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Anthocyanins extraction from Purple Sweet Potato (*Ipomoea batatas* (L.) Lam): The effect of pH values on natural color

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Abstract. Anthocyanins are widely used in the food industry as safe natural colorants thanks to their benefits, attractive colors and safety for consumption. The first objective of this study was to evaluate anthocyanins extraction from Vietnam purple sweet potato (Ipomoea batatas (L.) Lam) for use as food coloring. Different extraction temperatures of solvent ethanol (40 - 70%), duration of extraction (40 - 80 min), temperature extraction (30 - 70^{o}C) and liquid-solid ratios (4:1 - 8:1 mL/g) were selected in order to extract purple sweet potato. Second, this study examines the anthocyanin color behavior at various pH levels in aqueous solutions levels ranging from 1.0 to 14.0. At low pH (acidic conditions), anthocyanins are stable and gives a red color. Meanwhile, increasing the pH value of anthocyanin will change the color from red to pink, violet, blue, green and yellow. This variation suggested that anthocyanin could be utilized as a possible pH color indicator in commercial packaging industry and agriculture.

1. Introduction

Natural products chemistry plays a pivotal role in the physical and biological sciences. Developing new avenues by presenting novel applications, broad perspective, valuable inputs, and a new vision for the future is described [1-8]. Since color and texture of food are often perceptually associated with freshness, ripeness or safety, coloring alternatives from natural sources to synthetic dyes have been an interest of research [9,10]. Among many natural colorants, anthocyanins are commonly used due to their health benefits, attractive colors and consumption safety. Structures of anthocyanins are polar consisting aromatic rings bound with hydroxyl, carboxyl, methoxyl and glycolyl groups. In addition, anthocyanins also possess other various beneficial properties, including antioxidising activity and mitigation of risk of circulatory diseases [11,12]. Multiple physiological functions including antimutagenicity, antihyperglycemic effect and free radical-scavenging activity are exhibited from natural purple sweet

potato color. Back in time, purple sweet potato (Ipomoea batatas (L.) *Lam*) was derived from Japan and was considered as a safe and stable source of purple color thanks to its high volume of anthocyanin [13,14]. In the past few years, owing to the growing demand pressed by rapid development in the food industry, purple sweet potato varieties have been cultivated in many countries. This is in line with the current trend of countries around the world to study the use of natural colors used in food, because they are fairly safe for users.

One characteristic that has made anthocyanins utilizable in the food industry is their capability to change color. There are many factors that could alter color stability of anthocyanin including pH, storage temperature, chemical structure, concentration, light, oxygen, solvents the presence of enzymes, flavonoids, proteins and metallic ions. In food applications, pH also greatly impacts microorganisms in terms of denaturization, gelification, enzymatic activities, growth and mortality, muscle foods in terms of water-holding capability, bacterial spores and other chemical reactions such as the Maillard reaction. Therefore, it is crucial to control pH to produce safe and high-quality food products [15,16]. From the current trend, it can be anticipated that the use of natural food colorants relative to the synthetic ones will keep increasing. Therefore, the goal of the present study is two-fold. First, we attempt to investigate extraction conditions that affect anthocyanin extraction from Vietnamese purple sweet potato (*Ipomoea batatas* (L.) Lam) including ethanol solvent, duration of extraction, extraction temperature and liquid-solid ratios. Second, we also aim to determine the color behavior of anthocyanin towards various pH levels.

2. Material and Methods

2.1. Extraction of anthocyanins from Vietnam purple sweet potato

Purple sweet potato (*Ipomoea batatas* (L.) Lam) were grown in Lam Dong, Vietnam. The harvested potatoes were washed, cut into cubes (3-5mm), dried by microwave 400W for 30 min, pulverized, then the collected powder was stored in dark black botte in room temperature for further use. All chemical and solvent are purchased from Sigma Aldrich.

For extraction parameter study, 15g of purple sweet potato powder was placed in the two neck round bottom flask and was extracted by H_2O: Ethanol solutions, liquid/solid ratio was 4:1 - 7:1 (mL/g). The extraction temperature was adjusted to about 40 - 70 (^oC) and time was 40 - 70 (min). Then, centrifugation took place at 4000 rpm for 15 min by high speed centrifuge Model LACE16 (COLO lab expert, China). 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 by UV-Visible Spectrophotometer (Evolution 60S) calibrated with distilled water as the blank. For determination of total monomeric anthocyanin content, the pH-differential method is used [17].

2.2. Anthocyanin extract as pH indicator application

To develop a natural liquid pH indicator from PSP, different test tubes containing a specified volume of anthocyanin extract was prepared. Following that, HCl 1N or NaOH 1N was added into tubes accordingly to produce samples with pH ranging from 1 to 14. Before visual inspection of color, the contents in each tube were thoroughly mixed. [18].

3. Result and Discussion

3.1. Determination of anthocyanin content

Effects of four individual factors on the anthocyanin content were summarized in Table 1. The rapid increase in anthocyanin content with the increase of the ethanol concentration is recognized in Table 1A. Besides, it is shown that the content and quality of anthocyanin pigments strongly depend on the solvent concentration. When rising ethanol concentration from 40% to 70%, the anthocyanin content increased and reached the highest point with EtOH60 at 200,79 mg/L. Ethanol concentration strongly influences the composition of the scavengers from the material, changes the translation properties so it strongly influences the other parameters of the color solution, which contains anthocyanin content. The anthocyanin content in the fluid as well as the effect of color separation from the material was increasing with temperature and reached the highest temperature of 60 - 65^oC, as showed in Table 1B. However,

when the temperature increased to 70°C, the change in anthocyanin content became subtle. The temperature is so low that only small amounts of anthocyanin are present in sweet potato powder, and the high temperature makes the anthocyanin come out more. By increasing the liquid-solid ratio from 4:1 to 6:1 mL/g, the extracted anthocyanin content increased from 170,13 mg/L to 198,48 mg/L (Table 1C). However, the anthocyanin content decreased sharply after increasing the ratio from 6:1 to 7:1 mL/g. The liquid/solid ratio reaches a certain value (depending on the characteristics of the material), the cell, owing to its efficient water absorption, swells to the maximum and bursts out simultaneously, releasing the color within the vacuole. From Table 1D, the level of anthocyanin in the fluid tends to decrease with different extraction times. When extracting for 40 - 70 minutes, the anthocyanin content is relatively stable, but when the time is increased over 60 minutes, it decreases markedly due to anthocyanin decomposition caused by the long exposure to high temperature.

Table 1. Fixed and variable factors in the single factor investigation of each variable

Experiment / A	A11	A12	A13	A14
Solvent Concentration	Ethanol 40%	Ethanol 50%	Ethanol 60%	Ethanol 70%
Absorption	0.7371	0.7679	0.8016	0.7854
Total anthocyanin content (mg/L)	184.63	192.35	200.79	196.73
Experiment / B	**B11**	**B12**	**B13**	**B14**
Temperature (°C)	40	50	60	70
Absorption	0.7657	0.8308	0.8477	0.8506
Total anthocyanin content (mg/L)	191.82	208.15	212.33	213.06
Experiment / C	**C11**	**C12**	**C13**	**C14**
Liquid/solid ratio (mL/g)	4:1	5:1	6:1	7:1
Absorption	0.6792	0.7926	0.7804	0.6535
Total anthocyanin content (mg/L)	170.13	198.53	195.48	163.57
Experiment / D	**D11**	**D12**	**D13**	**D14**
Time (min)	40	50	60	70
Absorption	0.8497	0.8552	0.7977	0.7608
Total anthocyanin content (mg/L)	212.83	214.21	199.81	190.57

3.2. The effect of pH values on anthocyanin color

Changing the pH of the anthocyanin pigment significantly affects the tone of the colorant. To be specific, basic colors of anthocyanins including blue, purple, red and orange are directly associated with quantities of hydroxyl groups and are indirectly related to quantities of methoxyl groups [19]. In the liquid indicator pH scale development, the anthocyanin extract showed different colors under different pH conditions (Figure 1). Therefore, it can be used as a natural pH indicator. Visually, the color varies from red in acidic solution to purplish to blue to green in mildly alkaline solution to yellow in a high alkaline solution. These anthocyanin-exclusive changes of color range are similar to red cabbage purple, corn flowers blue, and poppies red. This is contrast with some commercialized indicator, such as phenolphthalein, whose color change only occurred from colorless or pink in acid base titration. It is because phenolphthalein undergoes a structural rearrangement of one-proton removal from one of its phenol group as the pH rises. Anthocyanin extract, on the other hand, was able to show multiple color changes due to more than one proton donation or acceptance group stability over a fairly wide pH range when they contain two or more acyl groups [20].

At the equilibrium state, anthocyanins are characterized by five different equilibrium structures, including red flavylium cation, colorless carbinol pseudo base, purple quinoidal base, blue quinoidal base and and yellowish chalcone, and ratio of such structural anthocyanins, determined by pH value, results in color change. Among those structures, flavylium form could not be recovered or formed by re-acidification.. At acidic medium of pH = 1 - 3, red flavylium cation (2-phenylchromenylium cation) is predominant in the solution. . However, increasing the pH leads to a decrease in in concentration of the flavylium cation due to the hydration which in turn reduces the color intensity and produces the colorless carbinol pseudobase (hemiacetal or chromenol). In the hydration process, the anthocyanidin skeleton of the conjugated 2-benzopyrilium system is disrupted at the 2 position by a nucleophilic attack of water (Figure 2). As the pH shifts higher, flavylium cation loses proton rapidly. At pH < 7, purple quinoidal anhydrobase is dominant in the equilibrium. at pH < 8, the color turned to the deep blue characterized by ionized anhydrobase. When pH exceeds 8, the central pyran ring opened, allowing the formation of carbinol form and the light yellow chalcone. At any point, the color could be altered by adjusting the pH of the solution to acidic or alkaline. In general, it is the transformation of ions that cause color change and pH adjustment could be utilized to make such changes.

Table 2. Results of pH application

pH	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Color	Red	Dark pink	Pinkish red	Pinkish red	Lavender	Faint pink	Faint violet

pH	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Color	Faint blue	Dark blue	Dark green	Faint green	Green	Yellowish green	Yellow

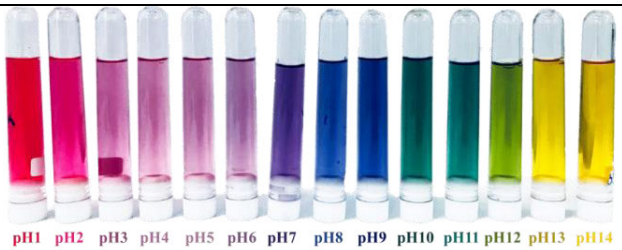

Figure 1. The color change of the anthocyanin corresponds to the different pH

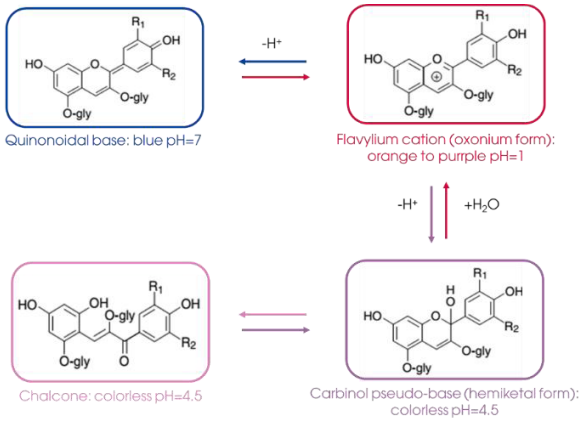

Figure 2. Predominant structural forms of anthocyanins present at different pH levels

4. Conclusion

We have successfully extracted anthocyanins from Vietnamese purple sweet potato (Ipomoea batatas (L.) Lam) and have obtained the set of condition variables including solvent ethanol of 60%, temperature of 70^{o}C, liquid-solid ratio of 5:1 (mL/g), and time of 50 (min), whose corresponding anthocyanin content was the highest at 214.21 mg/L. In addition, the study on purified anthocyanins as natural pH indicator revealed that the color of anthocyanin pigments was drastically altered with adjustment in pH value of the medium. To be specific, anthocyanins are red at low pH (acidic conditions) and higher pH value will alter the color from pink to violet, then blue, green and finally, yellow. These results suggest that anthocyanin extracts from Vietnam purple sweet potato (Ipomoea batatas (L.) Lam) represent a feasible solution for pigmentation for the food colorant market.

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