Investigation of pH varied anthocyanin pigment profiles of *Agapanthus praecox* and its potential as natural colourant

J. S. Yaacob*[1], A. I. M. Yussof[1], S. Abdullah[1], K. Ramesh[2] and R. M. Taha[1]

Anthocyanin pigment profiles, colours and pH values of *Agapanthus praecox* were investigated. The anthocyanin pigments from the blue flower petals were solvent extracted with 70% acetone, and the pH of the extracts was varied (pH 1, 2·5, 4·5 and 7). The colour of the pigment solution changed with pH, where it became pinkish purple at pH 1, dark red at pH 2·5, light purple at pH 4·5, dark turquoise at pH 7, dark green at pH 9·5, light green at pH 11·5 and yellow at pH 14. The extracts were also subjected to ultraviolet–visible spectrophotometric analysis, where a distinct peak at 544 nm was observed for both pH 1 and 2·5, while multiple peaks at 535, 571·5 and 627 nm were observed for pH 4·5 and at 535, 574 and 620 nm for pH 7. The anthocyanin extract with the most stable pH (at pH 1) was mixed with 20% poly(methyl methacrylate) and coated onto glass slides, which was then subjected to weathering tests to determine its durability.

Keywords: Anthocyanin, *Agapanthus praecox*, Solvent extraction, Weathering test

Introduction

Agapanthus praecox, commonly known as 'lily of the Nile' or the 'African lily', is a flowering plant that belongs to the genus *Agapanthus*, together with *Agapanthus africanus* and *Agapanthus inaperthus*.[1,2] *A. praecox* originated from South Africa[3] and has attractive violet/blue flowers that bloom on a single long stem.[1]

The blue coloured petals were believed to contain covalently linked anthocyanin–flavonol pigments.[3] Coloured anthocyanin pigments have always been an interesting candidate for organic colouring, and to date, various investigations to improve the stability and colour properties have been carried out on species such as radishes,[4] black carrots[5] and strawberries.[6]

The present study reports on pH varied anthocyanin pigments derived from *A. praecox* and their potential use as a natural colourant.

Experimental

Solvent extraction of anthocyanins

Agapanthus praecox plants with violet/blue flowers were used in the present study. The flower petals were excised from the stem and dried under natural conditions at room temperature for 3 days. Acetone (70%) was preprepared by adding distilled water.

The anthocyanin pigments from the flower petals were solvent extracted using 70% acetone, with dry weight/volume of acetone ratio of 1 : 5. The pH of the extracts was varied to pH 1, 2·5, 4·5, 7, 9·5, 11·5 and 14, and three replicates were made for each pH. The colour of each anthocyanin extract at its respective pH was also noted.

Ultraviolet–visible spectrophotometric analysis

Acidic anthocyanin extracts (pH 1, 2·5, 4·5 and 7) were subjected to UV spectrophotometric analysis in the visible region (between the wavelengths of 400 and 700 nm), and the spectrum profiles were compared. Three sample replicates were made for each pH, and for each replicate, the absorbance readings were measured three times. The mean values of the retrieved data were used to construct the spectrum profiles of each pH.

From the absorbance spectra of the acidic anthocyanin extracts, the differences in the number of peaks and the maximum absorbance values were compared and recorded. The extracts were stored under dark condition at 4°C, and the colours of the extracts were observed everyday. Among all the samples, the anthocyanin extracts at pH 1 were found to be the most stable and the last to fade; hence, it was used in the production of natural colourant and in coating application test in subsequent experiments.

Measurement of total anthocyanins

The total anthocyanins of *A. praecox* extracts (in mg L^{-1}) were also measured via pH differential method[7–9] as described in equation (1)

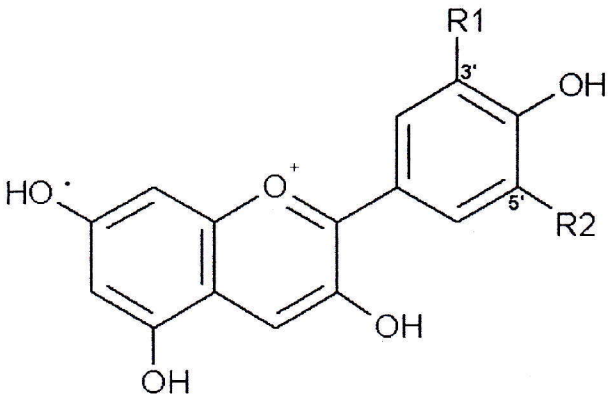
$$\text{Total anthocyanins} = \frac{A \times MW \times DF \times 10^3}{\varepsilon l} \quad (1)$$

where $A = (A_{max} - A_{700\ nm})pH_{1\cdot0} - (A_{max} - A_{700\ nm})pH_{4\cdot5}$,

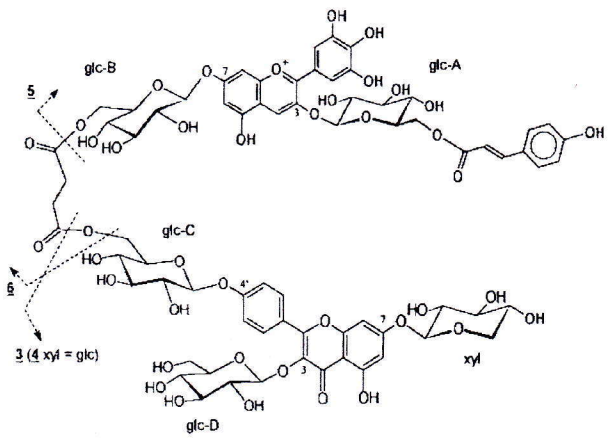
[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
[2]Center for Ionics University of Malaya, Physics Department, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia

*Corresponding author, email jam_sya@yahoo.com

Received 13 June 2010; accepted 1 April 2011
DOI 10.1179/143307511X13031890748236



1 Generalised anthocyanin structure



2 Anthocyanin structure of *Agapanthus praecox*[3]

MW is the molecular weight, *DF* is the dilution factor, ε is the molar extinction coefficient (L mol^{-1} cm^{-1}) and t is the path length (1 cm). The molecular weight was estimated to be 3225 based on the *m/z* values of the two major covalently linked anthocyanin pigments of *Agapanthus* sp.[3] The structure of *A. praecox* is slightly different from the generalised structure, as shown in Fig. 1. The structure of *A. praecox* anthocyanin is shown in Fig. 2, whereby two delphinidin 3-O-(6-O-p-coumaroyl glucoside) 7-O-(6-succinyl glucoside) are linked to kaempferol-3,4′-diglucoside 7-xyloside and kaempferol-3,7,4′-triglucoside.[3]

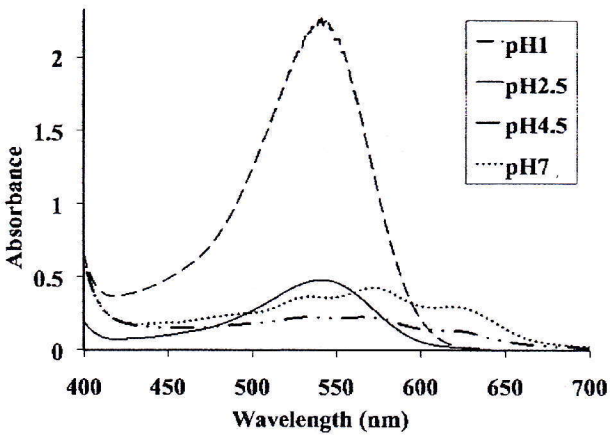
On the other hand, the molar extinction coefficient was estimated to be 26 900,[10] which is the molar extinction coefficient of cyanidin-3-glucoside (a general anthocyanin compound).[7] As shown in Fig. 1, the generalised anthocyanin structure constitutes an anthocyanin pigment with a carbohydrate (sugar groups, usually glucose) usually esterified at the 3′ or 5′ positions.[4,10,11]

Weathering tests

The potential of *A. praecox* anthocyanin to be used as a natural colourant was also investigated. A mixture of resin poly(methyl methacrylate) (PMMA) with the anthocyanin extracts at pH 1 (with resin/extract ratio of 1 : 5) was coated onto several glass slides and air dried for 1 h. The coated slides were then subjected to several weathering tests such as in NaCl solutions and under heated conditions.

Salt test

NaCl was used to mimic the content of seawater, while heating tests were performed to mimic the variable hot weather, which often occurs in nature. The outcomes of these tests were examined using an optical microscope to observe any occurrence of surface cracks and discolouration. The glass slides were dipped in 1, 5 and 10%NaCl solutions for 3 h before microscopic observations and absorbance measurements at 544 nm at 15 min interval.



3 Ultraviolet–visible spectrum profiles of *A. praecox* anthocyanin extracts

Heating test

In the heating test, the glass slides were heated to 60, 80 and 100°C for 3 h, and microscopic observations and absorbance measurements at 544 nm (λ_{max} for pH 1) were carried out at 15 min interval.

Results and discussion

Total anthocyanin content in *A. praecox*

The total anthocyanin content in *A. praecox* was calculated using the pH differential method, as shown in equation (1), with reference to the different structures and *m/z* values of *A. praecox* anthocyanin (Fig. 2) as compared to the general anthocyanin structure (Fig. 1). The total anthocyanin content in *A. praecox* was found to be roughly ~1227·578 mg L^{-1}.

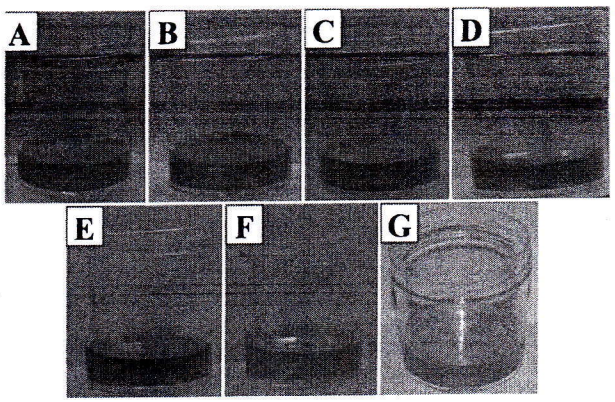
Spectrum and colour profiles of *A. praecox* anthocyanins

Based on the spectrum profiles obtained via UV–visible spectrophotometric analysis on the acidic coloured extracts, as shown in Fig. 3, a distinct peak at 544 nm was observed for both pH 1 and 2·5, while multiple peaks at 535, 571·5 and 627 nm were observed for pH 4·5 and at 535, 574 and 620 nm for pH 7. The graphs show maximum absorbance readings at a wavelength between 490 and 550 nm, which is apparent for anthocyanins.[11] These results are depicted in Table 1.

Apart from that, another strong indicator that the extracts contain anthocyanin pigments is the fact that

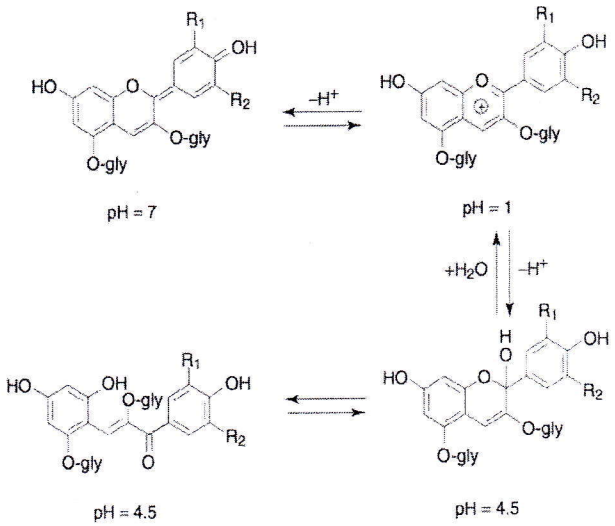
Table 1 Peaks A_{max} for each extract

pH of extract	Peaks/nm	A_{max}
1·0	544	2·24489
2·5	544	0·47957
4·5	535, 571·5 and 627	0·22700
7·0	535, 574 and 620	0·42717



A pH 1; *B* pH 2·5; *C* pH 4·5; *D* pH 7; *E* pH 9·5; *F* pH 11·5; *G* pH 14

4 Colour changes of anthocyanin extracts with pH



5 Structural transformations and predominant forms of anthocyanins according to pH[7]

they changed colour with pH.[7,10] The extract became pink purplish at pH 1, dark red at pH 2·5, light purple at pH 4·5, dark turquoise at pH 7, dark green at pH 9·5, light green at pH 11·5 and yellow at pH 14, as shown in Fig. 4.

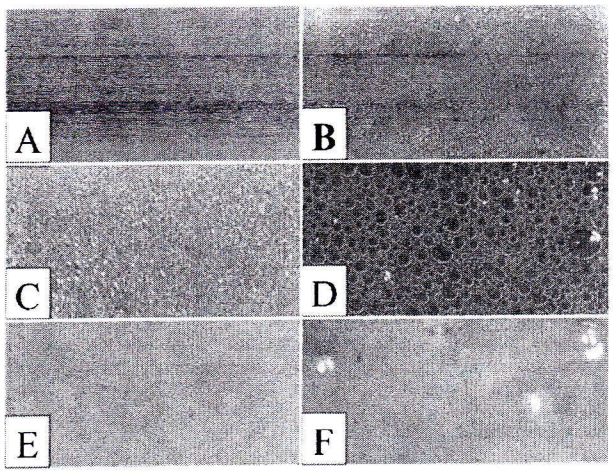
The changes in spectrum profiles and extract colours with pH were due to the change in the pigment's structure, as summarised in Fig. 5.

As the pH gets more acidic, the addition of cations (H^+ ions) changed the structure to be predominantly oxonium forms, while the addition of anions (OH^- ions) or loss of H^+ cations rendered the pigments to be predominantly in quinonoidal base forms.[7,12]

These therefore changed the colour of the extract according to the colour spectrum, where it became red as it acquires flavylium cation at acidic pH (example, at pH 1), colourless or lightly coloured when in hemiketal forms at pH 4·5, bluish when they are in quinonoidal base forms at pH 7 and became more bluish (or sometimes greenish) when in alkaline forms, as they became predominantly in anionic quinonoidal base.[12,13]

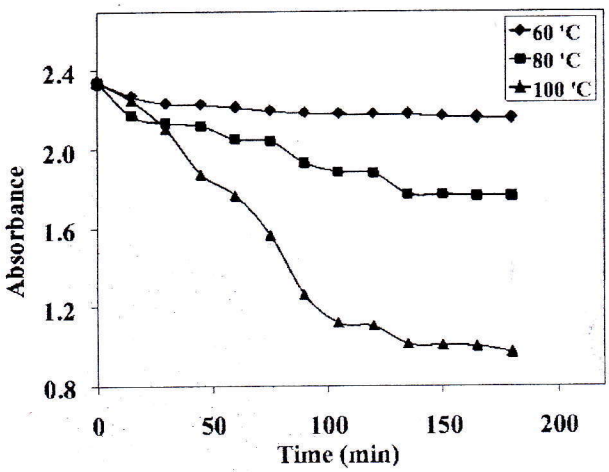
Weathering tests on PMMA mixed anthocyanins

The coated glass slides were subjected to two weathering tests: heating test and the salt test. The resin–anthocyanin mixture was uniformly mixed, and the surface of the



A before heat or NaCl treatment; *B* after 3 h of heat at 60°C; *C* after 3 h of heat at 80°C; *D* after 3 h of heat at 100°C; *E* after 3 h of treatment with 1%NaCl; *F* after 3 h of treatment with 10%NaCl

6 Effects of salt and heat treatments on anthocyanin coated glass slides



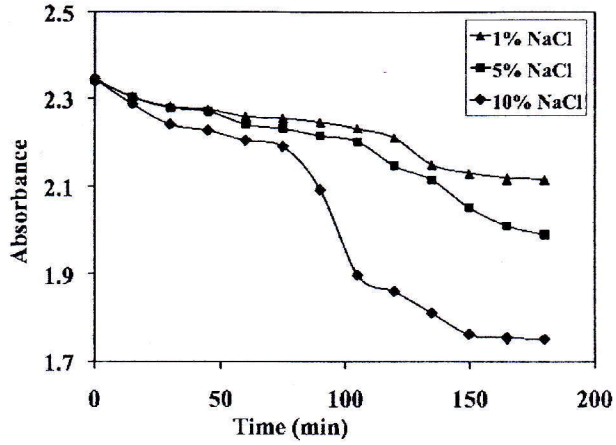
7 Effects of different heating temperatures on absorbance values (irradiated at 544 nm, λ_{max})

coated glass slides looked shiny and even, with no cracks observed. This is shown in Fig. 6.

Heating tests on PMMA–anthocyanin coated glass slides

The glass slides were heated to several temperatures (60, 80 and 100°C) in an oven for 3 h. Microscopic observations conducted on the glass slides showed more formation of air bubbles and occurrences of surface cracks as the temperature increased. The colour of the coatings also changed with temperature, where it became pink/orange at 60°C, creamy orange at 80°C and dark orange/brown at 100°C, as shown in Fig. 6.

Before the heating tests, the coated surface showed an even and shiny appearance. However, as depicted in Fig. 6, it was found that bubbles had formed at the edges of the glass slides after 3 h of heat at 60°C, but no cracks had occurred. At 80°C, small air bubbles accumulated on the entire surface of the coated slides after 3 h of heat, but only a small crack was observed. At 100°C, the entire coated surface was full with big air bubbles, and the surface was hardened; hence, a lot of surface cracks occurred after 3 h of heat.



8 Effects of different NaCl concentrations on absorbance values (irradiated at 544 nm, λ_{max})

In addition, the UV–visible spectrophotometric measurement showed a decrease in absorbance values with time. This was true to all samples, but different incubation temperatures yielded different rates, where coated slides incubated at 100°C showed a faster absorbance decrease than slides incubated at 80 and 60°C, and 80°C faster than 60°C respectively. This is summarised in Fig. 7.

Salt tests on PMMA–anthocyanin coated glass slides

Similar observations were also obtained in the salt tests, where more formation of air bubbles occurred on the coated slides dipped in 10%NaCl than in 5 and 1%NaCl. As observed in Fig. 6, for 1%NaCl, only three very tiny air bubbles were observed, and the coated surface was still smooth and did not change in colour after 3 h of treatment, while in 10%NaCl, a few large air bubbles had formed, but the coated surface remained smooth, and the colour remained intact after 3 h of treatment.

The decrease in absorbance values also occurred at a faster rate for coated slides dipped in higher NaCl concentrations, as shown in Fig. 8.

Conclusions

The UV–visible spectroscopic analysis on *A. praecox* anthocyanins proved that different pH yielded different spectrum profiles and that an acidic pH stabilised the anthocyanin molecules, therefore producing a distinct peak at 544 nm at pH 1 and 2·5. The differences in pH caused the structure of anthocyanin molecules to change, which in turn caused the spectrum profiles to differ from one another depending on the pH.[7]

Anthocyanin colours also changed with pH, where it became reddish with the addition of cations (H^+ ions) or bluish with the addition of anions (OH^- ions). This therefore made anthocyanin a very interesting molecule as it allows flowers and other natural sources to have different attractive colours, and sometimes in combination of colours, a result of the anthocyanin molecules existing at different values of pH.

In addition, it was found that *A. praecox* anthocyanins were also able to mix well with PMMA to produce a smooth and shiny texture when applied onto slick surfaces such as on glass slides. The heating tests showed that the colour did not fade with increasing temperatures, although it became darker as the temperature increased. However, there were occurrences of surface cracks, but only at extreme temperatures (100°C), which rarely occur in nature.

The salt tests also showed similar results, therefore proving that *A. praecox* anthocyanin is indeed a valuable source for a natural colourant, which can be used as environmental friendly decorative coatings.

References

1. S. Suzuki, K. Supaibulwatana, M. Mii and M. Nakano: *Plant Sci.*, 2001, **161**, 89–97.
2. Y. Mor, A. H. Halevy, A. M. Kofranek and M. S. Reid: *J. Am. Soc. Hortic. Sci.*, 1984, **109**, 494–497.
3. S. J. Bloor and R. Falshaw: *Phytochemistry*, 2000, **53**, 575–579.
4. M. M. Giusti and R. E. Wrolstad: *J. Food Sci.*, 1996, **61**, 688–694.
5. F. C. Stintzing, A. S. Stintzing, R. Carle, B. Frei and R. E. Wrolstad: *J. Agric. Food Chem.*, 2002, **50**, 6172–6181.
6. J. E. Abers and R. E. Wrolstad: *J. Food Sci.*, 1979, **44**, 75–78.
7. M. M. Giusti and R. E. Wrolstad: in 'Current protocols in food analytical chemistry', (ed. R. E. Wrolstad), F1.2.1–F1.2.13; 2001, New York, Wiley.
8. T. Fuleki and F. J. Francis: *J. Food Sci.*, 1968, **33**, 72–77.
9. T. Fuleki and F. J. Francis: *J. Food Sci.*, 1968, **33**, 78–83.
10. R. E. Wrolstad, R. W. Durst and J. Lee: *Trends Food Sci. Technol.*, 2005, **16**, 423–428.
11. L. Pauling: *Fortschr. Chem. Org. Naturst.*, 1939, **3**, 203–235.
12. F. C. Stintzing and R. Carle: *Trends Food Sci. Technol.*, 2004, **15**, 19–38.
13. O. Dangles, N. Saito and R. Brouillard: *J. Am. Chem. Soc.*, 1993, **115**, 3125–3132.