

## Determination of Anthocyanin Content of Purple Sweet Potato (*Ipomea batatas* L) Extract Using the Differential pH Method

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### ABSTRACT

Purple sweet potato (*Ipomea batatas* L) is a type of tuber that has many benefits in the food and health. The purple color in sweet potatoes is caused by the presence of anthocyanin purple pigments that spread from the skin to the tuber flesh. The content of sweet potato anthocyanins depends on the intensity of the color of the tubers. The purple the color of the tubers, the higher the anthocyanin content. Anthocyanins have various health benefits, namely as antioxidants, anti diabetic and other benefits, namely as natural dyes. This study aims to determine the levels of anthocyanins in purple sweet potato extract by using Differential pH method. From the research results, it can be concluded that the anthocyanin content of purple sweet potato (*Ipomea Batatas* L) is 4.26 mg/liter.

### INTRODUCTION

Purple sweet potato (*Ipomea batatas* L) is a type of sweet potato that has a variety of purple colors with different levels of color strength, this is due to its high anthocyanin content. Purple sweet potato has a high total phenol content and high antioxidant activity (Tang et al., 2023). Anthocyanins are

pigments in plants that can provide certain gradations of color (such as red, blue, or purple) found in fruits, tubers, vegetables and flowers. Anthocyanin is a molecule consisting of anthocyanin aglycone and several sugar groups (Guclu et al., 2023). The types of anthocyanins found in plants

are generally in the form of cyanidin, peonidin, degonidin, malvidin, delphinidin, petunidin, etc (Liu et al., 2023). Anthocyanins are organic molecules in the flavonoid group, part of the polyphenolic compounds (Sáenz-de la O et al., 2023). Anthocyanins have excellent health benefits namely, in chronic liver diseases such as non-alcoholic fatty liver disease (NAFLD) which is caused by oxidative stress due to lipid accumulation, anthocyanins can effectively inhibit the production of reactive oxygen species and increase oxidative stress (Hao et al., 2023). The stability of purple sweet potato anthocyanins is strongly influenced by the environment such as pH variations. Anthocyanin has a variety of colors ranging from red to purple, this is a consideration as a food coloring and its use has been permitted (Tena & Asuero, 2022). Anthocyanin as a red, blue, and purple coloring pigment is a possible alternative in the future, such as purple violacein and red pyranoanthocyanin, as a choice of natural food coloring that has high beneficial value for health (Novais et al., 2022). Research has been conducted that the stability of anthocyanins in purple sweet potatoes is influenced by the cooking process, in three types of anthocyanins, for example cyanidin 3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside, peonidin 3-caffeoyl sophoroside-5-glucoside, and cyanidin 3-(6"-caffeoyl-6"-feruloylsophoroside)-5-glucoside which is carried out through steaming, pressure cooking, microwave cooking and frying can reduce the total anthocyanin content significantly by 8-16% (Yan et al., 2022). Anthocyanin extraction can be done in various ways, the results of anthocyanin extraction are influenced by the optimal

temperature and extraction time. Different types of solvents can also affect the extraction results but do not have a significant effect (Clodoveo et al., 2022).

Determination of anthocyanin levels can be done using various methods including using ultra-performance liquid chromatography connected to a mass spectrometer (UPLC-MS). The results showed that the maximum total anthocyanin was 2.27 mg/g (Wang et al., 2022).

Anthocyanins have good stability in acidic conditions, and can be degraded at pH above 7 (Sobini et al., 2022). Anthocyanins are unstable compounds and are strongly influenced by the surrounding environment, especially pH. So that the appropriate extraction method needs to be considered in obtaining optimal anthocyanin content results. Optimization includes solvent, addition of HCl and plant particle size. Levels can be measured using the differential pH method using a visible spectrophotometer (Pham et al., 2022).

Anthocyanin concentration can be determined using the pH differential method, which is a fast and simple spectrophotometric method based on changes in anthocyanin structure due to changes in pH (at pH 1.0 it is colored and at pH 4.5 it is colored) (Karakashova et al., 2022).

## **METHOD**

### **Preparation Sample:**

In this study, the sample used was fresh purple sweet potato (*Ipomoea* Limits L). Samples were taken from farmers in Ciamis. The part used is the tuber. because the tubers of purple sweet potatoes contain higher anthocyanin pigments than other parts.

### **Making of purple sweet potato simplicia:**

To make purple sweet potato simplicia

(*Ipomoea batatas* L) it is done in several stages, namely wet sorting, washing with running water, washing several times so that the purple sweet potato is clean and free of dirt, then sliced crosswise and with a thickness of about 3-4 mm, then Dry in the sun covered with a black cloth until dry. Extraction :

Purple sweet potato simplicia was mashed using a blender. Then 240 gr of the powder was weighed, put into a maceration apparatus and soaked in 2400 ml of 70% ethanol. The maceration process was carried out for 24 hours and stirring was carried out every 6 hours.

Identificatin of Anthocyanin:

Identification of anthocyanin compounds in purple sweet potato extract was carried out by preparing 2M HCL and 2M NaOH in a measuring flask. Then the extract was added with 2M HCL and heated at 100°C for 5 minutes until the color turned red. Then the extract was taken with a drop of 2M NaOH and heated at 100°C for 5 minutes until the color changed to green and slowly faded.

Measurement of the total anthocyanin concentration was carried out using the pH differential method (Koraqi et al., 2023).

The pH-differential spectrophotometry method is a calculation by means of differences in the absorbance of visible light at different pHs, namely at pH 1.1 and pH 4.5. At pH 1,1 anthocyanins are in the form of flavilium cations which are pink in color, while at pH 4.5 anthocyanins are in the form of quinoidal bases which are green in color.

The anthocyanin structure is more stable at pH ranges 1 and 3, whereas at pH >4 the anthocyanin structure is unstable. This is due to the fact that in an acidic state the dominant structure of anthocyanin is in the protonated flavilium cation form and lacks electrons.

Research on anthocyanin content showed that the most abundant anthocyanin found in nature is cyanindin-3-glucoside with a molar absorptivity (A) of 26,900. In

general, cyanidin-3-glucoside is used as the reference compound for anthocyanins. The first step is to make a buffer solution pH 1.1 by means. Weigh 186 mg of KCl, then put it in a beaker glass and add 98 ml of distilled water. Then measure the pH to 1.1. If not shows a pH of 1.1, add HCl 2 N until it shows a pH of 1.1. After making a pH 1.1 buffer solution, then make a pH 4.5 buffer solution (Na Acetate 0.4 M) by weighing 272 mg of Na Sulfate, then putting it into a beaker glass then adding 50 ml of quadest. Then measure the pH to 4, 5 by adding HCl 2 N.

Furthermore, measuring the absorbance value of the buffer solution pH 1.1 with purple sweet potato extract by mixing the buffer solution pH 1.1 with a sample solution of purple sweet potato anthocyanin extract with a ratio (1:4). Then measure the absorbanceby using the following equation:  $A = (A_{\lambda 510} - A_{\lambda 700})_{pH\ 1.1} - (A_{\lambda 510} - A_{\lambda 700})_{pH\ 4.5}$  Calculation of anthocyanin levels is carried out using the following equation:

$$\text{Anthocyanin (ml/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times L \times Wt}$$

Were A = Absorbance

$\epsilon$  = Molar absorptivity of Cyanidin-3-glucoside = 26900 L/(mol.cm)

L = Width of cuvette = 1 cm

MW = Cyanidin-3-glucoside molecular weight = 449.2/mol

DF = Dilution factor

V = Pigment extract volume (L)

Wt = Weight of starting material (g)

## RESULTS AND DISCUSSION

### 1. Purple sweet potato extract

The extraction results of the purple sweet potato obtained 22 grams of extract, after that the yield was calculated, the results were obtained as much as 4.4%.

$$\text{Extract (\%)} = \frac{\text{extract weight obtained}}{\text{sample weight}} \times 100\%$$

$$= \frac{22 \text{ grams}}{24 \text{ grams}} \times 100\%$$

$$= 9,1 \%$$

## 2. Identification results of anthocyanin compounds of purple purple sweet potato extract (*Ipomoea Batatas L*)

Table.1 Identification result of anthocyanin in puple sweet potato.

Test	Result
Heated with 2M HCl for 5 minutes with a temperature of 100c	Red Positif antosianin
Dropped with 2M NaOH drop by drop for 5 minutes temperature 100C	Turns green and slowly fades away Positif antosianin

The purple sweet potato extract was carried out by adding 2M HCL to the extract, then heating it at 100°C for 5 minutes to change to a red color, while the extract was dripped with 2M NaOH and heated to 100°C for 5 minutes to change to a red color. green and slowly fading, and the results showed positive anthocyanin content.

## 3. Results of Determination of Anthocyanin Levels Using the Differential pH Method

Table. 2 Absorbance Value of anthocyanins by spectrophotometry UV Vis at pH 1.1, and wavelength 510 nm – 700 nm.

pH	Absorbance	
	510 nm	700 nm
pH 1.1	0,571	0.396
	0.582	0.394
	0.590	0.379

Table.3 Absorbance value of anthocyanins by Spectrophotometry UV-Vis at pH 4,5 in wavelength 510 nm – 700 nm

pH	Absorbansi	
	510 nm	700 nm
pH 4.5	0.427	0.301
	0.433	0.321
	0.462	0.310

Absorbance in differential pH fo three reflication:  
A1 = (  $A_{\lambda 510} - A_{\lambda 700}$  ) pH 1.1 – (  $A_{\lambda 510} - A_{\lambda 700}$  ) pH 4.5

$$= (0,571 - 0,396) - (0,427 - 0,301)$$

$$= 0,175 - 0,126$$

$$= 0,049$$

A2 = (  $A_{\lambda 510} - A_{\lambda 700}$  ) pH 1.1 – (  $A_{\lambda 510} - A_{\lambda 700}$  ) pH 4.5

$$= (0,582 - 0,394) - (0,433 - 0,321)$$

$$= 0,188 - 0,112$$

$$= 0,076$$

A3 = (  $A_{\lambda 510} - A_{\lambda 700}$  ) pH 1.1 – (  $A_{\lambda 510} - A_{\lambda 700}$  ) pH 4.5

$$= (0,590 - 0,379) - (0,462 - 0,310)$$

$$= 0,211 - 0,152$$

$$= 0,059$$

The results of the calculation of anthocyanin levels are as follows:

$$\text{Anthocyanin (mg/L)} = \frac{Ax MW x DFx 10^3}{\epsilon x Lx Wt}$$

=

$$\frac{0,049x 449,2 x 1 x 1000}{26,900x 1x240}$$

$$= 3,4 \text{ mg/L}$$

Anthocyanin (mg/L)

$$= \frac{Ax MW x DFx 10^3}{\epsilon x Lx Wt}$$

$$= \frac{0,076x 449,2 x 1 x 1000}{26,900x 1x240}$$

$$= 5,3 \text{ mg/L}$$

Anthocyanin (mg/L)

$$= \frac{Ax MW x DFx 10^3}{\varepsilon x Lx Wt}$$

$$= \frac{0,059x 449,2 x 1 x 1000}{26,900x 1x240}$$

$$= 4,1 \text{ mg/L}$$

The average anthocyanin level is 4,26 mg/L

## CONCLUSION

From this research, it can be concluded that the sweet potato extract showed positive results for anthocyanin, marked by a red color change after being dropped on HCl, indicating the presence of flavilium compounds, and after being dripped with NaOH, it changed to green, indicating the presence of quinonoid base compounds. Based on the results of determining the content of purple sweet potato with The differential pH method showed anthocyanin levels of 4.26 mg/liter.

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