of anthocyanin extracts drastically. The colour strength and total anthocyanin content steadily increased as the pH rose. Light exposure also influenced the stability of anthocyanin extract throughout the 70-day exposure to fluorescent light. To sum up, anthocyanin extracts from the C. nucifera are stable in a highly alkaline condition and must be stored away from light, extreme heat, and high pressure.

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1. INTRODUCTION

Anthocyanins are natural pigments found in plants that not only impart colour but may also have health advantages (Swer et al., 2019). Anthocyanin displays a wide range of colours from as dark as black to violet and as bright as red to pink, presenting the varied colours of fruits and vegetables. This spectrum of pigment has a special appeal to consumers and is indispensable to the dye industry. Anthocyanin has been discovered from various members of the plant kingdom, especially fruit and vegetable. Besides its usage as alternative colouring, it also offers pharmacological properties (Ramadan & El-Hadidy, 2015). Putta et al. (2017) provided evidence showcasing the antioxidative characteristics of anthocyanins. These properties enable anthocyanins to display anti-inflammatory, chemotherapeutic, cardioprotective, hepatoprotective, and neuroprotective activities. Moreover, anthocyanins have demonstrated therapeutic effects against various chronic diseases.

Coming from varied resources, its stability is debatable because it is susceptible to structural modification and environmental factors. Traditionally, anthocyanins are subjected to processing and storage practices that involve maintaining dark, cool, and anaerobic conditions to minimize their degradation (Cai et al., 2022). Anthocyanins possess a significant drawback due to their limited stability. Their stability is susceptible to various factors including pH levels, light exposure, temperature fluctuations, co-pigmentation, sulfites, ascorbic acid, oxygen, and enzymatic activity (Enaru et al., 2021).A decrease in anthocyanin concentration of black rice aqueous extract with increasing temperature of heat treatment and storage was observed by Sui et al., (2016). Anthocyanins are more stable in acidic conditions, but under normal processing and storage conditions, they easily convert to colourless hemiketal equilibrium forms of anthocyanins and then to insoluble brown pigments (Hernández-Herrero, &Frutos, 2014). The color expression of anthocyanins is influenced by the pH level of the solution due to the ionic nature of their molecular structure. Under acidic conditions, certain anthocyanins exhibit a red hue. In a neutral pH environment, anthocyanins display a purple color, whereas an elevated pH causes a shift towards a blue hue (Khoo et al., 2017).

Anthocyanin exists in the form of anthocyanidin glucosides and acylated anthocyanin. Anthocyanidin glucoside is a typical anthocyanin. Its conjugated form contributes in the colour of plants, which are red, blue and

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purple. Meanwhile, acylated anthocyanin can be further divided into acylated anthocyanin, coumarylated anthocyanin, caffeoylated anthocyanin and malonylated anthocyanin. The most common types of anthocyanidins are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Khoo et al., 2017).

Coconut (*Cocos nucifera*) husk is chosen as the source of anthocyanin in this study due to its abundance in Malaysia. Coconut is listed as one of the major industrial crops after oil palm and rubber. According to the most recent FAO statistics, global coconut production reach 62.06 million tonnes in 2019, with approximately 30 million tonnes of coconut mesocarp and coconut endocarp (Yang et al., 2021). Thus, it contributes to a significant amount of biomass and leads to environmental problems due to poor waste management (Rodiah et al., 2018a). Due to its reusability and renewability, coconut husk can be utilised and applied to the research of anthocyanin pigment after the flesh is obtained. Studies on the anthocyanin's stability after extraction process and during storage period are of great importance to better understand the degradation mechanisms of anthocyanins. Therefore, this study is aimed to determine the effect and optimum level of temperature, autoclaving condition, pH and light on the stability of anthocyanin extracted from coconut husk.

2. MATERIALS AND METHODS

2.1 Materials

Brown mature coconut husks (*C. nucifera*) were obtained from Tanjung Karang, Selangor, Malaysia. The mesocarps of the coconuts were cut into tiny pieces, dried (60 °C for 24 hours), and ground. The samples were sieved using a 0.5-mm mesh-sieve, kept in ziplock bags wrapped in aluminium foils, and stored in a desiccator, away from heat and humidity (Rodiah et al., 2018b).

2.2 Extraction Method

The mesocarp powder was mixed with 0.8 M of NaOH and heated at 300 W microwave power for 4 minutes. The samples were cooled down and then filtered using filter paper (CHM, Germany) (150 mm) in order to remove plant materials. Approximately, 1% of sodium benzoate ($C_7H_5NaO_2$) was added for preservative purpose before the samples were stored at 4°C in a refrigerator (Nur Asma Fhadhila et al., 2016). All the samples were further tested for the stability test.

2.3 Stability Test

2.3.1 The Effect of Temperature on the Stability of Anthocyanin

Samples in triplicates were put into thermostatic water baths according to the respective temperatures for 3 hours, i.e., 40, 50, 60, 70, and 80 °C. Later, the universal tubes were directly chilled in ice water to avoid a continuous increase in temperature (Sun et al, 2009).

2.3.2 The Effect of Autoclaving on the Stability of Anthocyanin

Anthocyanin extracts (100 mL) were filled in a schott bottle, and the extracts were autoclaved at a fixed temperature (121 °C) and pressure (15 psi). The duration of autoclaving was set to 15, 30, 45, and 60 minutes. This test was done in triplicate for each duration (Chatthongpisut, Schwartz & Yongsawatdigul, 2015).

2.3.3 The Effect of pH on the Stability of Anthocyanin

Anthocyanin extracts were mixed with 14 different pH buffer solutions, ranging from pH 1 to pH 14 (Brown, 2008) in separate test tubes at a ratio of 1:1. The mixtures were homogenizely shaken using a vortex machine for 2 minutes. This test was done in duplicate for each pH.

2.3.4 The Effect of Light on the Stability of Anthocyanin

Approximately, 10 mL of anthocyanin extract was filled in a small glass bottle (volume of 15 mL) and then put in a drawer with a built-in fluorescent lamp for 70 days at room temperature (30 °C). Number of bottles was prepared according to sampling time in triplicate for each sampling time. The samples were located 10 cm apart from the light source. The samples were examined every day for the first 7 days, and the next examination was started on the 14th day with one week interval until the 70th day ended.

2.4 Analysis

2.4.1 Determination of Colour

A colour reader (Konica Minolta, Japan) was used to determine the colour through CIELAB colour space coordinate. The colours were represented by L*, a*, b*, c* and h* values, where L* represents lightness (ranging from 0 = black to 100 = white), a* (red for positive value to green for negative value), b* (yellow for positive value to blue for negative value), c* for chroma, and h* for hue angle (Nur Asma Fhadhila et al., 2016).

2.4.2 Total Anthocyanin Content

The absorbance of anthocyanin at 520 nm was determined using a spectrophotometer (Hitachi, Japan). The total anthocyanin content was calculated according to Du and Francis (1973), as follows:

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$$Total\ anthocyanin\ content\ (mg/g): \frac{A \times Df}{Sample\ weight \times 55.9} \times 100$$

A stands for absorbance value and *Df* is the dilution factor.

2.4.3 Statistical Analysis

The samples were prepared in triplicates, and the results were conveyed as mean ± standard. The data was interpreted using one way ANOVA, and the Tukey test was applied to determine the significant difference ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 The Effect of Temperature on The Stability of Anthocyanin

Table 1 represents the colour coordinate of anthocyanin measured by the L*, a*, b*, c*, and h* values. The L* values decrease moderately (26.2 to 28.0), indicating that the colours of the samples became lighter with the increase in temperature. However, the values of a* (–4.7 to –2.2) and b* (–2.9 to –1.7) are negative with no significant difference ($p > 0.05$), exhibiting greener and bluer tones. Meanwhile, Table 2 shows the gradual decline values of total anthocyanin content from 35.51 mg/g to 29.83 mg/g without significant difference ($p > 0.05$) as the temperature rose at 10 °C intervals. It shown that anthocyanins become less stable at higher temperatures. Temperature significantly reduced the half-life of anthocyanins, according to research done by Bordignon et al. (2007) on the stability of anthocyanin colour extracted from Isabel grape. Based on a previous study, heat treatment at a maximum temperature of 35°C reduced the total anthocyanin content in common grape berries to less than half the amount in control berries at 25°C (Mori et al., 2007). Even though the pH of the solution was low, the colour of anthocyanin changed from red to orange at temperatures as high as 40°C (West & Mauer, 2013). In another study, heat treatment of an extract solution rich in anthocyanins might not result in anthocyanin pigment destruction. This is because phenolic compounds in the extract are commonly degraded enzymatically by polyphenol oxidase. However, mild heat treatment of the extract to temperatures as high as 50°C has been shown to inactivate the enzymatic reaction (Patras et al., 2010). The presence of the hydroxyl group led to anthocyanin instability. Upon exposure to intensive heat, the anthocyanin-copigment complex will dissociate, leading to colour degradation. The major factors in anthocyanin colour change are the ring opening from the hydroxyl group and the decline in anthocyanin content (He et al., 2015).

Table 1: The Effect of Temperature on The Colour Coordinate of Anthocyanin.

Temperature (°C)	Colour coordinate				
	L*	a*	b*	c*	h*
40	26.2 ± 0.3 [a]	-2.2 ± 0.1 [c]	-1.7 ± 0.3 [b]	2.7 ± 0.1 [a]	219.6 ± 3.2 [c]
50	26.8 ± 0.1 [b]	-4.7 ± 0.4 [a]	-1.7 ± 0.4 [b]	5.0 ± 0.3 [d]	199.9 ± 5.8 [a]
60	27.1 ± 0.2 [bc]	-3.4 ± 0.1 [b]	-2.2 ± 0.2 [ab]	4.1 ± 0.1 [b]	212.3 ± 2.9 [bc]
70	27.6 ± 0.2 [cd]	-3.9 ± 0.1 [b]	-2.9 ± 0.2 [a]	4.9 ± 0.2 [c]	216.5 ± 1.7 [bc]
80	28.0 ± 0.2 [d]	-3.9 ± 0.1 [b]	-2.1 ± 0.5 [ab]	4.4 ± 0.3 [bc]	208.0 ± 6.0 [ab]

Values are represented as mean ± standard deviation for each temperature, columns with different superscripts are significantly different at $p < 0.05$.

Table 2: The Effect of Temperature on the Total Anthocyanin Content.

Temperature (°C)	TAC (mg/g)
40	35.51 ± 3.59 [b]
50	35.49 ± 0.72 [b]
60	31.37 ± 1.03 [ab]
70	30.75 ± 1.50 [ab]
80	29.83 ± 1.25 [a]

Values are represented as mean ± standard deviation for each temperature, columns with different superscripts are significantly different at $p < 0.05$.

3.2 The Effect of Autoclaving Condition on The Stability of Anthocyanin

Table 3 shows that the autoclaving condition affected the colour coordinate of anthocyanin. L* values escalated moderately from 26.5 to 27.6, without significant difference ($p>0.05$), as the duration of the autoclaving condition increased. It could be concluded that the sample colour became lighter due to the decomposition of the extracts. The result is further supported by the reduction of total anthocyanin contents from 38.59 mg/g at the 15th minute to 33.19 mg/g at the 60th minute, as shown in Table 4.

The results obtained in the present study were also in close agreement with those reported by Marszalek et al. (2017) who observed that the pressure exerted less effect on anthocyanin degradation, while thermal conditions significantly affected its decomposition. However, the combination of pressure and thermal condition increases the chance of degradation significantly because monomeric anthocyanin turns into a more condensed compound, and the condensation activity changes the colour of anthocyanin. In the present study total anthocyanin content was reduced non-significantly ($p>0.05$)

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Table 3: The Effect of Autoclaving Condition on the Colour Coordinate of Anthocyanin.

Duration (min)	Colour coordinate				
	L*	a*	b*	c*	h*
15	26.5 ± 0.3 ^{a}	-2.7 ± 0.3^{c}	-1.3 ± 0.3^{b}	3.9 ± 0.5 ^{a}	205.8± 2.8 ^{a}
30	26.9 ± 0.2 ^{a}	-3.9± 0.1 ^{a}	-1.5 ± 0.4 ^{b}	4.2 ± 0.1 ^{ab}	201.4± 6.4 ^{a}
45	27.1 ± 0.4 ^{ab}	-3.2 ± 0.1^{b}	-2.2 ± 0.3 ^{b}	3.9 ± 1.2 ^{ab}	215.0 ± 3.4 ^{ab}
60	27.6 ± 0.1^{b}	-3.5 ± 0.2 ^{ab}	-3.6 ± 1.3 ^{a}	5.1± 1.1^{b}	224.2 ± 8.1^{b}

Values are represented as mean ± standard deviation for each duration, columns with different superscripts are significantly different at $p < 0.05$.

Table 4: The Effect of Autoclaving Condition on the Total Anthocyanin Content.

Duration (min)	TAC (mg/g)
15	38.59 ± 0.68^{b}
30	36.27 ± 2.15 ^{ab}
45	33.53 ± 0.94 ^{a}
60	33.19 ± 2.27 ^{a}

Values are represented as mean ± standard deviation for each duration, columns with different superscripts are significantly different at $p < 0.05$.

3.3 The Effect of pH on the Stability of Anthocyanin

Acidity and alkalinity affected the colour coordinate of anthocyanin pigment. Table 5 shows that the L*values insignificantly ($p > 0.05$) surged from 29.3 at pH 14 to 31.2 at pH 1. The rise in L* values indicates that the extracts turned lighter and are degraded with the decrease in pH values.The colour of anthocyanins is affected by the pH of the solution. This is due to the ionic nature of anthocyanins' molecular structure. Some anthocyanins turn red when exposed to acid. Anthocyanins have a purple hue in neutral pH, but the colour changes to blue as the pH rises. Anthocyanin pigments are mostly flavylium cations, which give them their red colour. At a lower pH solution, these anthocyanins are more stable. The flavylium cation formed at lower pH allows the anthocyanin to be highly soluble in water (Turturică et al., 2015).

The total anthocyanin content (Table 6) decreased from 54.42 to 46.80 mg/g with the reduction of pH values (pH 14 to pH 1). Results indicated that anthocyanin extract of *C. nucifera*is unstable in acidic conditions. Rodiah et al. (2016a) revealed that anthocyanin could be extracted from *C. nucifera* under high alkalinity due to glycoside and phenolic groups, which are soluble in alleviated pH solution. Thus, the alkaline condition could assist the hydrolysis of glycoside in the natural dye for optimum extraction and high yield colour.In accordance, Fan et al. (2008) reported that the structural alterations between the flavylium cation, quinonoidal bases, colourless carbinol pseudobases, and yellow chalcone caused by varying pH values or protonation or hydration reactions determine the colour stability of anthocyanins.

Table 5: The Effect of pH on the Colour Coordinate of Anthocyanin Content.

pH	Colour coordinates				
	L*	a*	b*	c*	h*
1	31.2 ± 0.1 ^{b}	-14.8 ± 0.0^{bc}	6.4 ± 0.1^{h}	16.1 ± 0.0^{def}	156.9 ± 0.3 ^{a}
2	30.6 ± 0.1 ^{ab}	-18.4 ± 0.0 ^{a}	5.9 ± 0.6^{gh}	19.3 ± 0.1^{g}	162.3 ± 1.6^{b}
3	30.1 ± 0.1 ^{ab}	-16.3 ± 1.1 ^{ab}	4.8 ± 1.2^{efgh}	16.9 ± 1.4^{efg}	163.9 ± 2.8^{bc}
4	30.0 ± 0.1 ^{ab}	13.2 ± 0.0^{cd}	3.0 ± 0.1^{bcd}	13.6 ± 0.1^{bc}	167.2 ± 0.3^{cd}
5	30.0 ± 0.2 ^{ab}	-16.2 ± 0.4 ^{ab}	4.2± 0.4^{cdef}	16.7 ± 0.4^{ef}	165.6 ± 1.6^{bcd}
6	29.9 ± 0.3 ^{ab}	-16.8 ± 0.1 ^{ab}	5.3 ± 0.1^{fgh}	17.7 ± 0.1^{efg}	162.5 ± 0.7^{b}
7	29.9 ± 0.5 ^{a}	-12.2 ± 0.8^{d}	2.7 ± 0.5^{bc}	12.6 ± 0.9^{b}	167.7 ± 1.3^{cd}
8	29.8 ± 0.2 ^{a}	-13.3 ± 0.4^{cd}	3.3 ± 0.2^{bcde}	13.7 ± 0.6^{bcd}	166.5 ± 0.5^{bcd}
9	29.7 ± 0.1 ^{a}	-14.7 ± 0.1^{bc}	4.2 ± 0.0^{cdefg}	15.3 ± 0.1^{cde}	164.0 ± 0.1^{bc}
10	29.7 ± 0.1 ^{a}	-17.4 ± 0.0 ^{a}	5.3 ± 0.1^{fgh}	18.2 ± 0.1^{fg}	163.2 ± 0.3^{bc}
11	29.6 ± 0.0 ^{a}	-12.0 ± 0.4^{d}	2.3 ± 0.1^{b}	12.2 ± 0.4^{b}	169.1 ± 0.2^{d}
12	29.5 ± 0.1 ^{a}	-15.0 ± 0.3^{bc}	4.2 ± 0.3^{cdefg}	15.6 ± 0.3^{cde}	164.3 ± 0.8^{bc}
13	29.4 ± 0.0 ^{a}	-15.0 ± 0.7^{bc}	4.6 ± 0.5^{defg}	15.7 ± 0.8^{cdef}	163.2 ± 0.9^{bc}
14	29.3 ± 1.0 ^{a}	-9.4 ± 1.2^{e}	0.5 ±0.3 ^{a}	9.5 ± 1.1 ^{a}	177.3 ± 1.5^{e}

Valuesare represented as mean ± standard deviation for each pH, columns with different superscripts are significantly different at $p < 0.05$.

Table 6: The Effect of pH on the Total Anthocyanin Content.

pH	TAC (*mg/g*)
1	46.80 ± 16.70 ^{a}
2	47.12 ± 4.39 ^{a}
3	47.87 ± 4.55 ^{a}
4	48.09 ± 4.85 ^{a}
5	49.59 ± 6.06 ^{a}
6	50.66 ± 5.77 ^{a}
7	51.20 ± 2.88 ^{a}
8	51.52 ± 5.162 ^{a}
9	51.63 ± 1.36 ^{a}
10	53.56± 8.34 ^{a}
11	53.67 ± 5.46 ^{a}
12	53.78 ± 6.23 ^{a}
13	54.42 ± 4.10 ^{a}
14	54.42 ± 3.79 ^{a}

Values are represented as mean ± standard deviation for each pH, columns with different superscripts are significantly different at $p < 0.05$.

3.4 The Effect of Light on the Stability of Anthocyanin

The L* values from day 0 to day 63 show an insignificant ($p > 0.05$) increase from 26.3 to 29.7 (Table 7). However, by day 70, a significant increment ($p > 0.05$) in the L* values were observed, i.e., 30.8. The longer the exposure of samples to light, the lighter the colour of the extracts. which was visually confirmed by differences in

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the colour of the solution. Moreover, the a* values moved towards the greener tones, while the b* values towards the yellow tones. Meanwhile, the total anthocyanin content in Table 8 showed a significant difference ($p < 0.05$) between the total anthocyanin contents on days 0 to 7(from 39.06 to 32.13 mg/g) and on days 14 to 70 (from 29.98 to 19.68 mg/g).

The decline in total anthocyanin content values is an indicator of indicates anthocyanin decomposition in the samples. The light could affect anthocyanin in two ways, by biosynthesis or degradation. Biosynthesis helps increase anthocyanin content, while degradation accelerates the loss of pigment. Hence, anthocyanin is normally preserved in the dark, away from light. The instability of anthocyanin in light depends on the plant sources and the structure of individual anthocyanin (Akhavan et al., 2016).

Table 7: The Effect of Light on the Colour Coordinate of Anthocyanin.

Day	Colour coordinates				
	L*	a*	b*	c*	h*
0	26.3 ± 0.1^{a}	-2.0 ± 0.2^{gh}	0.0 ± 0.1^{bcdef}	2.0 ± 0.2^{a}	178.9 ± 1.9^{abcd}
1	26.7 ± 0.3^{ab}	-7.8 ± 0.1^{ef}	0.3 ± 0.2^{bcdef}	7.9 ± 0.1^{b}	170.5 ± 4.9^{abc}
2	27.0 ± 1.0^{ab}	-9.3 ± 0.2^{de}	0.4 ± 0.5^{bcdef}	9.3 ± 0.2^{c}	173.1 ± 9.0^{abc}
3	27.0 ± 0.5^{ab}	-10.2 ± 0.2^{d}	1.0 ± 0.9^{def}	10.5 ± 0.5^{c}	198.9 ± 18.3^{cd}
4	27.0 ± 0.7^{ab}	-7.2 ± 0.3^{f}	0.6 ± 0.2^{cdef}	7.3 ± 0.3^{b}	165.1 ± 4.2^{a}
5	27.1 ± 0.6^{ab}	-1.9 ± 0.3^{gh}	-0.9 ± 0.4^{abcde}	1.9 ± 0.3^{a}	191.6 ± 6.4^{abcd}
6	27.3 ± 0.1^{ab}	-12.4 ± 0.5^{c}	1.1 ± 0.1^{def}	12.4 ± 0.6^{d}	170.0 ± 8.5^{abc}
7	27.4 ± 0.2^{abc}	-1.7 ± 0.2^{gh}	-1.1 ± 0.2^{abcd}	1.7 ± 0.2^{a}	192.7 ± 3.2^{abcd}
14	27.5 ± 0.6^{abc}	-12.4 ± 0.2^{c}	1.3 ± 0.4^{efg}	12.6 ± 0.2^{d}	193.3 ± 27.4^{abcd}
21	27.6 ± 0.2^{abc}	-2.3 ± 0.1^{gh}	-1.7 ± 0.3^{abc}	2.3 ± 0.1^{a}	201.6 ± 4.3^{cd}
28	27.6 ± 0.5^{abc}	-2.6 ± 0.3^{g}	-1.8 ± 0.6^{abc}	2.6 ± 0.3^{a}	197.2 ± 4.4^{bcd}
35	27.7 ± 0.4^{bc}	-1.7 ± 0.3^{gh}	-1.9 ± 1.3^{ab}	1.7 ± 0.3^{a}	205.8 ± 17.7^{d}
42	27.7 ± 0.6^{bc}	-0.7 ± 2.1^{h}	-1.0 ± 1.1^{abcde}	1.8 ± 0.1^{a}	194.5 ± 7.5^{abcd}
49	27.8 ± 0.2^{bc}	-2.1 ± 0.2^{gh}	-2.5 ± 1.0^{a}	2.1 ± 0.2^{a}	205.6 ± 8.2^{d}
56	28.7 ± 0.4^{cd}	-13.2 ± 0.4^{ab}	1.5 ± 1.0^{fg}	13.3 ± 0.5^{d}	172.9 ± 4.5^{abc}
63	29.7 ± 0.1^{de}	-14.7 ± 0.3^{b}	3.5 ± 0.5^{gh}	15.1 ± 0.4^{e}	166.6 ± 1.3^{ab}
70	30.8 ± 0.3^{e}	-16.8 ± 1.0^{a}	5.2 ± 1.8^{h}	17.7 ± 1.1^{f}	163.0 ± 5.7^{a}

Values are represented as mean ± standard deviation for each day, columns with different superscripts are significantly different at $p < 0.05$.

Table 8: The Effect of Light on the Total Anthocyanin Content.

Day	TAC (mg/g)
0	39.06 ± 1.90^{h}
1	38.41 ± 1.00^{gh}
2	34.98 ± 2.70^{fgh}
3	34.78 ± 1.50^{fgh}
4	34.22 ± 2.10^{fgh}
5	32.70 ± 0.40^{efgh}
6	32.59 ± 0.40^{efgh}
7	32.13 ± 4.40^{efg}
14	29.98 ± 5.20^{def}
21	28.79 ± 2.80^{cdef}
28	28.65 ± 0.50^{cdef}
35	27.17 ± 0.03^{cde}
42	26.66 ± 1.50^{bcde}
49	23.42 ± 0.70^{abcd}
56	22.90 ± 1.50^{abc}
63	20.51 ± 0.60^{ab}
70	19.68 ± 1.20^{a}

Values are represented as mean ± standard deviation for each day, columns with different superscripts are significantly different at $p < 0.05$.

4. CONCLUSION

In conclusion, the stability of anthocyanin is most affected by the pH and light. As expected, a slight degradation of anthocyanin occurred in all samples when exposed to acidic solution (pH 6-1) and prolonged storage with light exposure up to 70 days. Hence, it is best for anthocyanin extracts of *C. nucifera* to be stored under alkaline conditions and protected from light. Although the samples are still susceptible to heat and pressure, the factors do not significantly affect their stability. It should be highlighted that the anthocyanin extracts of *C. nucifera* could be an interesting functional natural colouring in batik and leather industry considering the colour stability to pH (7-14) and temperature (40-80 °C).

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