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# Purple sweet potato (*Ipomoea batatas* L.) extract as a pH-sensitive natural dye for the development of packaged food freshness indicator

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#### **Abstract**

Natural pigments from tubers are applied for the development of pH indicator for food freshness label. In this study, the extract from purple sweet potato was used for pH sensitive dye characterisation. The colour changes of the sample extract were measured using UV-Vis spectrophotometer at the absorbance range between 400 nm to 800 nm and also a chroma meter. The results showed that the dye from purple sweet potato exhibited pH sensitivity. The colour was red in acidic pH, purple in neutral and green to yellow in alkaline pH. The maximum absorbance peak was found at 475 nm for the pH below than 3. In pH range of 4 to 6, the absorbance peaks were found at 500 nm and 525 nm for neutral pH. An alkaline pH buffers show the absorbance peaks around 550 nm. There are significant (p < 0.05) differences in value of a\*, b\*, C\* and  $\Delta$ E\* with pH variation. The  $\Delta$ E\* value also shows a linear and quantitative relationship to a specific pH range. Due to the sensitivity characteristic of the dye at different buffer pHs, this dye has the potential to be used as an indicator for food freshness especially for the products that undergo pH changes during deterioration.

Keywords: purple sweet potato, anthocyanin, pH, natural dye, freshness indicator

#### Introduction

In the scope of food safety, it has been reported that there is an increase in microbial population and pH value during the period of food storage. Fisheries products such as fish and seafood produce volatile compounds containing trimethylamine, dimethylamine and ammonia that are associated with the activity of microorganisms and the concentration of these compounds is increasing in the packaging space causing an increase in the pH value (Zhang 2014). Therefore, monitoring pH changes in the packaging space has the potential to be used as an indicator of food freshness during distribution and storage. Several scientists have reported the results of their research about pH monitoring by using chemical reagents as pH-sensitive dyes such as methylene blue (Mills and Lawrie 2011), bromophenol blue (Kuswandi et al. 2013) and a mixture of bromocresol green, bromothymol blue and phenol red (Rukchon et al. 2014). However, these chemical reagents are not categorised as food grade. Therefore, it is not suitable to be used as a pH-sensitive dye or pH indicator in monitoring the freshness of packaged food because of the risk of toxicity and cause harm to human health.

Purple sweet potato (*Ipomoea batatas* L.) is one type of tuberous roots originated from South America, belongs to Convolvulaceae family, and usually grows in tropics and some warm temperate regions. The tuberous roots become fleshy and enlarged because of carbohydrates as food reserves (Levetin and McMahon 2012). The purple sweet potato is served as various dishes, nutritional brewing dry powder, stuffing and sweetmeat in food processing industry and has become a popular cultivar in Asia (Mu et al. 2016). As a staple crop, the purple sweet potato is rich in bioactive phyto pigments, namely anthocyanin and good antioxidant ability. Therefore, undoubtedly make it as the star variety of sweet potatoes (Low and van Jaarsveld 2008). According to Steed and Truong (2008), total anthocyanin content in purple sweet potatoes ranged between 51.5 - 174.7 mg/100 g.

It is reported that the content of anthocyanin in purple sweet potatoes is significantly higher than that in ordinary orange-fleshed sweet potatoes (Xu et al. 2015), similar to those of anthocyanin crops with the highest yield,

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such as blueberries, blackberries, cranberries, and grapes (Enicole et al. 2010). Moreover, purple sweet potato is an important source of natural anthocyanin pigments because of its low cost (Gisela and Wilhelm 2006). Purple sweet potato anthocyanins are a compound of many varieties of anthocyanins, whose chemical structure is mainly composed of cyanidins and peonidin in the form of monoacylation and diacetylation. Because of its acylation form, purple sweet potato has high heat resistance and ultraviolet stability, which is their advantage as natural pigments in food additives (Odake et al. 1994).

The use of natural pigments is considering substituting synthetic food colorants as the demand of consumers to natural, safer and healthier food products (Martins et al. 2016). Anthocyanin is a water-soluble natural pigment that is responsible for the red, purple and blue colorants in vegetables, fruits and tubers (Fang 2015; Liu et al. 2015). It is a glycoside of polyhydroxy and polymethoxy from 2-phenylbenzopyrylium (flavylium) derivates. It consists of two benzene aromatic rings combined with the heterocyclic ring contains an oxygen atom (Ma et al. 2020). The use of anthocyanin as the natural food ingredient can be obtained through an extraction process (Sinela et al. 2017; Chen et al. 2019). Besides the potential as the food colorant (Albuquerque et al. 2020), anthocyanin has antioxidant activity from the hydrogen atom donation to radical compounds (Ma et al. 2020), which contribute to health benefits (Guo et al. 2020; Peng et al. 2020). A study by Hwang et al. (2011) found that purple sweet potato anthocyanins could attenuate dimethylnitrosamineto in rat liver.

Anthocyanin pigments are unstable at high pH values and may present unique colours at different pH such as red (pH 1), purple (pH 7), blue (pH 10), green (pH 11) and yellow (pH 13). However, the yellow colour indicates the formation of chalcones, which is an anthocyanin degradation product (Khoo et al. 2017). Due to the instability of anthocyanin pigments at different pH makes it potentially used as a natural colorant in the development of freshness indicators based on pH changes when food has spoiled. Therefore, the main purpose of this study is to identify whether purple sweet potato extract can be used as a natural dye in the development of a colorimetric pH indicator for determining the freshness of packaged food products.

#### Materials and methods

# Preparation of purple sweet potato extract

Purple sweet potato (Ipomoea batatas L.) was purchased from the Taman Kekal Pengeluaran Makanan at Hulu Chuchuh, Sepang, Selangor, Malaysia. Upon arrival, the sweet potato was peeled, washed and steamed for 10 min in the steamer. Then, it was left to cool, packed in laminated aluminum bags and kept frozen until used. The extraction method used was referred to Devarayan and Kim (2015) with some modification. The sample was extracted by taking 50 g of ground purple sweet potato

and soaked in 100 mL of 60% ethanol. After that, it was kept under constant stirring using shaker (Labwit Model ZHWY-304, Australia) at 150 rpm for 12 h. The shaking process was conducted at room temperature in the dark condition. Then, the sample was filtered through 2 layers of muslin cloths to remove coarse particles resulting in the *Ipomoea batatas* L. crude extracts. The filtrates were centrifuged (Heraeus Model # 7590 centrifuge, England) at 8000 rpm for 10 min to remove the fine suspended particles. Then, 100 mL of clear extracts were concentrated under vacuum using rotary evaporator (Buchi Model rotavapor R-3, Switzerland) at 50 °C to filtrate volume of about 10 mL. The concentrated extracts were stored at -19 °C until ready to be use.

# Preparation of buffer solution

Buffer solutions from pH1 to pH13 were prepared according to the method of Devarayan and Kim (2015). The following are the chemicals used to produce buffer solutions: hydrochloric acid and potassium chloride were used to produce a pH 1 and pH 2 buffer solution; citric acid and sodium hydrogen phosphate for pH 3 to pH 8; carbonate and bicarbonate salts for pH 9 and pH 10. A mixture of sodium hydroxide and hydrochloric acid was used to prepare of pH 11 to 13 buffer solutions. The pH values were measured at 25 °C with pH meter and a glass electrode (Mettler Toledo Model FE20, Columbia, USA).

#### Spectroscopic analysis

Spectroscopic analysis was performed by adding 100  $\mu L$  of purple sweet potato extract into each 5 mL buffer solution from pH 1 to pH 13. Then, the absorption spectrum was recorded using a UV-Visible Spectrophotometer (Perkin Elmer Model Lambda 25UV/VIS, Shelton, USA) at a wavelength range of 400-800 nm.

## Colour measurement

Colour measurement was carried out by adding 100  $\mu L$  of purple sweet potato extract into each 5 mL buffer solution from pH 1 to pH 13 and measured by using a chromameter (Minolta Model CR300, Osaka, Japan). A total of five readings were taken on different parts of the buffer solution. The parameter values of L\*, a\*, b\* were used to calculate the colour saturation (C\*) and the overall colour difference ( $\Delta E^*$ ) by using the following equation (Schanda 2007; CIELAB Colour Space 2008; Anshika et al. 2012):

$$C^* = (a^{*2} + b^{*2})^{1/2}$$
  
 $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ 

Where:  $\Delta L^* =$  difference of lightness;  $\Delta a^* =$  red colour difference; and  $\Delta b^* =$  yellow colour difference.  $C^*$  and  $\Delta E^*$  values were expressed as the average of five readings on different parts and the experiment was repeated three times.

## Statistical analyses

Analysis of variance (ANOVA) was used to analyse the data. The average value of the obtained colour parameters was compared with Duncan's multi-range test for each change of pH solution. Differences between treatments were determined with a less significant difference test (LSD) at the p < 0.05 levels using a statistical analysis system (SAS Inst. 1985).

## Results and discussion

The colour change of purple sweet potato extract in different buffer solutions is shown in Figure 1. Generally, the colour change of the extract in pH 1 - 13 buffer solution includes red, purple, green and yellow. The extract displayed a bright red colour at pH 1 to pH 3 in buffer solutions and the colour intensity decreases at pH 4. Most anthocyanins produce a red colour in acidic conditions with their main structure being in the form of flavylium cations (Bakowska-Barczak 2005). At pH 5 to pH 7, the solution turns purple. According to Khoo et al. (2017), as pH increases, the flavylium cation deprotonates and hydrates, leading to the formation of the carbinol pseudo base (colourless) and ultimately the quinonoid base (purple/blue). This shift in structure results in a decrease in the red colour intensity of the solution. While at pH 8 - pH 12, the sweet potato extract produces a green colour and changes to a yellow at pH 12 to pH 13. At increasing pH conditions, colorless carbinol and chalcone pseudobase structures are formed (Fossen et al. 1998). The results of the study have shown that the range of colour changes is wide, which involves several colours and significant differences between acid, neutral and alkaline conditions.

Figures 2a and 2b shows the absorption spectrum of anthocyanin contained in purple sweet potato extract at pH 1 – 13 buffer solution. Maximum absorption occurs at wavelengths between 475 and 550 nm. The colour change of anthocyanin is expressed through the difference in the maximum wavelength position measured as the absorbance value (Cabrita et al. 2000; Fossen et al. 1998). In general, absorption values were found to decrease with increasing pH (Table 1). Kirca et al. (2007) reported that the stability of anthocyanins in black radish decreased significantly at higher pH values. The results of this study are equivalent to the findings reported by Kirca et al. (2007) with the maximum absorption value measured at pH 1 with a reading of 0.850 followed by pH 3 which is 0.492 at a wavelength of 475 nm (Table 1). At pH 1 to pH 6, hypochromic effects were found, followed by a bathochromic shift, in which the absorption intensity value decreased and the absorption moved towards longer wavelengths (475 – 500 nm). Increasing the pH of the solution causes continuous deprotonation and this will produce a bathochromic shift.

The L\*, a\*, b\*, C\* and  $\Delta E^*$  values of the purple sweet potato extract in the pH 1 – 13 buffer solution are shown in *Table 2*. The results of the study show that the





Figure 1. Purple sweet potato extract in pH 1-13 buffer solution

colour lightness (L\*) increases when the solution towards neutral. The highest L\* value was recorded at pH 6 with a reading of  $87.85 \pm 0.99$ . Purple sweet potato extract shows the presence of a\* value as positive in acidic and negative for alkaline solution. This indicates the change in colour from red to green when the pH value changes from acid to alkaline. The red colour decreases (p < 0.05) with increasing pH value of the solution. The higher of the pH solution, the greater of negative value of a\* which shows the green colour. The findings found that there was a significant difference (p < 0.05) for the value of a\* at pH 1 to pH 10.

Generally, b\* value decreases significantly (p < 0.05) until pH 7 but increases at alkaline pH. These results are illustrated in Figure 1 that shows that sweet potato extract produces purple colour at pH 7 and yellow colour for pH 12 and 13. The value of b\* also found that there was no significant difference (p > 0.05) between pH 5 and pH 6 (Table 2). This suggests that there is no significant change in the positive b\* value that refers to the yellow colour in weakly acidic solutions. The b\* value was found to be the highest at pH 13 (Table 2) which is  $37.77 \pm 0.38$  and it shows a clear yellow colour visually (Figure 1). The colour saturation value (C\*) of sweet potato extract the highest at pH 13 and low (p < 0.05) in weak acid, neutral and weak alkali (Table 2). Based on the significant difference (p < 0.05) detected in the C\* value, it shows that this extract has good colour diversity. According to Choubert and Baccaunaud (2006), the C\* value represented colour intensity which is the result of a combination of a\* and b\* values. Therefore, the colour measured was directly affect due to changes in one or both of those values.

The results of the study also found that the overall colour difference ( $\Delta E^*$ ) which is referring to the combination of difference values from changes in lightness, redness/greenish and yellowness/bluish ( $\Delta L^*$ ,  $\Delta a^* \Delta b^*$ ) has shown a significant difference (p < 0.05) in the pH 1 to pH 13 buffer ( $Table\ 2$ ). As shown in  $Figure\ I$ , the red colour decreases as it nearly neutral, and then changes to purple, green and then yellow in alkaline solution. This colour change causes a reduction and increase in  $a^*$  and  $b^*$  values at acid, neutral and alkaline pH respectively, as summarised in  $Table\ 2$ .

pH sensitive natural dye

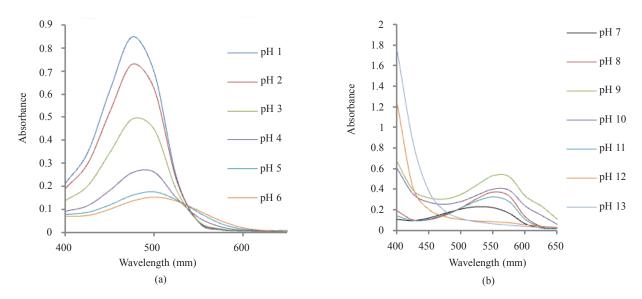


Figure 2. Absorption spectrum of purple sweet potato extract in buffer solution: (a) pH1-6 and (b) pH7-13

Table 1. The absorption value of purple sweet potato extract in buffer solutions of different pH

Buffer solution	Maximum wavelength, λ (nm)	Absorbance
pH 1	475	0.850
pH 2	475	0.730
pH 3	475	0.492
pH 4	500	0.261
pH 5	500	0.177
pH 6	500	0.154
pH 7	525	0.230
pH 8	550	0.372
pH 9	550	0.534
pH 10	550	0.400
pH 11	550	0.322

Table 2. Changes in color value (L\*, a\*, b\*, C\* and  $\Delta E$ \*) of purple sweet potato extract in pH 1 – 13 buffer solution

рН	L*	a*	b*	C*	$\Delta E^*$
1.0	$80.36 \pm 0.60^{\rm e}$	$27.32 \pm 0.80^{b}$	$3.00 \pm 0.06^{e}$	$27.49 \pm 0.80^{\circ}$	$30.71 \pm 0.96^{b}$
2.0	$81.21 \pm 0.57^{\rm e}$	$27.90 \pm 0.57^{a}$	$2.36\pm0.10^{\rm f}$	$28.00 \pm 0.58^{\circ}$	$30.88 \pm 0.73^{b}$
3.0	$82.83 \pm 0.55^{d}$	$19.94 \pm 0.58^{\circ}$	$1.09 \pm 0.04^{g}$	$19.97 \pm 0.58^{d}$	$23.16 \pm 0.75^{e}$
4.0	$83.49 \pm 1.18^{cd}$	$12.12 \pm 0.45^{d}$	$0.53\pm0.12^h$	$11.94 \pm 0.80^{e}$	$16.52 \pm 0.80^{g}$
5.0	$86.01 \pm 0.47^{b}$	$6.54 \pm 0.63^{\rm e}$	$0.87\pm0.26^{gh}$	$6.60 \pm 0.59^{\rm f}$	$10.84 \pm 0.67^{i}$
6.0	$87.85 \pm 0.99^{a}$	$5.07 \pm 0.20^{\rm f}$	$0.98\pm0.10^{gh}$	$5.12 \pm 0.29^{g}$	$8.56\pm0.84^k$
7.0	$86.08 \pm 0.62^{b}$	$3.07 \pm 0.24^{g}$	$-0.68 \pm 0.31^{j}$	$3.15\pm0.30^{\text{h}}$	$9.64 \pm 0.72^{j}$
8.0	$81.25 \pm 0.46^{\rm e}$	$-2.05 \pm 0.17^{j}$	$-0.95 \pm 0.53^{j}$	$2.30\pm0.34^i$	$13.75 \pm 0.56^{h}$
9.0	$77.19 \pm 0.85^{\rm f}$	$-4.93 \pm 0.20^{h}$	$11.64 \pm 0.39^{d}$	$12.38 \pm 0.88^{e}$	$19.27 \pm 0.90^{\rm f}$
10.0	$75.28 \pm 0.70^{g}$	$-0.52\pm0.04^k$	$19.51 \pm 0.17^{c}$	$19.52 \pm 0.17^{d}$	$24.58 \pm 0.61^{d}$
11.0	$83.71 \pm 0.72^{cd}$	$-0.59 \pm 0.05^{k}$	$-1.52 \pm 0.43^{i}$	$1.63 \pm 0.41^{i}$	$11.37 \pm 0.68^{i}$
12.0	$84.96 \pm 0.45^{\circ}$	$-3.75\pm0.04^{i}$	$30.50 \pm 0.73^{b}$	$30.26 \pm 0.73^{b}$	$28.57 \pm 0.79^{c}$
13.0	$84.35 \pm 0.29^{cd}$	$-3.64 \pm 0.29^{i}$	$37.77 \pm 0.38^{a}$	$37.34 \pm 1.05^{a}$	$35.64 \pm 0.41^{a}$

Means with the same superscript indicate no significant difference (p > 0.05) between the pH of the buffer solution

Table 3. Quantitative data on the linear correlation between chromametry parameters (L\*, a\*, b\*, C\*,  $\Delta$ E\*) and pH

Correlation parameters	pH range	Linear correlation equation (n = 5)	R <sup>2</sup>
L*	1-7	y = 1.2007x + 79.173	0.88048
a*	1-9	y = -4.3273x + 32.19	0.95756
b*	1-8	y = -0.51x + 3.195	0.8642
C*	1-7	y = -4.7196x + 33.489	0.93565
$\Delta E^*$	1-7	y = -4.2918x + 35.783	0.91465

The quantitative data displayed in *Table 3* shows that there is a clear correlation between all chromametry parameters (L\*, a\*, b\*, C\* and  $\Delta E^*$ ) and pH changes. Parameters a\* and b\* show a good correlation for both acid and alkaline conditions at a larger pH range (pH 1-9 and pH 1-8) with values of  $R^2=0.95756$ ,  $R^2=0.8642$ . Based on the significant difference of a\* and b\* values and also the colour change in a large pH range, making purple sweet potato extract potentially used as a natural dye in the development of a quantitative pH sensor through chromametry method. This method can be used to determine the freshness level of food products, especially packaged fish or seafood products because the freshness of such products is related to pH changes.

#### Conclusion

Purple sweet potato extract is able to produce a wide spectrum of colours and intensities at different pH. This study found that colour changes can be clearly measured through both spectrometric and chromametric methods. Therefore, purple sweet potato extract has the potential as a pH-sensitive dye to be used in developing pH sensors, especially in monitoring the packaged foods freshness such as seafood because the spoilage of such products is closely related to pH changes.

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