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Enzyme-Assisted Extraction of Anthocyanins Pigment from Purple Sweet Potatoes (*Prunusnepalensis*L.)

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ABSTRACT
Herein, anthocyanins pigment was extracted from purple sweet potatoes (*Prunusnepalensis*L.) with the assistant of the enzymes alpha-amylase in order to gather the natural colorants used in the food industry. To optimize the extraction conditions, the effect of extraction temperature and time was also investigated. The results showed that extraction temperature and time play a significant role in theextraction process. The optimum conditions are extraction temperature: 65 ^{o}C and time: 60 min, exhibited thehighest yield.
Keywords:Anthocyanins pigment, purple sweet potatoes, Extraction, Enzyme

I. INTRODUCTION

In recent years, public concern about the safety of synthetic pigments led to increasing interest in the development of food colorants from plant tissues, especially from some edible sources. Colour is an important attribute related to the visual appeal and the quality of food products[1–3]. More and more attentions are paid to the natural pigment, which could be served as a functional component. Interest in anthocyanin pigments in the consumer market has increased recently due to their potential health benefits as dietary antioxidants and the range of colors they produce with potential as a natural dye. Anthocyanins, as a group of phenolic compounds widely existing in the plant kingdom, which providescolors ranging from salmonpink through red, and violet to nearly black in a variety of plant sources[4–6]. They play a critical role in the color quality of many fruits, vegetables, cereal grains, and flowers. In addition to colorant properties, interest in anthocyanins has intensified because anthocyanins showed a number of biological functions, including antioxidant and anti-carcinogen activities, hepato-protection capacity and the ability to enhance memory. Therefore, extending the use of anthocyanin-rich extracts is significant in thefood industry because they can provide products with potential health benefits besides attractive colors.

Vietnam is known as an agricultural, tropical country with the ability to produce various crops and plantation products. Purple sweet potato contains a high level of anthocyanins and is an indigenous fruit crop widely grown in different parts of the Mekong Delta. Several studies have been carried out on theextraction of anthocyanins from plants, fruits, and vegetables using organic solvents.Anthocyanins are polar molecules and consequently more soluble in polar solvents, research on extracting anthocyanins from fruits and vegetables have shown that extraction conditions are also key factors in their overall solubility[2]. Several extraction methods have been proposed to obtain extracts rich in anthocyanin, usually based on solvents as methanol, ethanol, acetone, water or mixtures[7–10].

Though widely used, it poses some drawbacks; as the solvent used might pose toxicological and safety hazards thus attracting complications due environmental and health issues. Moreover, anthocyanins being vacuolar pigments may not be completely extracted as the solvent used may not completely disperse into the substrate, thus making them unavailable for extraction [1,4].Enzymatic treatment has long been used in food industries to increase the fruit juice yield and production of high-end quality products. The role of theenzyme is to degrade cell wall constituents and release intracellular contents. Usually, a plant cell wallcomprises cellulose, hemicelluloses, and pectin, while flesh has a significant content of pectin and proteins. Cell wall degrading enzymes such as cellulases, pectinases, proteases and –amylase can, therefore, be used for degradation and break down of cell structure, therefore, facilitating better results for extraction of intracellularcontents [11].

In this paper, we prepared anthocyanins pigment from purple sweet potatoes (*Prunusnepalensis*L.) using enzyme-assisted

extraction. Experiments were performed to evaluate the effects of solvent type, incubation time and temperature on total monomeric anthocyanin, phenolic concentrations and percent of dry matter during anthocyanin extraction from purple sweet potatoes.

II. MATERIALS AND METHODS

2.1.Materials.

All chemicals were used as received without further purification. The fresh purple sweet potato was harvested inVinh Long agricultural region in Vietnam. After harvested, potato samples were washed in water, cut into pieces of approximately 2-3 mm and dried by microwave irradiation. Then, the dried samples were ground into powder and kept in a brown desiccator. The enzymes alpha-amylase (Termanly) was procured fromNovozyme.

2.2. Enzyme-assistedextraction of purple sweet potato anthocyanins

The study of enzyme-assisted extraction of purple sweet potato anthocyanins was carried out.In the typical process: Firstly, the enzymes alpha-amylase was added and mixed thoroughly to the dried purple sweet potato powder with 1% enzyme/powder weight ratio. This mixture was incubated for 60 min in a thermostatically controller shake incubator kept at 65 ^{o}C and pH 4; Secondly, anthocyanins were extracted in water-ethanol mixed solution (water/ethanol volume ratio = 40/60 mL/mL).To optimize the extraction conditions the effect of varied extraction time (30-70 min) and temperature (5-70 min) was evaluated.

2.3. Color coordinates

The changes in tristimulus werecharacterized with a Minolta CR-300 chromameter (Japan). Results were given on the most common L*a*b* coordinates (CIE-Lab and CIE-LCh). Hunter lightness (L*), hue (H^{o} = artag b*/a*) and chroma ($C^* = \sqrt{a^{*2} + b^{*2}}$) parameters were estimated from a* and b*.

2.4.Determination of anthocyanins concentration.

Wet-basis moisture content was measured by a moisture analyzer (Sartorius MA35,Germany). The maximum absorbance of purple sweet potato anthocyanins was determined by a UV-Vis spectrophotometer.

The pH differential method was employed to determine the quantity of anthocyanins in extracts as described by Giusti and Wrolstad. The monomeric anthocyanin pigment concentration was calculated using following equations:

$$C_{anthocyanin}(mg/g) = \frac{A \times W \times DF \times 10^{3}}{\varepsilon \times l} \times \frac{0.025}{m \times (100 - w) \times 10^{2}} \quad (1)$$

$$C_{anthocyanin}(\%) = \frac{C_{anthocyanin}(mg/g) \times V}{m(100 - w) \times 10^{-2}} \times 100 \quad (2)$$

where,

$A = (A_{\lambda vis\ max} - A_{700nm})_{pH\ 1.0} - (A_{\lambda vis\ max} - A_{700nm})_{pH\ 4.5}$,

MW is the molecular weight of the cyanidin-3-glucoside = 449.2 g/mol, *DF* is the dilution factor = 100, ε is the molar absorptivity of cyanidine-3-glucoside = 26900 mg/L.cm, *l* is the pathlength = 1 cm, m is the weight of the powder (g), and *w* is the percent of dry matter of powder.

III. RESULTS AND DISCUSSION

In this study, the anthocyanins pigment extracts from purple sweet potato with assisted the enzymes alpha-amylase. The effect of extraction temperature and time was investigated to optimize the extraction conditions.

3.1. Effect of extraction temperature

Figure 1 showed the change in L*C*H^{o} parameters for the extract, indicating that extraction temperature has a slight effect on the change in visual color attributes. Lightness value of extracts at various temperature shows the same, fluctuating in the range of 43-48. The chroma and hue value have a slight decrease which would be related to the degradation of monomeric anthocyanins[12,13].

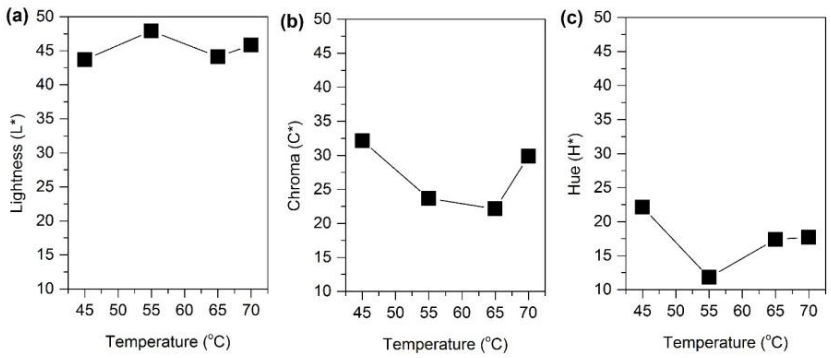

Figure 1. Effect of extraction temperature on the Hunter lightness (a), Chroma (b) and Hue (c) parameters of aqueous anthocyanin.

The percent of dry matter is shown in Figure 2. The results demonstrate that after treated with anenzyme the dry matter significantly increase. The percent of dry matter from the sample treated with the enzyme is 8.82 %, while the sample without enzyme accounting 5.66 % (not shown in thefigure). This increase may be conducive to the effect of the digestion of starch into sugar by the enzyme. Besides, there is a slight change in the percent of dry matter from the sample with different extraction temperature, fluctuating from 7.44% to 8.82 %.

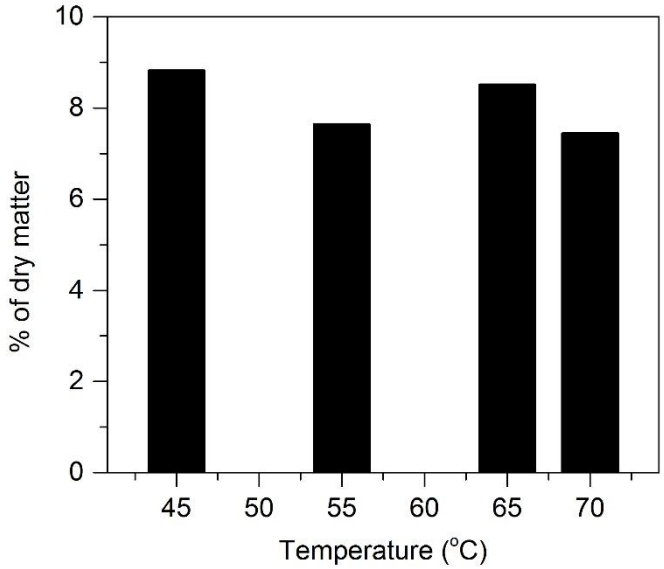

Figure 2. Effect of temperature on dry matter.

Variation in extraction temperature was evaluated to get the optimized temperature for maximal anthocyanins concentration (mg/L) and anthocyanin recovery (%) during the extraction process, as shown in Figure 3. The results showed that with an increase extraction temperature from 45 to 70 ^{o}C, the anthocyanins concentration (mg/L) and anthocyanin recovery(%)significantlyincrease. The maximal anthocyanins concentration (mg/L) was achievedin the range of 60 -70 ^{o}C. However, when increasing extraction temperature up to 70 ^{o}C, anthocyanins concentration have a slight decrease.

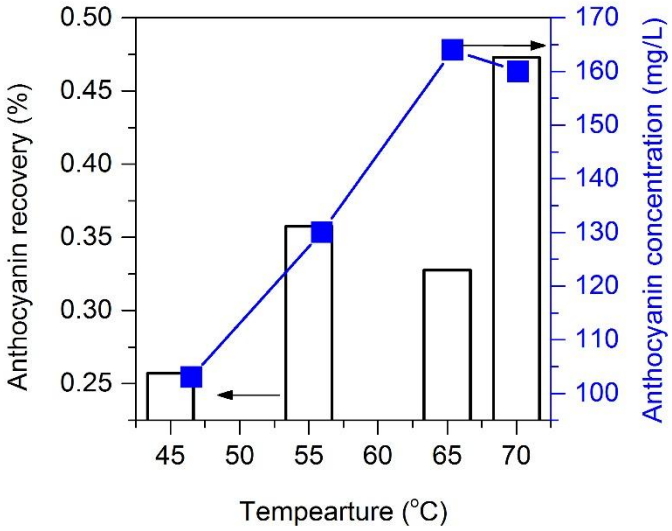

Figure 3. Effect of extraction temperature on anthocyanins concentration and anthocyanin recovery.

3.2. Effect of extraction time

Variation in extraction time was evaluated to get the optimized temperature for maximal anthocyanins concentration (mg/L) and anthocyanin recovery (%) during the extraction process. The change in L*C*H^{o} parameters for the

extract, indicating that extraction time has a slight effect on the change in visual color attributes. Lightness value of extracts at thevarious time shows the same, fluctuating in the range of 45-50, similar to the results of extracts at various temperature. The chroma and hue value have a slight decrease which would be related to the degradation of monomeric anthocyanins.

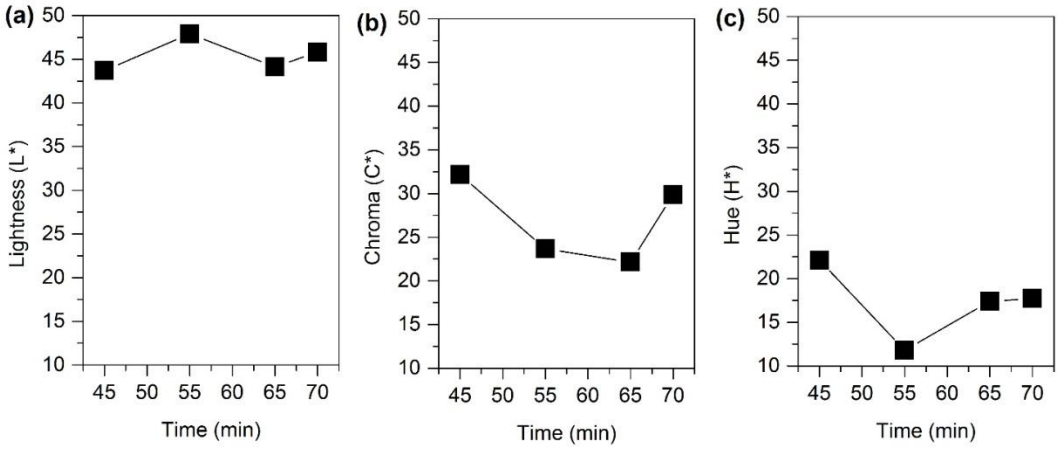

Figure 4. Effect of extraction time on the Hunter lightness (a), Chroma (b) and Hue (c) parameters of aqueous anthocyanin.

The percent of dry matter in extracts which were obtained at different time points of 30-70 min is shown in Figure 4. As shown in Figure 4, the percent of dry matter slightly increases when increasing extraction time. The extract obtained at 40 min stand at thehighest level (the percent of dry matter is 8.51%).

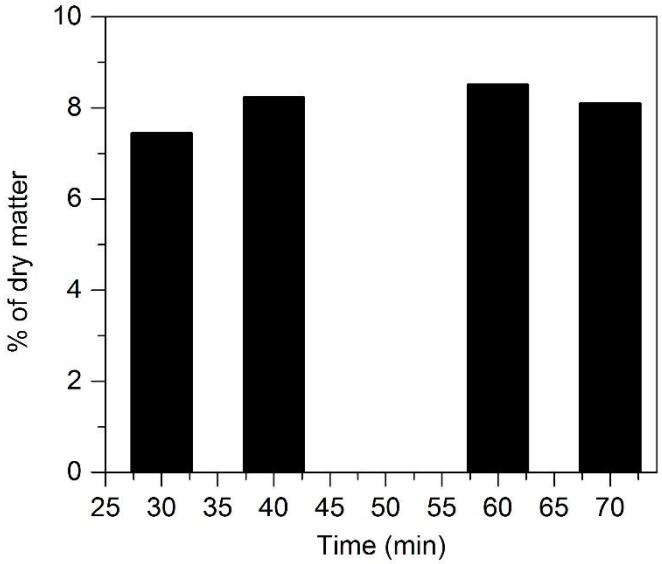

Figure 5. Effect of extraction time on thedry matter.

Figure 3 shows the effect of reaction time (30-70 min) on the anthocyanins concentration (mg/L) and anthocyanin recovery (%), clearly indicating a downtrend in the anthocyanins concentration (mg/L) and anthocyanin recovery (%)via extraction times. When the time of extraction process was prolonged to 40 min and 60 min, the anthocyanins concentration remain unchanged. However, with an increase of extraction time up to 70 min, the anthocyanins concentration significant decrease. This decrease may be attributed to the degradation of anthocyanins in longtime treatment and hight temperature. Similarly, anthocyanin recovery also sees a decreasing trend from 0.685% to 0.394%, corresponding to the increase of extraction time from 30 to 70 min.

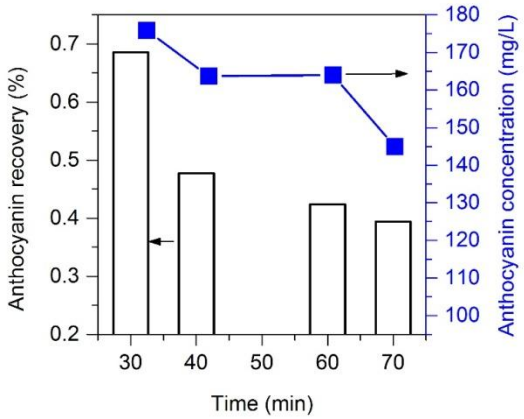

Figure 3. Effect of extraction temperature on anthocyanins concentration and anthocyanin recovery.

IV. CONCLUSION

We investigated the enzyme-assistedextractionanthocyanins pigment was extracted from purple sweet potatoes. We also investigated the effect of extraction temperature and time to optimize the extraction conditions. The results showed that extraction temperature and time play a significant role in theextraction process. The optimum conditions are extraction temperature: 65 ^{o}C and time: 60 min, exhibited thehighest yield.

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