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Preparation and Determination of Total Anthocyanins extraction from the Skin of Vigna cylindrica Skeels (Dolichos catjang Burm. f)

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Abstract. Because of antioxidative potential, anthocyanins become more and more popular in various fields including food products and pharmaceutical industries. The extraction technique has a great effect on the quality of natural color. Seed coat color is an essential part of customer choices for cowpea (Vigna unguiculata L. Walp). The experimental study was conducted to determine the effect of different extracting parameters such as duration of extraction, different extraction of temperature (20 - 90oC), duration of extraction (30 - 90 min) and solid-liquid ratios (2:1 - 10:1 mL/g) and solvent concentration (H2O : Ethanol) were selected in order to extract anthocyanin from Vigna cylindrica Skeels. The highest anthocyanin content of 205.58 mg/L was collected at 50% enathol, the solid/liquid ratio 6:1 (mL/g), extraction time 60 (min), and temperature 60 (oC). The results showed that different extraction media can significantly affected the anthocyanins content of the valuable source Vigna cylindrica Skeels extracts.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one the most popular beans worldwide as a food ingredient. It is most commonly called as "cow pea", is medicinal plant belonging to the family Fabaceae or Leguminosae, having lot of pharmacological properties. In the developing countries, especially Vietnam, the cowpea plays an important source of vitamin, minerals, antioxidants, proteins and so on [1-2]. The coloration of the bean skin from red or purple to even black is a consequence of anthocyanin biosynthesis. The previous studies showed that the amounts of flavonols in cowpea was high including anthocyanins, quercetin, and procyanindins. In addition, the seed coat color is the main determinant of flavonoid formation [3-5].

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In the natural food colorants, anthocyanins are the most popular family name was found in not only higher trees but also some fruits, nut, vegetables and so on. Anthocyanins maintain valuable pharmacological characteristics such as anti-inflammatory, antineurodegenerative and anti-oxidative. Nowadays, anthocyanins have advantageous health impacts because of their antioxidant activities. Therefore, it has application as anticancer agents and against cardiovascular diseases. Besides health advantages, anthocyanin pigments have a high trade in the commercial business such as textile industries and natural dyes in the food [6-8]

In term of extraction techniques, the research should maximize the recovery of anthocyanin and minimize the number of supplements and reduce the degradation of the natural state of colorants. Having the different process parameters impact on the extraction of the bioactive compound such as temperature, solvent composition, extraction time and solid to liquid ratio [9-14]. Aside from population studies, previous studies have highlighted the role of anthocyanins in different fields. However, relatively little is explored about the combination of different factors and measurement of optimal conditions in order to obtain a yield of anthocyanins. From a practical viewpoint, the study using random concentrations of solvents, extraction time, liquid-solid ratio and temperatures has been carried to extract anthocyanins from the skin of Vigna cylindrica Skeels (Dolichos catjang Burm. f) of some popular in Vietnam region varieties

2. Material and methods

2.1. Sample preparation

The cowpea samples were collected from Go Vap market (Ho Chi Minh City, Vietnam). Following with washing, soaking in water for about 30 minutes. Next, seeds were peeled and dried to constant volume and store in polyethylene vacuum bags at room temperature until extraction was taken. Ethanol (C2H5OH) is obtained from Sigma Aldrich (US). Distilled water was applied for the preparation of all solutions.

2.2. Anthocyanins extraction from Vigna cylindrica Skeels process

In order to extract anthocyanins, 10g CSC was put in the two neck round bottom flask as and was extracted by ethanol with concentration was investigated at conditions as concentrations of solvents (H_2O: Ethanol), the liquid/solid ratio: 2:1 - 10:1 (mL/g). The extraction temperature is adjusted to about 20–90 (^oC) and 30–90 (min). Then, centrifugation took place at 4000 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. The extraction process were done in dark color bottles with screwed caps. Monomeric anthocyanin was calculated as cyanidin-3-glycoside using a pH differential method [15]

3. Result and discussion

Effect of solvent concentration with extraction: Solvent concentration plays an important role in obtaining high extraction efficiency of anthocyanins. The choice of extracting medium is considered a vital factor for the extraction of natural colorants, which is the first crucial step for parameters optimization. Anthocyanins is the compound, which easy solute in water, so H_2O will take anthocyanin out of cell. Though, since H_2O is soluble sugar, starch during extraction should impede many anthocyanin escape processes. EtOH is more selective and therefore only dissolves certain compounds so limiting the solubility of some starch constituents, the sugar contained in pea pods, makes it easier for anthocyanins to come out, while also saving more solvent. Thus, ethanol/water is the common solvent used in the extraction of polar organic compounds. Therefore, the ethanol/water solution is the standard solvent applied in the extraction of polar organic compounds. Moreover, different ethanol concentrations were prepared as the solvent in order to examine the influence of ethanol concentration on the anthocyanin yield, and the results are shown in Figure 2A. The increasing

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ratio of ethanol to the raw material between 0 % and 100% could grow anthocyanins extraction. The anthocyanin yield was compared with the percentage of ethanol when the concentration from 25% (158.91 mg/L) to 50% (205.55 mg/L). Hence, in term of the cost and yield, 50 % ethanol in water was determined for the following experiments.

Effect of liquid-solid ratio: Liquid-solid ratio acts outstanding function in getting high extraction efficiency of anthocyanins in the method of standard extraction. In general, a larger solvent volume can dissolve components more effectively. In this research, the efficient liquid-to-solid ratio was estimated. Figure 2B presents the effect of liquid-to-solid ratio on the extraction yield of anthocyanins. TThe extraction with various different ratios of solvent to the skin of Vigna cylindrica Skeels including 2:1, 4:1, 6:1, 8:1, 10:1 mL/g were handled in a water bath at a temperature of 60^{o}C for 60 min.. The conclusions from this research revealed that the more the ratio of fresh calyxes to water the more the red color intensity perceived. Below the fixed conditions of different factors, it could be recognized that the extraction efficiency positively rose with the liquid-to-solid ratio, especially when the ratio raised from 2:1 (150.96 mg/L) to 6:1 (205.51 mg/L). However, the anthocyanin content decreased sharply after increasing that ratio from 8:1 to 10:1. This can be explained as when the solvent/material ratio reaches a certain value (depending on the characteristics of the material), the cell then swells to the maximum and bursts out simultaneously, releasing the color within the vacuole. Based on the graph, the ratio 6:1 with an extraction content of 205.51 mg/L was selected to produce the skin of Vigna cylindrica Skeels extract to be concentrated

*Effect of temperature***:** Besides solvent concentration and liquid-solid ratio, temperature extraction is also an essential factor in the extraction of heat compounds. In this research, extraction was taken at various temperatures (30-90 ℃) While other extraction parameters did not change. Particularly, other conditions were fixed at 50% ethanol, 6:1 liquid-to-solid ratio, 60 min extraction time. The influence of temperature on the extraction yield of anthocyanins is presented in Figure 2C. When the temperature raised from 20 to 60 ℃, the yield of anthocyanins rise from 118.08 to 205.60 mg/L, following with falling at the temperature from 60 to 90 ℃. Too low temperatures make only small amounts of anthocyanins available in the black bean shell, and high temperatures cause anthocyanins to come out more, but large amounts of anthocyanins are also decomposed. Therefore, with a low temperature of 20 ℃, only a small amount of anthocyanin can be attracted. At 60^{o}C the appropriate temperature for anthocyanin diffusion is relatively large and does not decompose much due to the effect of temperature. Thus, the optimal temperature for obtaining sugar content as well as anthocyanin is 60^{o}C.

Effect of time: Time duration plays vital functions in getting high extraction efficiency of anthocyanins as well. After the yield of anthocyanins extraction rises with time, the establishment of equality for the objective components in and out of plant cells. Furthermore, the impact of time on the extraction yield, different time points were examined from 20 to 100 min. Figure 2D presents the extraction results taken under various time duration. Anthocyanins content was highest at 60 minutes and reached 205.57 mg/L, but when the time is increased over 80 minutes, it decreases markedly. Because of its high temperature at long periods of time, the anthocyanin is decomposed. This suggests that extraction time does not increase the anthocyanin content. The appropriate time to extract anthocyanin is 60 minutes.

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Figure 1. The process of the total anthocyanins extraction from the skin of Vigna cylindrica Skeels (Dolichos catjang Burm. f)

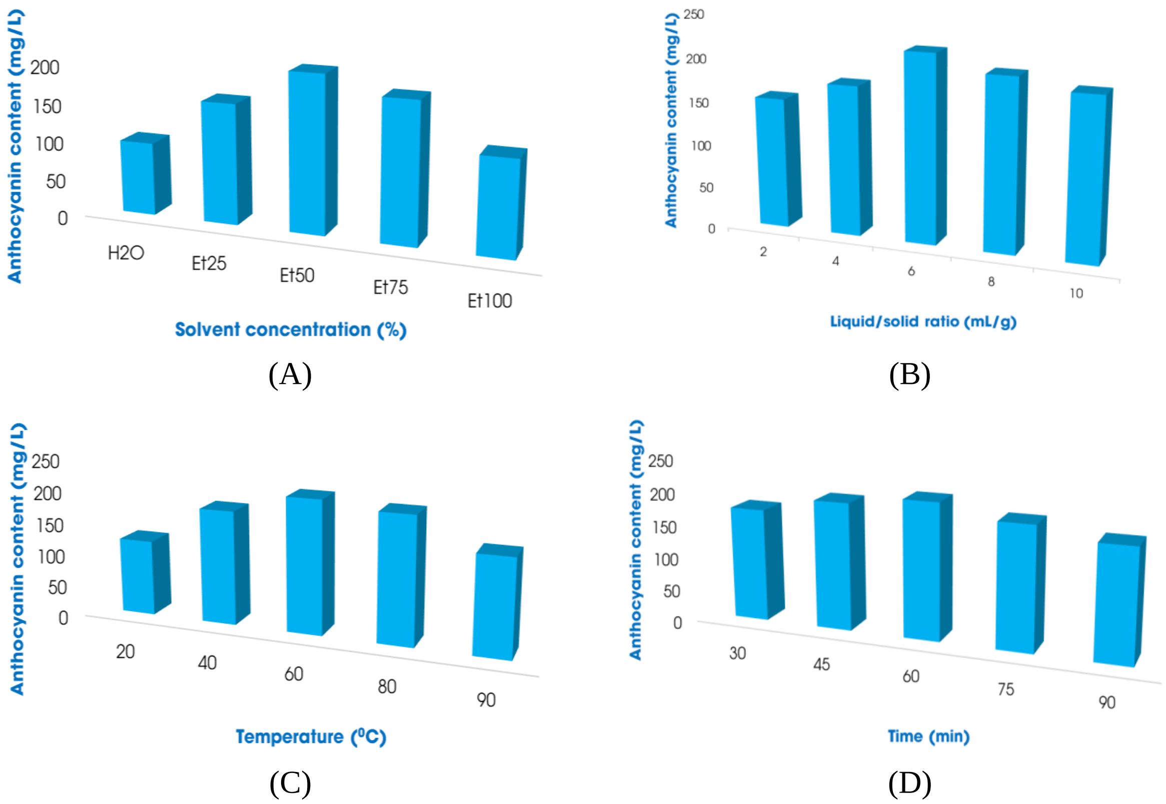

Figure 2. Effects of (A) solven ethanol concentration, (B) liquid/solid ratio, (C) temperature and (D) time factor on the content of anthocyanin

4. Conclusion

In this studies, Though extraction of anthocyanins from the skin of Vigna cylindrica Skeels (Dolichos catjang Burm. f), we genetically analyzed the temperature, extraction time, and liquid/solid ratio had significant impacts on anthocyanin extraction. Various extracting media were applied and the mixture of 50% (v/v) ethanol resulted in maximum anthocyanin content (205.60 mg/L). The increasing of time, regardless of the other variables, led to a reduction in the total anthocyanin content. When antagonize with other verve of natureal anthocyanins, the Vigna cylindrica Skeels is rich in anthocyanin and it can be used as a natural color resources of anthocyanin for food and pharmaceutical industries.

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