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Colour development and quality of mangosteen (*Garcinia mangostana* L.) fruit during ripening and after harvest

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ABSTRACT

The colour of mangosteen (*Garcinia mangostana* L.) fruit changes from green to purple black after harvest as the fruit ripens, and is used as a quality guide for growers and consumers. We determined the relationship between anthocyanin composition and content during fruit colour development in relation to fruit maturity and postharvest quality. Fruit at different stages of maturity (light greenish yellow with 5% scattered pink spots to purple black) were harvested and kept at 25 °C (85–90% RH). Fruit from each maturity stage all developed to the final purple black stage. During the postharvest period, hue angle values and pericarp firmness decreased significantly, while soluble solids contents increased. Anthocyanin contents in the outer pericarp were higher than in the inner pericarp and increased to a maximum at the final colour stage. Sensory evaluation and fruit quality (hue angle values, soluble solids and titratable acidity) of fruit harvested at the different stages did not differ once the fruit had finally developed to the purple black stage. The anthocyanins in the outer pericarp mainly consisted of five compounds, identified by HPLC/MS as cyanidin-sophoroside, cyanidin-glucoside, cyanidin-glucoside-pentoside, cyanidin-glucoside-X, cyanidin-X_2 and cyanidin-X, where X denotes an unidentified residue of m/z 190, a mass which does not correspond to any common sugar residue. Cyanidin-3-sophoroside and cyanidin-3-glucoside were the major compounds and the only ones that increased with fruit colour development.

1. Introduction

The mangosteen (*Garcinia mangostana* L.) fruit has an edible aril that is white, juicy, sweet and slightly acid with a pleasant flavor, located inside a dark purple pericarp that is rich in bioactive secondary metabolites including anthocyanins, oligomeric proanthocyanins and xanthones (Fu et al., 2007; Ji et al., 2007). Fruit colour is a major criterion used to judge maturity and for grading of mangosteen fruit. The fruit are usually harvested at different stages according to colour, from light greenish yellow with scattered pink spots to dark purple. After harvest, the purple colour continues to develop very quickly. For high fruit quality, the minimum harvest colour stage is that of distinct irregular, pink–red spots over the whole fruit. If fruit are harvested with a light greenish yellow with scattered pink spots, the fruit do not ripen to full flavor (Tongdee and Suwanagul, 1989; Paull and Ketsa, 2004).

The purple colour of the mangosteen fruit pericarp is mainly due to anthocyanins. In the only published analytical work to date on the colour pigments, Du and Francis (1977) identified by thin layer chromatography (TLC), two anthocyanins in the mangosteen pericarp, cyanidin-3-sophoroside and cyanidin-3-glucoside. However, more detailed analysis of anthocyanins and their changes during fruit colour development has not been reported, nor a comprehensive study of the relationship between colour development and fruit ripening, the latter being important for successful marketing of this fruit. In this work, we report on new anthocyanin compounds in mangosteen pericarp that are associated with colour development, and show how fruit colour changes after harvest at different maturities in relation to fruit quality.

2. Materials and methods

2.1. Plant material

Fruit were selected for colour and size (75–90 g) uniformity at a commercial grower in Chanthaburi, and then transported by refrigerated truck (15 °C) to the laboratory within 6 h. The fruit

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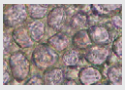
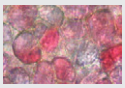
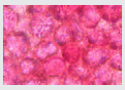
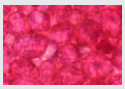
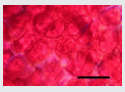
Table 1
Light microscopy, time, quality and sensory evaluation of mangosteen fruit

Fruit stage	Outer pericarp section	Time (d)	Firmness (N)		a^*/b^* ratio		% SSC		% TA		SSC/TA		Sensory
			A	B	A	B	A	B	A	B	A	B	B
1			0	6.66a	44.8	0.03b	11.06b	15.2c	17.2	0.77bc	0.81	19.8bc	21.2b	4.2
2			1	5.30b	43.6	0.44b	12.09ab	15.3bc	17.3	0.78b	0.81	19.6bc	21.3b	3.7
3			2	4.91c	46.0	1.16b	12.10ab	16.3ab	17.5	0.84a	0.80	19.3c	21.9ab	3.8
4			3	4.59d	42.9	2.28b	10.04b	16.6a	17.9	0.80ab	0.78	20.8bc	23.0ab	4.0
5			5	4.20e	45.0	3.71b	9.63b	17.1a	17.5	0.79b	0.75	21.7ab	23.5a	3.7
6			9	3.84f	47.9	16.69a	13.95a	17.2a	17.4	0.73c	0.74	23.7a	23.7a	4.1

Fruit were either harvested at stage 1 (A) and allowed to ripen at 25 °C, or harvested at the six different maturity stages and measurements made when the fruit of each maturity had reached stage 6 (B). The bar in the outer pericarp section shows 0.1 mm. The firmness values in A column are log(ln) transformed data, with original data provided in Section 3. Means within any column followed by the same letter are not significantly different ($P > 0.05$).

were separated by skin colour into six stages, using a colour index slightly modified from that of Tongdee and Suwanagul (1989): yellowish white or yellowish white with light green (stage 0), light greenish yellow with 5–50% scattered pink spots (stage 1), light greenish yellow with 51–100% scattered pink spots (stage 2), spots not as distinct as in stage 2 or reddish pink (stage 3), red to reddish purple (stage 4), dark purple (stage 5) and purple black (stage 6) (see Table 1). To study fruit colour development (A), fruit were harvested at stage 1 and analyzed immediately for quality and anthocyanin content. The remaining fruit were then kept at 25 °C (85–90% RH). Quality and anthocyanin assessments were made at regular intervals until the fruit reached stage 6. To study fruit maturity relationships (B), fruit were harvested at stages 1–6 and stored at 25 °C (85–90% RH) and when fruit reached stage 6, the fruit were transferred to 15 °C (85–90% RH) to preserve fruit quality. When all fruit reached stage 6, they were analyzed for quality and anthocyanin contents with three replicates of seven fruit in each replicate.

2.2. Quality assessment and sensory evaluation

Fruit colour was measured using a Minolta CR-300 chromameter (Minolta, Osaka, Japan) as L^*, a^*, b^* values (CIE $L\,a\,b$) and converted to hue angle (colour wheel, with red–purple at an angle of 0°, yellow at 90°, bluish-green at 180°). The a^*/b^* ratio was calculated. The colour reading was taken twice at the equatorial region of each fruit and averaged to give a value for each fruit.

Pericarp firmness was measured using a hand-held fruit firmness tester (Effegi, Alfonsine, Italy) equipped with a cylindrical plunger 0.5 cm in diameter. The plunger was inserted to a depth of 0.5 cm and the force recorded in Newtons.

To measure soluble solids content (SSC) and titratable acidity (TA) on the flesh juice, the white flesh of the arils, with seeds, was wrapped in cheesecloth, and squeezed by hand to separate juice from seeds. SSC was measured with a hand-held refractometer (Atago, Tokyo, Japan) and calibrated with distilled water. TA was determined from a 5 mL aliquot by titration with 0.1 mol L^{-1} NaOH with phenolphthalein as an indicator and results are given as grams of citric acid per 100 mL. The SSC/TA ratio was calculated.

Ten judges conducted a sensory panel evaluation of mangosteen flavor for all fruit from different harvest maturities (stages 1–6) that had reached stage 6. The stems and calyxes of the fruit were removed to reduce possible bias due to visible appearance. The judges rated the overall flavor and acceptability on a scale of 1–5, where 5 = excellent, 4 = very good, 3 = good, 2 = poor, and 1 = very poor.

2.3. Ethylene production

Ten fruit at stage 1 were individually weighed and place into separate 0.8 L plastic containers at 25 °C (85–90% RH). The air was flushed before closing the container. After 30 min, air samples were taken from the headspace using a syringe. Concentrations of ethylene within containers were measured by sampling through a sampling port with a syringe and measured with a gas chromatograph (Shimadzu GC 8A, Kyoto, Japan), equipped with a flame ionization detector and a 2.1 m × 2.4 mm stainless steel column filled with activated alumina of 177–149 μm. The column tempera-

ture was 80 °C. Injector and detector temperatures were 150 °C. The data were the average values from 10 fruit.

2.4. Light microscopy

Fruit pericarps at different stages of colour development were hand-sectioned using a razor blade and mounted with a drop of distilled water. The slides were examined in the bright field using a light microscope (Carl Zeiss, Gottingen, Germany) equipped with a digital camera.

2.5. Anthocyanin analysis

The fruit pericarp was separated into outer and inner pericarps (Fig. 2A). Total anthocyanins were extracted from both tissues using methanol–HCl (Piccaglia et al., 2002). One gram of outer pericarp or two grams of inner pericarp were homogenized with 20 mL of methanol:HCl (99:1, v/v), and the homogenates then shaken for 6 h at 4 °C. The aqueous phase was removed and the pellets were re-extracted four times within 24 h and then adjusted to final volume to 100 mL with methanol:HCl. The combined aqueous extracts were centrifuged at 8000 × *g* for 10 min (4 °C) and anthocyanin contents then measured at an absorbance of 530 nm. The anthocyanin contents were expressed as cyanidin. The samples were kept at −80 °C until the individual anthocyanins were analyzed.

Individual anthocyanin compounds were analyzed by HPLC and LCMS as described by Stevenson et al. (2006). Aliquots of 500 μL (A) were dried down in a Labconco Centrivap Concentrator (Labconco, Kansas City, MO, USA). Samples were resuspended in 20% methanol (250 μL). Forty microlitres of the sample were analyzed using a Shimadzu analytical HPLC with a column oven, auto-sampler, vacuum solvent degas module and diode-array detector (Shimadzu, Kyoto, Japan). Separations were achieved on a 250 mm × 4.6 mm column, Synergi®, 4 μm particle size, Polar-RP, 8 nm pore size (Phenomenex, Auckland, NZ), using (A) acetonitrile + 0.1% formic acid, and (B) acetonitrile/water/formic acid (5:92:3). Flow rate was 25 $\mu L\,s^{-1}$ at a column temperature of 45 °C. The content of solvent A was 0% at zero time and ramped linearly to 20% at 20 min, 30% at 26 min, 50% at 28.5 min, 50% at 28.5 min, 95% between 32 and 35 min and back to 0% between 36 and 42 min.

LC–MS analysis of a selected sample was carried out to confirm the compound. Identification was based on both mass (M+) of molecular ions and characteristic fragments, and comparison of retention times and fragmentation with authentic standards; cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside (Polyphenols, Norway). Quantification was achieved by reference to standards of anthocyanin compounds at 520 nm.

2.6. Statistics

All data were at least repeated once and analyzed statistically by ANOVA and mean differences estimated by Duncan's new multiple range test (DMRT).

3. Results

3.1. Fruit colour development and quality

During ripening of fruit harvested at stage 1 (Table 1, A column), the fruit developed rapidly to the purple black stage (6) within 9 d, with colour development from stage 5 to 6 being slower than between the other stages. During colour development, the a^*/b^* values increased slightly from stage 1 to 3, and then increased sharply to stage 6. The increase in the a^*/b^* values correlated well with colour development. Pericarp firmness decreased sharply from stage 1 to 6, 779.3, 201.3, 136.0, 98.4, 66.5 and 46.5 N, respectively, whereas SSC increased slightly and TA decreased slightly during colour development from stage 3 to 6 [Table 1, stage 1 (A column)].

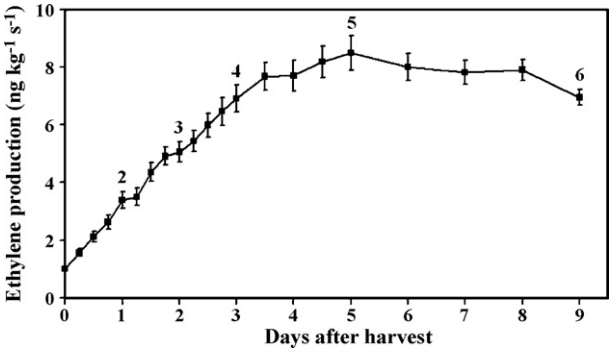


Fig. 1. Ethylene production of mangosteen fruit harvested at stage 1 (light greenish yellow with 5% scattered pink spots). The numbers 1–6 in the graph represent maturity stages of mangosteen fruit at stages 1–6 as described in Section 2. Data are means of 10 fruit ± S.E.

When fruit at the six different stages of maturity were harvested and kept at 25 °C, each stage fully developed to the purple black stage 6 [Table 1, stage (B columns)]. No matter at what stage the fruit were harvested, they all ripened such that there were no significant differences in sensory evaluation and fruit quality, including hue angle values (data not shown), firmness, SSC and TA, when the fruit were assessed at stage 6 (Table 1, B columns).

3.2. Ethylene production of mangosteen fruit after harvest

Ethylene production of mangosteen fruit harvested at stage 1 increased linearly until stage 5 (dark purple) by 5 d, then decreased slightly thereafter (Fig. 1).

3.3. Anatomy of mangosteen skin

The outer pericarp (fruit skin) anatomy from fruit at stages 1–6 was examined (Table 1). The red pigment released from the sectioned outer pericarp was more intense in the purple black (stage 6) than other stages due to breakdown of cells while cutting. The density of red pigment was higher in cells of purple black outer pericarp (stage 6) than for other stages.

3.4. Anthocyanins of mangosteen pericarp

During fruit colour development, the total anthocyanin contents in the outer pericarp increased more than 20-fold from stage 1 to stage 6, each value being significantly different from the preceding ($P \leq 0.001$), whereas hue angle values decreased sharply (Fig. 2B). The total anthocyanin contents in the inner pericarp tissue increased following the same trend, although the contents at all stages were less than those in the outer pericarp (Fig. 2B). Hue angle values and total anthocyanin content were closely associated with fruit colour development.

The anthocyanins in the outer pericarp mainly consisted of five compounds (Table 2 and Fig. 3). These compounds were identified by HPLC/MS as cyanidin-sophoroside (M+611, major fragment, *m*/*z* 287), cyanidin-glucoside (M+449, *m*/*z* 287), cyanidin-glucoside-pentoside (M+581, *m*/*z* 287), cyanidin-glucoside-X (M+639, *m*/*z* 287), cyanidin-X_2 (M+667, *m*/*z* 287) and cyanidin-X (M+477, *m*/*z* 287). X denotes an unidentified residue of *m*/*z* 190, a mass which does not correspond to any common sugar residue. The two major compounds from HPLC and LCMS analyses corresponded to those

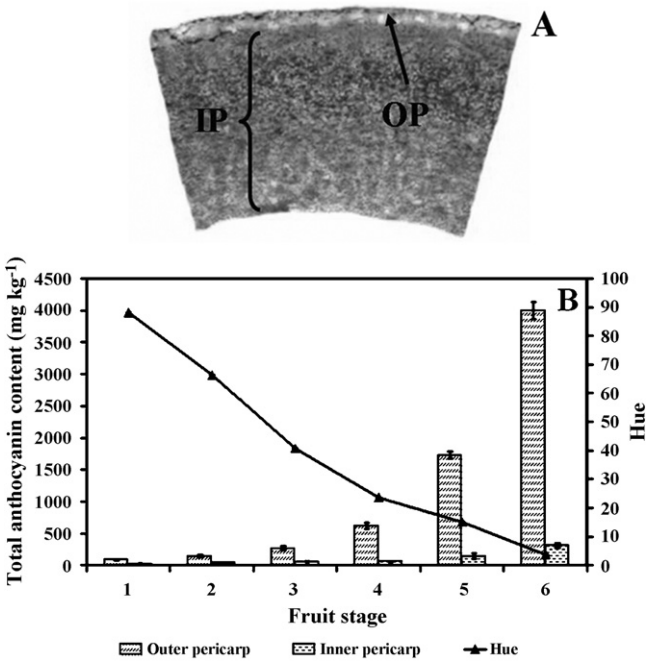


Fig. 2. (A) Cross section of mangosteen pericarp showing outer pericarp (OP ▨) and inner pericarp (IP ▨). The bar denotes 2 mm. (B) Hue values (▲) of skin colour and total anthocyanin contents of mangosteen pericarp during colour development from light greenish yellow with 5% scattered pink spots to purple black (stages 1–6).

of authentic standards of cyanidin-3-sophoroside and cyanidin-3-glucoside. The concentration of these two compounds increased steadily during fruit colour development, approximately doubling between each stage. In addition, cyanidin-glucoside-pentoside was found at low levels, with patterns similar to those of the two major anthocyanins (Table 2). The other anthocyanins had initial low concentrations and decreased further by stage 6. The inner pericarp contained essentially the same compounds, but at much lower concentrations and there was little increase during colour development (data not shown).

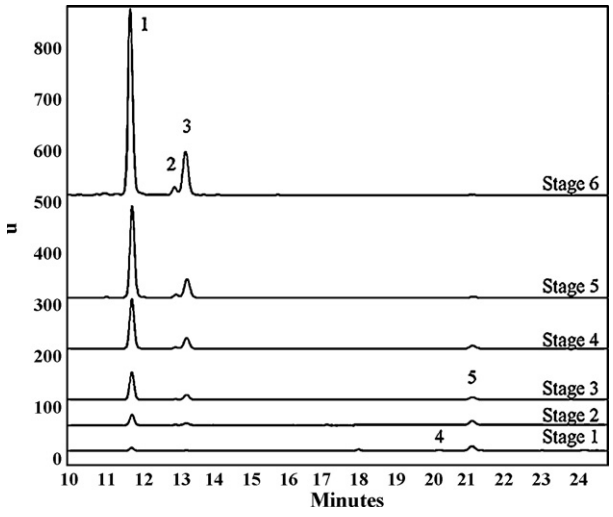


Fig. 3. Anthocyanin profiles in outer pericarp of mangosteen fruit harvested at stage 1–6 (light greenish yellow with 5% scattered pink spots to purple black) during colour development. Peak identity was as follows: (1) cyanidin-sophoroside, (2) cyanidin-glucoside-pentoside, (3) cyanidin-glucoside and cyanidin-glucoside-X (overlapping peak), (4) cyanidin-X_2, and (5) cyanidin-X. X denotes a residue of *m*/*z* 190 which is unified atomic mass units (u).

Table 2
Anthocyanin contents (g kg^{-1}) in outer pericarp of mangosteen during colouration from light greenish yellow with 5% scattered pinkish spots to purple black (stage 1–6)

Fruit stage	Unknown 1	Unknown 2	Unknown 3	Cy-sop	Cy-glu-pent	Cy-glu + Cy-glu-X	Unknown 4	Unknown 5	Cy	Cy-(190)$_2$	Cy-(190)	Total
1	0	0	0	52 ± 6.6	0	11 ± 1.9	0	0	11 ± 5.8	11 ± 1.8	83 ± 12.5	167 ± 21.8
2	0	0	0	143 ± 24.2	0	36 ± 5.4	0	0	3 ± 2.9	10 ± 0.4	87 ± 3.5	278 ± 35.5
3	0	0	0	359 ± 74.8	9 ± 1.8	81 ± 17.4	0	0	0	2 ± 2.0	58 ± 1.5	509 ± 90.6
4	0	6 ± 3.2	3 ± 2.7	823 ± 53.1	27 ± 3.0	191 ± 21.1	0	0	0	6 ± 3.0	56 ± 3.5	1111 ± 64.1
5	10 ± 1.6	20 ± 2.0	14 ± 1.4	1403 ± 122.9	62 ± 5.2	290 ± 27.4	0	0	0	0	26 ± 0.3	1824 + 132.1
6	15 ± 3.4	44 ± 10.0	30 ± 5.3	3126 ± 207.7	125 ± 7.9	842 ± 21.9	9 ± 0.8	16 ± 4.0	0	0	27 ± 3.3	4235 ± 203.5

Total values represent the sum of the individual compounds. The data are mean ± S.E. Cy-sop: cyanidin-3-sophoroside; Cy-glu-pent: cyanidin-glucoside-pentoside; Cy-glu: cyanidin-3-glucoside; Cy-gluc-X: cyanidin-glucosideX; Cy-X: cyanidin-X (X denotes a residue of *m*/*z* 190 which has not been identified).

4. Discussion

Mangosteen is one of the few species of fruit that develops red colour after harvest, similar to the dark purple changes observed in 'Hass' avocado (Cox et al., 2004) and Chinese bayberry (Zhang et al., 2005). While most of the mangosteen fruit in the current study had started development of red colour on the tree, colour development proceeded quickly during the postharvest period at 25 °C. The colour changes correlated well with ethylene production (Fig. 1), as has also been found in grapes (El-Kereamy et al., 2003), suggesting that a useful study could be made on the regulatory role of ethylene in stimulating the anthocyanin biosynthetic pathway in fruit such as mangosteen, grape or avocado. The pattern of ethylene production in mangosteen fruit is similar to that in other reports (Kanchanapom and Kanchanapom, 1998; Paull and Ketsa, 2004; Noichida et al., 2007). Hue angle values and pericarp firmness decreased rapidly during fruit colour development, these changes being general phenomena of ripening processes. Although intensity of anthocyanins depends on pH in the cell sap (Mol et al., 1998), pH of outer pericarp from stage 1 to 6 ranged between 4.1 and 4.5 (data not shown). This suggests that increased intensity of anthocyanins in outer pericarp is not due to an increase in pH.

Postharvest quality of fruit is generally dependent on the stage of maturity at harvest. We found that fruit harvested at any of the defined stages, 1–6, ripened such that at stage 6 for each of them, there were no significant differences in sensory evaluation and fruit quality (Table 1, B columns). This suggests that ripening development was already stimulated and underway at harvest for all stages. There is a practical advantage from this. Current growing practice in Thailand is to harvest fruit at stage 1 (light greenish yellow with 5% scattered pink spots) for export. Our results confirm that this has no detrimental effect on final fruit quality, with the advantage of a slightly longer shelf-life over fruit harvested at later stages. In Malaysia, a guideline for exporting mangosteen recommends harvesting fruit when showing a colour of reddish-yellow with patches of red, which is equivalent to stage 1 for Thailand fruit (Osman and Milan, 2006).

The major anthocyanins found in the pericarp were cyanidin-3-sophoroside and cyanidin-3-glucoside, confirming the brief report of Du and Francis (1977). Our data show at least three cyanidin-3-glycosides, including the pentoside and a further cyanidin with an unidentified residue. Small amounts of the other cyanidin derivatives were detected. Colour development in the outer pericarp was closely correlated with the strongly increasing concentrations of cyanidin-3-sophoroside and the cyanidin-3-glucosides (Fig. 3). The rapid elevation of anthocyanic colour suggests that precursor polyphenolics are readily available for conversion to cyanidins. In the absence of other pigments, the dramatic increase in levels of anthocyanin pigments alone explains the final appearance. Cox et al. (2004) reported a similar increase in cyanidin-3-glucoside which correlated closely with skin colouration of 'Hass' avocado.

In conclusion, the results show that ripening in mangosteen fruit is triggered and underway by the time of harvest at the first stage identified here, with no disadvantages to ultimate fruit quality. This allows growers a window for handling and retailing the fruit. The fruit also provides a model for further investigation into the control of red colour in relation to ethylene and other ripening stimulants. A further study of the cyanidin compound derivatives with the 190 mass residue may be important in terms of producing commercial products from mangosteen. As well, regulation of enzymes and genes associated with the anthocyanin pathway in mangosteen are currently underway.

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