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Research Paper

Study of Anthocyanin Extraction from Red Banana (*Musa sapientum* L. *var Rubra*) Waste and Characteristics of Light Effects

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Abstract
Anthocyanins are compounds responsible for plants' blue, purple, violet, magenta, red, and orange colours. Anthocyanins are found in tropical fruits. Generally, anthocyanins are found in the peel tissues of plants. The increasing interest in anthocyanins, especially in the field of food and health, supports the development of anthocyanin exploration research. One of the uses of anthocyanins that are widely developed today is the addition of anthocyanins as indicators in smart packaging. For application on the packaging, in addition to extraction techniques, it is also necessary to assess the characteristics of anthocyanins in the environment. This study aims to examine the anthocyanin potential of red banana waste and the effect of light on anthocyanin stability. The anthocyanins observed are the result of anthocyanin extraction from the red banana peel and bracts using the maceration method. The solvent used is water acidified with citric acid. The study results showed that the total anthocyanin content in red banana bracts extract was higher than in extract from red banana peel. A concentrated extract from the bracts of a red banana contains 114.26 μg/g FW of total anthocyanins. In comparison, the concentrated extract of red banana peel contains 110.27 μg/g FW of total anthocyanins. Identification of concentrated extracts of red banana peel and flower through FTIR test, maximum wavelength test with UV-Vis and discolouration test showed that the extract contains anthocyanin compounds. Irradiation with a 25-watt bulb lamp, UV lamp and sunlight on concentrated extracts of the red banana peel and bracts showed degraded anthocyanin content. The results of this study show that the peel and flower of red bananas have the potential to be developed as a source of anthocyanins.

Keywords
Red Banana (*Musa sapientum* L. *var Rubra*), Red Banana Peel, Characteristics

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1. INTRODUCTION

Anthocyanins are compounds belonging to the flavonoid group. The colour and stability of anthocyanin pigments are influenced by pH, oxygen, light, temperature, ascorbic acid, co-pigmentation, metal ions and structures (Horbowicz et al., 2008; Khoo et al., 2017; Yang et al., 2015). The stability of anthocyanins is also influenced by the B ring on the anthocyanin structure and the presence of hydroxyl or methoxyl groups (Castañeda-Ovando et al., 2009). The stability of the thermal properties of anthocyanins is influenced by pH and temperature. The colour of anthocyanins will be stable at a lower pH. In addition to pH, anthocyanin discolouration also occurs as the temperature increases during storage (Wu et al., 2018).

Interest in anthocyanins is increasing, both in the field of food and health (Martín et al., 2017; Pina, 2014; Santos-Buelga et al., 2014). In the food industry, anthocyanins are no longer just a source of natural food colouring, but anthocyanins are starting to be widely applied as indicators of products in packaging. The development of technology demands a better packaging function. Packaging no longer serves as a protector of the product, but also as a means of communication between producers and consumers. Anthocyanins are compounds that have good performance in the development of active packaging. This is because anthocyanins contain potent antioxidants and have the potential to be antimicrobials. The addition of anthocyanins to the active packaging is perfect for pH-sensitive products.

Several research results show that anthocyanins are successfully used as indicators on the packaging. Using chitosan-purple potato not only improves the physical properties of the film produced but also has antioxidant properties. So, it is very suitable for product packaging that is easily oxidized

(Li et al., 2019). Intelligent pH-sensor indicators based on nanocellulose bacteria and anthocyanins from black carrots show discolouration as the freshness of fish fillets decreases (Moradi et al., 2019). The use of anthocyanins from blueberry waste, red cabbage extract and Aqueous hibiscus extract has been successfully used as a pH indicator in smart packaging and intelligent food packaging (Andretta et al., 2019; Jiang et al., 2020; Peralta et al., 2019).

With the growing use of anthocyanins in the non-food industry, it is necessary to study potential anthocyanin sources. Anthocyanins are abundant in tropical fruits (Khoo et al., 2017). Some sources of anthocyanins that are commonly found in Indonesia include purple sweet potatoes contain 2.57±0.86 mg/g total anthocyanin (Arisanti et al., 2020), dragon fruit containing 104.58 mg/Kg total anthocyanin (Kwartiningsih et al., 2016), rosella flowers contain 9.43 mg/g total anthocyanin (Lestari et al., 2019), red spinach contain 93.03 mg/g total anthocyanin (Narayanan et al., 2018) and and banana bracts contain 57.49 mg/100g total anthocyanin (Begum and Deka, 2017). According to Martín et al. (2017) anthocyanins are abundantly contained in the fruit's peel. Research Sujithra and Manikkandan (2019) shows that banana leaves and other banana plant waste have the potential to be used as a source of anthocyanins and natural dyes. The study's results Fu et al. (2018) red banana peel contains six times more anthocyanins than yellow banana peel, which is 154.57 μg/g FW. Red bananas have a proportion of fruit flesh of 59.05±9.01 and fruit peels of 40.95± 9.01. The proportion of fruit skin is large enough that it has the potential to be used as a raw material in phytochemical processing. The red banana plant has the Latin name *Musa acuminata Colla Red/Musa acuminta colla Red Dacca* (Lim, 2012). This plant has the synonym *Musa sapientum* L. *var Rubra* (Kasrina and Zulaikha, 2013). In Indonesia, red bananas have several names, including pisang merah, pisang udang, and pisang kidang. Red banana plants are identified through the red colour on petioles and bones of leaves, tree trunks and flowers are red. Unripe red banana fruits will be dark red and will turn into a red-orange colour by the time the fruit ripens. The fruit's flesh is milky white with a sweet taste when the fruit is ripe.

Therefore, it is necessary to begin to develop the use of anthocyanins from the waste of the red banana plant. The use of waste in the non-food industry will be more profitable because it will not interfere with food sources. The purpose of the study was to examine the anthocyanin potential of red banana waste and the effect of light on anthocyanin stability. The anthocyanins observed result from anthocyanin extraction from the peel and bracts of a red banana.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this study were fresh peel and bracts of red banana (*Musa sapientum* L. *var Rubra*) taken from a farmer's farm in Bengkulu Tengah Regency, Bengkulu Province, Indonesia, distilled water, KCl, $CH_3CO_2Na.3H_2O$, HCl, citric acid and NaOH. The tools used are a pH meter, glassware, UV-vis (Thermo Scientific Genesys 150), FTIR (Alpha FT-IR Spectrometer) and blender.

2.2 Methods

2.2.1 Physical and Physico-chemical Characterization of Red Banana Fruit

Physical characterization is carried out by measuring the fruits of red bananas using callipers. The physicochemical characterization of red banana fruit is carried out through proximate tests of red banana fruit using the AOAC method.

2.2.2 Anthocyanin Extraction

Anthocyanin extraction of red banana peel and bracts used a modification method (Rosalina et al., 2022). Fresh ingredients weighing 200 grams are mashed using a blender. Extraction is carried out by mixing 200 gram of fresh material in 400 mL of water solvent. The solvent is acidified by adding 16 grams of citric acid. The extraction process is carried out by the maceration method for 36 hours. Next, the solution is filtered using filter paper to remove coarse particles. The filtering process is continued by using a vacuum filter with Whatman filter paper (No.01) to remove fine particles that are still dissolved. Then it was centrifuged using a centrifuge at 3000 rpm for 15 minutes. The extract purification process is carried out using a vacuum rotary evaporator at a temperature of 50°C.

2.2.3 Identification of Anthocyanin Compounds

Identification of anthocyanin compounds in concentrated extracts of red banana peel and bracts was carried out using: FTIR Test, UV-Vis Test and colour change test using HCl solvents 2 M and NaOH 2 M method Harboone (1997) cited in Lestario et al. (2014).

2.2.4 Total Anthocyanins

Measurement of the total anthocyanin content was calculated using the spectrophotometer method based on the pH difference. This method was adopted by (Giusti et al., 1999; Giusti and Wrolstad, 2001). Red banana peel and bracts extract is diluted in a pH buffer of 1.0 and a buffer of 4.5. Determine the dilution factor of the diluted extract at a pH buffer of 1.0. The exact dilution factor of the sample is obtained maximum absorbance at a wavelength of 510 nm. Furthermore, the absorbance of the solution at wavelengths of 510 nm and 700 nm was measured, using a UV-Vis spectrophotometer. The absorbance value is calculated using Equation 1.

$$A = [(A_{510} - A_{700})\text{pH } 1.0 - (A_{510} - A_{700})\text{pH } 4.5] \quad (1)$$

Information:

A = absorbance value

The total anthocyanin content is calculated using Equation 2.

$$\text{Total anthocyanins (mg/L)} = \frac{(A \times Df \times MW \times 1000)}{\varepsilon \times l} \quad (2)$$

Information:

A = absorbance value; Df= dilution factor; MW= molecular weight ($C_{12}H_{21}ClO_{11}$. 449.2 g/mol); ε= koef. molar absorpivity (26,900 L/mol.cm); l= width of the cuvet (1 cm).

2.2.5 Light Influence Test

Taken 3 mL of diluted anthocyanin extract. Then the sample is illuminated with a light derived from a UV lamp, a 25watt incandescent lamp and sunlight. Absorbance is measured every 6 hours and observed for 2 days. Absorption is measured at a wavelength of 510 nm.

3. RESULT AND DISCUSSION

3.1 Physical and Physico-Chemical Characterization of Red Banana Fruit

The raw materials used in this study were fresh red banana peels and bracts. Bananas are harvested when the fruit is ripe on the tree. When harvested, the skin colour of red bananas is still purple, slightly green at the base of the fruit. The bananas are ripened until the bananas are ripe, which is indicated by the colour of the skin turning red orange (Figure 1).

Figure 1. Red Banana Plant a) Tree b) Unripe Fruit and Ripe Fruit c) Banana Flower Bud

The number of fruits is 12-15 pieces on each comb. The amount of hands of Red Banana are relatively less than other kind of banana. Generally, it has 4-6 hands of banana. Red bananas have a proportion of fruit flesh of 59.05±9.01 and fruit peels of 40.95±9.01. The proportion of fruit peel is large enough that it has the potential to be studied as a raw material in phytochemical processing (Kibria et al., 2019). Proximate analysis is used to see the chemical content of the fruit. Differences in the degree of maturity of bananas affect the chemical content of the flesh and peel of the fruit. The carbohydrate content shows significant differences in banana kepok from various levels of maturity (Nurhalimah et al., 2019). Research on the tongka banana fruit peel shows that the ripe peel has a higher protein, fat, and carbohydrate content than raw tongka banana peel (Wakano et al., 2020). Differences in maturity levels also affect the anthocyanin content. Research Aurelia et al. (2021) shows that the more mature the peel of the coffee fruit used, the more anthocyanin content also increases. The same results were also obtained in the study Moradinezhad et al. (2018) the anthocyanin content of barberry fruit increased as the maturity index of the fruit increased. A proximate analysis of red banana fruit is presented in Table 1.

Table 1. Characteristics of Unripe Banana Fruit *Musa sapientum* L. *var Rubra*

Physical Characteristics	
Weight	226.520±16 g
Long	15.750±0.64 cm
Diameter	4.125±0.25 cm
Thick	4.750±0.50 cm
Physico-Chemical Characteristics	
Moisture Content (%)	3.58
Ash Content (%)	3.00
Total Protein (%)	1.23
Total Carbohydrates (%)	90.47
Total Fat (%)	0.48
Fibre (%)	1.24

3.2 Identification of Anthocyanin Compounds

Anthocyanins are water-soluble colour pigments. Anthocyanin stability is influenced by pH, light, temperature, and anthocyanin pigment structure. The presence of hydroxyl and methoxyl groups, as well as the presence of rings, also affect the stability of anthocyanins (Khoo et al., 2017). The anthocyanin structure consists of three carbon atoms bonded by oxygen atoms to link the benzene ring (Figure 2).

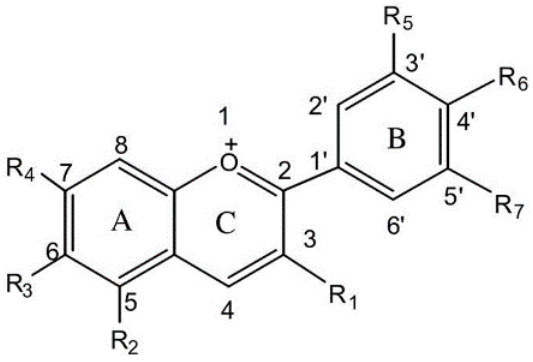

Figure 2. Structure Anthocyanin (Castañeda-Ovando et al., 2009)

3.2.1 FTIR Test

The results of spectrum analysis using FTIR are shown in Figure 3 and Table 2. FTIR analysis aims to identify the content of compounds in the extract based on functional groups. According to Swer et al. (2018), the characteristic anthocyanin compounds on the FTIR spectra are characterized by the presence of functional groups O-H, C=O, C=C and C-O-C. The

emergence of peak C=O vibration absorption on FTIR spectra shows the characteristic anthocyanins (Adu et al.).

The functional group characteristics in the concentrated extract of the red banana are carried out at a wave number of 4000–600 cm^{-1} (Figure 3). The concentrated extract of red banana shows O-H strain vibration in the absorption range of wave number 3596-3201 cm^{-1}. The C=C group is observed in the absorption of wave numbers 1682–1502 cm^{-1}. Wave number absorption in the range of 1756–1691 cm^{-1} indicates the presence of the C=O group. C-O and C-H groups were identified in the absorption of wave numbers 1305-1059 cm^{-1} and 2963-2857 cm^{-1}. The peak of absorption showing the C=O vibration typical of anthocyanins is detected at a wave number of 1732 cm^{-1} (Table 2).

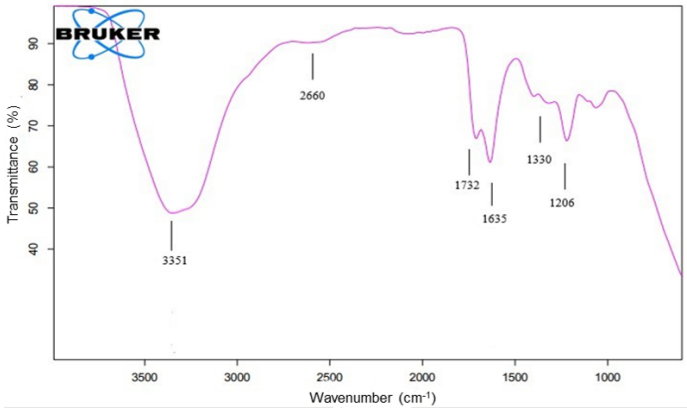

Figure 3. Spectra IR Extract Concentrated (FTIR analysis)

The identification of the O-H, C=O, C=C and C-O-C functional groups on the FTIR spectra shows that the concentrated extract of the red banana peel and bracts contains anthocyanin compounds.

Table 2. Characteristics of Anthocyanin Absorption in FTIR Spectra

Absorption Area	Literature*	Functional Clusters
3351	3200–3600	O-H
1635	1500–1600	C=C aromatic
1732	1690–1760	C=O
1206	1050–1300	C–O alcohol

*source: (Skoog et al., 2018)

3.2.2 UV-Vis Test

Anthocyanins belong to the flavonoid compound group, with the typical spectrum of band II being in the range of 230-295 nm and a band I in the range of 300-560 nm. Anthocyanin UV absorption is in the range of 270-280 nm (band II) and 465-560 nm (a band I) (Neldawati et al., 2013). The results of the UV-Vis spectrophotometer analysis of the red banana peel and flower extract are presented in Figure 4. Figure 4 shows that the maximum wavelength of red banana peel and bract extract is 510 nm. These results show that the maximum wavelength of the extract corresponds to the characteristics of anthocyanins. It can be stated that the extract of red banana peel contains anthocyanin compounds.

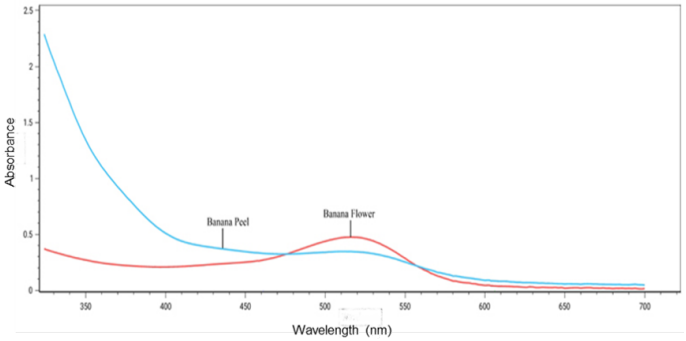

Figure 4. Maximum Wavelength of Concentrated Extract of Red Banana Peel and Bracts

3.2.3 Colour Change Test

The colour change test is is to determine whether there is anthocyanin compounds in the concentrated extract of the red banana peel and bracts. The colour test was carried out using HCl 2 M and NaOH 2 M compounds. Concentrated extracts that experienced a steady red change if added HCl 2 M and the colour became green-blue when dripped with NaOH 2 M showed that the extract contained anthocyanins (Sani et al., 2018; Meganingtyas and Alauhdin, 2021; Lestario et al., 2014; Surianti et al., 2019). Discolouration in anthocyanin extracts is associated with the acidic and alkaline conditions of the solution.

The discolouration test on the concentrated extract of the red banana peel and bracts is presented in Table 3. Observational data showed that the colour of the concentrated extract of red banana peel and bracts changed after adding HCl and NaOH. These results show that concentrated extracts contain anthocyanin compounds.

Based on the identification of anthocyanins in the concentrated extract of red banana peel through the FTIR test, UV-Vis test, and extract discolouration test, it can be concluded that the extract contains anthocyanin compounds.

3.3 Total Anthocyanin Content

According to Castañeda-Ovando et al. (2009) the use of methanol solvents in the extraction of anthocyanins is more toxic. So, the use of anthocyanins in the food and pharmaceutical industries is limited. Research Tensiska and Natalia (2006) shows that the use of acidified water as a solvent can extract anthocyanins with the highest value compared to using ethanol and ethyl acetate solvents in the anthocyanin extraction of arben fruit. The use of water as a solvent can be considered in green extraction.

Table 3. Colour Change Test

Treatment	Change	Anthocyanins*
The extract was heated at a temperature of 100°C with HCl 2 M±5 min	The red colour does not fade	+
Furthermore, the extract is dripped with NaOH 2 M	The red colour becomes bluish-green which is land-fading	+
Visible spectrum	λ_{max} 505-535 nm	λ_{max} 510nm

*+: *appropriate changes occur*

Extraction using a water solvent acidified with citric acid on the red banana peel yielded a total anthocyanin of 110.27 μg/g FW. This result was slightly lower than the total anthocyanins of red banana peel in the study Fu et al. (2018) using methanol solvent, which was 154.57 μg/g FW. This result show that the use of water acidified with citric acid was the potential to be applied to the anthocyanin extract of red banana peel. This was because the total anthocyanin is only a little bit different for the same type of banana though it used different solvent. The total anthocyanins extracted from red banana bracts using water as solvent were 114.26 μg/g FW. This result is lower than previous studies that used ethanol and methanol as solvents. Total anthocyanins from the extraction of banana bracts (*Musa paradisiaca*) were obtained by 321.4 μg/g FW (Sujithra and Manikkandan, 2019). Anthocyanin extraction from banana bracts (Musa ABB) was processed with total anthocyanins of 569.8 μg/g FW (Begum and Deka, 2017), and research Lestario et al. (2014) resulted in a total anthocyanin of 332 μg/g FW from the extraction of banana flower bracts (*Musa paradisiaca* L). This is due to the difference in the polarity properties of the solvent and the type of banana used, thus affecting the total anthocyanins extracted (Kitdamrongsont et al., 2008). The results of this study show that red banana fruit waste, namely red banana peel and bracts, has the potential to be a source of anthocyanins. Red banana peel has a proportion of 40.95% per red banana. According to Pazmiño-Durán et al. (2001), during the harvest season, as much as 300 kg/Ha of banana bract is disposed of as waste. The use of water as a solvent produces anthocyanins that are safe for food applications.

The results of the study obtained that the total anthocyanin content in red banana bracts was higher than extract from red banana peel. Table 4 shows the anthocyanin content of the peel and the bracts of the banana. This difference is due to differences in the colour density of the extract in the peel extract and red banana bracts. This can be seen from the absorbance value of the anthocyanin extract of the red banana bracts, which is higher than the peel red banana extract (Figure 4). According to Simmonds (1954), the colour of the banana bracts is related to variations in anthocyanin.

Table 4. Total Anthocyanin Content in Concentrated Extract of Red Banana Peel and Bracts

Source	Anthocyanin Total
Peel red banana	110.27 μg/g FW
Bracts red banana	114.26 μg/g FW

3.4 Light Effects

The environment greatly affects the stability of anthocyanins. One of the factors that affect the stability of anthocyanins is light. The results showed that the rate of anthocyanin degradation from the peel and bracts of the red banana experienced a faster decrease when illuminated by light from a UV lamp compared to the light of a 25-watt bulb lamp. This result is in line with previous research, and here UV light is the main factor in the degradation of anthocyanins due to the presence of light (Bąkowska et al., 2003; Mohammadi et al., 2022). This shows that anthocyanins will degrade more quickly at higher ambient temperatures. Based on observations, the ambient temperature illuminated by UV lamps is 36°C, while the environment illuminated by 25-watt bulb lamps has an average temperature of 33°C.

The degradation of anthocyanins due to the presence of light followed the first-order reaction model (Figures 5, 6, and 7). The effect of light on anthocyanin degradation has been widely studied. Storage of the anthocyanin extract of C. hirsutus fruit in light conditions drains faster than storage in dark conditions (Rakkimuthu et al., 2016). Research Boo et al. (2012) showed that the anthocyanin degradation rate reached 30% when the anthocyanin extract was exposed to sunlight for only six hours. The degraded rosella anthocyanin extract showed a significant colour change (Wu et al., 2018). Research Mohammadi et al. (2022) shows that anthocyanin degradation depends on the number of methoxyl groups or hydroxyl groups in. The highest anthocyanin degradation occurred in Delphinidin 3-O-glucoside. Delphinidin contains more hydroxyl groups. The number of dominant hydroxyl groups causes the colour to tend to be unstable. Anthocyanin irradiation causes anthocyanin to be degraded. According to Bąkowska et al. (2003) irradiation of anthocyanins in direct sunlight causes the highest rate of anthocyanin degradation. Energy from light can trigger photochemical reactions (photooxidation) so that anthocyanins are degraded (Nurtiana, 2019). The photochemical mechanism of anthocyanins due to light is due to the opening of carbon ring number 2 which will result in the formation of colourless compounds such as chalcone (Hendry, 1996). This can be seen from the decrease in the absorbance value of the anthocyanin extract. The decrease in the absorbance value of the extract indicated the degradation process of anthocyanins. The degradation of colour in anthocyanins is caused by a change in red potassium flavylium to colourless alkaline

carbinol and ends up becoming colourless chalcone (Markakis, 1982). Visually, the degradation of anthocyanins can be seen from the reduction of the red colour in the extract to a light colour or fading. If there is an oxidizing agent, the colour of the extract will turn brown. In the anthocyanin structure, light has two different effects, namely increasing the rate of thermal degradation and triggering product formation through excited flavylium (Furtado et al., 1993). According to Markaris et al. (1957), the initial stage of anthocyanin degradation is the opening of the pyrylium ring and the formation of chalcones. Furthermore, the ring will be lost along with the formation of coumarin glycoside derivatives (Patras et al., 2010).

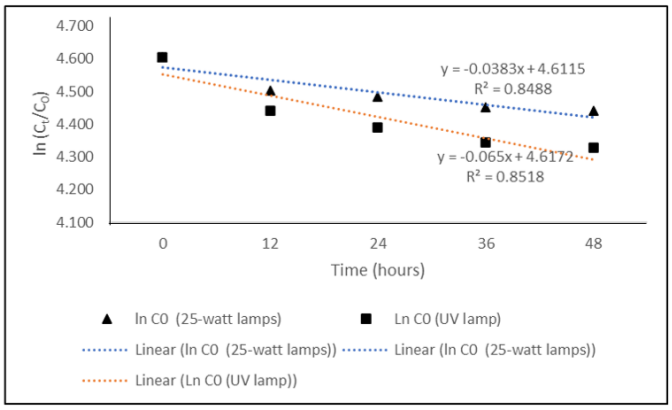

Figure 5. The Degradation Rate of Red Banana Peel Anthocyanin Extract

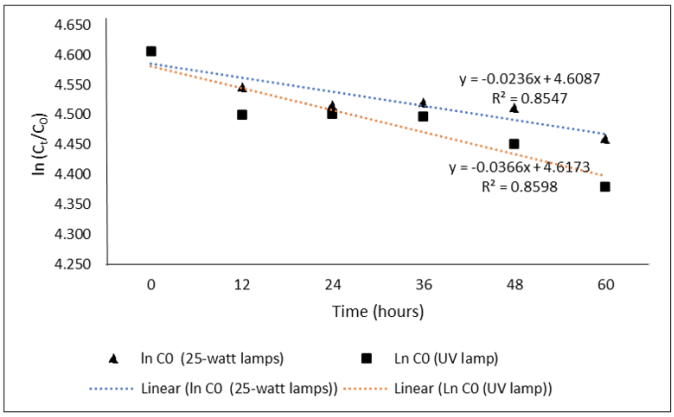

Figure 6. The Degradation Rate of Red Banana Bracts Anthocyanin Extract

The rate of degradation of anthocyanins in the peel and bracts of the red banana is higher if given direct sunlight. These results show that anthocyanins in the peel extract and bracts of the red banana are greatly affected by their stability in the presence of light in the environment. These results are in line with research (Bakhshayeshi et al., 2006; Mohammadi et al., 2022; Rakkimuthu et al., 2016).

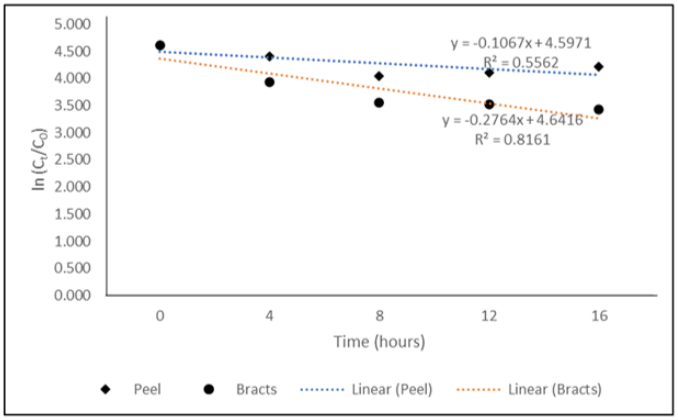

Figure 7. The Degradation Rate of Anthocyanin Anthocyanin in Red Banana Peel And Bracts in Sunlight

4. CONCLUSION

Extracts of red banana peel and bracts using water solvents acidified with citric acid are proven to contain anthocyanin compounds. The total anthocyanins in the concentrated extracts of the peel and bracts of the red banana were 110.27 μg/g FW and 114.26 μg/g FW, respectively. These results show that the peel and bracts of a red banana have the potential to be a source of anthocyanins. Light affects the stability of anthocyanins. This is shown by the rate of anthocyanin degradation, which shows a downward trend.

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