Effects of various solvent concentration, liquid-solid ratio, temperatures and time values on the extraction yield of anthocyanin from Vietnam Hibiscus sabdariffa L. (*Roselle*)

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Effects of various solvent concentration, liquid-solid ratio, temperatures and time values on the extraction yield of anthocyanin from Vietnam Hibiscus sabdariffa L. (*Roselle*)

Tri Nhut Pham[1,2], Thuong Nhan Phu Nguyen[1], Tri Duc Lam[1], Thien Hien Tran[1], Duy Chinh Nguyen[1], Dai-Viet N. Vo[3], Xuan Tien Le[4], Sy Trung Do[5], Long Giang Bach[1,2,*]

[1]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[2]Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[3]Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Malaysia
[4]Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam
[5]Institute of Chemistry, Vietnam Academy of Science and Techology, Hanoi City, Vietnam
*Email: blgiang@ntt.edu.vn , labasm2013@gmail.com

Abstract. Nowadays, the trends in human health and fossil fuel dependent markets present opportunities for agricultural crops as renewable sources in several substitutes of synthetic components in clothing, fuels, and food. This research focused on anthocyanins along with protocatechuic acid and quercetin, which have been recognized as bioactive compounds in Hibiscus sabdariffa L. aqueous extracts. Laboratory extraction study was conducted on various solvent concentration, liquid-solid ratio, temperatures and time values on the extraction yield of anthocyanin from Vietnam Hibiscus sabdariffa L. (Roselle). On theother hand, total anthocyanins wereinvestigated in Hibiscus sabdariffa L. extract as μg/mL cyanidin-3-glucoside (cyd-3-glu). Extraction temperature of 60◦C using solvent concentration 50%, extraction time of 30 min at liquid-solid ratio of 8:1 showed the highest anthocyanin recovery at 180,821 mg/L. Overall, Vietnam Hibiscus sabdariffa L. (Roselle) are responsive to anthocyanin extraction, making it an appropriate substrate for the development of industrial colorants and dyes.

1. Introduction

Nowadays, the application of natural ingredients become more and more popular in various fields such as global food, beverages, cosmetic and pharmaceutical industries [1-3]. Generally, food pigments know dangerous degeneration during processing, and recovery of the missing color is a method to preserve the freshness perspective of foods [4,5]. In food colors of natural origin, anthocyanins are the most common color family. Anthocyanin is a water-soluble phenolic compound that functions as a color from red to dark green to plants that have long been used as a natural coloring ingredient for food safety. Anthocyanin colorants can be extracted from a variety of materials. Anthocyanins, apart from being recognized as natural plant pigments from the flavonoid family, possess valuable pharmacological properties such as anti-oxidative, anti-inflammatory, and antineurodegenerative effects [6,7].

Hibiscus Sabdariffa is one of the Malvaceae family of the Hibiscus genus, also identified as Roselle. This plant can tolerate with short terms of drought and can be grown throughout the subtropics and tropics during rainy and hot seasons. The different parts in a Roselle plant including calyces, flower, and petals are wealthy in anthocyanins which one of the chemical components can be located naturally in the plant [8,9]. The various extraction conditions affect on extraction yields of anthocyanins from plant tissues [10,11]. By using solvent extraction processes, isolation of anthocyanin pigments from plants is completed. Anthocyanins belong to polar molecules group and more dissolved in polar solvents, however, extraction conditions also play an important role in their overall solubility. The previous studies on extracting anthocyanins from vegetables and fruits such as purple corn, red and black currants, grapes and so on have presented that ethanol extraction is appropriate. The extraction requirements such as incubation temperature and time, solvent concentration, and solid-liquid ratio are significantly critical in the concentration and stability of anthocyanins. The objective of this work was to find a suitable and potential alternative to replace cochineal as the organic dye for red color, which focuses on the extraction of Roselle. Therefore, an examination of the efficient extraction of anthocyanins from Hibiscus sabdariffa L. (*Roselle*) grown in Viet Nam requires evaluation under various solvent type, solid loading, incubation temperature and time valuesThe property parameters analyzed included the color of the extracts by applying the pH-differential method. The results of the project hope to bring out the potential for the application of natural pigments in the food industry and improve the value of using raw materials in Vietnam.

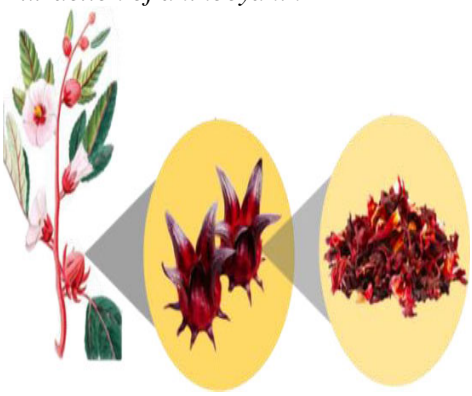
2. Material and methods

2.1 Sample repairation

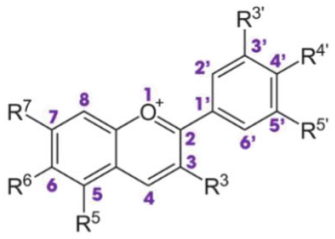
Hibiscus sabdariffa L. dried calyces were used, provided by farmers of Lam Dong Province, Viet Nam. Dried petals of roselle were ground using a commercial grinder and were weighed to 10g, put in the two neck round bottom flask and was extracted by Ethanol 50^{o} solutions. The various particle diameter ground samples were kept in polyethylene vacuum bags at room temperature until extraction was carried out.

2.2 Extraction of anthocyanin

(A)



(B)



Anthocyanidin	R_3'	R_4'	R_5'	R_3	R_5	R_6	R_7
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH
Petunidin	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH

Figure 1. Extraction of the anthocyanin (A) Hibiscus sabdariffa L dried. (B) The structure of anthocyanin.

First of all, each experimental condition will be tested individually to determine the optimum condition. For extraction parameter study, 10g of roselle powder was placed in the two neck round bottom flask as and was extracted by ethanol with concentration at 50. The liquid/solid ratio in this experiment ranges from 2:1 to 10:1 (mL/g). The extraction temperature is adjusted from 40 to 80 (^{o}C) and time varies from 15 to 35 (min). Then, centrifugation took place at 4500 rpm for 15 min by high speed centrifuge Model LACE16 (from COLO lab expert). The supernatant was collected and the extract, after being filtered with filter paper, was transferred into plastic bottle to estimate anthocyanin content. The pH scanning of supernatant ranges from λvis_{max} to 700 nm were recognized for anthocyanin content. The pH-differential method uses to determine the total anthocyanin level [12]. The maximum absorption is 519nm (Figure 1).

2.3. Total Anthocyanin Content Measurement.

The total anthocyanin content was determined according to the spectrophotometric pH differential method. Samples were diluted separately with 0.025M potassium chloride buffer (pH 1) and 0.4M sodiumacetate buffer (pH 4.5). Absorbance of the mixture was measured at 519 (λvis-max) and 700 nm using a UV-Vis spectrophotometer. Absorbance was calculated as A = [($A511-A700$) pH 1.0 − ($A511-A700$) pH 4.5. The total anthocyanin content was calculated as cyanidin-3-glucoside equivalents as in the following equation, anthocyanin content (mg/100 g)

$$= \frac{A \times \mathrm{MW} \times \mathrm{DF} \times \mathrm{V} \times 100}{a \times l \times m}$$

With A is the absorbance, MW is the molecular weight of cyanidin-3-glucosode (449.2g/mol), DF is the) dilution factor, V is the solvent volume (ml), a is the molar absorptivity (26900), l is the cell path length (1cm), and m is the freeze-dried sample weight.

3. Result and discussion

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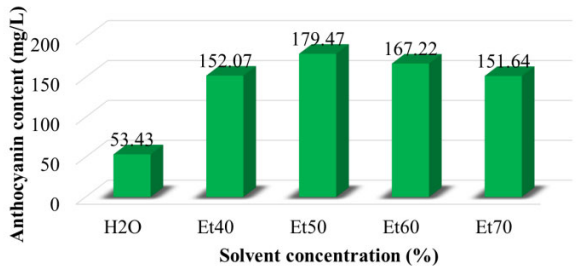

Figure 2. The effects of various solvent concentration on extraction yield of anthocyanin from Vietnam Hibiscus sabdariffa L. (*Roselle*)

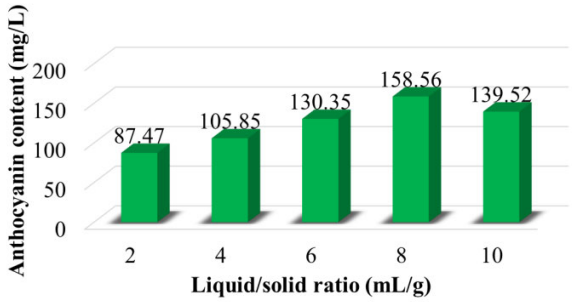

Figure 3. The effects of various liquid-solid ratio on extraction yield of anthocyanins from Vietnam Hibiscus sabdariffa L. (*Roselle*)

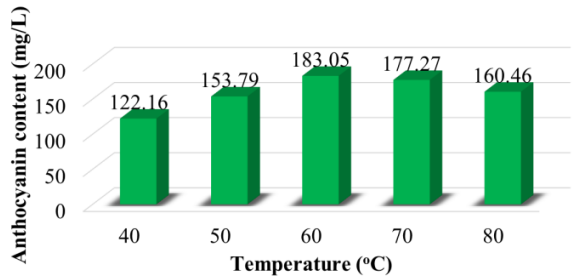

Figure 4. The effects of various temperature on extraction yield of anthocyanin from Vietnam Hibiscus sabdariffa L. (*Roselle*)

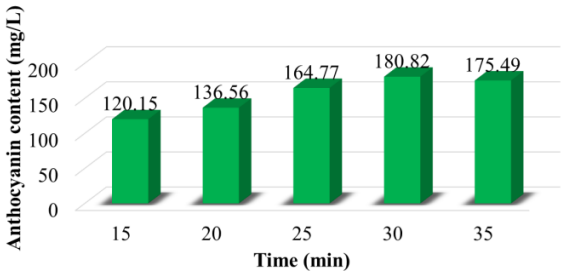

Figure 5. The effects of various time on extraction yield of anthocyanins from Vietnam Hibiscus sabdariffa L. (*Roselle*)

3.1. Effect of solvent concentration

As extraction anthocyanins from red onions may have a promising to be applied as eco-friendly dyestuffs and nutraceuticals. In term of stability, the factors affecting their applications are addressed in this study.

Z determining the solvent influence on anthocyanins extracted from Vietnam Hibiscus sabdariffa L. (*Roselle*), the following solvent systems were used water, ethanol/water (40/60), ethanol/water (50/50), ethanol/water (60/40), ethanol/water (70/30). the pH differential spectrophotometric method is applied in order to evaluation of the anthocyanin concentration. As shown in Figure 2, regarding the extraction yield of anthocyanin, 50% ethanol solution demonstrated more efficient than the other systems. The results presnted that the anthocyanin content in the color solution tended to increase with the alcohol content and reached the maximum at EtOH 50, then gradually decreased to the EtOH 70 concentration. With EtOH 50, the anthocyanin content in the color solution was 1.2 times higher (179.47 mg/L) than with EtOH 70 (133.76 mg/L). The best solvent system is 50% ethanol solution which also minimizes the pigment decomposition favoring the extraction of anthocyanin in their original form. EtOH is more selective, so it only dissolves certain compounds, so limiting the solubility of some starch components, the sugar contained in the roselle, makes it easier for the anthocyanin to come out, while also saving more solvent. In addition, increasing the concentration of EtOH will increase the selectivity of the extraction solvent, thus attracting more anthocyanins and reducing other impurities [13]. Using EtOH 50 due to the alcohol content is enough to entice a large amount of anthocyanin out simultaneously while helping to save solvent.

3.2. Effect of liquid-solid ratio

Figure 4 demonstrated the effect of liquid-solid ratio on anthocyanin content. By increasing the liquid-solid ratio from 2:1 to 8:1, the extracted anthocyanin content increased from 87.47 mg/L to 158.56 mg/L. However, from 8:1 to 10:1 of liquid-solid ratio, the anthocyanin content decreased sharply. This can be explained as follows. When the ratio of materials/solvent is insufficient to fill up the material, the hypertonic environment could not be created, detaining the color in the vacuole of the material. However, when the solvent/material ratio reaches a certain value (depending on the characteristics of the material), the cell, owing to its capability to rapidly absorb water, swells to the maximum and bursts out simultaneously, releasing the color within the vacuole [14,15]. Therefore, based on the graph we choose the liquid/solid ratio of 8/1.

3.3. Effect of temperature

As showed in Figure 4, the anthocyanin content in the fluid as well as the effect of color separation from the material increased with increasing temperature and reached the highest level at the temperature of 60^{o}C. There are significant differences from 30 to 60°C but did not observe between 60 and 70 °C. Therefore, in practice experiment, an extraction temperature of 60 °C could be applied, according to the effect of the good extraction yield of anthocyanins from Vietnam Hibiscus sabdariffa L. (*Roselle*). However, from 60 to 70^{o}C, the anthocyanin content in the extract did not change significantly and rising temperature past 70^{o}C caused the content to diminish due to decomposition. Long exposure at high temperatures would be harmful to the wanted compound as it would undergo the process of oxidation and polymerization [16]. The appropriate temperature will allow the anthocyanin to come out of the solvent. Excessive temperature causes only a small amount of anthocyanin in the roselle to come out. High temperatures cause anthocyanin to come out more but also a large amount of anthocyanin has decomposed [17]. Therefore, with low temperature as 40^{o}C can only attract small amount of anthocyanin so the anthocyanin content in the color is low. At 60-70^{o}C proper temperature makes the anthocyanin diffusion volume relatively large and does not decompose much due to the effect of temperature. At 80^{o}C the temperature is so high that large quantities of anthocyanin are released but also decompose many colors. So, we choose the temperature of 60^{o}C as the optimal temperature for obtaining sugar content as well as anthocyanin.

3.4. Effect of time

From Figure 5, the level of anthocyanin in the fluid tends to decrease with prolonged extraction time. Anthocyanin content was highest at 30 minutes, but as the time increased past 35 minutes, the content decreases markedly due to anthocyanin decomposition caused by the long exposure to high temperature [18]. When the extraction temperature was increased, the anthocyanin yield can be raised [19].Therefore, the appropriate time to extract anthocyanin is 30 minutes. As the extraction capability and anthocyanin

stability depend mostly on the mixed effects caused by solvent concentration liquid-solid ratio, temperatures and time values. Herein, concentration of ethanol 50%, liquid/solid ratio at 8:1 (mL/g), temperature 60^{o} C and reaction time 30 (min) was considered to be optimal for anthocyanins extraction from Vietnam Hibiscus sabdariffa L. (*Roselle*)

4. Conclusion

In this paper, the extraction of Hibiscus sabdariffa L. anthocyanin, which is increasingly used as a natural and safe coloring agent was investigated. After examining different liquids to support in extraction, the results have shown that the extraction of anthocyanin was higher with the use of non-toxic media composed of water/ethanol mixtures extracts of 50:50 (179.47 mg/L) than with water (53.43 mg/L). An increase in extraction time created with a greater quality of extracts. The solution extracted using 50% ethanol for 30 min, at 60^{o}C was suitable for receiving colorant extracts with high quantities of anthocyanins from Hibiscus sabdariffa L. This research recommends that anthocyanin extraction from Vietnamese *Hibiscus sabdariffa* L. is a usable process, reasonable price and relatively quick, presenting a final extracted dye solution of such a high concentration, making it a hopeful substrate for the development of industrial dyes and colorants.

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