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Original Research Article

Exploring Purple Sweet Potato Pigment as An Eco-Friendly Titration Indicator for Acid Determination

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ABSTRACT

Ipomea batatas L., or purple sweet potato (PSP) tubers, are sources of anthocyanin, a pigment that can show colour changes at different pH values. The aims of this study are to explore the phytochemical content, colour characteristics, stability, precision, and accuracy of PSP pigment (PSPP) as a titration indicator. Further, PSPP was applied as a titration indicator for acid (H^+) determination. PSPP was extracted with medical-grade ethanol (PSPP-MG) and analysis-grade ethanol (PSPP-AG). This study showed PSPP-MG and PSPP-AG contain alkaloids, flavonoids, saponins, tannins, and steroids. They showed red colour at pH 1-2, pink at pH 3-6, and purple at pH 7. At pH 8–9, they were blue, turned to green at pH 10–11, and yellow at pH 12–14. These pigments were stable for 90 days at room temperature in tropical areas (25–30 ^{0}C) and showed clear colour changes at the endpoint of titrations with good precision and accuracy. Furthermore, PSPP-MG and PSPP-AG are used as titration indicators for H^+ determination. The results of this study highlight the performance of PSPP-MG and PSPP-AG for H^+ determination in HCl samples. Both of these pigments were able to show clear colour changes at the endpoint of the HCl sample titration. They were able to show a similar value of H^+ concentration as determined using phenolphthalein. Thus, PSPP can be used as titration indicator replacing phenolphthalein. Using this pigment as a titration indicator is cheaper, easier to obtain, easier to prepare, and environmentally friendly.

***Keywords*:** Colour change, phytochemical, natural dye, pigment, *Ipomea batatas L*

Introduction

Currently, efforts are being made to support green chemistry. Chemical research and other related fields are directed and designed to be environmentally friendly, reduce the use of chemicals, and sustainable resources.[1-5] Titrations is a method for volumetric determination of substance by endpoint detection visually.[6] In an acid-base titration, there is a substance known as an indicator. It plays a role in giving a signal (in the form of a colour change) when one of the substances involved in the reaction is in excess. Chemical compounds that are widely used as indicators in acid-base titrations are synthesis indicators such as phenolphthalein, methyl orange, and methyl red. These indicators are toxic, not environmentally friendly, and expensive.[7] To overcome this problem, it required other chemical compounds that could replace them, and plant pigments are the solution.[8, 9]

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PSPP contains anthocyanins.[10-12] an eco-friendly natural pigment.[13] It can show colour changes at different pH values.[13-15] PSPP has been widely used as a smart food packaging system,[16-20] but as titration indicator for acid-base titration, it has not been widely used.[21-24] Its use as an indicator for acid-base titrations in chemistry experiments is also not common.[8, 25]

In various studies that have been reported, the solvent for extracting the pigment of PSP is methanol or ethanol of analysis grade; not many reports have used methanol or ethanol of medical grade or other grades.[26, 27]

PSPP has the potential to be developed as an acid-base titration indicator because it contains anthocyanins. PSP is an agricultural product that is spread throughout Indonesia, including Kupang. The use of PSPP as a titration indicator has not been studied in depth. Its application as a titration indicator is limited to HCl-NaOH and HCl-NH_4OH titrations.[28, 29] Thus, it is important to carry out an in-depth exploration of PSPP as an indicator for determining H^+, including in HCl samples.

Afandi et al., and Jenimat et al., have studied the use of PSPP extract as a titration indicator,[28, 29] but only for titration of HCl-NaOH solutions and HCl-NH_4OH solutions. They have not studied the use of PSPP extract as an indicator for determining H^+ in samples. The novelty of this research is to examine the performance of PSPP extract as an indicator for determining H^+ in HCl samples. The overall study related to PSPP as an indicator and the contribution of this study can be seen in Table 1. The objectives of this study include assessing the phytochemical content, colour characteristics change, stability, precision, accuracy, and performance of PSPP as a titration indicator for H^+ determination in HCl samples.

Table 1: Overall study related to PSPP as an indicator and the contribution of this study

Authors	Indicator	Tipe of titration
Afandi et al.[32]	PSPP	HCl – NaOH solutions
Jenimat et al.[33]	PSPP	HCl – NaOH solutions
		HCl –NH_4OH solutions
This study	PSPP	HCl-NaOH solutions
		HCl –NH_4OH solutions
		Sample of HCl – NaOH solution
		Sample of HCl–NH_4OH solution
		Sample of CH_3COOH–NaOH solution

Materials and Method

Chemicals and equipment

The chemicals used were HCl 37%, CH_3COOH 99,8%, NH_3 99%, NaOH 99%, H_2SO4 98%, $FeCl_3$ 99%, $CHCl_3$ 98%, Mayer's reagent, Wagner's reagent, ethanol 96% (analytical-grade, Merck, Indonesia), ethanol 95% (medical-grade, One Med, Indonesia), distilled water, and purple sweet potato tubers were purchased from a local market whose location is at the following latitude and longitude positions -10.1494796,123.6429464. The equipment used were standard glassware used in chemical laboratories, burette (pyrex, Indonesia), digital pH meter (Mediatech P-2Z – B1900126, Indonesia), rotary evaporator (Buchi Rotavapor R-210).

Collection of plant material

Fresh purple sweet potato (PSP) tubers were purchased from a local market in Kupang City, Indonesia, whose location is at the following latitude and longitude positions 10°08'34.8"S 123°39'12.7"E.

Purple sweet potato pigment (PSPP) Extraction

PSP tubers were thoroughly washed, cleaned, thinly sliced, dried, and blended. A number of 100 grams of blended sample were macerated in 300 mL of medical-grade ethanol in an acidic condition for 24 hours.[30] The same procedure was repeated using analytical-grade ethanol. Both extracts were concentrated to dryness using a rotary evaporator. The pigment extracted with medical-grade ethanol was referred to as PSPP-MG, while the one extracted with analytical-grade ethanol was referred to as PSPP-AG. The pigments were stored at room temperature (25–30°C) until ready for use.

Phytochemical content of PSPP

Test for alkaloids (Mayer's test) : PSPP-MG 1 mL was placed in the test tube, and a few drops of HCl 0.1 M were added. Then, a few drops of Mayer's reagent were added.

Test for alkaloids (Wagner's test): PSPP-MG 1 mL was placed in another test tube, and a few drops of Wagner's reagent were added. Alkaloid was indicated by the white precipitate formed in the Mayer test and the brown precipitate formed in the Wagner test. [9]

Test for flavonoid : PSPP-MG 1 mL was put in the test tube, 5 drops of 2 M HCl were added and shaken. Then, a few powders of Mg were added. The presence of flavonoids was indicated by the formation of oranye, yellow, red[9, 31], or a green-blue colour .[32]

Test for saponin : PSPP-MG 1 mL was put in the test tube, 2 mL of hot water was added, and it was shaken for 30 seconds. The presence of saponin was indicated by the formation of foam that was stable after adding 1 mL of 2 M HCl.[33]

Test for tannin : PSPP-MG 1 mL was put in the test tube, and 3 drops of $FeCl_3$ 1% were added[31]. Tannin presence was indicated by the formation of a greenish-black or dark blue precipitate.[33]

Test for triterpenoid and steroid : PSPP-MG 1 mL was put in the test tube, 2 mL of chloroform 98% was added, and the mixture was shaken. The chloroform layer was taken, dripped onto the drip plate to dry, and 5 drops of acetic acid anhydride 98% were dripped and added 3 drops of H_2SO_4 98%. Triterpenoid was identified by the formation of a red, yellow, or orange colour . Steroids were identified by the formation of a green colour .[33]

The same procedure was repeated for PSPP-AG.

Colour character test of PSPP

Solutions of different pH range were prepared from hydrochloric acid (HCl), sodium hydroxide (NaOH), and distilled water as follows; Solution pH 1-6 was prepared by dilution of 1 M HCl, solution pH 8–14 was prepared by dilution 1 M NaOH, solution pH 7 was prepared from distilled water.[14] The solutions (1 mL each) were placed in separate test tubes, and to each solution was added one drop of PSPP-MG, and the colour change was immediately observed.[14] The same procedure was carried out for PSPP-AG.

Preparation of standard solution for titration

A total of 1.26 g oxalic acid ($H_2C_2O_4.2H_2O$) was accurately weighed, dissolved and diluted in a 100 mL volumetric flask. This solution was used to standardize NaOH and NH_4OH solutions. The NaOH and NH_4OH solutions were used to determine the concentration of H^+.

Preparation of sample for titration

HCl sample without spike: One tablet of drug sample which containing HCl was crushed and dissolved in 50 mL of distilled water. The mixture was filtered, the filtrate was put into a 100 mL volumetric flask, and distilled water was added to the mark of 100 mL and homogenized. The sample solution is ready to be used for titration.[23]

HCl sample spike: One tablet of drug sample which containing HCl was crushed and dissolved in 50 mL of distilled water. The mixture was filtered, the filtrate was put into a 100 mL volumetric flask. Concentrated HCl (density 1.19 g/mL, concentration 37%, molecular weight 36.5 g/mol) was taken as 1 mL and put into a volumetric flask containing the sample. Add distilled water up to 100 mL to the volumetric flask and homogenize. The spiked HCl concentration was 0.1206 M.[23]

CH_3COOH sample without spike: A number of 1 mL vinegar was taken and put into a 100 mL volumetric flask; distilled water was added up to the 100 mL mark. The mixture was homogenized and ready to be used in the titration.[23]

CH_3COOH sample spike: A number of 1 mL vinegar was taken and put into a 100 mL volumetric flask. Concentrated CH_3COOH (density 1.05 g/mL, purity 99.8%, molecular weight 60.05 g/mol) was taken in 1 mL and then put into a volumetric flask containing the vinegar sample. Add distilled water up to 100 mL to the volumetric flask and homogenize. The spiked CH_3COOH concentration was 0.1745 M.[23]

Stability, accuracy, and precision test of PSPP as a titration indicator

Stability test : the stability of PSPP as a titration indicator was studied based on its colour stability during storage. PSPP was stored at room temperature in tropical areas (25^0C–$30\ ^0$C), and it was observed for 90 days. It was used as a titration indicator, and the colour change at the end point was observed.

Accuracy test : the accuracy was determined by sample titration. This titration used PSPP-MG, PSPP-AG, and phenolphthalein as indicators. The titration was carried out for samples without spikes (HCl samples and CH_3COOH samples) and spike samples (HCl samples spiked with concentrated HCl and CH_3COOH samples spiked with concentrated CH_3COOH).

Precision test : for precision test, all of these titrations were carried out repeatedly.[34]

Determination of H^+ in sample using PSPP as a titration indicator

HCl-NaOH/NH_4OH titration: A number of 10 mL of HCl solution was placed in the titration flask, dripped with three drops of PSPP-MG, and titrated with NaOH 0.1025 M. The titration process was carried out until the PSPP-MG changed from red to blue. This titration was repeated seven times. The same titration was also carried out for PSPP-AG and phenolphthalein as indicators. The same procedure was carried out for the NH_4OH 0.1024 M.[23, 29] HCl sample titration: this titration follows the HCl-NaOH/NH_4OH procedures.

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HCl sample titration: The same procedure as described above was carried out for H^+ determination in HCl sample.

Data Analysis

Stability

PSPP stability was analyzed descriptively based on its colour stability over a certain period of time.[9]

Precision

The precision of PSPP was analyzed based on repeated titrations. Precision was analyzed using the coefficient of variation, CV (%), shown in equation 1. PSPP is declared precise as an indicator if the coefficient of variation obtained is at most 2%. This is required by the Association of Analytical Communities.[34]

$$\text{CV}\ (\%)\ \frac{\text{SD}}{\bar{x}}\ .\ 100\%\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Where,

CV : coefficient of variation (%)

SD : standard deviation

$\bar{x}$: the average of titrant volume (mL)

Accuracy

The Accuracy of PSPP was analyzed based on the recovery, R (%) of the analyte, shown in equation 2. PSPP is declared accurate as an indicator if the percentage recovery obtained is 90%–108%. This is required by the Association of Analytical Communities.[34]

$$\text{R}(\%) = \frac{\text{concentration of spike sampel} - \text{concentration of sample without spike}}{\text{spike concentration}}\ .\ 100\%\ \ldots\ldots\ldots\ldots\ (2)$$

Mean Values of NaOH and Standard Deviation

The mean values of NaOH and the standard deviation data were analyzed using descriptive statistical analysis.

Results and Discussion

Phytochemical content

PSPP-MG and PSPP-AG were obtained show the same of phytochemical content (Table 2). This indicates that the chemical content of the two pigments is the same despite being extracted with different grades of ethanol. This suggests that the extraction solvent used has no effect on the chemical components extracted but only affects to amounts of chemical components.[29]

Colour character of PSPP

The colour character of PSPP-MG and PSPP-AG in solution of different pH are show in Figure 1. PSPP-MG and PSPP-AG gave the same colour change in solution pH 1-14. They are red in a strong acid, pink in a weak acid, purple in neutral, blue to green in a weak to moderate base, and yellow in a strong base. PSPP colour change is unique compared to phenolphthalein. This observation is similar to that reported by Bria *et al*. (2021).[14] The similar colour change pattern of PSPP-MG and PSPP-AG may be attributed to the similarity in their phytochemical constituents,[29] as confirmed in Table 2. The phytochemical component that contributed to the performance of purple sweet potato pigments as indicators are flavonoids of which anthocyanins are a major culprit.[10,19,27]

Anthocyanins in PSPP are good proton donors and proton acceptors, and as such can show multiple colour changes in variable pH media,[16,17] as shown in Figure 1. In strong acids (pH 1-2), anthocyanins protonate to flavylium cations (AH^+). It is the most dominant species and stable at this pH. In a weak acid pH 3-6, the flavylium cations are hydrated to carbinol (B), a colour less species. The formation of this species causes a decrease in AH^+ concentration, which led to a colour change from red to pink. In neutral solution (pH 7), flavylium cations are deprotonated to a quinoidal base (A), which is observed as a purple colour ed species.[35, 36] At pH 8–9, the quinoidal base is ionized, and so changes colour to blue. At pH 10-11, it changes to green. According to Março et al. (2011),[37] on weak to moderate bases, quinoidal bases ionize into quinodial base ions (A^-) and quinodial base diions (A^{2-}). In this study, quinodial base ions were observed as blue species, and quinodial base diions were observed as green species. In a strong base, at pH 12-14, it exists in yellow chalcone (C). Figure 2 shows the main anthocyanin pH equilibria.

Stability, precision, and accuracy of PSPP as a titration indicator

PSPP-AG and PSPP-MG were stable for 90 days of storage. They also gave a constant colour change at the end point of the titration (Table 3). Precision is expressed as the coefficient of variation, CV (%). The coefficient of variation of the phenolphthalein indicator was found to be 0.18% and 0.61% for the weak and strong acid titrations, respectively, and these values were lower than that obtained for PSPP-AG and PSPP-MG, which ranged from 0.89 to 1.17% (Table 4). Although the coefficient of variation of PSPP-AG and PSPP-MG were higher than phenolphthalein, they were below below 2%, which indicated that PSPP-AG and PSPP-MG have good precision for all types of titrations.[34]

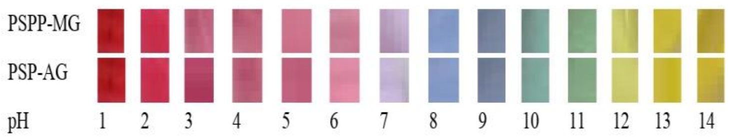

Figure 1: Colour change of (a) PSPP-MG and (b) PSPP-AG in solutions of pH 1-14

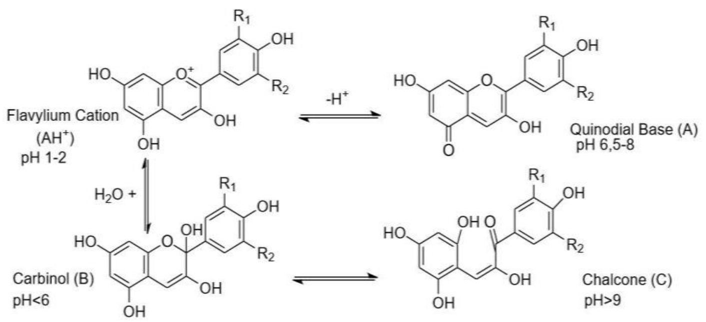

Figure 2: The main anthocyanin equilibria based on pH [41]

Table 2: Phytochemical content of PSPP

Phytochemical content	Observation	PSPP-MG	PSPP-AG
Alkaloids	White precipitate with Mayer reagent and blue precipitate with Wagner reagent	+	+
Flavonoids	A bluish green formed	+	+
Saponins	A foam-formed	+	+
Steroids	A green colour formed	+	+
Tannins	Greenish black precipitate	+	+
Triterpenoids	No yellow, red, or orange colour was observed	-	-

(+): Present, (-): Absent

Table 3: Stability of PSPP

Time (Day)	Colour of PSPP	Colour change at the end point of titration		
		HCl vs NaOH	HCl vs NH4OH	CH_3COOH vs NaOH
0	Red	Red to blue	Red to blue	Pink to blue
1	Red	Red to blue	Red to blue	Pink to blue
3	Red	Red to blue	Red to blue	Pink to blue
7	Red	Red to blue	Red to blue	Pink to blue
30	Red	Red to blue	Red to blue	Pink to blue
60	Red	Red to blue	Red to blue	Pink to blue
90	Red	Red to blue	Red to blue	Pink to blue

Table 4: Precision of PSPP

Type of Titrations	CV (%)		
	PP	PSPP-AM	PSPP-MG
HCl Sample-NaOH	0.61	0.90	0.90
HCl Sample-NH_4OH	0.61	0.97	0.97
CH_3COOH Sample- NaOH	0.18	0.89	1.17

Table 5: Accuracy of PSPP

Type of Titrations	Recovery (%)		
	PP	PSPP-AM	PSPP-MG
HCl Sample-NaOH	102.48	102.44	102.44
HCl Sample-NH_4OH	102.54	102.49	102.38
CH_3COOH Sample-NaOH	70.18	70.34	70.34

On the other hand, the accuracy is expressed as recovery (%). Table 5 shows the recovery of H^+ concentration from each type of titration. In the titration of HCl sample vs NaOH and HCl sample vs NH_4OH, the recovery of H^+ concentration using the three indicators was almost the same, namely; 102.48%, 102.44%, and 102.44% for PP, PSPP-AG, and PSPP-MG, respectively for titrations of HCl vs NaOH. For the titration of HCl vs NH_4OH, the recovery of H^+ concentration using the three indicators were 102.54%, 102.49%, and 102.38% for PP, PSPP-AG, and PSPP-MG, respectively. It is important to note that the recovery of H^+ concentration using the three indicators is between 90% and 108%, which means that PSPP-AG and PSPP-MG are accurate indicators for this titration.[23,34] The recovery of H^+ concentration in the titration of CH_3COOH vs NaOH with PP as indicator was 70.18%, while for the PSPP-AG and PSPP-MG as indicators was 70.34% each. This data indicates that these indicators are not accurate for this type of titration.[34]

Determination of H+ in sample using PSPP as a titration indicator

The colour changes of PSPP-MG and PSPP-AG as titration indicators are shown in Tables 6 and 7. They both displayed a red colour in HCl solution before titration. They became blue at the endpoint of the titration. The colour change occured because at the endpoint, there is an excess of NaOH or NH_4OH in the solution, which causes an increase in the pH of the solution. According to Março et al. (2011),[37] in a weak to moderate alkaline solution, anthocyanins exist in quinodial base ionized species (A^- and A^{2-}), which are observed as blue at the endpoint. The colour change of PSPP before titration and at the endpoint of the titration is confirmed by the data in Fig. 1.

Table 6, shows the volume of NaOH used in titration and the concentration of H^+ based on the calculated data. It can be seen that the mean volume of NaOH used in the titration with the three indicators gives similar values, as well as the standard deviation. The concentration of H^+ for the titrations with the three indicators also gives similar values. Likewise with the data in Table 7. These data show that the performance of PSPP-MG and PSPP-AG were identical to phenolphthalein as an indicator for HCl-NaOH and HCl-NH_4OH titration. [21]

The use of PSPP as a titration indicator was extended to determine the concentration of H^+ in the HCl sample. This is important because it was envisaged that there could be matrices in the sample that might interfere with the performance of PSPP as an indicator. The concentration of H^+ in the HCl sample are presented in Tables 8-9. The HCl sample was titrated with NaOH (Table 8) and NH_4OH (Table 9). The concentrations of H^+ were the same in both titrations using PP, PSPP-AG, and PSPP-MG as indicators. This observation shows that PSPP-AG and PSPP-MG have the same performance as phenolphthalein. In addition, the results indicate that the matrices in the samples analyzed do not affect the performance of PSPP as a titration indicator. It also indicates that both grade of ethanol used for extraction do not affect the PSPP performance as an acid-base titration indicator because the two grades of ethanol were capable of extracting similar chemical components.[29]

Conclusion

The study has shown that PSPP exhibit different colours in different pH ranges. The pigments (PSPP-MG and PSPP-AG) extracted with different grades of ethanol had the same phytochemical constituents, they were stable at room temperature, they showed good precision, accuracy, and performance, which were comparable to phenolphthalein for H^+ determination in HCl solutions. Therefore, PSPP-MG and PSP-AG can be applied as titration indicators for the determination of H^+ in HCl sample. PSPP is not expensive, can easily be obtained, and is environmentally friendly. Hence, its use as a titration indicator can reduce the use of synthetic chemicals, and promote green chemistry.

Conflict of Interest

The authors declare no conflict of interest.

Table 6: Titration of HCl solution with NaOH 0.1025 M

Titrant	Indicator	Mean Values of NaOH ± SD (mL)[a]	Colour change	Concentration of H^+ (M)
10 mL HCl	PP	10.0286 ± 0.0488	Colour less to pink	0.1028
	PSPP-AG	9.9286 ± 0.0488	Red to blue	0.1018
	PSPP-MG	9.9286 ± 0.0488	Red to blue	0.1018

[a]All values are means ± SDs from seven replications
SD – Standard deviation

Table 7: Titration of HCl solution with NH_4OH 0.1024 M

Titrant	Indicator	Mean Values of NH_4OH ± SD (mL)[a]	Colour change	Concentration of H^+ (M)
10 mL HCl	PP	10.0143 ± 0.0378	Colour less to pink	0.1026
	PSPP-AG	9.9286 ± 0.0488	Red to blue	0.1017
	PSPP-MG	9.9286 ± 0.0488	Red to blue	0.1017

Table 8: Titration of HCl sample with NaOH 0.1025 M

Titrant	Indicator	Mean Values of NaOH ± SD (mL)[a]	Colour change	Concentration of H^+ (M)
10 mL of sample	PP	4.0157 ± 0.0270	Colour less to pink	0.0412
	PSPP-AG	4.0143 ± 0.0244	Red to blue	0.0411
	PSPP-MG	4.0143 ± 0.0244	Red to blue	0.0411

Table 9: Titration of HCl sample with NH_4OH 0.1024 M

Titrant	Indicator	Mean Values of NH_4OH ± SD (mL)[a]	Colour change	Concentration of H^+ (M)
10 mL of sample	PP	4.0143 ± 0.0244	Colour less to pink	0.0411
	PSPP-AG	4.0143 ± 0.0244	Red to blue	0.0411
	PSPP-MG	4.0143 ± 0.0244	Red to blue	0.0411

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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