The Influence of Process Parameters on Anthocyanin Content in Ipomoea Batatas Extract

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Abstract. Anthocyanin content reaches high value in hot extraction conditions of Ipomoea Batatas at 60°C, using a mixture of ethanol: water at ratio of 4:1. The sizes of the material had significantly affected the anthocyanin content in Ipomoea Batatas solution. The colour of the anthocyanin-rich extracted solution varied significantly in range of pH from 1-12. So, anthocyanin was used as a colour indicator. Qualitative method by colorimetric showed that the colour of anthocyanin extracted solution was very little change during the 30-day survey period (without preservatives). However, quantitative analysis by UV-Vis, with a maximum wavelength of $\lambda_{max} = 523$ nm, shows that the absorbance of anthocyanin decreased markedly with time of storage. Experimental results showed that the storage environment also influences anthocyanin colour and uptake in Ipomoea Batatas extracted solution.

Keywords: Anthocyanin, Ipomoea Batatas, Hot Extraction, Colour, Wavelength.

1. Introduction
Anthocyanin is widely distributed in nature of 27 plant families, present in many vegetables, flowers, fruits such as red rose, red beet, eggplant, strawberry, purple cabbage [1-3]. In addition, anthocyanin is also contained in some leaves such as mulberry leaves and perilla leaves. Anthocyanins range in colour from deep red, bright pink to charcoal purple [4-7]. Because of its outstanding natural colour, anthocyanins are used as a colouring for the beverage, jam and food industries, as well as increasing the bright red of sausages without being toxic to users [8-11].

Intensity and fastness of anthocyanin colour depends on many factors such as structural composition, pigment concentration, pH, temperature and light. In addition, the colour of anthocyanin is also affected by metal ions, enzymes, oxygen, vitamin C, sugar and SO₂. Its color have a wavelength of absorption in the visible region (400-700nm), high absorption at wavelengths of about 510-540nm [2-3].

According to the National Agriculture and Food Research Organization of Japan, Ipomoea Batatas contain a high and stable purple colour due to the presence of large anthocyanin content [3]. In addition, Ipomoea Batatas is considered a nutrient-rich food such as amino acids, vitamins A, B, C and E, calcium, zinc, iron, and others.

A number of the previous studies have created natural colours for cosmetics through extracts from dragon fruit skin and intestines, from red rose as well as strawberries [5-7]. In this article, the study evaluated to the effect of extraction parameters on the anthocyanin content in Ipomoea Batatas extract. From there, there were several suggestions for appropriate storage conditions for the extracted solution when not using the preservative.
2. Methodology

2.1. Preparation of raw materials
Ipomoea Batatas were from Binh Tan commune, Binh Minh district, Vinh Long province (Viet Nam) where is a region specializing in Ipomoea Batatas cultivation. Ipomoea Batatas roots used for experiments are round, slender, long, smooth skin without defects like concave waist. Ipomoea Batatas were washed, peeled and pretreated in dilute salt water.

2.2. Effects of extracted conditions
The effects of several parameters to extraction of anthocyanin content in Ipomoea Batatas was carried out including solvent type, solvent content ratio, extraction temperature and raw material shapes. To extract anthocyanin from Ipomoea Batatas, the organic solvents usually use with high polarization or polar solvent systems. Water and ethanol are good polar solvents suitable for extracting anthocyanins. Using mixture of ethanol and water to make solvent extraction system with the ratio of ethanol: water is at 1:4, 2:3, 1:1, 3:2 and 4:1. The selected extraction temperature was at 30, 45, 60, 75 and 90°C. Ipomoea Batatas material was prepared into 2 shapes: thinly sliced with a thickness of 3 mm and solid blocks with dimensions of about 3x3x3 mm³.

![Figure 1. Ipomoea Batatas material was prepared into 2 shapes: solid blocks (on the left) and thinly sliced (on the right)](image)

pH meter and acetic acid were used to investigate anthocyanin colour change in the range of pH from 1-12. Analysis method of UV-vis spectra was used to determine the maximum wavelength and investigate changes in anthocyanin uptake in extracts over time, temperature and storage conditions. Moreover, the method was to measure the absorbance of the extract at pH=1 and pH=4.5 at the maximum absorption wavelength, compared to the absorbance at 700 nm [3].

Based on the formula of Lambert Beer law:

\[
\lg \frac{I_0}{I} = \varepsilon.l.C
\]  

in which:
- \( \lg \frac{I_0}{I} \): value of the absorbance (optical density), symbolized as A.
- I: Light intensity after passing through the solution.
- \( I_0 \): Light intensity projected into solution.
- C: concentration of solution, mol/L.
- l: Thickness of solution layer that light passes through.
- \( \varepsilon \): Molecular absorption coefficient, L.mol⁻¹.cm⁻¹.

Determination of anthocyanin content:

\[
Sc = \frac{A.M.K.V}{\varepsilon.l}
\]  

(2)
in which:

\[ A = (A_{\text{max}}^{\text{pH=1}} - A_{700\text{nm}}^{\text{pH=1}}) - (A_{\text{max}}^{\text{pH=4.5}} - A_{700\text{nm}}^{\text{pH=4.5}}) \]

Sc: content of anthocyanin (g/g).

\[ A_{\text{max}}, A_{700\text{nm}}: \text{maximum absorption at maximum wavelength and 700 nm, at pH=1 and pH=4.5.} \]

M: Molecular weight of anthocyanin, (g/mol).

l: Thickness cuvet (cm).

K: Dilution.

V: Volume of extracted solution, (L).

Anthocyanin content was determined:

\[
\text{Anthocyanin content} = \frac{Sc}{m(100-w)} \times 100 \quad (3)
\]

in which:

Sc: content of anthocyanin was determined in formula (2) (g).

m: mass of initial materials (g).

w: Water content of raw materials (%).

2.3. Process of extracting anthocyanin from Ipomoea Batatas

Process of preparation of raw Ipomoea Batatas and anthocyanin extraction is shown in Figure 2 below:

![Figure 2. Process of extracting anthocyanin from Ipomoea Batatas material](image-url)
3. Results and Discussions

3.1. Determine anthocyanin content

Anthocyanin solution was poured in glass cuvet and put into chamber of UV-Vis equipment. The UV-Vis system was set wavelength ($\lambda$) in range of 400-600 nm with the maximum wavelength at 523 nm ($\lambda_{\text{max}} = 523$ nm). This result is consistent with previously published studies [10-14]. Using the UV-Vis system, the absorbance (optical density) of anthocyanin extracted solution from Ipomoea Batatas were measured at wavelength of $\lambda = 523$ nm and $\lambda = 700$ nm with pH values at 1.0 and 4.5 as shown in Table 1.

Table 1. Absorbance (A) and anthocyanin content in the extracted solution from Ipomoea Batatas

| Sample | pH=1.0 | pH=4.5 | A | Anthocyanin (mg/100 g) |
|--------|--------|--------|---|------------------------|
|        | $A_{523}$ | $A_{700}$ | $A_{523}$ | $A_{700}$ |                |
| 1      | 0.95    | 0.033  | 0.301  | 0.020  | 0.636  | 92.53          |
| 2      | 0.98    | 0.030  | 0.307  | 0.022  | 0.665  | 96.75          |
| 3      | 0.96    | 0.031  | 0.302  | 0.020  | 0.647  | 94.13          |
| Average values | 0.95 | 0.031 | 0.303 | 0.021 | 0.629 | 94.47±1.52 |

The anthocyanin content obtained from the extracted solution of Ipomoea Batatas is high (94 mg/100 g). In comparison with the previous research results, anthocyanin content obtained in Ipomoea Batatas is lower than in mulberries (126.4 mg/100 g) but higher than in perilla leaves (80.48 mg/100 g), grapefruit (60.01 mg/100 g) and eggplant peel (46.92 mg/100 g) [3,6,9]. Moreover, anthocyanin content in Ipomoea Batatas is much higher than fresh rose (3.34 mg/100 g) and dried rose (3.14 mg/100 g) [5].

3.2. Effects of solvent content ratio

Ethanol was increased its concentration in ratio of solvent and the values of absorbance and anthocyanin content were listed in Table 2.

Table 2. Anthocyanin content obtained from the ratios of ethanol:water

| Run No. | Ratio of ethanol:water | A ($\lambda$=523 nm) | Anthocyanin (mg/100 g) |
|---------|------------------------|----------------------|------------------------|
| 1       | 1:4                    | 0.565                | 77.6                   |
| 2       | 2:3                    | 0.596                | 82.9                   |
| 3       | 1:1                    | 0.638                | 89.0                   |
| 4       | 3:2                    | 0.629                | 89.7                   |
| 5       | 4:1                    | 0.677                | 98.5                   |

Experimental results show that the content of anthocyanin in extracted solution of Ipomoea Batatas increased gradually as the ratio of ethanol:water were increased from 1:4 to 4:1. At ratio of 4:1 anthocyanin content reached the highest value at 98.5 mg/100g. The experimental results are consistent with previous research on Ipomoea Batatas [10].

3.3. Effects of shapes and sizes of raw materials to anthocyanin content

Raw material of Ipomoea Batatas was prepared in two shapes and sizes: thinly sliced and solid blocks as shown in Fig.1. After extracted, the solutions were measured absorbance and determined anthocyanin content in Table 3 and Table 4.
Table 3. Absorbance (A) and anthocyanin content of the extracted solution of Ipomoea Batatas in thinly sliced shapes

| Samples | pH=1.0 |  | pH=4.5 |  | Absorbance | Anthocyanin |
|---------|--------|---|--------|---|------------|-------------|
|         | $A_{523}$ | $A_{700}$ | $A_{523}$ | $A_{700}$ | $A$ | (mg/100 g) |
| 1       | 0.95   | 0.033 | 0.412  | 0.02 | 0.525 | 78.13       |
| 2       | 0.98   | 0.030 | 0.408  | 0.022 | 0.564 | 83.02       |
| 3       | 0.92   | 0.031 | 0.413  | 0.020 | 0.496 | 75.03       |
| Average | 0.95   | 0.031 | 0.411  | 0.021 | 0.528 | 78.73±2.86  |

Table 4. Absorbance (A) and anthocyanin content of the extracted solution of Ipomoea Batatas in shapes of solid blocks

| Sample  | pH=1.0 |  | pH=4.5 |  | Absorbance | Anthocyanin |
|---------|--------|---|--------|---|------------|-------------|
|         | $A_{523}$ | $A_{700}$ | $A_{523}$ | $A_{700}$ | $A$ | (mg/100 g) |
| 1       | 1.065  | 0.030 | 0.410  | 0.038 | 0.663 | 94.88       |
| 2       | 1.050  | 0.033 | 0.436  | 0.024 | 0.605 | 84.06       |
| 3       | 1.030  | 0.032 | 0.450  | 0.038 | 0.586 | 85.30       |
| Average | 1.048  | 0.032 | 0.43   | 0.033 | 0.618 | 88.08±4.53  |

Table 3 and 4 showed that anthocyanin content obtained from the Ipomoea Batatas in shapes of solid blocks is at 88.08 mg/100g that is higher than the Ipomoea Batatas in thinly sliced shapes is at 78.73 mg/100g. Hence, in the same conditions, sizes and shapes of the raw materials effected to anthocyanin content. This is related to surface areas of raw materials in the dissolved process. In this case the raw materials with shapes of solid blocks has smaller sizes and higher surface areas that another. Therefore, the research group suggests that raw materials of Ipomoea Batatas should be ground to conduct experiments of extraction for future research.

3.4. Effects of extracted temperature to anthocyanin content

The extracted processes were carried out at 30°C, 45°C, 60°C, 75°C, and 90°C with experimental results as shown in Table 5 and Fig. 3.

| No. | Temperature (°C) | Absorbance, A ($\lambda=523$ nm) | Anthocyanin (mg/100g) |
|-----|------------------|-------------------------------|-----------------------|
| 1   | 30               | 0.584                         | 82.14                 |
| 2   | 45               | 0.606                         | 90.71                 |
| 3   | 60               | 0.663                         | 94.88                 |
| 4   | 75               | 0.618                         | 85.93                 |
| 5   | 90               | 0.501                         | 79.98                 |

Experimental results show that anthocyanin content increased gradually with the extracted temperature range of 30-60°C, and then the values decreased in the range of extracted temperature from 75–90°C. Hence, temperature of 60°C is the best condition for high anthocyanin content at 92.16 mg/100 g.
From the experimental results, the study showed some parameters to carry out extracted anthocyanin from the *Ipomoea Batatas*: Solvent of ethanol and water with ratio of 4:1; the extracted temperature of 60°C and the *Ipomoea Batatas* in fine powdered materials.

3.5. Effects of pH values to changes of anthocyanin colour
The study was carried out experiments to test colour changes of anthocyanin extracted solution in pH conditions from 1 to 12 as shown in Fig. 4.

It is easy to see that the colour of anthocyanin extracted solution changed from fresh red to purple in range of pH from 1-7. With pH from 8 to 10, the anthocyanin extracted solutions have colour from dark purple to green and then it was changed to dark orange at pH values of 11 and 12. There are reports show that anthocyanin rich extracted solution will turn from red to yellow when the pH increases from 1-12 [5-7,11-14]. The modification of anthocyanin-rich extracted solution increased significantly with increasing of pH in the presence of oxygen [11]. This result proves that the extracted solution with high anthocyanins has the ability to be used as a colour indicator.

3.6. Effects of temperature and time of storage
Anthocyanins are often unstable when exposed to visible light rays and their decomposition occurs mainly due to photochemical oxidation reactions [9]. Therefore, the experiments were conducted in the absence of direct light. The experiments on the effect of temperature on anthocyanins were carried out at 30°C and 30°C in a period of 30 days, without preservatives with the results as shown in Table 6 and Fig. 5.
Table 6. Effects of temperature and time of storage to parameters of anthocyanin extracted solution

| Parameters | 0 | 10 | 20 | 30 |
|------------|---|----|----|----|
| Temperature at 30°C Colour | Purple | Purple | Purple | Purple |
| Absorbance, A | 0.441 | 0.382 | 0.301 | 0.239 |
| Temperature at 3°C Colour | Purple | Purple | Purple | Purple |
| Absorbance, A | 0.441 | 0.401 | 0.382 | 0.369 |

Figure 5. Effect of storage temperature to absorbance of anthocyanin during 30 days

Visually, the colour of anthocyanin extracted solution had no change when stored at 30°C and 3°C (in Table 6). However, the absorbance (A) of anthocyanin changed after stored for 30 days using UV-Vis spectra method. Specifically, the absorbance decreased slightly (about 16.32% in comparison with the first day) with storage temperature at 3°C. When the extracted solution was stored at 30°C, the value of absorbance was down to 0.239 mg/100g (around 45.80% compared to initial samples). This indicates
the anthocyanin extracted solution should be stored in condition of low temperature during long time without preservatives and avoid visible light.

4. Conclusions
The study has identified some important parameters of Ipomoea Batatas extraction process so that anthocyanin content reaches high value. Materials treated at smaller sizes will obtain high anthocyanin content in the extracted solutions of Ipomoea Batatas. Experiments show that the colour of extracted solutions changes from magenta (in acid condition) to green (neutral condition) and dark yellow (alkaline condition). They indicated that anthocyanin can be used as a colour indicator. Qualitative analysis by UV-vis spectroscopy showed that anthocyanin extracted solutions should be stored at low temperatures for long time without preservatives and avoid visible light.

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