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Contributed Paper

The Effects of Sugar Application on the Concentrations of Anthocyanin and Flavonol of 'Mahajanaka' Mango (*Mangifera indica* Linn. cv. Mahajanaka) Fruit

Kanyarat Lueangprasert*[a], Jamnong Uthaibutra [a], Kobkiat Saengnil [a] and Osamu Arakawa [b]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
[b] Faculty of Agriculture and Life Science, Hirosaki University, Aomori, 036-8561, Japan.
*Author for correspondence; e-mail: keng023@hotmail.com; kanyarat_l@yahoo.com

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ABSTRACT

The relationship between contents of anthocyanin, flavonol, sugars and sunlight were explored in Mahajanaka mango (*Mangifera indica* Linn. cv. Mahajanaka) fruit exocarp in Thailand. Sugars including fructose (5 or 10% weight/volume; w/v) and sucrose (10% w/v) were applied three times at 77, 84 and 91 days after full bloom before red colour development. Anthocyanin and flavonol contents increased throughout fruit development, especially after the application of 10% fructose with a greater response in the sunny exposed sections of the fruit than in shaded sections. The concentrations of sucrose and fructose were generally higher in the exposed sections after applications of the sugars compared with the control. There was a positive correlation between anthocyanin and flavonol contents across the sugar treatment in the exposed side of the fruit. However, there were strong correlations between the concentrations of anthocyanin and sugars in the shaded side of the fruit. These results suggest a strong relationship between colour development, light and exposure to exogenous sugars in this mango cultivar.

Keywords: Mahajanaka mango**,** sugar, anthocyanin, flavonol.

1. INTRODUCTION

Mahajanaka mango is a hybrid cultivar between 'Nang Klang Wan' and 'Sunset', and has a distinctive red or yellow-red exocarp due to the presence of anthocyanins. The two main factors affecting anthocyanin synthesis in plants are light and sugars. There is a positive correlation between phenols and flavonols with fruit skin reddening [1]. In the biosynthesis of flavonoid, phenylalanine is converted by phenylalanine ammonia lyase (PAL) into coumaroyl CoA, which is first condensed by chalcone synthase and subsequently with malonyl CoA. Eventually, these products are combined with different sugar molecules with the aid of UDP-glucose: flavonoid 3-o-glucosyltransferase (UFGT) to form flavonols and anthocyanins [2].

Bakhshi and Arakawa [3] reported that light stimulated quercetins (flavonols) and anthocyanin synthesis in 'Jonathan' apple.

Other studies showed that this response was related to wavelengths of light radiation. UV-B and red light irradiation stimulated anthocyanin synthesis in 'Jonathan' apple [4], UV-B and white light was effective in 'Jonathan', 'Fuji' and 'Orin' apples [1]. Likewise, the sunlight was effective in 'Early Black' cranberry [5], 'Jonagold', 'Elstar' [6], 'Starkrimson', and 'Golden Delicious' apples [7].

The biosynthesis of anthocyanins and flavonols is also dependent on a regular supply of sugars in the plant. Anthocyanins and flavonols in the cell vacuole are found as solutes in the form of glycosides [8]. The main sugars associated with anthocyanins were glucose in blackberry [9], galactose in 'Winesap', 'Grimes Golden' and 'Jonathan' apples [10, 11] and fructose in grape [12]. Applications of sugar increase anthocyanin synthesis and red colour development in several species. Ten percent (w/v) fructose was effective in grape berry [12], 175 mM sucrose and glucose, and 88 mM fructose under constant illumination (~50 $\mu E/m^2s$) were each effective in radish [13], and 0.05-0.20 M sucrose, glucose and fructose were each effective in 'Black Mexican Sweet' maize [14].

The objective of this study was to determine whether light and sugars affected anthocyanin synthesis in Mahajanaka mango. Fructose and sucrose were applied to fruit of this cultivar before they started to colour. Exocarps were sampled from exposed and shaded parts of the fruit to evaluate the impact of 'light' on the response.

2. MATERIALS AND METHODS

2.1 Plant Material

Forty trees of 7-year old 'Mahajanaka' mango (*Mangifera indica* Linn. cv. Mahajanaka) from an orchard in Chiang Mai province, Thailand were used for this investigation. Inflorescences from the east side of the tree canopy were tagged on the day of full bloom, thereafter fruit ages were counted with the full bloom date used as day 0. After fruit development, 200 mangoes were selected. The fruits at 77 days after full bloom were dipped in either 5% or 10% (w/v) fructose solution or 10% (w/v) sucrose solution for 1 min. Non-dipped fruit acted as the control making a total of 4 treatments (5% fructose, 10% fructose, 10% sucrose and control) with 50 fruits per treatment. The fruits were dipped again on days 84 and 91. Thereafter, 40-fruits were sampled at weekly intervals from day 98 until maturity on day 119. The sunny exposed side and shaded side that faces inward towards the tree trunk of each fruit were marked on the fruit exocarp. The exocarp of each fruit was peeled (1 mm thickness) and divided into sunny exposed and shaded sides, then was frozen in liquid nitrogen, freeze-dried and kept in a cool room. Exocarp samples were ground into a fine powder prior to analysis.

2.2 Anthocyanin and Flavonol Analyses

Anthocyanins and flavonols were analysed by high performance liquid chromatography (HPLC) as described by Bakhshi and Arakawa [1]. Extraction was carried out by adding 1 ml of a solution containing 15% (v/v) acetic acid and 85% (v/v) methanol to 0.5 g of finely ground exocarp samples, and kept at 4°C for 1 h. The extract was centrifuged at 8,000 × g for 10 min at 4°C (Himac CR15, Hitachi, Japan). The supernatant was filtered through a 0.5 μm disposable syringe filter (Advantec, Toyo Roshi Kaisha, Ltd., Japan) and used for HPLC coupled to a diode array detector with the wavelength set at 350 nm detection for flavonol and 530 nm for anthocyanin detection. The column was 1.5 mm I.D. × 250 mm (Grand C18-UG 120-5 SE, MASIS, Inc., Aomori, Japan) with 1.5 mm I.D. × 35 mm guard column. Elution solvents were (a)

1.5% phosphoric acid in milli-Q water with a flow rate of 90 µl min^{-1} and (b) 1.5% phosphoric acid, 20% formic acid and 25% acetonitrile solution with the flow rate of 10 µl min^{-1}. The sample (5 µl) was injected into the column which was maintained at 40°C. Results were expressed as absorbance unit (ABU) at 350 nm for flavonols and 530 nm for anthocyanins. Moreover, the anthocyanin and flavonol contents were calculated in terms of peak areas of HPLC Chromatograms as shown in figure 3.

2.3 Sugar Analysis

The method used for endogenous sugar determination was that of Thammawong [15]. One gram of fine powder was extracted three times each for 30 min with 8 ml of 80% (v/v) ethanol at 80°C and the suspension was centrifuged (Centrifugal Vaporizer CVE-200D; EYELA, Japan) at 10,000 rpm at 60°C. The remaining ethanol-insoluble fraction was filtered through a 0.45 µm disposable syringe filter (Advantec) and injected into HPLC (Shimadzu, Co., Ltd.). The HPLC was operated using a Shim-Pack SPR-Ca (7.8 mm I.D. × 250 mm column, Shimadzu Co., Ltd.) with water as the mobile phase at 0.5 ml min^{-1}, a column temperature of 80°C and analyzed with a RI detector (RI-200, ISILABO). Sugar contents were compared with sucrose and fructose standards. Analysis of sugar content was carried out in triplicate.

2.4 Statistical Analysis

The statistical analysis of anthocyanin and flavonol contents as well as sugar content was carried out in triplicate. The Statistical Packages for the Social Science (SPSS) software for windows was used for ANOVA and LSD with a completely randomized design in this experiment.

3. RESULTS AND DISCUSSION

3.1 Sugars

The concentrations of fructose and sucrose in the mango fruit were highest on day 112. The endogenous sugar contents in the exocarp of the exposed side were also significantly higher than those in the shaded side. The effects of exogenous sugar concentration were reported for day 112 (Table 1).

The concentrations of both endogenous fructose and sucrose were lower in the shaded side of the fruit than in the exposed side. Fructose at 5 and 10% increased the concentration of endogenous fructose in the exposed side. The sugar treatments also increased the concentration of endogenous sucrose in the exposed side, whereas they were

Table 1. The effects of sugar application on the concentrations of endogenous fructose and sucrose (mg/g dry weight) in exposed and shaded sides of mango fruit exocarp 112 days after full bloom. Data are the means of three replications per treatment. Column means for exposed and shaded sides of each sugar followed by different letters are significantly different (P = 0.05).

Treatments	Fructose		Sucrose	
	Exposed side	Shaded side	Exposed side	Shaded side
Control	68.8bc	29.8a	53.8pq	35.1p
Fructose 5%	88.0de	57.0b	82.7r	42.5p
Fructose 10%	95.9e	37.0a	76.7qr	35.7p
Sucrose 10%	75.8cd	38.4a	84.8r	37.4p

ineffective in the shaded side. The relative result was also reported in radish, which rapidly takes up exogenous sugars increasing the concentration of endogenous sugars. Especially, the sucrose is metabolized to fructose and glucose, which increases the proportion of fructose [13]. Further, the simultaneous application of sugars and light, via immersion in sucrose solution, to 'Royal Purple' (*Eustoma grandiflorum*) petals caused increased purple coloration suggesting an increase in endogenous sucrose. Light was essential for the transfer of carbohydrates from leaves to petals, while low light conditions reduced the soluble sugar content of petals [16]. Light also facilitated the uptake of external carbohydrates into strawberry leaf tissue [17].

3.2 Anthocyanin and Flavonol

Applications of sugars increased the concentrations of anthocyanin and flavonol (Figures 1 and 2) in both the exposed and shaded sides throughout fruit development. There were two peaks for anthocyanin and two peaks for flavonol in the HPLC analysis of the mango tissue (Figure 3).

The concentrations of anthocyanin generally increased during fruit development, with higher levels in the exposed side of the fruit than in the shaded side (Figure 1). Applications of sugar increased the concentrations of anthocyanin in the exposed side compared with control levels, with a greater response in the exposed side than in the shaded side. In the exposed side, fructose at 10% was the most effective, followed by

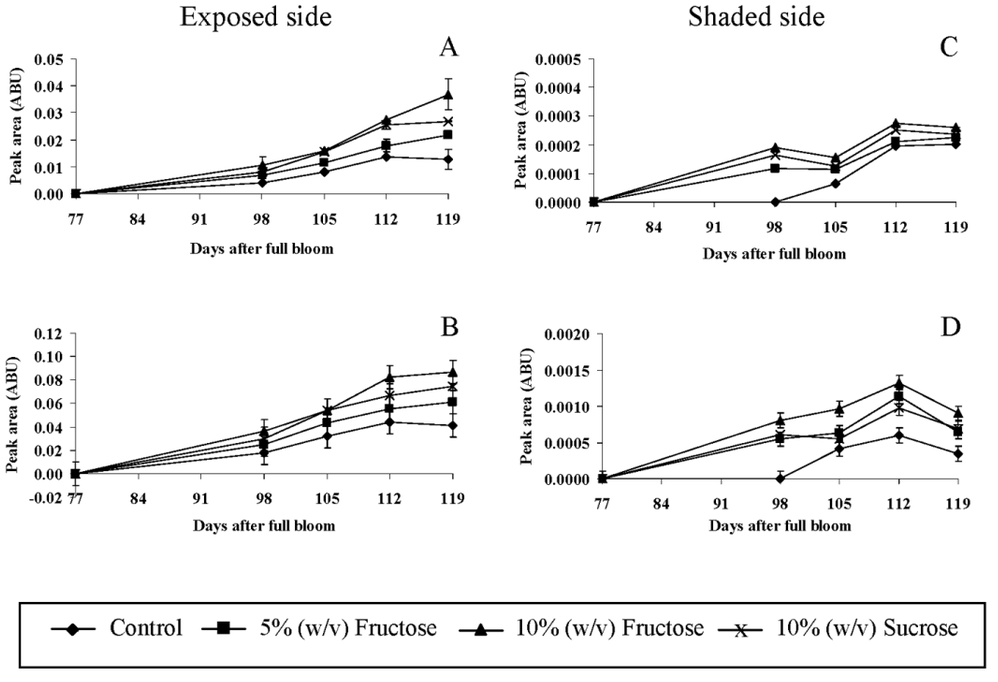

Figure 1. The effects of sugar application on anthocyanin content (ABU) in exposed and shaded sections of 'Mahajanaka' mango. Anthocyanins identified as peaks 1 (A and C) and 2 (B and D) from HPLC (Figure 3). Vertical bars indicate ± SD.

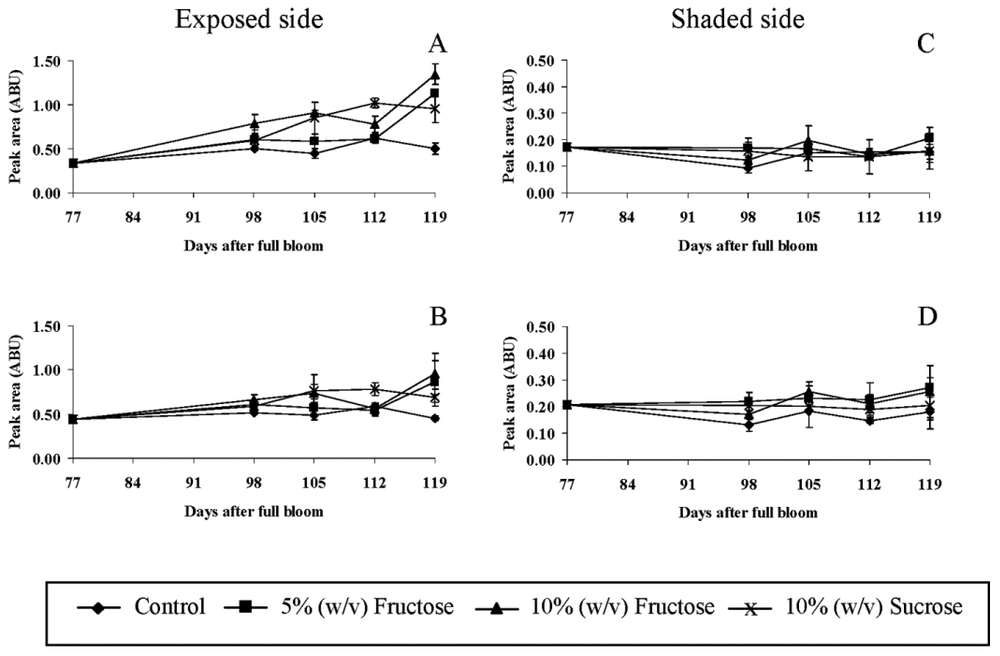

Figure 2. The effects of sugar application on flavonol content (ABU) in exposed and shaded sections of 'Mahajanaka' mango. Flavonols identified as peaks 5 (A and C) and 6 (B and D) from HPLC (Figure 3). Vertical bars indicate ± SD.

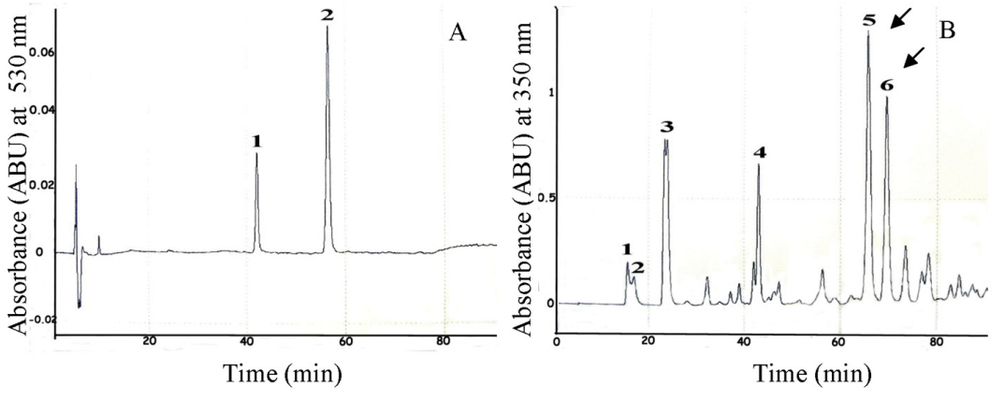

Figure 3. Chromatograms of anthocyanins (A) and flavonols (B) from the HPLC analysis.

sucrose at 10% and then fructose at 5%.

The changes in the concentrations of flavonol were less dramatic than the changes in anthocyanin. The concentration of between flavonol (peaks 5 and 6) and anthocyanin (peaks 1 and 2) increased over time in the exposed side, whereas the concentrations in the shaded side were relatively stable (Table 2 and Figure 2). The effect of applying exogenous sugars (10% fructose > 5% fructose > 10% sucrose) increased the concentrations of flavonol in the exposed side

compared with levels in the control especially peak 5. In contrast, the sugars had little effect in the shaded side of the fruit. All exogenous sugar treatments increase the higher levels of flavonol and anthocyanin in the exposed side than in the shaded side.

The results showed that generally sugars enhanced anthocyanin and flavonol contents during red colour development only in the exposed side of the fruit. Hara *et al.* [13, 18] suggested that the exogenous sugars provided to radish seedlings were not only energy sources but also signal molecules to elicit anthocyanin biosynthesis as the seedling were incubated under constant illumination which increased the radish's red colour. Phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone flavonone isomerase (CHI), flavonone 3-hydroxylase (F3H), dihydroflavonol reductase (DFR) and anthocyanidin synthase (ANS) were enhanced by sucrose in the hypocotyl of red radish. In transgenic *Arabidopsis* [19] and *Vitis vinifera* cell [20], a transcription factor regulated the genes involved in anthocyanin production [21]. This is perhaps how sugars, especially fructose operated in the mango fruit. Fructose was effective in coloration and anthocyanin biosynthesis in grape [12]. Each sugar enhanced flavonol content differently which was observed based on the specific sugar in the flavonol aglycone, as reported by Lister *et al.* [22] who examined 'Granny Smith' and 'Splendour' apples. For instance, the galactose caused a high relative activity of myricetin, kaempferol and quercetin, while glucose and xylose caused the highest relative activity of quercetin.

Exposed sections of the mango exocarp had higher concentrations of anthocyanin and flavonol. Sunlight increased cyanidin (anthocyanin) and quercetin (flavonol) synthesis in apples [3]. Light-induced enzymes including DFR, ANS and UFGT may be needed for leucocyanidin and cyanidin glycoside synthesis, eg. 'Granny Smith' and 'Golden Delicious' apples [23].

Using HPLC analysis, two peaks of anthocyanins were found in the Mahajanaka mango exocarp (Figure 3A). Peak 2 was cyanidin 3-galactoside [24, 25] and peak 1 was probably a related substance. For flavonols (Figure 3B), peak 5 was similar to standard quercetin 3-galactoside, while peak 6 was probably quercetin glucoside. Thus, quercetins were closely linked to cyanidins in Mahajanaka mango. The result suggested that the metabolism of both substances was a light dependent process causing more red colour development in the exposed side than shaded side as also reported in apple [3].

There was a positive correlation between anthocyanin content (peaks 1 and 2) and flavonol content (peaks 5 and 6) across the sugar treatment in exposed side of the fruit (Table 2). In contrast, there were no significant correlations in the shaded side of the fruit (Table 2). The result of this study showed that

Table 2. Coefficient of determination (R^2) between anthocyanins (peak 1, 2) and flavonols (peak 5, 6) in exposed and shaded sides of mango fruit exocarp (n=20).

Anthocyanins	Flavonols			
	Peak 5		Peak 6	
	Exposed side	Shaded side	Exposed side	Shaded side
Peak 1	0.81**	0.00ns	0.62**	0.07ns
Peak 2	0.75**	0.00ns	0.57**	0.12ns

** significant at $p = 0.01$; ns, not significant.

the sugars and light effectively increased anthocyanin and flavonol contents. As concordantly reported with 'Fuji' and 'Jonathan' red skin apples, light irradiation induced an increase of both anthocyanins and flavonols, which showed positive correlation between the two substances. The positive correlation between anthocyanin and flavonols showed that the expression of genes controls the synthesis [1].

There was a positive correlation between the concentration of anthocyanin as peak 1 and the concentration of endogenous fructose and sucrose in the shaded side of the fruit (Table 3). In contrast, there were no significant correlations with peak 1 and endogenous sugars in the exposed side, or peak 2 in either side (Table 3).

Table 3. Coefficient of determination (R^2) between anthocyanins (peak 1, 2) and endogenous sugars content (mg/g dry weight) in exposed and shaded sides of mango fruit exocarp (n=20).

Anthocyanin	Fructose		Sucrose	
	Exposed side	Shaded side	Exposed side	Shaded side
Peak 1	0.36ns	0.59**	0.01ns	0.48*
Peak 2	0.33ns	0.41ns	0.01ns	0.36ns

*,** significant at $p = 0.05$ and $p = 0.01$, respectively; ns, not significant.

4. CONCLUSIONS

In conclusion, parts of the mango fruit exposed to light had higher concentration of sugars and higher concentrations of anthocyanins and flavonols than parts of the fruit that were shaded. Application of sugars especially 10% fructose increased sugar content and colour components in exposed fruit, but were less effective in shaded fruit. Moreover, application of sugars can also increase colour development in Mahajanaka mango. Effects of increasing light exposure of the fruit by appropriate pruning of the canopy should be explored.

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