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Anthocyanins from *Hibiscus sabdariffa*, *Melastoma malabathricum* and *Ipomoea batatas* and its color properties

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Abstract

There are many factors influencing the stability and color variation of natural colorant anthocyanin and pH is among the most significant factor. This study aims to determine the stability of the anthocyanins in freeze-dried *Hibiscus sabdariffa*, *Melastoma malabathricum* and *Ipomoea batatas* in various acidic pH (pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5). Total monomeric anthocyanin, degradation index, color density and percent polymeric color were analyzed to determine anthocyanins degradation and their color variations. Among the samples, *H.sabdariffa* contain the highest monomeric anthocyanins (163.3 mg/L) followed by *M. malabathricum* (49.9 mg/L) and the lowest is *I.batatas* (13.8 mg/L). Monomeric anthocyanins from *I.batatas* were found to be very stable and not affected by changes in pH than in *H. sabdariffa* and *M. malabathricum*. However, degradation index (DI) of H. sabdariffa was the lowest with value of 0.365 ± 0.049 at pH 3.5. The lowest percentage of polymeric color (4.94 ± 0.64) was also shown by *H. sabdariffa* at pH 2.5 and maintained a deep red color with increasing pH indicating higher color stability compared to *M. malabathricum* and *I. batatas*. Overall, natural pigment in *H. sabdariffa* was found to be the most stable in both monomeric anthocyanin content and chromaticity properties. These results provided information that will further proven the potential usage of *H. sabdariffa*, *M. malabathricum* and *I. batatas* as natural coloring agents to replace the synthetic colorant in food and beverage industries.

Introduction

Anthocyanins are an important group of water-soluble plant pigments commonly found in various fruits and vegetables. The interest arises due to the pigments wide range of attractive color spectrum from shiny orange, pink, red, purple and blue that has great potential for use as natural food colorant to replace synthetic dyes. However, the pigment is not stable due to processing and storage conditions. Several factors influencing the pigment stability have been investigated such as species, environmental and agronomic conditions; extraction and processing parameters such as pH, storage temperature, concentration, chemical structure, light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions (Rein, 2005; Patras *et al*., 2010; Cavalcanti *et al*., 2011). Among these factors, pH is one of the major factors significantly influenced the pigment color variations and stability.

In general, anthocyanins are more stable in acidic media at low pH values than in alkaline solutions (Rein, 2005). Cavalcanti *et al*. (2011) explained the anthocyanins' color shift based on their chemical structures. In aqueous solutions, anthocyanins exist basically in the form of four species in equilibrium depending on the pH: quinonoidal base (QB), flavylium cation (FC), carbinol or pseudobase (PB) and chalcone (CH). Under acidic conditions (pH < 2), the anthocyanins exist primarily in the form of deep-red flavylium cation (FC). Increasing pH values causes rapid loss of the proton producing blue or violet quinonoidal base forms (QB). At the same time hydration of flavylium cation (FC) occurs, generating the carbinol or pseudobase (PB) which reaches equilibrium slowly with the colorless chalcone (CH). The relative amounts of FC, QB, PB and CH forms at the equilibrium condition vary according

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to pH (Cavalcanti *et al*., 2011). In other words, the anthocyanins ionic nature enables reversible changes of the molecule structure according to the prevailing pH, resulting in different colors and hues at different pH values. It is believed that through pH adjustment, the natural stabilization process for anthocyanins can be achieved, thus the knowledge may become of great value to food manufacturers.

Hibiscus sabdariffa L. also known as roselle is widely grown in Malaysia and other countries such as Indonesia, Africa and America. Its calyxes were brilliant red in color due to its anthocyanins compound; delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside which are the non-methylated type (Castaneda-Ovando *et al*., 2009). *Melastoma malabathricum* L. is a plant use in traditional Malay folk medicine for treatment of diarrhea, post-partum treatment, dysentery, toothache, flatulence and haemorrhoids (Susanti *et al*., 2007; Sunilson *et al*., 2008). The pulp of *M.malabathricum* fruit is dark red-blue or purple in color and contains many small white colored seeds. Two major anthocyanins aglycon in *M. malabathricum* are cyanidin-3-glucoside and cyanidin-3, 5-diglucoside (Koay, 2008). Anthocyanins content in *Ipomoea batatas* L. varies depending on the varieties with purple sweet potatoes having the highest value compared to the white, yellow and orange variety (Terahara *et al*., 2004). Majority of anthocyanins in *I.batatas* are of the mono- or di-acylated forms of cyanidin and peonidin (Pascual-Teresa *et al*., 2002).

There are many research been conducted on *H.sabdariff*a extract, however most focused on its antioxidant activity (Tsai *et al*., 2002; Tsai and Huang, 2004; Hirunpanich *et al*., 2006; Norhaizan *et al*., 2010) rather than on their anthocyanins pigment stability and application in food products. While for *I.batatas* and *M.malabathricum*, most of the research conducted on the identifications and characterization of their anthocyanins structure (Goda *et al*., 1997; Giusti and Wrolstad, 2003; Terahara *et al*., 2004; Janna *et al*., 2006). Hence, the objective of this study is to determine the stability of the anthocyanins in freeze-dried *H. sabdariffa*, *M. malabathricum* and *I. batatas* at various acidic pH.

Materials and Methods

Raw materials

H. sabdariffa was obtained from FAMA Rengit, Johor, *M. malabathricum* was collected from a farm in Muar, Johor while *I. batatas* was purchased from Banting, Selangor. Analytical chemicals such as sodium acetate and potassium chloride were purchased from Merck (Darmstadt, Germany), sodium carbonate anhydrous from Fluka (Buchs, Switzerland), cyanidin-3-glucoside standard from Sigma Chemical Co. (Steinheim, Germany), sodium metabisulphite from Ajax Finechem (Auckland, New Zealand), concentrated hydrochloric acid from R&M Chemicals (Essex, United Kingdom).

Sample preparation

All the samples were washed and cleaned prior to further treatments. For *M. malabathricum* and *I. batatas*, the outer skin of the fruit and root were peeled off manually, while for *H. sabdariffa*, the seeds were removed. The aqueous extraction was carried out according to the method of Pin-Der and Gow-Chin (1997) with slight modification in water: sample ratio. Two hundred milliliter distilled water was added to 100 g of sample and boiled for 10 mins. After boiling, the sample was blended and filtered using Whatman No. 4 filter paper in a Buchner funnel under vacuum. The filtrate was then evaporated using a rotary evaporator (R-200, Buchi Rotavapor, Switzerland) at 60^{o}C until less than 10% of the initial volume remained. The anthocyanins' content and pH value of the natural aqueous extract from each sample were recorded. The extracts were then frozen at -25^{o}C and freeze-dried (Alpha 1-4 LD plus, Christ, Germany). The dried product obtained was ground into fine powder and kept refrigerated at 4°C in an air-tight amber bottle prior to further analysis.

Analysis of monomeric anthocyanins

The monomeric anthocyanins contents of samples were determined using the pH-differential method as described by Giusti and Wrolstad (2001). The wavelength of maximum absorption was 520 nm for anthocyanins. Spectrophotometric measurements were carried out using a double-beam spectrophotometer (Helios-α, ThermoSpectronic, Cambridge, England) at 420 nm, 524 nm, and 700 nm. Calculations were based on cyanidin-3-glucoside with extinction coefficient of 26900 and molecular weight of 449.2. Quartz cuvettes of 1 cm pathlength were used and all measurements were carried out at room temperature (±25^{o}C). Absorbance readings were made against distilled water as a blank.

$$\text{Anthocyanin pigment (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

where $A = (A_{524\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{524\ nm} - A_{700\ nm})_{pH\ 4.5}$

DF = dilution factor l = pathlength in cm

ε = molar extinction coefficient 10^3 = factor for conversion from g to mg.

Determination of degradation index (DI)

DI is the ratio between monomeric and total anthocyanins content in sample, calculated at absorption 420 nm, 524 nm and 700 nm using the following formula:

$$\text{Degradation index} = [(A_{524nm} - A_{700\ nm})_{pH1}] - [(A_{524nm} - A_{700\ nm})_{pH1} - (A_{524nm} - A_{700\ nm})_{pH\ 4.5}]$$

Determination of polymeric color percentage and color density

Polymeric color was determined using the bisulfite-bleached treatment and calculated using the following equation using absorption at 420 nm, 524 nm and 700 nm.

$$\text{Polymeric color (bisulfite-treated)} = [(A_{420\ nm} - A_{700\ nm}) + (A_{524nm} - A_{700\ nm})] \times DF$$

Color density is defined as the sum of absorbencies at the maximum wavelength and at 420 nm, calculated using the following formula:

$$\text{Color density} = (A_{420\ nm} - A_{700\ nm}) + (A_{524nm} - A_{700\ nm})$$

The ratio between polymerized color and color density is used to determine the percentage of the color that is contributed by polymerized material (Giusti and Wrolstad, 2003). Percent polymeric color was calculated using the formula:

$$\%\ \text{Polymeric color} = (\text{Polymeric color} / \text{color density}) \times 100$$

Chromaticity determination

The changes in color of the extracts were determined using chromameter (CR 400, Konica Minolta, Japan) based on CIELab color space with standard illuminator of D65, observation angle of 2° visual field and 1 cm pathlengh quartz cell. Within the CIELAB uniform space, a psychometric index of lightness, L^* (ranging from 0, black, to 100, white), and two color coordinates, a^* (which takes positive values for reddish color and negative values for greenish ones) and b^* (positive for yellowish color and negative for bluish ones) are defined. From these coordinates, other color parameters are defined: the chroma (C^*) is the quantitative attribute of color intensity and the hue angle (H^o) is the qualitative attribute of the color. The measurements were repeated five times for each samples and averaged for statistical analysis.

$$\text{Chroma, } C = (a^{*2} + b^{*2})^{½}$$
$$\text{Hue angle, } H^o = (\tan^{-1} a^*/b^*)$$

Statistical analysis

All data were reported as mean ± standard deviation of triplicate readings in every analysis. Experimental data were analyzed by the one-way analysis of variance (ANOVA) SPSS version 15.0 computing program. Significant differences between means were determined by using the Tukey's test.

Results and Discussion

The effect of pH on the anthocyanins content of three plants samples (*H. sabdariffa*, *M. malabathricum* and *I. batatas*) was studied at six different pH (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5) at 25°C. pH of samples were adjusted using citric acid-Na_2HPO_4 buffer. The range of acidic conditions were selected within the pH range of acidic food products such as preserves, jams, jellies, beverages and juices.

Monomeric anthocyanin content in H. sabdariffa, M. malabathricum and I. batatas at various acidic pHs

The monomeric anthocyanins content were determined using the pH-differential method that relies on the structural transformation of the anthocyanin chromophores as a function of pH (Giusti and Wrolstad, 2003). All three samples have different anthocyanins structural forms and therefore to avoid confusion when comparing the anthocyanins content between samples, cyanidin 3-glucoside standard was used. The molar extinction coefficient and molecular weight was based on the standard properties, hence the calculated monomeric content is expressed as cyanidin 3-glucoside equivalents.

Figure 1 shows the effect of pH on the content of monomeric anthocyanins in *H. sabdariffa*, *M. malabathricum* and *I. batatas*. The initial pH value of the natural aqueous extract of *H. sabdariffa*, *M. malabathricum* and *I. batatas* were 2.2, 4.6 and 6.0, respectively. Among the samples, *H. sabdariffa* contained the highest monomeric anthocyanins (163.32 ± 8.17 mg/L), which was 3 times higher than *M. malabathricum* (49.90 ± 2.50 mg/L) and 12 times higher than I. batatas (13.80 ± 1.25 mg/L).

For freeze-dried *H. sabdariffa*, reduction in pH to 2.0 from the initial pH value of aqueous extract (pH 2.2) caused 2% decrease in monomeric anthocyanins content. While, increase in pH to 2.5 caused 6% decrease in the monomeric anthocyanins. Further increase in pH from 2.5 to 4.0 showed no significant different ($p>0.05$) in the monomeric content. However, the monomeric anthocyanins at pH 4.5 gave almost similar amount to those obtained at pH 2.0, indicated that the anthocyanins structure were reversible between the pH. Overall, the result

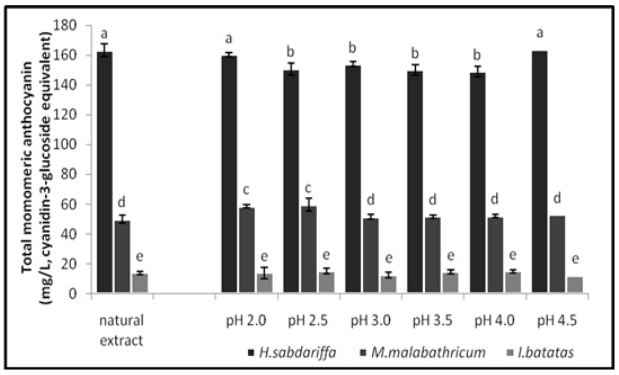

Figure 1. Monomeric anthocyanin content in *H. sabdariffa*, *M. malabathricum* and *I. batatas* at acidic pH values

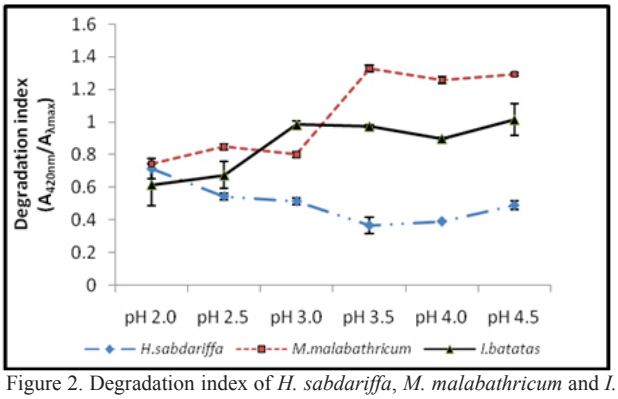

Figure 2. Degradation index of *H. sabdariffa*, *M. malabathricum* and *I. batatas* at increasing pH

showed that freeze-dried *H. sabdariffa* extract was stable in the acidic pH range studied. The monomeric anthocyanin content was only slightly affected, less than 10% by pH changes. This finding was in contrast with the results obtained by Chumsri *et al*. (2008), where they reported that there was a considerable anthocyanin destruction caused by the narrow change in pH during optimization treatment of *H. sabdariffa* extraction.

The pH of freshly prepared aqueous extract of *M. malabathricum* had pH of 4.6, which is close to subacid range. Lowering the pH to 2.0 for freeze-dried *M. malabathricum* caused the monomeric anthocyanins content (58.5 ± 2.93 mg/L) to increase about 15%. This shows that *M. malabathricum* anthocyanins were stabilized either due to lowering of pH or the freeze-drying process that caused dehydration of the extract. Further increase to pH 2.5 showed no significant different ($p>0.05$) in the monomeric anthocyanin content. However increase in pH from 3.0 to 4.5 showed reduction of monomeric anthocyanins value about 12% compared to pH 2.0 and 2.5. Similar results obtained by Janna *et al*. (2006) for anthocyanins pigments at different flower developmental stages of *M. malabathricum*. The suitable storage conditions suggested for the flower anthocyanin pigments is in a very low acidic solution of pH 0.5 to 1.0 (Jana *et al*, 2006). However, results in this study revealed that anthocyanins in *M. malabathricum* fruit were stable at pH below 2.5.

For *I. batatas*, the initial fresh aqueous extract contains 13.80 ± 1.25 mg/L of monomeric anthocyanins at pH 6.0. pH adjustment to lower acidic values from 2.0 to 4.5 in freeze-dried *I.batatas* extract did not show any significant different ($p>0.05$) in monomeric anthocyanins content. Therefore, monomeric anthocyanins from I. batatas were very stable and not affected by changes in pH. Similar finding were reported by Fan *et al*. (2008a), where anthocyanins from *I. batatas* were more stable under acid conditions (pH 2.0 to 4.0) than in the subacid range (pH 5.0 to 6.0).

Overall, monomeric anthocyanins in *I.batatas* were more stable to pH changes than in *H. sabdariffa* and *M. malabathricum*. *H.sabdariffa* anthocyanidin are a mixture of cyanidin and delphinidin structures, while *M. malabathricum* anthocyanidin is composed of only cyanidin structures. Anthocyanidins in *I. batatas* were of cyanidin and peonidin structures acylated with caffeic, ferulic or p-hydroxybenzoic acids (Terahara *et al*., 2004). Extracts containing acylated anthocyanins are known to be more stable than those extracts with non-acylated anthocyanins. Giusti and Wrolstad (2003) stated that acylation of anthocyanins molecule improve their stability through intramolecular and/or intermolecular copigmentation and self-association reactions. The presence of acyl groups in an anthocyanin molecule hinders hydrolysis of the red flavylium cationic form of the colorless carbinol base. According to Hee *et al*., (2011), acidic pH below 3.5 is necessary to obtain the desired red color and stability of anthocyanins. However, results in this study indicates that anthocyanins from *H. sabdariffa* and *M. malabathricum* were only slightly affected by pH and quite stable up to pH 4.5 with no great reduction in their monomeric content while I. batatas was not affected at all by pH changes.

Degradation Index of H. sabdariffa, M. malabathricum and I. batatas at various acidic pH

Degradation index (DI) is the ratio between monomeric and total anthocyanins contents which includes the monomeric and polymeric compounds in the sample. Basically, DI accounts for three components of degradation, first by an increase in absorbance due to browning, secondly by a decrease in absorbance due to formation of colorless carbinol bases and finally by the effect of bathochromic shifts due to anthocyanin structure evolving into less stable forms (Patras *et al*., 2010). Increase in DI is indicated by the large increased in absorbance at 420 nm (λ

Table 1. Color density of *H. sabdariffa*, *M. malabathricum* and *I. batatas* at various pHs

Sample	*H. sabdariffa*	*M. malabathricum*	*I. batatas*
natural extract	43.76±7.11^{c}	5.56±0.30^{d}	1.39±0.15^{b}
pH 2.0	69.44±2.57^{b}	4.07±1.51^{f}	7.49±1.51^{a}
pH 2.5	112.99±1.82^{a}	5.35±0.61^{e}	6.10±0.46^{a}
pH 3.0	54.25±8.32^{c}	14.66±2.12^{b}	5.99±1.21^{a}
pH 3.5	22.89±5.14^{e}	14.66±0.30^{c}	6.74±0.45^{a}
pH 4.0	31.67±0.30^{d}	14.77±1.21^{ac}	5.56±0.61^{a}
pH 4.5	19.02±2.72^{e}	13.48±0.13^{a}	2.89±0.45^{b}

Data are mean ± SD (n=3).
Means within same column having different letters showed a significant different ($p<0.05$)

Table 2. Polymeric color percentage of *H. sabdariffa*, *M. malabathricum* and *I. batatas* at various pH values

Sample	*H. sabdariffa*	*M. malabathricum*	*I. batatas*
Natural extract	5.38±1.21^{e}	19.40±0.85^{d}	98.81±21.89^{a}
pH 2.0	7.55±0.49^{d}	7.76±0.22^{f}	22.08±7.66^{c}
pH 2.5	4.94±0.64^{e}	13.82±1.00^{e}	57.22±10.07^{b}
pH 3.0	10.16±1.28^{c}	42.25±7.34^{c}	27.60±9.58^{c}
pH 3.5	27.65±4.89^{a}	77.90±6.88^{a}	32.12±11.14^{c}
pH 4.0	7.43±0.88^{d}	73.45±1.41^{a}	42.26±0.84^{c}
pH 4.5	16.28±2.23^{b}	59.73±4.42^{b}	121.67±20.07^{a}

Data are mean ± SD (n=3).
Means within same column having different letters showed a significant different ($p<0.05$)

for brownish/colorless absorbance) and a decrease of absorbance at 520 nm (λ for highest red color absorbance).

Figure 2 shows the DI values of *H. sabdariffa*, *M. malabathricum* and *I. batatas* at different pH. From the figure, *M. malabathricum* and *I. batatas* showed an increasing trend in DI with an increased in pH values but the opposite trend was shown by *H. sabdariffa*. The *M. malabathricum* anthocyanins was the most susceptible to degradation since the DI showed an increment up to 78% at higher pH (pH>3.5) which indicates that pH significantly ($p<0.05$) intensifies monomeric degradation rate. For *I.batatas*, the DI value increased to 64% at pH 4.5 from the initial pH 2.0. The DI values for *H. sabdariffa* showed an opposite trend; it decreases with the increase in pH. The *H. sabdariffa* DI decreased as much as 32% from pH 2.0 to pH 4.5. These results pointed out that anthocyanins in *H. sabdariffa* are more stabilized as the pH increases. This observation is consistent with the monomeric anthocyanins content shown in Figure 1. The DI for *M. malabathricum* also shows consistent results with the monomeric content in Figure 1 in which the DI increase and monomeric content decrease with the increase in pH. This indicates the formation of browning compounds in *M. malabathricum* anthocyanins with the increase in pH hence causes DI value to increase. However for *I. batatas,* the DI value was inconsistent with the results shown in Figure 1. Based on Figure 1, monomeric anthocyanins in I.batatas were not affected by the increase in pH. Therefore the DI value should either shows a constant or a decreasing trend. This inconsistency may be explained by the different degradation components that contributed to the DI value as stated by Patras *et al*. (2010). Perhaps, the DI obtained in *I. batatas* was not entirely contributed by the increase in absorbance due to browning but rather by the combinations of evolving anthocyanins structure to colorless carbinol and bathochromic shift. Further detailed studies should be conducted to ascertain the actual factors contributing to the inconsistency in results between DI and monomeric content for *I. batatas*.

Color density and polymeric color percentage of H. sabdariffa, M. malabathricum and I. batatas at various acidic pH

Color density is defined as the sum of absorbencies at the maximum wavelength. Table 1 shows the color density of the natural extract of *H. sabdariffa*, *M. malabathricum* and *I. batatas* and their freeze-dried extracts at different pH. The color density values of natural aqueous extract shows that *H.sabdariffa* possessed the highest color density followed by *M. malabathricum* and *I. batatas*.

Table 1 shows that freeze-dried *H. sabdariffa* have the highest color density at pH 2.5 (112.99 ± 1.82) and the lowest at pH 4.5 (19.02 ± 2.72). For *M. malabathricum*, higher color density was observed within the pH range of 3.0 to 4.5 with a drastic increase about 3 fold between pH 2.5 (5.35 ± 0.61) and pH 3.0 (14.66 ± 2.12). For *I. batatas*, color density values remain constant between pH 2.0 to pH 4.0 and then decrease by half at pH 4.5. This indicates that color density may not be a suitable measurement to demonstrate the effects of pH on the anthocyanins colors. According to Tsai and Huang (2004), the decrease in color density can be associated with high DI value. But, based on these results, no such relationship exists between color density and DI in the sample.

The polymeric color percentage was calculated to determine the level of polymerization as it was part of the reactions that contribute to the increase in DI. Initially, fresh sample has most of its anthocyanins in the monomeric form and upon extraction and processing; polymerization occurs (Cevallos-Casals and Cisneros-Zevallos, 2004). Polymeric color percentage would give an indication of the red color contribution before polymerization of monomeric pigments and finally turn into a brownish color. Therefore, the higher percentage of polymeric color indicates higher degradation rate and reduction in color intensity.

Table 2 shows that polymerization reactions was most severe in *M. malabathricum* compared to *H.*

Table 3. Color properties of roselle, senduduk and PSP at different pH values

	Lightness			Chroma			Hue angle		
Sample	roselle	*senduduk*	PSP	roselle	*senduduk*	PSP	roselle	*senduduk*	PSP
Natural extract	20.95±0.33^{de}	21.45±0.19^{d}	30.95±0.08^{b}	14.55±0.48^{b}	5.04±0.14^{g}	1.35±0.06^{f}	28.64±0.34^{a}	9.27±0.44^{f}	323.72±2.08^{g}
pH 2.0	21.49±0.19^{cd}	22.77±0.11^{c}	29.48±0.29^{d}	15.84±0.48^{a}	14.11±0.31^{a}	8.94±0.18^{a}	28.02±0.32^{b}	31.27±0.16^{d}	354.48±0.28^{a}
pH 2.5	20.3±0.46^{e}	22.98±0.40^{c}	29.77±0.65^{cd}	16.4±1.90^{a}	13.34±0.31^{b}	8.75±0.30^{a}	27.96±0.35^{b}	30.59±0.16^{e}	351.02±0.39^{b}
pH 3.0	22.18±0.11^{bc}	24.06±0.34^{b}	30.95±0.09^{b}	16.19±0.30^{a}	11.14±0.30^{c}	6.65±0.21^{b}	26.56±0.19^{c}	30.62±0.52^{e}	347.17±0.22^{c}
pH 3.5	21.87±0.09^{c}	24.63±0.09^{b}	30.01±0.55^{c}	14.05±0.29^{b}	9.77±0.15^{d}	4.63±0.06^{c}	20.46±0.23^{d}	34.41±0.41^{c}	342.33±0.55^{d}
pH 4.0	22.79±0.64^{b}	25.62±0.19^{a}	32.29±0.25^{a}	12.37±0.35^{c}	7.37±0.08^{e}	3.63±0.09^{d}	13.37±0.27^{e}	37.31±0.43^{b}	336.35±1.38^{e}
pH 4.5	25.99±1.21^{a}	25.4±1.50^{a}	30.96±0.39^{b}	6.48±0.43^{d}	6.76±0.09^{f}	2.23±0.09^{e}	5.89±0.99^{f}	42.83±0.50^{a}	332.73±1.00^{f}

Data are mean ± S.D. (n=6)
Means within same column having different letters showed a significant different ($p<0.05$)

sabdariffa and *I. batatas*. The lowest polymeric color of *M. malabathricum* was at pH 2.0 (7.55 ± 0.49%) increased about 10 fold (77.90 ± 6.88%) at pH 3.5. For *I. batatas*, the maximum increase of polymeric color is about 6 fold, while for *H. sabdariffa* about 4 fold. The *H. sabdariffa* natural extract had the lowest polymeric anthocyanin compared with *M. malabathricum* and *I. batatas*. Study by Tsai and Huang (2004) showed that anthocyanins extracts from *H. sabdariffa* exist mostly as monomers and after 2 hours of heating *H. sabdariffa* in model solution at 90^{o}C, the polymeric color was only 2.66%. Although their study indicates that anthocyanins structure in *H. sabdariffa* are thermally stable but our results showed otherwise. The polymerization in *H. sabdariffa* is higher than that reported by Tsai and Huang (2004).

For *I. batatas*, the percentage polymeric anthocyanin in the extract was 98.81±21.89 and increased to 23% at pH 4.5. These high values may presumably due to the denaturation process in *I. batatas* during extraction which involves loss of color followed by formation of brownish degradation products and insoluble compounds as stated by Fan *et al*. (2008b). The high value in its plant extract represent the anthocyanin that did not change color with increase of pH or bisulfite treatment (Wrolstad *et al*., 2005) plus its acylated anthocyanin properties assured to stabilize throughout pH changes. However, an increased in polymeric color percentage value is an indicative of either condensation reactions of anthocyanins with other phenolic compounds or oxidative degradation as reported by Patras *et al*. (2010).

Lightness, chroma and hue angle of H. sabdariffa, M. malabathricum and I. batatas at different pHs

The *H. sabdariffa*, *M. malabathricum* and *I. batatas* showed a decreasing trend of chroma value with increasing pH, indicating a slight decreased in the saturation of the red color in every sample (Table 3). The color changes of the sample could also have indicated a degradation of anthocyanin at increasing pH. This result was consistent with the DI of anthocyanin as in Figure 2. The *H. sabdariffa* had the highest chroma value at pH 2.0 compared to *M. malabathricum* and *I. batatas*. *H. sabdariffa* chroma value decreased to about half of the initial chroma at pH 4.5. The lowest chroma value was obtained in *I.batatas* with initial pH 2 (8.94±0.17) and decreased to 76% at pH 4.5. These changes of color in *I.batatas* were mainly due to formation of polymeric anthocyanin and some non-enzymatic browning pigments as suggested by Yang and Gadi (2008).

All samples exhibited a gradual increasing trend in lightness (L) with an increasing pH values (Table 3). The increased in L values could be related to the formation of translucent extracts due to the color fading. The *H. sabdariffa* sample showed a dark red color at pH 2.0 to 4.0 and was the darkest color among all samples as its lightness value was in the range of 20.30±0.46 to 25.99±1.21. The *M. malabathricum* sample also showed a dark shade of red color in the range of 22.77±0.11 to 25.40±1.50. The *I. batatas* recorded the highest L value at pH 4.0 (32.29±0.25) indicating a light colour solution was observed and the lowest at pH 2.0 (29.48±0.29). Both *M. malabathricum* and *H. sabdariffa* exhibited a reddish color and *I.batatas* exhibited a hue angle of purplish color (Table 3). The *H. sabdariffa* and *M. malabathricum* had reddish nuances with increasing pH values. It is noticeable that both *M. malabathricum* and *H. sabdariffa* displayed a red tone at all pH and had similar values at pH 2.0, 2.5 and 3.0. By increasing the pH until pH 4.5, the color of *M. malabathricum* extract gradually increased while that of *H. sabdariffa* decreased. In *I. batatas*, the hue angle decreased with increasing pH. The caffeoyl group acylated to anthocyanin and phenolic compound in *I. batatas* clearly influence the aglycone chromophore by inter/intramolecular association, creating more purple or bluish tones as shown by the hue angle. Based on both results, the *H. sabdariffa* extract was the most stable based on the highest chroma value and the lowest in lightness value as observed in (Table 3).

Conclusion

Results from this study showed that stability of anthocyanins in *H. sabdariffa*, *M. malabathricum* and *I. batatas* extract were not greatly affected by pH range between 2.0 to 4.5. The *H. sabdariffa* contained the highest monomeric anthocyanins while anthocyanins from *I. batatas* showed better stability at different acidic pH attributed by its acylation anthocyanins structure. The DI values have indicated that *H. sabdariffa* had the lowest degradation rate than *M. malabathricum* and *I. batatas*. The *H. sabdariffa* also showed the lowest % polymeric color formation and highest chroma values. Overall, the *H. sabdariffa* had good stability in both monomeric anthocyanin content and chromaticity properties hence should make anthocyanins in *H. sabdariffa* competitive as natural food color replacer.

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