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Lipote fruit anthocyanin-rich extracts as food colourants: extraction optimization and stability of natural and copigmented anthocyanins

[1,2,*]Rodriguez, R.G.,[1]Sagum, R.S. and [1]Trinidad, T.P.

[1]*The Graduate School, University of Santo Tomas, España, Manila, Philippines*
[2]*Department of Science and Technology – Food and Nutrition Research Institute, Bicutan, Taguig City, Philippines*

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Abstract

Natural pigments such as anthocyanins are currently being explored for food colouring applications due to safety issues associated with synthetic colourants. Lipote (*Syzygium polycephaloides*), an underutilized fruit in the Philippines, is found to contain high amounts of this natural pigment. Thus, this study aimed to optimize parameters for maximum anthocyanin extraction from these fruits by mixture design and response surface methodology followed by the evaluation of the extracts' stability using model beverage systems. Results revealed that the optimum solvent for maximum anthocyanin recovery was 55% acidified ethanol while optimum extraction temperature and time were 45.8°C and 92 mins, respectively. Stability tests showed that the lipote fruit extract, in its natural form, was most stable in the dark, at low pH (pH 3.0) and low storage conditions (4°C) with an extrapolated half-life ($t_{1/2}$) of 169.06 days. Anthocyanin stability was further enhanced by copigmentation with caffeic acid resulting in an even higher t½ of 223.60 days.

1. Introduction

Colour is one of the important characteristics of food affecting consumer acceptability (Gras *et al.*, 2017). It provides food with its distinct identity and also serves as a determinant of food quality (Sui *et al.*, 2016). Thus, the addition of regulated colourants or dyes is an acceptable practice for food colour improvement or restoration, particularly for colour changes or losses brought about by different processing conditions (Gras *et al.*, 2017).

Owing to their stability and intense colour, synthetic colourants are commonly used to improve food colour properties (Gordillo *et al.*, 2018). However, its use has been associated with several health safety concerns from attention disorders and hyperactivity in children to potential carcinogenicity (Zhang *et al.*, 2006; McCann *et al.*, 2007; Soares *et al.*, 2015). This has led to the exploration of natural pigments as food colourants with a demand even foreseen to increase at an annual rate of 10-15% in the global market (Tan *et al.*, 2018).

Among these natural pigments are anthocyanins whose fruit and vegetable sources are plentiful in nature (Chung *et al.*, 2016). Their red to blue vivid colours along with their water-soluble and non-toxic properties make this natural pigment suitable for food applications (Lao and Giusti, 2018). Aside from this, anthocyanins also exhibit antioxidative properties that have been linked to the reduction of risk of several diseases (Yamashita, *et al.*, 2017).

Lipote (*Syzigium polycephaloides*) are red to dark purple-coloured fruits considered among the indigenous and underutilized fruits in the Philippines (DA-BAR, 2012; DENR-ERDB, 2017; Ilano *et al.,* 2021). Neglected and/or underutilized crops are often indigenous plant species that are often used minimally compared to their innate potential (Mayes *et al.,* 2012). To improve the utilization of lipote, the Department of Agriculture – Bureau of Agricultural Research (DA-BAR) (2012) and the Department of Environment and Natural Resources - Ecosystems Research and Development Bureau (DENR-ERDB) (2017) promote the use of lipote in different food applications which would eventually translate into higher demand for its production and preservation.

As lipote fruits have been reported to contain high amounts of anthocyanins (Reynertson *et al.*, 2008), among its possible applications is its utilization as a source of food colourant. Its red colouration may possibly be used as an alternative to synthetic colourants with red hues such as Allura Red. However, there are

*Corresponding author.
Email: *regina.rodriguez.gs@ust.edu.ph*

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limited studies about the stability of the lipote fruit extract which would help establish food colouring potential. This information is of particular importance as anthocyanins' stability, in general, is affected by different factors such as temperature, light, pH and storage conditions (Sari *et al.*, 2012; Weber *et al.*, 2017; Fan *et al.*, 2019). Moreover, the addition of copigments as possible improvements of stability has not been explored from anthocyanins of this source. Several studies have reported copigmentation of anthocyanins with phenolic acids and flavonoids resulted in a more intense and stable colour through the copigment's protective action on the flavilium moiety towards hydration (Sari *et al.*, 2012; Weber *et al.*, 2017; Fan *et al.*, 2019). Caffeic acid, a hydroxycinnamic acid, was reported to be among the best copigments (Sari *et al.,* 2012; Fan *et. al.,* 2019).

Ideally, optimum conditions for maximum recovery of anthocyanins should also be known. Response surface methodology (RSM) is often used for optimization as this utilizes designed experiments in obtaining optimal response (Chaudhary and Mukhopadhyay, 2013). Instead of trying all possible experimental combinations, RSM will provide an experimental design or mathematical models useful to generate information on process interactions thereby resulting in improved product quality while reducing solvent consumption and other production costs (Chaudhary and Mukhopadhyay, 2013; Pedro *et al*., 2016). However, although numerous studies have been reported to optimize anthocyanins using the design of experiments, none, to the knowledge of the researcher have utilized lipote fruits as raw material.

Based on the aforementioned, this study explored the potential of lipote fruits as a source of anthocyanin-based food colourant. Specifically, this was through the optimization of solvent and process parameters for maximum extraction of anthocyanins from lipote fruits and evaluation of the stability of the extracted natural anthocyanins as affected by pH, storage conditions, light and heat using model beverage systems. Moreover, the stability of lipote anthocyanins when copigmented with caffeic acid was also assessed.

2. Materials and methods

2.1 Plant material preparation

Fully ripe lipote (*Syzygium polycephaloides*) fruits were collected from a residential town in Lopez, Quezon province, Philippines. These were then submitted to the Museum of Natural History, University of the Philippines, Los Baños, Laguna for identification and authentication.

Lipote fruits were washed in running water and allowed to dry. The seeds were manually removed using a knife and the remaining skin and pulp was subjected to freeze-drying (Heto Drywinner, 6551). Dried lipote fruits were ground into powder form using a food blender (Oster 10-speed blender) and homogenized by mixing thoroughly in a clean container. Homogenized samples were transferred to clean dark-coloured polyethylene sample bottles and stored at -20°C until extraction.

2.2 Extraction optimization of anthocyanins from lipote fruits

2.2.1 Solvent optimization

Solvent optimization was carried out according to the method of Arici *et al.* (2016) with minor modifications. Anthocyanins from powdered lipote fruits were extracted using mixtures of ethanol and deionized water acidified with 0.1M HCl (pH = 3.0). Using Design Expert Version 7.0 software, a simplex lattice mixture design was created treating ethanol and water as independent variables, and total anthocyanin content as the dependent response. The concentration of both ethanol and water for extraction ranged from 0-100%, adjusting each component such that the total composition remained 100%. Design points generated by the software for modelling are presented in Table 1.

Table 1. Anthocyanin content of lipote fruits extracted using different solvent combinations determined by simplex lattice mixture design

Run	Solvent Concentration		Total anthocyanin content
	Ethanol (%)	Water (%)	(mg C3G/g DW)
1	75	25	17.35±0.10^{d}
2	25	75	15.73±0.09^{c}
3	0	100	5.70±0.11^{a}
4	100	0	9.16±0.10^{b}
5	50	50	19.40±0.07^{e}
6	0	100	5.80±0.07^{a}
7	50	50	19.30±0.12^{e}
8	100	0	9.26±0.04^{b}

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$). C3G = Cyanidin-3-glucoside.

A total of 25 mL of each solvent combination was added to 3.0 g of powdered lipote fruit and was mechanically shaken for 2 hrs at 25°C. After shaking, extracts were subjected to filtration using Whatman filter paper no.1 and diluted with the acidified solvent combination (pH = 3.0) to a final volume of 50 mL. Immediately after extraction, all solutions were analyzed for total monomeric anthocyanin content (TMA).

2.2.2 Process optimization

Using the solvent identified to provide the highest anthocyanin yield from lipote fruits, the optimum temperature and time were also assessed through

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response surface methodology (RSM) as in Arici *et al.* (2016) with modifications. A central composite design with two factors and three levels was used with extraction temperature and time as the two independent factors. The experimental design also included five (5) central points utilized for the estimation of pure error. Extraction time was set at 30, 75, and 120 mins and temperature at 25, 37.5, and 50°C (Prakash Maran *et al.*, 2015, Pedro *et al.*, 2016; Arici *et al.*,2016).

Similarly, 3.0 g powdered lipote fruits were added with 25mL solvent identified in 2.2.1 and extracted using time and temperature combinations set by RSM. After which, extracts were filtered through Whatman filter paper No.1, diluted to 50 mL, and analyzed right away for TMA.

For the succeeding parts of the study, anthocyanins were extracted from lipote fruits using conditions identified in the aforementioned optimization. Extracts were concentrated through rotary evaporation at 40°C and stored at -20°C until analysis.

2.3 Determination of total monomeric anthocyanins

The total monomeric anthocyanin (TMA) content of lipote fruit extracts was quantified using AOAC Official Method 2005.02 pH differential method (AOAC, 2019). Test extracts were diluted in two sets of buffers: 0.025M KCl, pH 1 (±0.05) and 0.4M CH_3COONa, pH 4.5 (±0.05). The absorbances of the diluted test solutions were determined at 520nm and 700nm using a UV-VIS spectrophotometer (UV-1900, Shimadzu). TMA was calculated using equations 1 and 2 and was reported as mg cyanidin-3-glucoside/g (mg C3G/g):

$$A = (Abs_{520} - Abs_{700})_{pH1.0} - (Abs_{520} - Abs_{700})_{pH\ 4.5} \quad (1)$$

$$TMA\ (mg/L) = (A \times MW \times DF \times 1000) / (\varepsilon \times l) \quad (2)$$

Where A = absorbance in equation 1, MW = 449.2 g/mol (molecular weight of cyanidin-3-glucoside), DF = dilution factor, ε = 26,900 L/cm mol (molar absorptivity of cyanidin-3-glucoside) and l = 1 cm (path length)

2.4 Evaluation of colour and anthocyanin stability

2.4.1 Effects of pH on natural anthocyanins

Buffer solutions of different pH conditions were prepared according to Sari *et al.* (2012) with minor modifications. Lipote fruit extract solutions were prepared at 500mg/L concentration using 0.025 M KCl buffer solution for pH 3-4 and 0.4 M CH_3COONa buffer for pH 5-7. Potassium sorbate (0.02%) was added as a preservative. Solutions were left at room temperature (25°C) for 1 hr followed by measurement of colour (CIELab/LCh) and TMA. These solutions were also stored in the dark and at ambient temperature (25°C) for 2 weeks and monitored for anthocyanin content and colour changes on days 1, 2, 7, and 14 (Reyes and Cisneros-Zevallos, 2007; Sipahli *et al.*, 2017).

2.5 Effects of storage conditions, light, and heating temperature on natural and copigmented anthocyanins

2.5.1 Preparation of model beverage system and copigmented anthocyanins

A model beverage system as described by Sari *et al.* (2012) was prepared with slight modifications. To prepare this, lipote fruit extract was diluted with 0.1M citrate buffer maintained at pH 3 to a final concentration of 500 mg/L with 0.02% potassium sorbate preservative. This was prepared to simulate the stability of lipote fruit extract in actual beverages that fall within pH 3 and thus, assess the potential application.

For the preparation of copigmented anthocyanins, the caffeic acid solution was prepared separately using 0.1M citrate buffer (pH 3) and was added to attain a final molar ratio of 1:10 (anthocyanin: copigment).

Model systems containing natural and copigmented anthocyanins (with caffeic acid copigments) were transferred to several test tubes and were subjected to different conditions of storage, light, and heating temperatures. Test solutions were first equilibrated at ambient temperature for 1 hr. After which, colour (CIELab/LCh) and TMA were measured and were denoted as initial reading or time zero.

2.5.2 Effects of storage conditions

This parameter was conducted as previously described by Sari *et al.* (2012) and Fan *et al.* (2019) with some modifications. Samples contained in test tubes were placed under two temperature storage conditions: 4°C and 25°C which were refrigerated and ambient temperatures, respectively. Evaluation of colour and TMA changes was for a period of 60 days with measurements conducted every 10^{th} day.

2.5.3 Effects of light

Colour and anthocyanin stability in the presence and absence of light was based on the method previously described by Sari *et al.* (2012). Samples contained in transparent test tubes were placed in two different conditions: fluorescent (Philips CDL, 1100 lumens) illuminated box or a dark box both maintained at ambient temperature (25°C) for 10 days. Measurements of colour and TMA were conducted every two days.

2.5.4 Effects of heating temperature

Heat treatment stability measurements were conducted as previously described by Fan *et al.* (2019) with some modifications. Solutions containing natural

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and copigmented anthocyanins were heated at temperatures of 60°C, 75°C, and 90°C for 5 hrs. Samples were collected every 60 mins for the measurement of TMA and colour parameters.

The colour properties of lipote fruit extract solutions were assessed through the CIELAB/LCh system using a chromameter (CM-5, Konica Minolta) as described by Sari *et al.* (2012). L*, a*, and b* values which are lightness, redness-greenness, and yellowness-blueness attributes, respectively were recorded. Corresponding chroma (C*) and hue angles (h°) were calculated using equations 3 and 4, respectively. The parameter of colour change used was the total colour difference (△E) calculated using equation 5.

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (3)$$

$$h^\circ = \arctan (b^*)/(a^*) \quad (4)$$

$$\triangle E = [(\triangle L^*)^2 + (\triangle C^*)^2 + (\triangle H^*)^2]^{1/2} \quad (5)$$

In terms of anthocyanins, numerous studies found these pigments to degrade following first order kinetics (Sari *et al.*, 2012; Arici *et al.*, 2016; Fan *et al.*, 2019), the reaction rate constant (k) and half-life time ($t_{1/2}$) or the time wherein 50% of the anthocyanins remain in the solution were calculated accordingly based on the following equations:

$$\ln (A_t / A_0) = -k \times t \quad (6)$$

$$t_{1/2} = -\ln 0.5 \times k^{-1} \quad (7)$$

Where t denotes time and A_t and A_0 correspond to final and initial anthocyanin concentrations at specified t, respectively.

2.6 Spectral characteristics of copigmented lipote anthocyanins

Change in spectra of the model solutions as a result of copigment addition was assessed as described by Klisurova *et al.* (2019) with some modifications. Absorbances at 400-800 nm of model beverage solutions containing lipote fruit extract with and without caffeic acid copigments were recorded using a UV-VIS spectrophotometer (UV-1900, Shimadzu). Copigmentation effect was expressed as hyperchromic effect (△A at $\lambda_{vis\text{-}max}$) and bathochromic shift ($\triangle\lambda_{vis\text{-}max}$).

2.7 Data analysis

All analyses were conducted in triplicates and were presented as mean±standard deviation (SD). Design expert software (Version 7) was used for the extraction optimization through simplex lattice mixture design and response surface methodology (RSM). Data for RSM was modeled according to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k} \beta_{ij} X_i X_j \quad (8)$$

Where Y is the predicted response while X_i and X_j are the independent variables. β_0 is the intercept model. β_i, β_{ii} and β_{ij} are the linear, quadratic, and interactive term constants, respectively (Arici *et al.,* 2016; Maciel *et al.,* 2018). The adequacy of the model was evaluated based on percentage of variability (R^2, adjusted R^2 and predicted R^2), lack of fit test (where $P_{lack\ of\ fit} > 0.05$ denotes adequacy of model), and significance ($p \leq 0.05$).

For stability testing, data were analyzed using analysis of variance (ANOVA) ($p \leq 0.05$) with Tukey's post hoc test through SPSS Statistical Software Version 27.

3. Results and discussion

3.1 Extraction optimization

3.1.1 Solvent optimization

Several studies have extracted anthocyanins from plant sources using different extraction mediums. However, ethanol solutions are commonly used when the extraction is intended for food applications as these solvents are deemed safer than other organic solvents (Chaudhary and Mukhopadhyay, 2013). Hence, the study focused on utilizing ethanol and water – another safe and cost-effective solvent, for extraction. The solvents were also acidified as low pH levels or acidic conditions (pH < 4.0) make anthocyanins appear in their most stable form, the flavylium cation (Ngamwonglumlert *et al.*, 2015).

Using the combinations randomized using the simplex lattice mixture design, anthocyanins extracted from lipote fruits ranged from 5.70±0.11 to 19.40±0.07 mg/g DW (Table 1). Pure ethanol extracted more anthocyanins than pure water, but combinations of ethanol and water further improved lipote fruit anthocyanin yields (Table 1 and Figure 1).

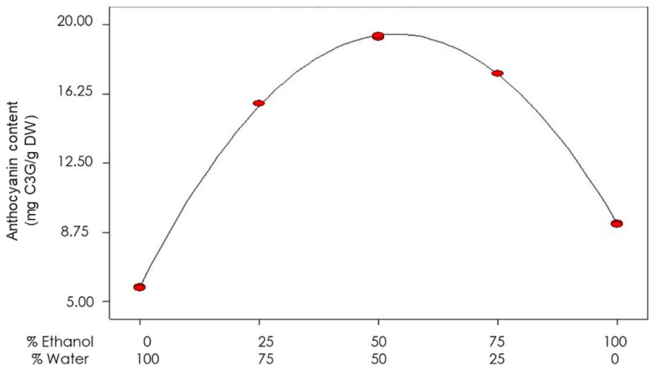

Figure 1. Solvent concentration effects on lipote fruit extract anthocyanin yield

These findings agree with several studies in which mixtures of water and ethanol were found to extract more anthocyanins rather than utilizing each solvent individually (Chandrasekhar *et al.,* 2012; Arici *et al.*,

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2016; Mojica *et al.,* 2017). In a mixture design by Arici *et al.* (2016), maximum anthocyanin content was extracted from tulip petals using acidified 35% ethanol. Chandrasekhar *et al.* (2012) also extracted higher amounts of anthocyanins using 50% ethanol rather than water alone. Higher amounts of anthocyanins were also extracted from black beans using 24% ethanol rather than pure water in a study by Mojica *et al.* (2017).

According to Chaudhary and Mukhopadhyay (2013), both ethanol and water are polar solvents, thereby facilitating the extraction of water-soluble anthocyanins. However, although ethanol can disrupt cell membranes resulting in higher anthocyanin yield than water when used individually, water is still needed for better dissolution of the hydrophilic anthocyanins (Meziant *et al.,* 2018). This explains why higher amounts of anthocyanins were extracted when the two solvents were combined. The important contribution of even small amounts of water in anthocyanin extraction was also reported by Patil *et al.* (2009). The exact concentrations however depend on the nature of raw material (Meziant *et al.,* 2018), justifying the importance of optimization.

Anthocyanin results were encoded in the design expert software to generate empirical model which can be used for anthocyanin content prediction and optimization. Below was the quadratic equation generated:

$$Y = 9.21A + 5.77B + 47.73AB \quad (9)$$

Where Y = total anthocyanin content, A = ethanol concentration and B = water concentration.

The adequacy of this model was evaluated using the results of the ANOVA, where the model, linear mixture components and ethanol and water interaction (AB) were all found to be significant terms ($p<0.05$). The lack of fit was found not significant which was desirable as the goal was to fit the generated model. R^2 (0.9998), adjusted R^2 (0.9997), and predicted R^2 (0.9995) values were close to 1 and were in agreement with each other, indicating that the generated model was accurate and can be used in predicting anthocyanin content. Based on this model, 55% acidified ethanol was found the best solvent combination for maximum anthocyanin extraction.

3.1.2 Process optimization

Using the optimized solvent combination, the optimum time and temperature conditions for maximum anthocyanin extraction from lipote fruits were also identified. Based on different time (30-120 mins) and temperature (25-50°C) combinations obtained using response surface methodology (RSM) with central composite design, anthocyanin content from lipote fruits ranged from 17.19±0.03 to 21.29±0.01 mg/g DW (Table 2). As shown in Figure 2, anthocyanin increased with increasing temperature and time. However, anthocyanin yield increase was no longer rapid at extraction times above around 75 mins.

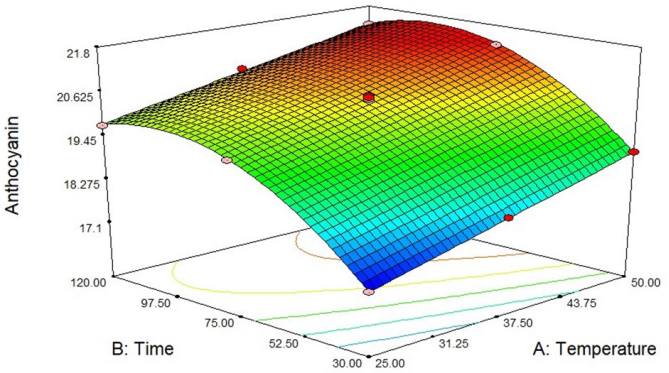

Figure 2. Effects of temperature and time on lipote fruit anthocyanin yield

Table 2. Anthocyanin content of lipote fruits extracted under time and temperature conditions as determined by Central Composite Design

Run	Temperature (°C)	Time (min)	Total Anthocyanin Content (mg C3G/g DW)
1	25	30	17.19±0.03[a]
2*	37.5	75	20.52±0.04[e]
3*	37.5	75	20.50±0.07[e]
4*	37.5	75	20.42±0.09[e]
5	25	75	19.61±0.06[d]
6*	37.5	75	20.49±0.07[e]
7*	37.5	75	20.53±0.09[e]
8	50	75	21.27±0.03[f]
9	50	30	19.03±0.07[c]
10	25	120	19.74±0.07[d]
11	37.5	30	18.11±0.11[b]
12	37.5	120	20.60±0.05[e]
13	50	120	21.29±0.01[f]

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$). C3G = Cyanidin-3-glucoside.
*Central points for estimation of pure error

Similar findings were seen by Prakash Maran *et al.* (2015) in the extraction of anthocyanins from Jamun fruit (*Syzigium cumini L.*), a fruit similar to and of the same family as lipote. Increases in both temperature and time were found to increase anthocyanin yield and increases in yield were also rapid at the initial stages of extraction. Such a trend was also observed in the anthocyanin extraction yields of tulip petals based on a study by Arici *et al.* (2015). The temperature was also found to affect anthocyanin yield from black rice (*Oryza sativa L.*) and roselle flowers (*Hibiscus sabdariffa*) based on studies by Pedro *et al.* (2016) and Maciel *et al.* (2018), respectively. The increase in temperature results in softening of the plant tissue which then allows penetration of solvent, thus enhancing the solubility. An increase in time also allows sufficient contact between

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raw material and solvent needed to facilitate the release of anthocyanins. The rapid increase at the initial stage can be explained by the higher concentration content of the starting material for penetration by the solvent and release into the solution (Prakash Maran *et al.*, 2015).

As in the solvent optimization, analyzed values were encoded in the design expert software to generate model which can be used for the prediction of anthocyanin yield after evaluation of its accuracy. This equation was the quadratic model generated:

$$Y = 20.48 + 0.84A + 1.22B - 0.072AB - 1.15B^2 \quad (10)$$

Where Y = total anthocyanins content, A = temperature and B = time. Based on the ANOVA results, this model, A, B (linear), AB (interactive), and B^2 (quadratic) terms were all significant ($p<0.05$). Other parameters also showed the suitability of the model for anthocyanin prediction - lack of fit was not significant and the R^2 (0.9991), adjusted R^2 (9986), and predicted R^2 (0.9970) were all close to unity and were in agreement with each other. Using the above model (10), the maximum anthocyanin content from lipote fruits was found to be extracted under conditions of 45.80°C and 92 mins, temperature and time, respectively.

3.2 Stability of natural anthocyanins

3.2.1 Effects of pH

3.2.1.1 Colour characteristics at pH 3-7

The pH of most food products is within pH 3-7 (Reyes and Cisneros-Zevallos, 2007), thus observations were limited within this pH range. Based on the results, the h° values of lipote fruit anthocyanin extract solutions maintained at different pH conditions were all close to 0° (Table 3) hence, fall within the red hue (Sui *et al*.,2016). However, as shown by a* and C* values, the redness and colour intensity of solutions at pH 3-4 were found higher than pH 5-7. In fact, at around pH 5-7, low C* values were recorded indicating that extracts were almost translucent (C* = 4.59±0.03 to 7.78±0.03) (Table 3).

These results are in agreement with the established characteristics of anthocyanins. The manifestation of red colour at low pH is due to the predominance of the flavylium cation, the most stable form of anthocyanin (Ngamwonglumlert *et al*., 2015; do Carmo Brito *et al.,* 2017). Upon increase to around pH 5 (or pH 4 in some anthocyanins), colourless carbinol pseudobases start to form, due to the nucleophilic attack by water molecules, justifying the combination of low C* and high L* values as shown in Table 3. At pH ≥ 6, quinoidal bases are formed manifesting unstable blue or violet colouration (Ngamwonglumlert *et al.,* 2015).

The colour characteristics found in the present study are similar to the findings of Sari *et al.* (2012) for jambolan anthocyanins (*Syzygium cumini*) wherein decreases in colour intensity were also observed with increasing pH. Moreover, Reyes and Cisneros-Zevallos (2007) also obtained red coloured solutions up to pH 3 for grape anthocyanins and pH 4 for purple carrot extracts and translucent to bluish hues at around pH 5-8.

3.2.1.2 Changes during storage

The total colour change (△E) increased with increasing pH, with pH 3 showing the lowest △E (5.03±0.03) after 14 days, thus demonstrating the highest stability (Table 4). On the contrary, pH 7 had the highest △E (44.76±0.48). Similar findings were also observed in *Hibicus sadariffa*, red and purple flesh potato, and grape anthocyanin extracts, which demonstrated the highest pigment retention at low pH conditions (Reyes and Cisneros-Zevallos, 2007; Sipahli *et al*., 2016). Likewise, purple corn anthocyanins also displayed increasing colour changes with increasing pH (Luna-Vital *et al*., 2017).

In terms of anthocyanins, increasing degradation rates (k) and decreasing half-life ($t_{1/2}$) time values were calculated with increasing pH (Table 4). This trend agrees with the findings of Arici *et al.* (2016) for tulip petal anthocyanins wherein the highest k was recorded for pH 6, the highest pH condition set in the study. Likewise, increasing pH also promoted the increase of anthocyanin degradation for rosella and purple sweet potato anthocyanins based on studies by Askar *et al*. (2015) and Jiang *et al.* (2019), respectively.

As mentioned, changes in colour and anthocyanin content can be explained with anthocyanins being the most stable at low pH conditions due to the appearance

Table 3. Initial colour characteristics of lipote fruit anthocyanin extract solutions at different pH conditions

pH	L*	a*	b*	C*	h°
3	80.18±0.04^{a}	32.47±0.02^{e}	5.55±0.04^{e}	32.94±0.02^{e}	9.69±0.06^{c}
4	83.89±0.04^{c}	23.86±0.04^{d}	2.98±0.03^{d}	24.04±0.04^{d}	7.11±0.06^{b}
5	90.97±0.02^{e}	5.48±0.04^{b}	0.55±0.03^{a}	5.51± 0.04^{b}	5.70±0.22^{a}
6	90.20±0.04^{d}	4.47±0.03^{a}	1.05±0.03^{b}	4.59±0.03^{a}	13.19±0.37^{d}
7	80.37±0.02^{b}	7.47±0.03^{c}	2.18±0.03^{c}	7.78±0.03^{c}	16.25±0.13^{e}

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$). L*: lightness, a*: redness-greenness, b*: yellowness-blueness, C*: chroma, h°: hue angle.

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of anthocyanins in their most stable form – as flavylium cations. At higher pH, the structure is disrupted and becomes more susceptible to nucleophilic attack prompting the formation of unstable forms (Ngamwonglumlert *et al.*, 2015; Maciel *et al.,* 2018).

Based on the results, lipote fruit anthocyanins demonstrated the highest stability at pH 3. Hence, this was used as the pH of model beverage solutions for the succeeding parts of the study. Moreover, most beverages are within this pH (Mojica *et al.,* 2017).

Table 4. Total colour change (△E), degradation constant (k) and half-life time ($t_{1/2}$) of anthocyanins in lipote fruit extract solutions maintained at different pH conditions

pH	△E*	k (day $^{-1}$)	$t_{1/2}$ (days)
3	5.03±0.03^{a}	0.0146±0.0004^{a}	47.48
4	7.63±0.07^{b}	0.0205±0.0004^{b}	33.81
5	8.06±0.34^{c}	0.0442±0.0006^{c}	15.68
6	24.34±0.83^{d}	0.3201±0.0011^{d}	2.16
7	44.76±0.48^{e}	0.5398±0.0044^{e}	1.28

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$).
*After 14 days of storage

3.2.2 Effects of storage conditions

The stability of lipote fruit anthocyanins contained in model beverage systems was observed at room temperature (25°C) and refrigerated storage conditions (4°C) for 60 days. Based on the results, solutions maintained at 4°C exhibited lower △E (8.45±0.02) than the 25°C-maintained model beverage solutions (△E = 27.45±0.01) after the 60-day observation period ($p<0.05$) (Table 5). In terms of anthocyanins, the degradation rate of those stored at 4°C (k = 0.0041±0.0002/day) was found significantly lower while $t_{1/2}$ (169.06 days) about three times higher than those stored at 25°C (k = 0.0147±0.0007, $t_{1/2}$ = 47.15days) (Table 5), implying noticeable improvement of anthocyanin stability (Table 5).

Similar findings were observed for jambolan fruit anthocyanins wherein refrigeration at 7°C decelerated colour change and prolonged half-life of anthocyanins (Sari *et al.,* 2012). Improvement of black bean anthocyanin stability upon refrigeration at 4°C was also observed by Mojica *et al.* (2017). Likewise, anthocyanins from blackberry and raspberry/strawberry jams also exhibited higher degradation rates and shorter half-lives when stored at 35°C and 23°C, respectively, than at 4°C (Weber *et al.,*2017; Martinsen *et al.,* 2020). The half-life of lipote fruit anthocyanins ($t_{1/2}^{25°C}$ = 47.15 days; $t_{1/2}^{4°C}$ = 169.06 days) was comparable with grape anthocyanins ($t_{1/2}$ = 47 days) and slightly higher than jambolan fruit anthocyanins ($t_{1/2}$ = 21.59 weeks (~151 days) at room temperature and refrigerated conditions, respectively (Reyes and Cisneros-Zevallos, 2007; Sari *et al.,* 2012). Higher temperatures cause higher tendencies of anthocyanin structure disruption resulting in degradation (Patras *et al.,* 2010). Thus, the greater stability demonstrated at low storage temperature (4°C) can be explained by the non-contribution of heat to anthocyanin degradation upon storage (Patras *et al.,* 2010).

3.2.3 Effects of light

Solutions exposed to light had significantly higher △E (15.15±0.03) than those maintained in the dark (△E = 5.20±0.01) after the 10-day-observation period ($p<0.05$) (Table 5). Exposure to light also resulted in significantly higher k (0.0382±0.0017/day) and lower $t_{1/2}$ (18.15 days), implying a higher extent of anthocyanin degradation as compared to those stored in the dark (k = 0.0147±0.0007, $t_{1/2}$ = 47.15 days) (Table 5).

The results of this study agree with the findings of Fan *et al.* (2019) for blackberry wine residue anthocyanins which showed higher anthocyanin degradation and colour changes for model beverages exposed to light than those kept in the dark. In the same way, jambolan fruit anthocyanins also exhibited slower degradation of anthocyanins and colour change when maintained in a dark environment (Sari *et al.*, 2012). The degradation of mulberry anthocyanins also quickened after exposure to light in a study by Aramwit *et al.* (2010). According to Verduin *et al.* (2020), light also promotes formation of chalcones, thus explaining the changes in colour and anthocyanin levels.

3.2.4 Effects of heating temperature

The thermal stability of lipote fruit anthocyanins was investigated by heating model beverage solutions at three temperatures: 60, 75, and 90°C. Results proved that heating and temperature affected colour and anthocyanin stability (Table 5). It can be seen that as the temperature increased from 60°C to 90°C, △E also increased, denoting higher colour losses at higher temperature conditions (Table 5). Additionally, the k of lipote fruit anthocyanins also increased and $t_{1/2}$ values decreased in response to increasing temperature (Table 5). Model beverage systems with lipote fruit anthocyanins heated at 60°C had the lowest △E (3.41±0.01) and k (0.0181±0.0006/hr) and highest $t_{1/2}$ (38.30 hrs) among the three temperatures tested (Table 5).

Similar findings were reported by several studies (Reyes and Cisneros-Zevallos, 2007; Arici *et al.*, 2016; Mojica *et al.*, 2017; Fan *et al.*, 2019; Maciel *et al.*, 2018). Black bean anthocyanins heated at 90°C showed

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Table 5. Total colour change (△E), degradation constant (k) and half-life time ($t_{1/2}$) of lipote fruit anthocyanins in model beverage solutions (citrate buffer, pH = 3) at different testing conditions

Testing conditions		△E	k	$t_{1/2}$
Stored at room temperature, 25°C*	LA	27.45±0.01^{d}	0.0147±0.0007^{d}	47.15 d
	LA+C	19.97±0.01^{c}	0.0117±0.0005^{c}	59.24 d
Stored at refrigerated temperature, 4°C*	LA	8.45±0.02^{b}	0.0041±0.0002^{b}	169.06 d
	LA+C	2.49±0.01^{a}	0.0031±0.0001^{a}	223.60 d
Kept in the dark**	LA	5.20±0.01^{b}	0.0147±0.0007^{b}	47.15 d
	LA+C	3.39±0.02^{a}	0.0117±0.0005^{a}	59.24 d
Exposed to light**	LA	15.15±0.03^{d}	0.0382±0.0017^{d}	18.15 d
	LA+C	11.11±0.01^{c}	0.0267±0.0018^{c}	25.96 d
Heated at 60°C***	LA	3.41±0.01^{b}	0.0181±0.0006^{b}	38.30 hrs
	LA+C	2.39±0.01^{a}	0.0149±0.0003^{a}	46.52 hrs
Heated at 75°C***	LA	6.62±0.01^{d}	0.0346±0.0017^{d}	20.03 hrs
	LA+C	4.81±0.02^{c}	0.0297±0.0010^{c}	23.34 hrs
Heated at 90°C***	LA	26.61±0.01^{e}	0.1529±0.0026^{e}	4.53 hrs
	LA+C	27.68 ±0.03^{f}	0.1614±0.0016^{f}	4.29 hrs

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$). Comparisons were carried out between 4°C and 25°C storage conditions, between light-exposed and dark-maintained, and between solutions heated at different temperatures. LA: Lipote fruit anthocyanins, LA+C: Lipote fruit anthocyanins with caffeic acid copigments.

*Stored for 60 d, **Stored for 10 d, ***Heated for 5 hrs.

significantly higher k and lower $t_{1/2}$ values than those heated at 80°C, and the latter at 70°C based on a study by Mojica *et al.* (2017). Fan *et al.* (2019) also reported increasing degradation and colour losses with increasing temperature up to 90°C. The differences in anthocyanin degradation and colour changes may be attributed to having higher temperatures promote disruption of anthocyanin ring, leading to ring opening and formation of chalcone moieties (Patras *et al.*,2010).

3.3 Stability of copigmented anthocyanins

Model beverage solutions containing lipote fruit anthocyanins with caffeic acid copigments were also subjected to different heating treatments (60°C, 75°C, and 90°C), exposed to fluorescent light (1100 lumens) and stored at different conditions (4°C and 25°C). For all conditions, total colour change (△E) and anthocyanin degradation constant (k) of natural and copigmented anthocyanins in model beverage solutions were all found significantly different ($p<0.05$) (Table 5). In general, the introduction of caffeic acid copigment resulted in more stable solutions as demonstrated by significantly lower anthocyanin degradation rates, lesser colour change and improved $t_{1/2}$ with the exception of model solutions heated at 90°C (Table 5). Initial results also showed solutions containing copigmented anthocyanins as darker and redder than solutions with natural lipote anthocyanins as shown by lower L* and higher C* values of the former (Table 6).

In detail, solutions copigmented with caffeic acid improved stability of lipote fruit anthocyanins stored at 4°C and 25°C. Copigmented solutions stored at 4°C showed the highest stability as demonstrated by the lowest k (0.0031±0.0001/day) and △E (2.49±0.01) values recorded for all conditions (Table 5). These results were similar to the findings of Weber *et al.* (2017) and Sari *et al.* (2012) who also observed the contribution of copigments in improving anthocyanin stability during storage. Caffeic acid further decreased overall change in colour and anthocyanin degradation for both storage conditions (28°C and 7°C) for jambolan fruit anthocyanins (Sari *et al.,* 2012), whereas preservation of anthocyanins was also more evident for blackberry anthocyanins copigmented with ferulic acid especially those stored at 4°C (Weber *et al*., 2017).

Table 6. Initial colour properties of natural and copigmented lipote fruit anthocyanins in model beverage solutions (citrate buffer, pH = 3)

	L*	C*	h°
LA	70.08±0.03^{b}	53.19±0.04^{a}	16.18±0.03^{b}
LA+C	68.32±0.02^{a}	55.33±0.03^{b}	14.67±0.02^{a}

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$). L*: lightness, C*: chroma, h°: hue angle, LA: Lipote fruit anthocyanins, LA+C: Lipote fruit anthocyanins with caffeic acid copigments.

Similarly, lipote fruit anthocyanins exposed to light showed improvement of anthocyanin and colour retention when copigmented with caffeic acid (Table 5). This agrees with the findings of Fan *et al.* (2019) and Sari *et al.* (2012), for blackberry wine residue and jambolan fruit anthocyanins, respectively, wherein solutions also exhibited slower degradation of anthocyanins and colour change when caffeic acid

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copigmented even when exposed to light. Pedro *et al.* (2016) also reported the increase in $t_{1/2}$ of black rice anthocyanins when copigmented with gallic acid.

In terms of heating treatments, caffeic acid also lowered degradation rate of anthocyanins and overall colour change when heated at 60°C and 75°C (Table 5). However, after increasing the temperature to 90°C, copigmentation no longer demonstrated anthocyanin stability improvement as shown by slightly higher k and $\triangle$E and lower $t_{1/2}$ values of copigmented solutions than solutions with lipote fruit anthocyanins alone (Table 5). This was also the case for blackberry wine residue anthocyanins that showed significantly lower rates of degradation and total colour change and higher $t_{1/2}$ when copigmented with caffeic acid upon heating at 50 and 70°C in a study by Fan *et al.* (2019). However, at 90°C, Fan *et al.* (2019) found higher rates of degradation upon copigmentation.

The improvements resulting from copigmentation could be attributed to the π-conjugated systems found in copigments (Pedro *et al.*, 2016; Maciel *et al.*, 2018). These π-conjugated systems are found in hydroxycinnamic acids such as caffeic acid, and together with their OH and C = O reactive groups, were reported as among the best copigments (Klisurova *et al.*, 2019). Through these, copigments possess the ability to enhance anthocyanin stability by stacking anthocyanins in a sandwich configuration through π-π interaction, thereby protecting the flavylium cation from hydration and conversion into unstable forms (Bimpilas *et al.*, 2017; Kanha *et al.*, 2019). However, at temperatures near 100° C, copigments also undergo degradation which may explain the non-contribution to anthocyanin stability at the studied temperature of 90°C (Maciel *et al.*, 2018). The high temperature may have also caused disruption of anthocyanin-copigment complexes leading to the formation of colourless degradation products, thus resulting in colour and anthocyanin losses (Sari *et al.*, 2012).

The occurrence of copigmentation can be confirmed through the changes in the anthocyanin's spectral properties. Such spectral property changes - a bathochromic shift (maximum wavelength) and hyperchromic effect (change in absorbance)- are initiated by the π-π complex formed during anthocyanin-copigment interaction (Pedro *et al.,* 2016). In this study, the caffeic acid addition caused the maximum wavelength to move from 513 to 514.5nm, and the absorbance spike by 5% at this maximum wavelength (Table 7). These effects were also seen by Klisurova *et al.* (2019) in black chokeberry (*Aronia melanocarpa*) (6.2% hyperchromic effect; 2nm bathochromic shift) anthocyanins when caffeic acid copigments were introduced.

Table 7. Wavelength at maximum absorbance (λmax) and variations in wavelength (bathochromic shift) and absorbance (hyperchromic effect) of natural and copigmented lipote fruit anthocyanins in model beverage solutions ((citrate buffer, pH = 3)

	λmax, nm	Bathochromic shift (Δ λmax)	Abs. reading at *λmax*	Hyperchromic effect (ΔA)
LA	513.0	-	0.4927	-
LA+C	514.5	1.5±0.0	0.5189	0.03±0.00

Values are presented as mean±SD, n = 3. LA: Lipote fruit anthocyanins, LA+C: Lipote fruit anthocyanins with caffeic acid copigments.

4. Conclusion

The study presented the feasibility of obtaining and optimizing anthocyanin-rich extracts from lipote fruits. Moreover, the study highlighted the stability characteristics of this extract that would help substantiate and maximize its potential for use as a natural food colourant. Stability tests showed that lipote anthocyanin extract, in its natural form, was most stable at low pH conditions, in the dark and at refrigerated storage temperature. Additionally, the incorporation of caffeic acid copigments did not only resulted in darker and more intense colouration but also further improved the stability of anthocyanins under most of the conditions tested.

Future studies may therefore explore translating the identified extraction parameters and stability conditions into actual food systems. For instance, by consideration of the high stability demonstrated at pH 3, possible food applications include water-based beverages such as fruit-flavored, energy, and sports drinks which all fall within this pH range. Furthermore, other means of enhancing anthocyanin stability such as the use of other types of copigments or employment of microencapsulation may also be explored.

Overall, data generated from the present work is deemed useful for the characterization and possible food application of lipote extract as research on lipote fruits is currently scarce. Moreover, the study may serve as a contribution to expanding the value of lipote by adding natural food colourant among its many other potential products in the food industry.

Conflict of interest

The authors declare no conflict of interest

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References

AOAC. (2019). Official Methods of Analysis of AOAC International. Method 2005.02. 21st ed. Maryland, USA: AOAC.

Aramwit, P., Bang, N. and Srichana, T. (2010). The properties and stability of anthocyanins in mulberry fruits. *Food Research International,* 43, 1093–1097. https://doi.org/10.1016/j.foodres.2010.01.022

Arici, M., Karasu, S., Baslar, M., Said Toker, O. and Sagdig, O. (2016). Tulip petal as a novel natural food colorant source: Extraction https:// doi.org/10.1016/j.indcrop.2016.07.003 optimization and stability studies. *Industrial Crops and Products,* 91, 215–222.

Askar, K.A., Alsawad, Z.H. and Moayad, K.N. (2015). Evaluation of the pH and thermal stabilities of rosella anthocyanin extracts under solar light. *Beni-Suef University Journal of Basic and Applied Sciences,* 4(3), 262-268. https://doi.org/10.1016/ j.bjbas.2015.06.001

Bimpilas, A., Panagopoulou, M., Tsimogiannis, D. and Oreopoulou, V. (2016). Anthocyanin copigmentation and color of wine: the effect of naturally obtained hydroxycinnamic acids as cofactors. *Food Chemistry,* 197(Part A), 39–46. https:// doi.org/10.1016/j.foodchem.2015.10.095

Chaudhary, B. and Mukhopadhyay, K. (2013). Solvent optimization for anthocyanin extraction from *Syzygium cumini L.* Skeels using response surface methodology. *International Journal of Food Sciences and Nutrition,* 64(3), 363-371. https:// doi.org/10.3109/09637486.2012.738647

Chandrasekhar, J., Madhusudhan, M.C. and Raghavarao, K.S. (2012). Extraction of anthocyanins from red cabbage and purification using adsorption. *Food and Bioproducts Processing,* 90(4), 615-623. https:// doi.org/10.1016/j.fbp.2012.07.004

Chung, C., Rojanasasithara, T., Mutilangi, W. and McClements, D. (2016). Enhancement of colour stability of anthocyanins in model beverages by gum arabic addition. *Food Chemistry,* 201, 14-22. https:// doi.org/10.1016/j.foodchem.2016.01.051

Department of Agriculture - Bureau of Agricultural Research (DA-BAR) Philippines. (2012). Promoting less-known Pinoy fruits. *BAR Research and Development Digest,* 14, 4.

Department of Environment and Natural Resources - Ecosystems Research and Development Bureau (DENR-ERDB). (2017). Lipote *Syzygium polycephaloides* (C.B.Rob) Merr. *Research Information Series on Ecosystems,* 29, 2.

do Carmo Brito, B.N., da Silva Pena, R., Santos Lopes, A. and Campos, C.R. (2017). Anthocyanins of Jambolao *(Syzigium cumini)*: Extraction and pH-dependent color changes. *Journal of Food Science,* 82(10), 2286-2290. https://doi.org/10.1111/1750-3841.13847

Fan, L., Wang, Y., Xie, P., Zhang, L., Li, Y. and Zhou, J. (2019). Copigmentation effects of phenolics on color enhancement and stability of blackberry wine residue anthocyanins: Chromaticity, kinetics and structural simulation. *Food Chemistry,* 275, 299-308. https://doi.org/10.1016/j.foodchem.2018.09.103

Gordillo, B., Sigurdson, G.T., Lao, F., González-Miret, M., Heredia, F.J. and Giusti, M. (2018). Assessment of the colour modulation and stability of naturally copigmented anthocyanin-grape colourants with different levels of purification. *Food Research International,* 106, 791-799. https://doi.org/10.1016/ j.foodres.2018.01.057

Gras, C.C., Nemetz, N. and Carle, R. (2017). Anthocyanins from purple sweet potato (*Ipomoea batatas* (L.) Lam.) and their colour modulation by the addition of phenolic acids and food-grade phenolic plant extracts. *Food Chemistry,* 235, 265-274. https://doi.org/10.1016/j.foodchem.2017.04.169

Ilano, M., Sartagoda, K., Flandez, L., Compendio, M., Morales, D. and Castillo-Israel, K. (2021). Antioxidant properties of lipote (*Syzygium polycephaloides (C.B. Rob.) Merr.*) flesh and seeds as affected by maturity and processing method. *Food Research*, 5(2), 475–484. https://doi.org/10.26656/ fr.2017.5(2).558

Jiang, T., Mao, Y., Sui, L., Yang, N., Li, S., Zhu, Z., Wang, C., Yin, S., He, J. and He, Y. (2019). Degradation of anthocyanins and polymeric colour formation during heat treatment of purple sweet potato extract at different pH. *Food Chemistry*, 274, 460-470. https://doi.org/10.1016/ j.foodchem.2018.07.141

Kanha, N., Surawanga, S., Pornsiri, P., Regenstein, J. and Laokuldilok, T. (2019). Copigmentation of cyanidin 3-O-glucoside with phenolics: Thermodynamic data and thermal stability. *Food Bioscience,* 30, 100419. https://doi.org/10.1016/ j.fbio.2019.100419

Klisurova, D., Petrova, I., Ognyanov, M., Georgiev, Y.,

Kratchanova, M. and Denev, P. (2019). Co-pigmentation of black chokeberry *(Aronia melanocarpa*) anthocyanins with phenolic co-pigments and herbal extracts. *Food Chemistry,* 279, 162-170. https://doi.org/10.1016/j.foodchem.2018.11.125

Lao, F. and Giusti, M. (2018). Extraction of purple corn (*Zea mays* L.) cob pigments and phenolic compounds using food-friendly solvents. *Journal of Cereal Science,* 80, 87-93. https://doi.org/10.1016/j.jcs.2018.01.001

Luna-Vital, D., Li, Q., West, L., West, M. and Gonzalez de Mejia, E. (2017). Anthocyanin condensed forms do not affect colour or chemical stability of purple corn pericarp extracts stored under different pHs. *Food Chemistry,* 232, 639-647. https://doi.org/10.1016/j.foodchem.2017.03.169

Maciel, L.G., Vieira do Carmo, M.A., Azevedo, L., Daguer, L., Molognoni, L., Mendes de Almeida, M., Granato, D. and Deliberali Rosso, N. (2018). *Hibiscus sabdariffa* anthocyanins-rich extract: Chemical stability, *in vitro* antioxidant and antiproliferative activities. *Food and Chemical Toxicology,* 113, 187-197. https://doi.org/10.1016/j.fct.2018.01.053

Martinsen, B.K., Aaby, K. and Skrede, G. (2020). Effect of temperature on stability of anthocyanins, ascorbic acid and colour in strawberry and raspberry jams. *Food Chemistry,* 316, 126297. https://doi.org/10.1016/j.foodchem.2020.126297

Mayes, S., Massawe, F.J., Alderson, P.G., Roberts, J.A., Azam-Ali, S.N. and Hermann, M. (2012). The potential for underutilized crops to improve security of food production. *Journal of Experimental Botany,* 63(3), 1075-1079. https://doi.org/10.1093/jxb/err396

McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., Kitchin, E., Lok, K., Porteous, L., Prince, E., Sonuga-Barke, E., Warner, J.O. and Stevenson, J. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *Lancet,* 370, 1560-1567. https://doi.org/10.1016/S0140-6736(07)61306-3

Meziant, L., Boutiche, M., Bachir Bey, M., Saci, F. and Louaileche, H. (2018). Standardization of monomeric anthocyanins extraction from fig fruit peels (*Ficus carica* L.) using single factor methodology. *Journal of Food Measurement and Characterization,* 12, 2865–2873. https://doi.org/10.1007/s11694-018-9901-6

Mojica, L., Berhow, M. and Gonzalez de Mejia, E. (2017). Black bean anthocyanin-rich extracts as food colourants: physicochemical stability and antidiabetes potential. *Food Chemistry,* 229, 628-639. *https://doi.org/10.1016/j.foodchem.2017.02.124*

Ngamwonglumlert, L., Devahastin, S. and Chiewchan, N. (2015). Natural Colourants: Pigment Stability and Extraction Yield Enhancement via Utilization of Appropriate Pretreatment and Extraction Methods. *Critical Reviews in Food Science and Nutrition,* 57 (15), 3243-3259. https://doi.org/10.1080/10408398.2015.1109498

Patil, G., Madhusudhan, M.C., Ravindra, V. and Ragvaharao, K.S.M.S. (2009). Extraction, dealcoholisation and concentration of anthocyanin from red radish peels. *Chemical Engineering and Processing,* 1(48), 364-369. https://doi.org/10.1016/j.cep.2008.05.006

Patras, A., Brunton, N.P., O'Donnell, C. and Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science and Technology*, 21(1), 3-11. https://doi.org/10.1016/j.tifs.2009.07.004

Pedro, A.C., Granato, D. and Rosso, N.D. (2016). Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa* L.) by modeling and assessing their reversibility and stability. *Food Chemistry,* 191, 12-20. https://doi.org/10.1016/j.foodchem.2015.02.045

Prakash Maran, J., Sivakumar, V., Thirugnanasambandhan, K. and Sridhar, R. (2015). Extraction of natural anthocyanin and colours from pulp of jamun fruit. *Journal of Food Science and Technology,* 52(6), 3617-3626.

Reyes, L.F. and Cisneros-Zevallos, L. (2007). Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (*Solanum tuberosum* L.). *Food Chemistry,* 100, 885-894. https://doi.org/10.1016/j.foodchem.2005.11.002

Reynertson, K.A., Yang, H., Jiang, B., Basile, M.J. and Kennelly, E.J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry,* 109(4), 883-890. https://doi.org/10.1016/j.foodchem.2008.01.021

Sari, P., Wijaya, C.H., Dondin, S. and Supratman, U. (2012). Colour properties, stability and free radical scavenging activity of jambolan (*Syzygium cumini*) fruit anthocyanins in a beverage model system: Natural and copigmented anthocyanins. *Food Chemistry,* 132, 1908-1914. https://doi.org/10.1016/j.foodchem.2011.12.025

Sipahli, S., Mohanlall, V. and Mellem, J.J. (2017).

https://doi.org/10.26656/fr.2017.7(1).1003

Stability and degradation kinetics of crude anthocyanin extracts from *H. sabdariffa*. *Food Science and Technology Campinas,* 37(2), 209-215. https://doi.org/10.1590/1678-457x.14216

Soares, B.M., Araujo, T.M., Ramos, J.A., Pinto, L.C., Khayat, B.M., De Oliveira Bahia, M., Montenegro, R.C., Burbano, R.M. and Khayat, A.S. (2015). Effects on DNA repair in human lymphocytes exposed to the food dye tartrazine yellow. *Anticancer Research,* 35(3), 1465-1474.

Sui, X., Bary, S. and Zhou, W. (2016). Changes in the colour, chemical stability and antioxidant capacity. *Food Chemistry,* 192, 516–524. https:// doi.org/10.1016/j.foodchem.2015.07.021

Tan, C., Celli, G.B., Selig, M. and Abbaspourrad, A. (2018). Catechin modulates the copigmentation and encapsulation of anthocyanins in polyelectrolyte complexes (PECs) for natural colourant stabilization. *Food Chemistry,* 264, 342-349. https:// doi.org/10.1016/j.foodchem.2018.05.018

Verduin, J., Den Uijl, M.J, Peters, R.J.B. and Van Bommel, M.R. (2020). Photodegradation products and their analysis in food. *Journal of Food Science and Nutrition,* 6, 100067. https://doi.org/10.24966/ FSN-1076/100067

Weber, F., Boch K. and Shieber, A. (2017). Influence of copigmentation on the stability of spray dried anthocyanins from blackberry. *LWT-Food Science and Technology,* 75, 72-77. https://doi.org/10.1016/ j.lwt.2016.08.042

Yamashita, C., Mi Song Chung, M., dos Santos, C., Roberta, C., Mayer, M., Moraes, I.C.F. and Branco, I.G. (2017). Microencapsulation of an anthocyanin-rich blackberry (*Rubus* spp.) by-product extract by freeze-drying. *LWT-Food Science and Technology,* 84, 256-262. https://doi.org/10.1016/ j.lwt.2017.05.063

Zhang, H.C., Zhan, J.X., Su, K.M. and Zhang, Y.X. (2006). A kind of potential food additive produced by *Streptomyces coelicolour*: Characteristics of blue pigment and identification of a novel compound, k-actinorhodin. *Food Chemistry,* 95(2), 186–192. https://doi.org/10.1016/j.foodchem.2004.12.028

