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The Effect of Preparation Method on Anthocyanin Contents from *Lpomoe Batatas (L.) Lam* as Intelligent PH Indicator

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ABSTRACT

Intelligent pH indicators have been gain interested in food industry as its acts as intelligent packaging where can be monitored and providing information and warning customer on the food condition in real time pH indicator. Calorimetric pH indicator used as indicator system in the packaging as in the most cases, pH changes can be detected when food deterioration occurs. However, the current pH indicator has lower colour changes and lower stability. In this research, new pH indicator was produced and have pronounced colour changes in wide range of pH that have higher stability to temperature and light from natural colorant sources. Two method of extract's preparation was conducted to evaluate the effect of anthocyanin contents which is with or without steaming. The characterization was done by observing the physical appearance, total anthocyanin contents, UV-Vis analysis and colour response of anthocyanin to various PH solution. As the results, steaming of sweet potatoes preparation method was found able to produce dark purple colour with high value of total anthocyanin contents. Anthocyanin colour produced using steaming process results more intents colour at pH 1 to pH 13 compared to without steaming. These finding was supported with UV-Vis peak that showing different wavelength which corresponding to the various colour produced. The results obtained show the anthocyanin extracts is a promising component to be used as pH indicator since its stimuli responsive with the pH of the environments.

1. Introduction

Consumer preferences and demand have shifted on the requirement for safe and high-quality food goods that has prompted new advancements and modifications to various types of packaging materials. Packaging is significant in the food sector because it protects the product from environmental effects, offers the customer with the better ease of use and time-saving convenience and includes items of varied sized and form [1]. The quality and safety of a good product could be

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affected at various aspects during its distribution and transportation. As a result, any mishandling of the food product especially if the package's integrity is not maintained, can have a significant effect on its safety and overall quality [2]. As a reaction to customer's increased worries about food safety and quality, new solution for intelligent packaging have been develops. The term intelligent packaging refers to a food packaging system that can be monitored and educate consumers on food conditions in real time [3]. It is able to provide information about the nature of food inside a package, such as quality, maturity, or contamination status. pH are important factors to inform spoilage of many food products. Changes in the concentration of metabolites such as glucose, organic acids, ethanol, carbon dioxide, biogenic amines, volatile nitrogen compounds or sulphur derivates during storage indicates microbial growth where will increase or decrease the pH of the food. pH sensitive dyes can be used to create pH indicators included bromophenol blue and chlorophenol red which is chemical reagents. However, these dyes are potential gives a harmful effect to human beings. For instance, the usage of synthetic dyes has been linked to a number of concerns, including allergy, toxicity, and carcinogenic effects [4]. In terms of environment, synthetic dyes may cause severe effect such as impairing photosynthesis system and increase biochemical oxygen demand (BOD) [5]. Therefore, the usage of synthetic chemical compound is highly recommended to be avoided in the food applications. Since natural dyes are rarely toxic, easy to manufacture and pollutant free. Many efforts have been made to develop visual pH indicators using anthocyanin as one types of intelligent food packaging system due to various advantages such as save, having sensitivity and low prices [6]. Therefore, there are increasing number of studies focusing on fabricating pH indicator by using anthocyanin.

Anthocyanin is a type of plant-based natural dye with various application. Anthocyanin had been used as nanoparticles synthesizing agent [7] and active ingredients in conventional herbal remedies [8] and functional food [9]. Meanwhile, as pH indicator, properties such as non-toxicity, water solubility, visibility to human eye, calorimetric pH sensitivity, anthocyanin-rich extracts were increasingly being used in the food business as replacement for synthetic pH indicator like FD&C Red No.40 (Allure red) [10]. For example, pH indicator films were developing with butterfly's pea flower anthocyanin and red cabbage anthocyanin. The films with butterfly's pea flower anthocyanin must be kept away from light at low temperature and only covered pH between 1-9 [11]. Therefore, suitable colourant should be introduced such as to increase stability to temperature and light. Also, able to show pronounced colour changes in wide range of pH.

Thus, the aims of this paper are prepared anthocyanin from different preparation method which is steaming and without steaming. The anthocyanin produced were characterized using colour changes, total anthocyanin contents (TAC), pH changes and UV-VIS. As conclusion, this anthocyanin from purple sweet potatoes is suitable to be used as the pH sensitive pigments as it has a higher stability to temperature and light, in comparison to dyes extracted from other plants and covered a wide range of pH.

2. Methodology

2.1 Materials

The purples sweet potatoes were purchased from local supermarket. All the chemical such as ethyl alcohol (95% v/v), hydrochloric acid (fuming 37%) and sodium hydroxide pellet were bought from R&M Chemicals using the analytical grades. Deionized water was used for all the sample preparation except for washing the sweet potatoes and apparatus.

2.2 Preparation of Purple Sweet Potato Powder

Purple sweet potatoes were washed using tap water and cut into thin slices. These slices of potatoes were divided into two groups to determine the intensity of anthocyanin extracts produced. The first group was steamed at boiling water for 7 minutes and then dried in oven at 70 °C for 48 hours for drying purpose. After dried, these samples were blend using blender to make it powder. Meanwhiles, the second group of potatoes was directly dried without steaming in oven with same temperature and time before being blended. The processes powder was finally stored in amber glass storage bottle with additional of silica gels pouches to absorb moisture in the environment until for further used.

2.3 Extraction of Anthocyanin from Purple Sweet Potatoes Powder

The purple sweet potatoes powder was extracted according to previous journal with slightly modification [12]. The powders were mixed with ethanol solvent (60% v/v) in a beaker with a solvent - solid ratio 5:1 (ml/g). Then, the beaker was close with aluminium foil and seal with plaster to avoid the evaporation of the alcohol. The samples were sonicated using sonicator (Elmasonic Easy 60H, Elma, Germany) for 50 minutes at 70 °C. This process is important to make sure all the anthocyanin contents were extracted to the ethanol solution. The extracts were then cool to the room temperature and filtered using vacuum filtration. The filtrates were transfer into vacuum rotary evaporator (Hei-Vap Core, Heidolph, Germany) until become concentrated. The resulting solvent were stored in an opaque glass bottle coated with aluminium foil at 4 °C to avoid it exposes to excess light and remain stable.

2.4 Characterization

2.4.1 The physical appearance of purple sweet potatoes powder

The physical appearance of purple sweet potatoes powders produced by using two step of preparation method were evaluated based on the colour observation. About 10 grams of powder were placed onto the disposable plastic petri dish and the physical appearance were evaluated to supports the future findings.

2.4.2 The total anthocyanin content (TAC) of the extract

The total anthocyanin contents in purple sweet potatoes were measured via the pH differential method using UV-VIS spectrometer (UV-2600, Shimadzu, Japan) following the methodology of previous research with slightly modification [13]. pH 1.0 buffer of 0.025M potassium chloride and pH 4.5 of 0.4M sodium acetate were prepared. The absorbance of test portion of purple sweet potatoes diluted with pH 1.0 buffer and pH 4.5 buffer at 520 nm and 700 nm were measured.

The dilution factor was measured by diluting the test portion of each sample by using pH 1.0 buffer until the absorbance of the spectrophotometer measured at 520 nm was within 20 to 50 min of preparation. The diluted test solution was found to be excessively turbid was filtered before measuring the absorbance using 0.45 μm of nylon filter membrane to ensure that anthocyanins were not be absorbed. The diluted test portions are read versus a blank cell filled with distilled water. Absorbance is also measured at 700 nm for the correction of haze. The anthocyanin pigment concentration (as cyanidin-3-glucoside equivalents) was calculated using the following Eq. (1):

$$\text{Anthocyanin pigment} = \frac{\text{A} .\text{MW} .\text{DF} .10^3}{\varepsilon .l} \quad (1)$$

where; A= ($A_{520\ nm} - A_{700\ nm}$) pH 1.0 – ($A_{520\ nm} - A_{700\ nm}$) pH 4.5, MW= 449.2 g/mol for cyanidin-3-glucoside, DF= dilution factor, l= 1 cm of path-length, ε = 26,900 molar extinction co-efficient, in L/mol.cm for cyanidin-3-glucoside and 10^3 = factor for conversion from g to mg.

2.4.3 Colour responses of anthocyanin to pH changes

Measurement of the colour changes intensity of anthocyanin extracts of purple sweet potatoes were performed on 13 pH conditions and the resultant solution were photographed. Buffer solution were prepared at range of pH from 1 to 13 with distilled water by adjusting the pH with an aqueous solution of 1.0 N hydrochloric acid and 1.0 N sodium hydroxide. The purple sweet potatoes anthocyanins were added to buffer solution and the colour changes were visible spectrophotometer (UV-2600, Shimadzu, Japan). The pH values of 1 to 13 were measured in the range of 400 – 800 nm.

3. Results and Discussion

3.1 The Physical Appearance Powder and Anthocyanin Extracts

In this research, purple sweet potatoes powders were prepared using two different methods in order to evaluate the physical properties and anthocyanin contents after the preparation process. First sample (sample A) were steamed, dried and powders meanwhile the second sample (sample B) were directly dried and being powdered (Figure 1 and Figure 2). Different techniques show the different colour were obtained for both powder where sample A gave dark purple colour and sample B gave purplish brown colour similar to Wirasuta and co-researcher (Figure 1).

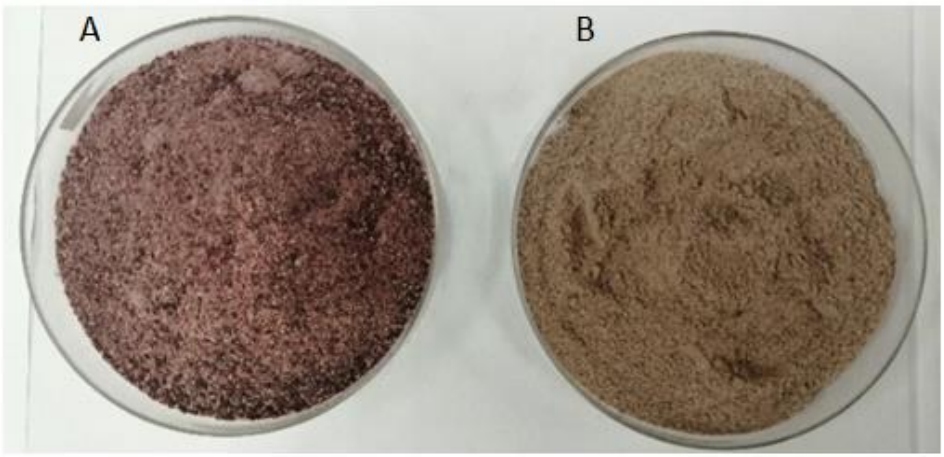

Fig. 1. The physical appearance of purple sweet potatoes powder (A) steaming (B) directly dried

The purple colour of purple sweet potatoes is still in contact for sample A although undergo steaming process but the colour turns slightly brown for sample B. The colour of anthocyanin extracted from purple sweet potatoes powders obtained shows the similar colour to the colour of the powder (Figure 2). It has been reported that drying thermal processing the anthocyanin can be directed to the thermal degradation and form colourless or undesirable brown-coloured pigments [14]. The reason behind is because the steaming process can denature proteins, so the enzyme anthocyanase, the peroxide enzyme and polyphenol oxidase enzyme become inactive in oxidizing

the anthocyanin. Due to this breaking of bonds between dye and protein, the colour become more intense than without steaming.

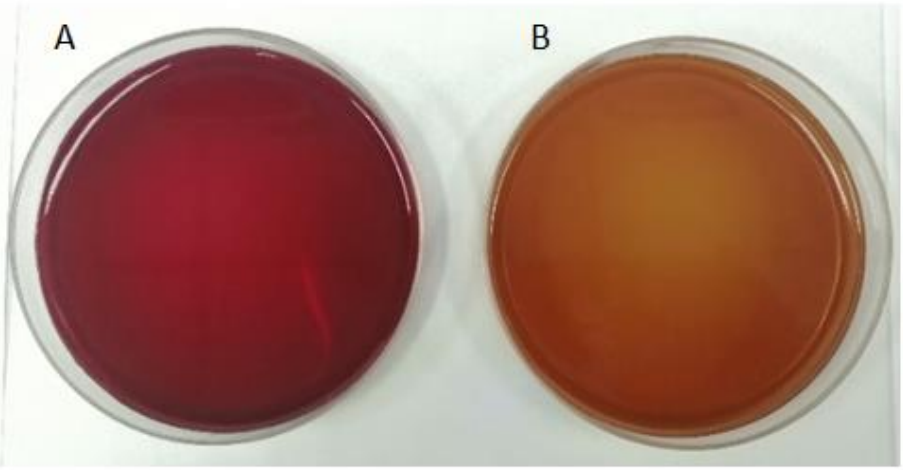

Fig. 2. The colour of the anthocyanin extract from (A) steaming powder (B) directly dried

3.2 The Total Anthocyanin Contents Analysis

Based on the date obtained in Figure 3, sample A (steamed) gave the high TAC value ta 534.36 mg/L compared to sample B at 92. 18 mg/L. The difference of TAC in both samples show that it has a correlation between the colour of the powder produced in Figure 1. Colour of the powder of sample A is more pronounced than sample B. The colour of anthocyanin extract in Figure 2 also supports the finding where the intensity of anthocyanin is more intense in sample A compared to sample B. These colour response to purple sweet potatoes anthocyanin have similar trend with other literature, however the intensity is referred to the suitable method used.

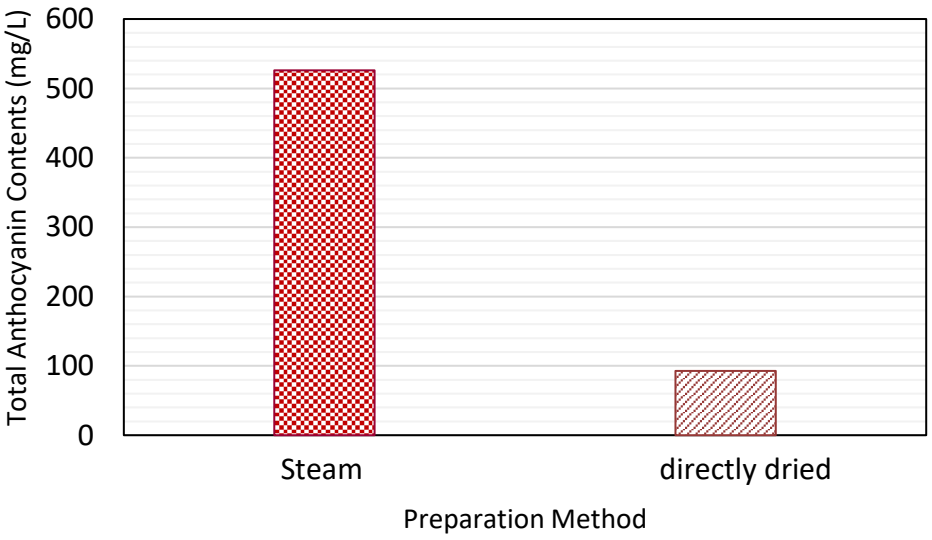

Fig. 3. The TAC data obtained from purple sweet potatoes anthocyanin extracts

3.3 The Colour Response of Anthocyanins to pH Changes

The colours of purple sweet potatoes anthocyanin extract in pH 1 to 13 buffer solutions were measured using colour observation and UV-VIS spectra. Based on the result show in the Figure 4, purple sweet potatoes anthocyanin extracts showed different colours under different pH condition. Visually, the anthocyanin extract in sample A, the colour is varying from red in acidic to purplish to blue to green in mildly alkaline solution and to yellow in a high alkaline solution similar to the result observed in the literature [13].

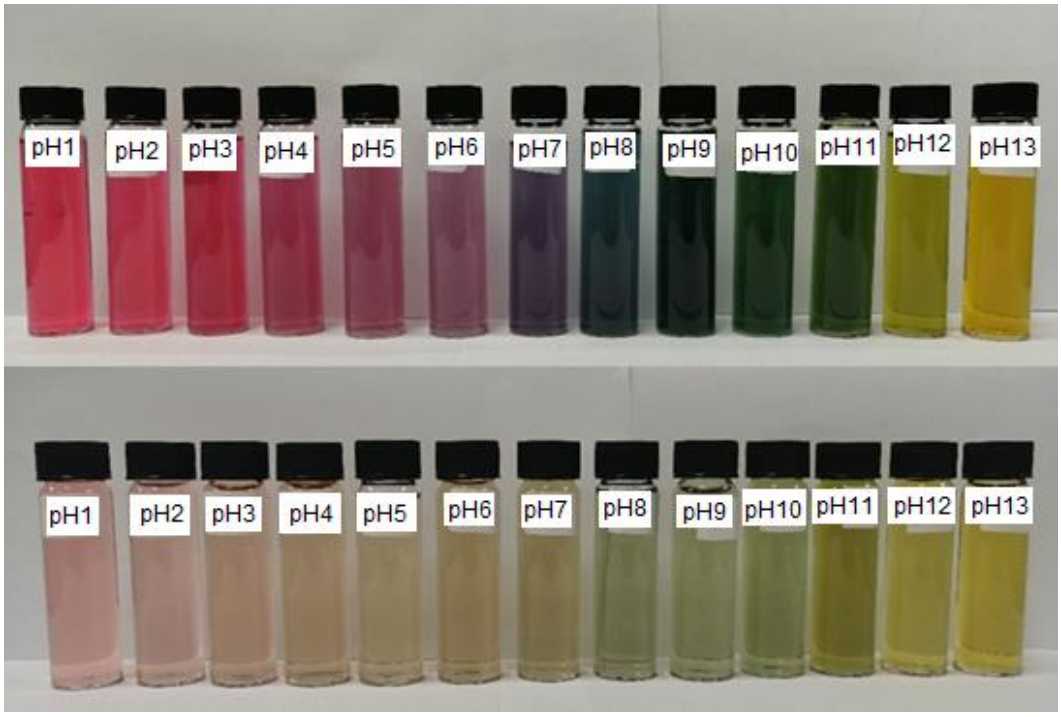

Fig. 4. The colour changes of purple sweet potatoes anthocyanin extract at pH 1 to pH 13

While anthocyanin extract from sample B colour is varies from faint red in acidic solution to faint brown to sage green in mildly alkaline solution and to yellow in a high alkaline solution (Table 1). The colour response of anthocyanin in sample A showed more pronounce colour compared to sample B. Those different might be due to the TAC in each sample. As TAC in sample A is higher than sample B, thus sample A able to show more different colours under different pH conditions since the anthocyanin pigments significantly affects the tone of the colorant.

Table 1
The summary of visual observation based on different pH application

pH	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Colour Sample A	Red	Red	Red	Pinkish Red	Pinkish Red	Lavender	Violet
Colour Sample B	Faint Red	Faint Red	Faint Red	Faint Brown	Faint Brown	Light brown	Light brown
pH	**8**	**9**	**10**	**11**	**12**	**13**	
Colour Sample A	Blue	Dark Blue	Dark Green	Green	Yellowish Green	Yellow	
Colour Sample B	Sage Green	Sage Green	Sage Green	Yellowish Green	Yellow	Yellow	

The reason behind this colour changes occurs is actually due to more than one proton donation or acceptance group stability over a fairly wide pH range when they contain two or more acyl group [14]. Anthocyanins have five different equilibrium structures in their equilibrium states, including the red flavylium cation, colourless carbinol pseudo base, purple quinoidal base, blue quinoidal base, and yellowish chalcone, and the ratio of these structural anthocyanin which is determine by pH value, causes colour changes (Figure 5). The red flavylium cation (2-phenylchromenylium cation) dominates the solution in an acidic medium with a pH of 1 to 3. However, increasing the pH causes the concentration of the flavylium cation to decrease due to hydration, resulting in a reduction in the colour intensity and the formation of the colourless carbinol pseudobase (hemiacetal or chromenol). During the hydration process, a nucleophilic attack of water disrupts the anthocyanin skeleton of the conjugated 2-benzopyriliym system at two positions. The flavylium cation rapidly loses proton when the pH rises. At pH 7, purple quinoidal anhydrobase is dominant in the equilibrium. At pH 8, the colour turned to the deep blue characterized by ionized anhydrobase. When pH exceeds 8, the core pyran ring opens, allowing carbinol and pale yellow chalcone to develop. The colour of the solution can be changed at any time by altering the pH to an acidic or alkaline level. In general, the colour changes are caused by ion transformations, and pH adjustments can be used to achieve this.

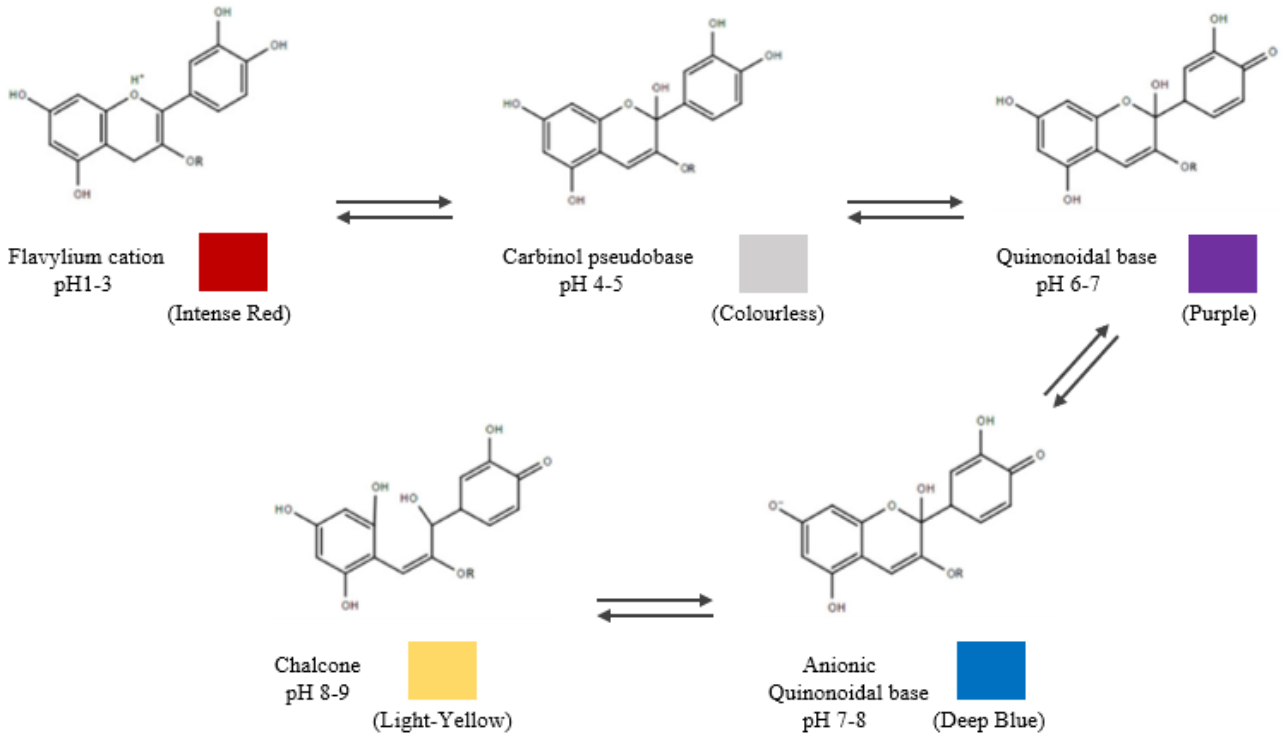

Fig. 5. The structural transformation of anthocyanins due to the different pH changes [15]

The colour variation and different structures of anthocyanins with the increasing pH can be seen through the peak shift in absorption as shown in Figure 6.

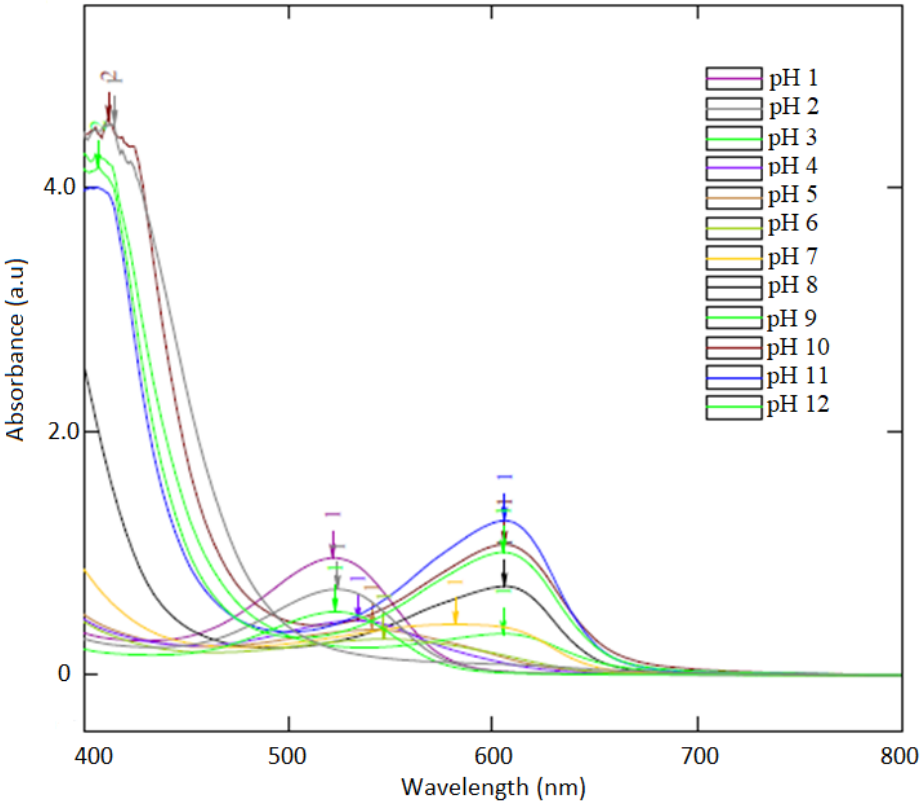

Fig. 6. The UV-VIS spectra of anthocyanin colour changes at different pH with steam process

The maximum absorption peak appeared at around 520 nm at pH 2, and the absorbance decrease with the increase of pH in the range of 2-4. When the pH was higher than 5, the maximum absorption peak appeared at 548 nm (in the range of pH 5-6) and the corresponding absorbance increased. Furthermore, the absorbance at 597 nm decreased in range of pH 7-13.

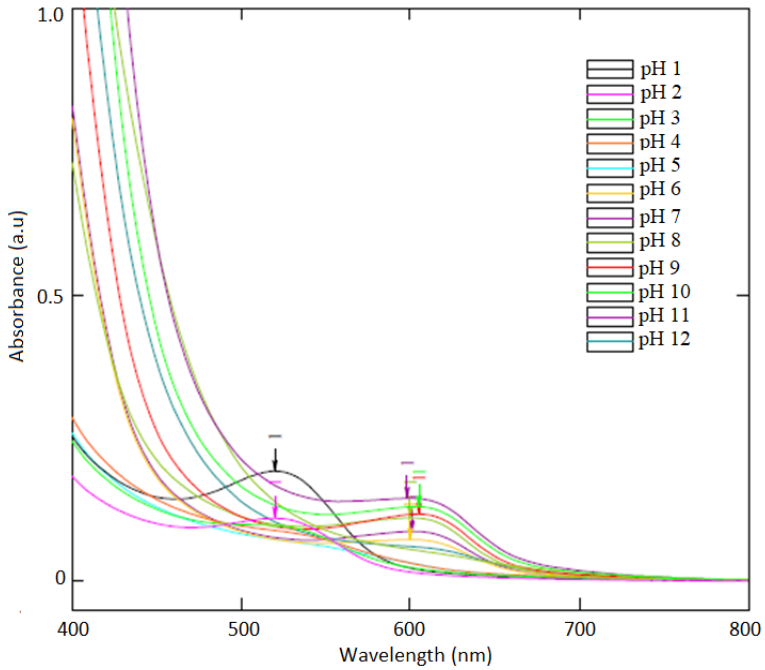

Fig. 7. The UV-VIS spectra of anthocyanin colour changes at different pH without steam process

4. Conclusions

In this project, two methods for preparation of purple sweet potatoes powder are being evaluated either starting with steaming process or directly dried to get in the dried powder form. Based on the colour observation, the intensity of colour was detected more intense for steamed sweet potatoes powder compared to directly dried. The solution extract dark purple colour showing higher contents of anthocyanin that supported by the data of total anthocyanin contents. The ability of the anthocyanin extract response with pH was evaluated using different pH values from pH 1 to pH13 and more wide and darker colour is produced when using the steaming process. Therefore, anthocyanin extract produced using steaming process is proven can produce high intensity of colour and this plant extract methodology is suggested to be used for further research for intelligent packaging of pH indicator.

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