Advanced Journal of Microbiology Research Vol. 2006
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Flower pigment analysis of Melastoma malabathricum

Janna O A[1]*, Khairul A[1], Maziah M[2] and Mohd Y[2]

[1]Department of Microbiology and [2]Department of Biochemistry, Faculty of Biotechnology and Molecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Accepted 8 November, 2005

Anthocyanin is among the permitted pigments that can be used for food colourant and having been considered a potential replacement for synthetic dyes. The objective of this study is to analyse the colour pigment, anthocyanin, that can be detected in flower and their stability in extracted form. All the analysed results will be used in the next study for the production of new food colouring material. From the observation, it shows that S3 flower developmental stage contains the highest anthocyanin concentration. Simple linear regression method was used to find the significant level correlating the anthocyanin concentration and storage time. Throughout the whole experiments, the extracted anthocyanin contents are quite stable. The graphs show a small degradation rate in the anthocyanin total concentration, except for the extracts that were exposed to the light where the degradation level reached more than 50%. At different pH values, the anthocyanin concentration decreased and the colour faded at higher pH. Extracts that were stored at high temperatures (31°C) showed higher degradation levels compared to the one kept at lower temperatures (25°C). From the study we find that the suitable storage condition for coloured anthocyanin pigments is in acidic solution (pH 0.5 and 1.0) kept in the dark and at low temperature (4°C).

Key words: Anthocyanin, colourant, pigment, Melastoma malabathricum.

INTRODUCTION

Melastoma malabathricum is a shrub that belongs to the family Melastomatacea. It comes with beautiful pink colour flowers and can be easily found around the world. In Malaysia, it grows wild along almost every highway. The pink flower makes it a valuable ornamental plant and a potential source for extraction of natural colourants. Colour is important for plant development because it attracts pollinator such as birds and insects (Harbone and Grayer, 1993).

The commercial value and the buyer's acceptance of any food products markedly depends on the quality and physical appearance, especially the colour of the products. Because of the legistative actions resulting in the continuous withdrawal of artificial dyes approved earlier, the interest in the development of food colourants from natural sources enormously increased (Morais et al., 2002). For the past decade, anthocyanins have become well known alternatives to synthetic dyes (Downham and Collins 2000).

Anthocyanins (glycosylated polyhydroxy derivatives of 2-phenylbenzo pyrylium salts) are natural, water soluble, nontoxic pigments responsible for some colours of fruits, vegetables, flowers and other plant tissue (Mazza and Brouilard, 1990). Anthocyanin provides attractive colours such as orange, red and blue. They are water soluble, which facilitates their incorporation into aqueous food system (Alexandra et al., 2001). Besides the colour attractions, anthocyanins are also beneficial to health, with potential physiological effect (Mazza and Mather's 1993) and have been observed to posses potent anti-oxidant properties (Wang et al., 1997). However, some factors, such as the low stability when compare to synthetic dyes have limited their use (Markakis, 1982).

New source of anthocyanin with high tinctorial power, stability and low cost are desired as natural food colourants. From an economic perspective, the best

*Corresponding Author. E-mail: janna@biotech.upm.edu.my, Tel: 603-89466697. Fax: 603-89430913.

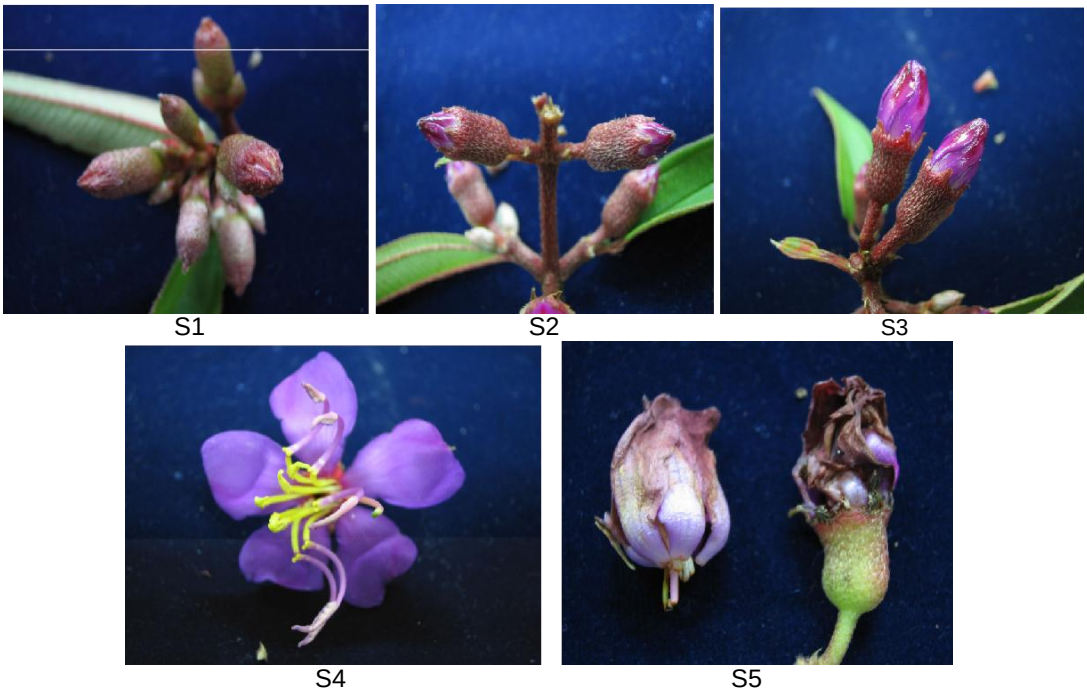


Figure 1. Flower development stages in Melastoma malabathricum. S1 (bud), S2 (early stage of petal development), S3 (fully-formed petal observed, not yet opened), S4 (fully-opened flower) and S5 (wilted flower).

potential commercial source of anthocyanin are those from which the pigment is a by-product of other manufactured value-added products, such as grape skin extracts (Jackman and Smith, 1996).

So far, anthocyanins have not been broadly used in foods and beverages because they are not as stable as synthetic dyes. To our knowledge, there has been no study of anthocyanin pigment in Melastoma malabathricum. In this study we attempted to analyse the stability of anthocyanins in different flower developmental stages of Melastoma malabathricum under different processing and storage conditions.

MATERIALS AND METHODS

Plant materials

Melastoma malabathricum flowers were obtained from wild grown plant found along route to Taman Bukit Serdang, Serdang.

Anthocyanin extraction

To prepare Melastoma malabathricum extract, petals at different stages of flower development were collected. S1 stage (bud) was not included because no petal formation was observed. Data obtained from this study came from stages S2 (early stage of petal development), S3 (fully formed petal observed, not yet opened), S4 (fully-opened flower), and S5 (petals starting to decay) as shown in Figure 1. Anthocyanin was extracted from 5 g of fresh petals with 50 ml methanol, 1% (v/v) HCL (Harbone, 1984) for an hour. The extract was then filtered with Whatmann 110 nm filter paper for subsequent treatments.

Determination of anthocyanin concentration

Total anthocyanin content was determined by measuring the absorbance of the extract at 505 nm using a spectrophotometer (PRIM, SECOMAM, France). All measurement were done in three replicates. In analyzing the effect of light and temperature on anthocyanin stability, the filtered extract was first exposed to light given by a 1.5 W lamp or kept in the dark, and then stored at different temperatures (25^{o}C, 31^{o}C). The absorbance of the extract was taken daily for 26 days or otherwise indicated. In analyzing the effect of pH on pigment stability, the extraction was carried out at different pHs ranging from 0.5 – 3.0, following which absorbance of the filtered extract was taken hourly for 24 h.

Storage condition experiment

Absorbance taking of the extract was carried out daily and hourly. The extract was placed into flasks with the following conditions: exposed to the light using a lamp of 1.5 W and in the dark with different temperatures (25^{o}C, 31^{o}C), and analyzed for 26 days; different pH ranging from 0.5-3.0 were analyzed hourly for 24 h.

Statistical analysis of data

Data were subjected to simple linear regression analysis using SPSS, release 5.0.

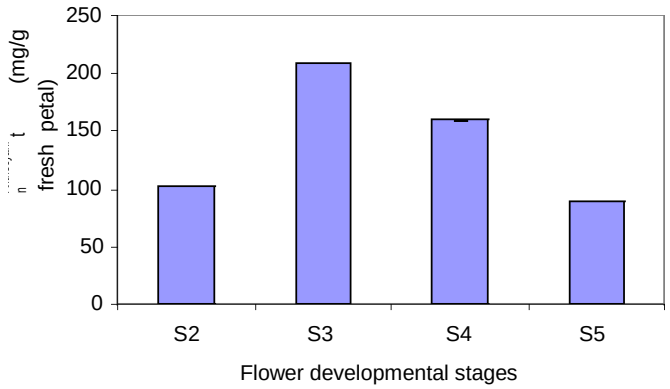


Figure 2. Total anthocyanin content in Melastoma malabathricum. The amount of anthocyanin was calculated based on per gram fresh weight of petal used in the extraction.

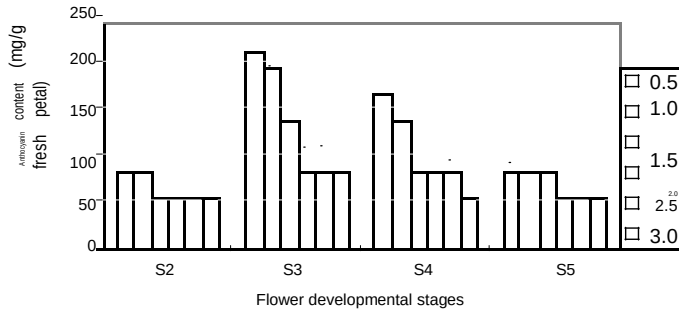


Figure 3. Melastoma malabathricum anthocyanin concentration at different pH.

RESULT AND DISCUSSION

Figure 2 show that S3 has the highest anthocyanin content. This is followed by S4, S2 and S5. Anthocyanin can be extracted with organic solvents from solid matrices before analysis. The total anthocyanin concentration can be easily measured by spectrophotometric after extraction (Morais et al., 2002). Anthocyanin concentration was detected to be the highest in S3 during the flowering development. It shows that the anthocyanin biosynthesis is at maximum level at this stage. Anthocyanin can be extracted with organic solvents from solid matrices before analysis. The development of anthocyanin in leaves, fruits and flowers is characterized by a slow increase at early the stage of organ growth, followed by a sharp abrupt and substantial rise to a maximum and finally, by a sharp slow decrease until the death of plant cells (Stickland, 1972; Woodward, 1972). Maximum anthocyanin accumulation take late in maturity when plastid pigments are undergoing rapid decomposition (Stickland, 1972; Woodward, 1972). This implies that M. malabathricum flower at developmental stage S3 is at its full-matured state. It can be observed physically that the colour of the petals at stage S3 is darker than the other flower stages in this species.

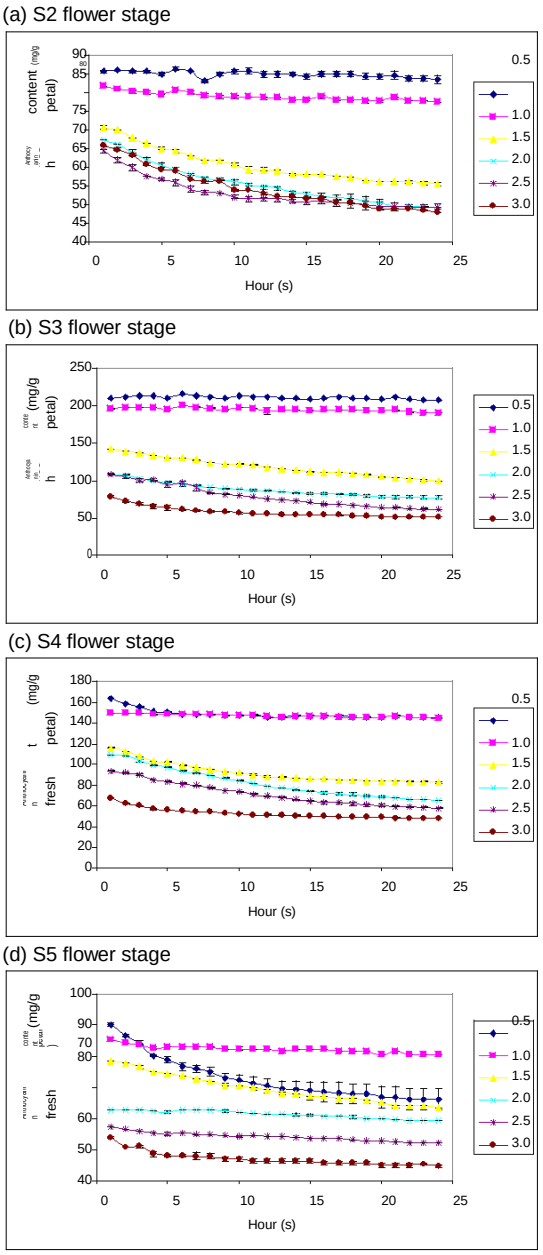


Figure 4. Melastoma malabathricum anthocyanin content in the original extract among the flower developmental stages S2, S3, S4 and S5 at different pH values in room temperature for 24 h. Simple linear regression was performed on the data, followed by F and t tests at α = 0.05.

The total concentration of anthocyanin content is greatly affected by the pH in the extract buffer as shown in Figure 3 where the total anthocyanin contents were measured from different developmental stages of the flower extracted at different pH. In acidic solution, increasing pH affect the concentration of the anthocyanin as well as the colour. The colour faded and became lighter. The extracted colour faded with increasing pH. The colour changes from red (pH 0.5), fading and finally turned to light red (pH 3.0) . Harbone (1984) and

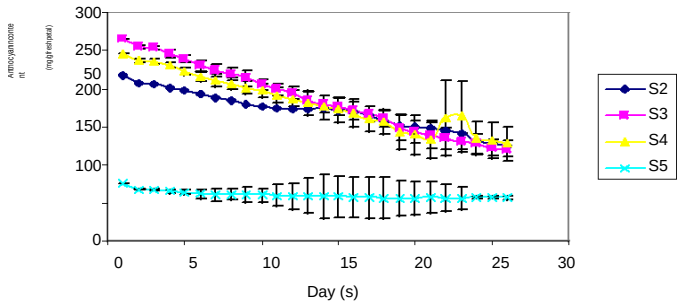
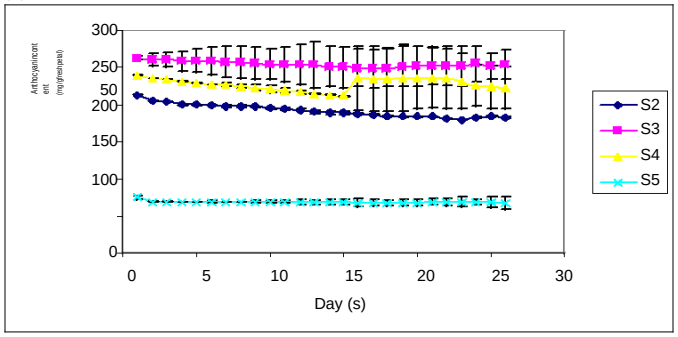


Figure 5. Melastoma malabathricum anthocyanin concentration in the original extract exposed to the light at 25^{o}C for 26 days. Simple linear regression was performed on the data, followed by F and t tests at α = 0.05.

(a) 25^{o}C



(b) 31 ^{o}C

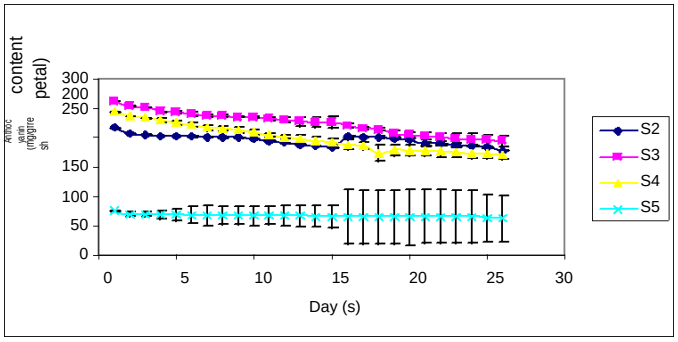
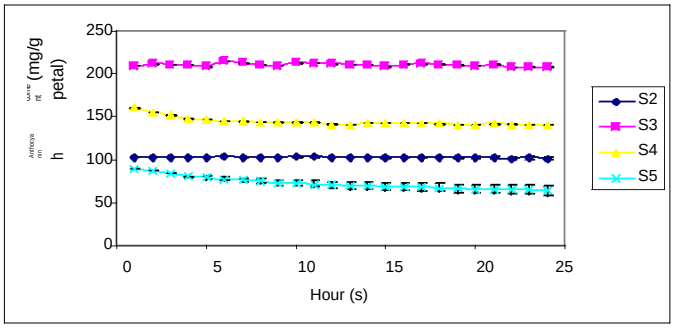


Figure 6. Melastoma malabathricum anthocyanin concentration in original extract among all flower developmental stages kept in darkness at (a) 25^{o}C and (b) 31^{o}C for 26 days. Simple linear regression was performed on the data, followed by F and t tests at α = 0.05.

Brouillard (1988) explained that anthocyanin extracts were red, orange or purple in acid and blue in alkali. Between pH1.0 to 3.0 the pigment existed as the deep red flavylium ion, and increasing pH hydrated this ion slowly to the thermodynamic pseudobase (Harbone, 1984). Brouillard (1988) observed that as the pH is raised, kinetic and thermodydynamic competition occurs between the hydration reaction on position two of the flavylium cation and the proton transfer reaction related to its acidic hydroxyl groups. This shows that anthocyanin is

(a) Hourly



(b) Daily

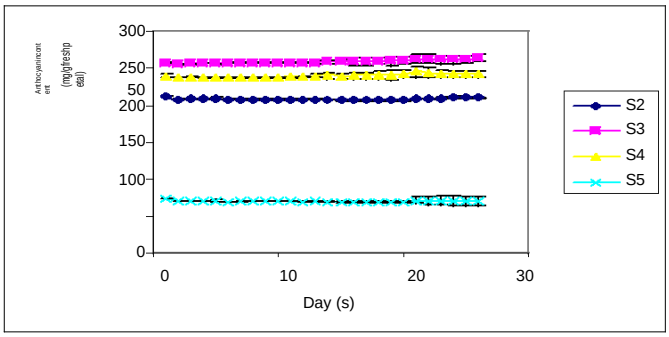


Figure 7. (a) Hourly and (b) daily detection of Melastoma malabathricum anthocyanin concentration in original extract that kept in darkness at 4^{o}C. Simple linear regression was performed on data followed by F and t tests at α = 0.05.

undergoing several reactions as the pigment is destroyed, which affects the colour. Thus, by manipulating the pH in the solution, a flower extract colour can be made to change accordingly.

In order to detect the effect of different pH on the stability of anthocyanin during the storage time, absorbance of the extract were taken every hour for 24 h. Figure 4 (a, b, c, d) shows the degradation of anthocyanin content in the extract with different pH among the four flower developmental stages S2, S3, S4 and S5. The figures explain that acidic extract is more stable. From the regression test conducted, the reduced value is not significantly correlated to the storage time.

Major decrease in anthocyanin concentration is observed in the extract after been exposed directly to 1.5 W lamps at 25^{o}C (Figures 5a, b, c, d). The highest reduction is shown by S3 (265.77 mg/g), followed by S4 (245.22 mg/g), S2 (218.39 mg/g) and S5 (75.98 mg/g), but the colour still remained only it was lighter except for S5 where the colour turned to yellow. Although the decrease may be high, it is not significantly correlated to the storage time. These results are supported by the observation of Ochoa et al. (2001) who reported that in samples kept in the presence of light the concentration of anthocyanin pigments decrease more strongly than samples kept in dark. Kucharska (1998) also reported similar results.

To compare the effects of temperature on anthocyanin content, data were taken at two different temperatures, 31 and 25^{o}C. The results obtained in Figure 6a and b indicated a smaller decrease in anthocyanin content at lower temperatures for all flower stages. In live plant, low temperature increases while elevated temperature decreases anthocyanin concentration; high temperatures decrease the synthesis of the anthocyanin pigments. In addition to its affect on anthocyanin synthesis, temperature may also affect the pigment stability, with elevated temperature causing increased degradation (Shaked-Sachray et al., 2002; Weshce-Ebeling et al., 1996). Kirca and Cemeroglu (2003) also observed that anthocyanin degrades with increasing temperature. El Gindy (1972) suggested that to produce well coloured products, the lowest storage temperature and low pH should be used. Figures 7a and b show that the extract including the colour is stable at 4^{o}C for 24 h and 26 days indicating that the storage condition performed in this study is suitable for the anthocyanin colour and concentration stability.

In conclusion, the results of this study show that M. malabathricum's highest anthocyanin concentration is obtained S3 at the flowering development stage. Furthermore, a suitable storage condition for the coloured anthocyanin pigments is acidic soloution (pH 0.5 and 1.0) in the dark at low temperature (4^{o}C).

ACKNOWLEDGEMENT

The project was supported by the IRPA Grant (09-02-04-0548) from the Ministry of Science, Technology and Innovation, Malaysia.

REFERENCES

Alexandra P-DE, Mónica GM, Wrolstad RE, Glóri MBA (2001). Anthocyanins from Oxalis triangularis as potential food colorants. Food Chem. 75: 211-216.

Bakowska A, Kucharska AZ, Oszmianski J (2003). The effect of heating, uv irradiation, and storage on stability of the anthocyanin-polyphenol co-pigment complex. Food Chem. 81: 349-355.

Bride P, Timberlake CF (1997). Anthocyanin as natural food colour–selected aspect. Food Chem. 58:103-109.

Brouillard R (1988). Flavonoids and flower colour. In: harbone JB, ed. The Flavonoids. Advance in research since 1980. London: Chapman and Hall.

Downham A, Collins P (2000).. Colouring our food in the last and next millennium. Int'l. J. Food Sci. and Tech. 35: 5-22.

Harbone JB (1984). Phytochemical method. London: Chapman and Hall.

Harbone JB, Grayer RJ (1993). Flavanoids and insects. In The Flavanoids: Advances in Research since 1980, (ed) Harbone JB, London: Chapman and Hall.

Jackmann RL, Smith JL (1996). Anthocyanin and betalains. In Hendry GAF Houghton JD (Eds). Natural food colorants (2nd ed.) Londres: Chapman and Hall. pp. 245-3092

Kirca A, Cemeroglu B (2003). Degradation kinetics of anthocyanin in blood orange juice and concentrate. Food Chem. 81: 583-587.

Kucharska AZ, Oszmianski J, Kopacs M, Lamer-Zarowska E (1998). Application of flavonoids for anthocyanin stabilization. II Conerence flavonoids and their employment. Polland: Rzeszow.

Mazza G, Brouillard R (1990). The mechanism of co-pigmentation of anthocyanin in aqueous solution. J. Phytochem. 29: 1097-1102.

Markakis P (1982). Stability of anthocyanin in foods. In: P Markakis (Ed). Anthocyanin as food color. New York: Academic Press. (pp. 163-180).

Morais H, Ramos C, Forgacs E, Cserhati T, Olivira J (2002). Influence of storage condition on stability of monomeric anthocyanin studied by reverse phase high-performance liquid chromatography. J. Chromatography B: Anal. Tech. in the Biomed. and Life Sci. 770: 297-301.

Ochoa MR, Kesseler AG, De Michelis A, Mugridge A, Chaves AR (2001). Kinetics of colour changes of raspberry, sweet (Prunus avium) and sour (Prunus cerasus) cherries preserve, packed in glass containers: light and room temperature effect. J. Food Eng. 49: 55-62.

Shaked-Shachray L, Weiss D, Reuveni M, Nissim-Levi A, Oren-Shamir M (2002). Increased anthocyanin accumulation in Aster flower at elevated temperature due to magnesium treatment. J. Physiol. Plant 114: 559-565.

Stickland RG (1972). Changes in anthocyanin, caretenoid, chlorophyll and protein in developing floretsof the Chrysanthemum. Ann. of Bot. 36: 59-469.

Wang H, Coa G, Prior RL (1997). Oxygen radical absorbing capacity of anthocyanin. J. Agric. Food Chem. 45: 304-309.

Wesche-Ebeling P, Argaíz-Jamet A, Hernández-Porras LG, López-Malo A (1996). Preservation factors and processing effects on anthocyanin pigments in plums. Food Chem. 57: 399-403. Woodward JR (1972). Physical and chemical changes in developing Strawberry fruits. J. Sci. Food Agric. 23: 465-473.