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Metabolic profiling of *Garcinia mangostana* (mangosteen) based on ripening stages

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Metabolomics is an emerging research field based on exhaustive metabolite profiling that have been proven useful to facilitate the study of postharvest fruit development and ripening. Specifically, tracking changes to the metabolome as fruit ripens should provide important clues for understanding ripening mechanisms and identify bio-markers to improve post-harvest technology of fruits. This study conducted a time-course metabolome analysis in mangosteen, an economically important tropical fruit valued for its flavor. Mangosteen is a climacteric fruit that requires an important plant hormone ethylene to regulate ripening processes and rate. We first categorized mangosteen samples in different ripening stages based on color changes, an established indicator of ripening. Using gas chromatography/mass spectrometry, small hydrophilic metabolites were profiled from non-ripened to fully ripened (ripening stages 0–6). These metabolites were then correlated with color changes to verify their involvement mangosteen ripening. Our results suggest that the increase of 2-aminoisobutyric acid, psicose, and several amino acids (phenylalanine, valine, isoleucine, serine, and tyrosine) showed a correlation with the progression of mangosteen ripening. This is the first report of the application of non-targeted metabolomics in mangosteen.

[**Key words:** Mangosteen; *Garcinia mangostana*; Ripening stages; Metabolomics; Gas chromatography–mass spectrometry]

Fruit ripening increases fruit palatability through changes in metabolite composition across ripening stages. Ripening in climacteric fruits (e.g., banana, papaya, mango, and mangosteen) depends on ethylene bursts that trigger metabolite production or breakdown to influence fruit color, taste, and firmness (1–4). Ethylene production in several climacteric fruits commence in the early ripening stages, which is characterized by an immediate increase followed by a rapid decrease in ethylene levels (3,5–7). Because excessive ethylene production during ripening process can drastically drop fruit quality, understanding the chemical mechanisms during ethylene production of fruit ripening stages is important for quality control and postharvest treatment of commercial fruits.

Mangosteen (*Garcinia mangostana*), often known as the Queen of Fruits, is an economically important tropical fruit desired for its distinctive appearance and unique taste. A rich source of vitamin C, mangosteen also has potential health benefits for humans, making it an attractive nutraceutical. For Southeast Asian countries such as Indonesia, Thailand, Malaysia and the Philippines, mangosteen is a primary export commodity. These qualities have resulted in considerable efforts to control mangosteen fruit ripening so as to achieve optimal fruit maturity for harvesting (8), as well as to devise appropriate postharvest packaging and handling strategies (9,10). Understanding the physiological mechanisms underlying ripening processes is necessary to better predict and control ripening processes.

Food quality is greatly affected by both pre-harvest (e.g., genetic origin, cultivation area, physicochemical properties of growing environment) and post-harvest processes (e.g., storage method, logistic condition). The complex, synergistic, non-linear interactions between many components define the sensory characteristics of food products. Therefore, the elucidation and scientific manipulation of these characteristics by targeting a single or just a few components become a very complex task (11,12). Existing studies on mangosteen fruit ripening have been focused on transcriptome-level changes or targeting only a few metabolites in the pericarp (9,13–17). In recent years, non-targeted metabolomics is a powerful approach to monitor global metabolite changes that occur during fruit development and ripening. Through the use of metabolic profiling approach, metabolic changes during ripening stages can be determined to aid in the development of postharvest packaging technologies and handling strategies.

In this study, gas chromatography–mass spectrometry (GC–MS)-based metabolite profiling was performed in peels, flesh and seed parts of mangosteen to gain insight on metabolic changes during mangosteen ripening stages. In addition, multivariate analysis was performed to correlate specific metabolites with color changes that occur during the ripening process. This work is the first to report the use of metabolomics approach for the study of mangosteen and the involvement of several metabolites in its ripening process.

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MATERIALS AND METHODS

Plant materials Mangosteen fruit from Indonesia corresponding to 7 different ripening stages (stages 0–6) were used in this study. Five samples (replicates) from 3 different trees were collected for each stage from the period of January–March 2016 in Center of Tropical Fruit Studies, Bogor Agricultural University (CENTROFS, IPB), Bogor, Indonesia. Color changes in fruit peels were measured using Konica Minolta CM-2500d before homogenization and extraction of mangosteen.

Measurement of color changes was calculated using the previously established CIELAB method with illuminant D65, observer angle 10° (17,18). The measured data was proceed using Spectramagic NX software (Konica Minolta, Tokyo, Japan). ΔL*a*b* color space value (CIELAB method) was used as color solid representatives value to represent mangosteen color changes in ripening process. L* indicates the lightness of the samples that were measured. a* and b* values are chromacity diagram that describes red-green color for a* values and yellow-blue color for b* values.

Samples extraction Three different parts of mangosteen, namely flesh, peel and seed were separated and homogenized. Homogenized mangosteen samples were mixed with methanol (Wako Pure Chemical Industries, Osaka, Japan), chloroform (Kishida Chemical Co. Ltd., Osaka, Japan), ultrapure water (Wako Pure Chemical Industries); 2.5/1/1 (v/v/v) together with an internal standard (Ribitol 100 μg/ml). Samples were sonicated for 1 min and incubated at 37 °C, 1200 rpm for 30 min. Incubated samples were centrifuged for 3 min at 4 °C, 16,000 rcf. Four hundred microliters of supernatant was transferred to a new 1.5 ml microtube and added with 200 μL of water. The mixture was centrifuged for 3 min at 4 °C, 16,000 rcf. Four hundred microliters of the polar phase was transferred to a new tube and vacuum evaporated for 2 h at room temperature before lyophilization overnight.

Derivatization One hundred microliters of methoxyamine hydrochloride (20 mg/ml in pyridine) were added and incubated for 90 min, 1200 rpm, 30 °C into lyophilized samples. Subsequently, 50 μL *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) (GL Sciences) were added and incubated for 30 min, 1200 rpm, 37 °C to incubated samples before analyzed using GC–MS.

Gas chromatography–mass spectrometry analysis Intracellular metabolites were measured by Shimadzu Ultra QP-2010 gas chromatography–mass spectrometry (GC–MS) with an InertCap 5 MS/NP (30 m, 0.25 mm i.d., 0.25 μm film thickness, GL Sciences). Tuning and calibration of the mass spectrometer was done prior to analysis. The derivatized samples were injected to GC–MS. GC and MS conditions were performed by following the procedure as described in the previous study (19).

Data analysis The raw data was processed using GC–MS solution software package (Shimadzu, Kyoto, Japan) for GC–MS analysis. Peak alignment was executed using metAlign (Wageningen; can be downloaded freely at http://www.wageningenur.nl/). The pre-processed data was then subjected to peak annotation using AIOutput and using our laboratory's in-house library.

Multivariate analysis was done using SIMCA-P+ version 11 (Umetricts, Umea, Sweden) for principal component analysis (PCA) and partial least square projections to latent structures (PLS). PCA was used as non-supervised multivariate analysis of mangosteen ripening stages which can help us to understand the relationship between the samples and the metabolites features. PLS was used to describe several variables of PCA and projected to regression by using the explanatory variable. PLS analysis is a supervised multivariate analysis that commonly uses to predict the significant metabolites using an observed value such as color changes. Hierarchical cluster analysis (HCA) was performed to further clarify metabolite distribution during mangosteen ripening using MeV: MultiExperiment Viewer (download freely at http://www.tm4.org/mev.html). The data was performed using Pearson's correlation to see the correlation between ripening stages.

RESULTS AND DISCUSSION

Assessment of mangosteen ripening stages The six mangosteen fruit ripening stages were classified following previous studies (13), and were based on color changes from yellow-green (unripe) to purple-black (fully ripe) (Fig. 1). These changes occur due to ethylene production, which begins from stage 1 (9 weeks post-blooming) and reaches maximum levels at stage 5 (12 weeks post-blooming), before decreasing slightly (10,13). Ethylene signaling networks increase plant pigments such as anthocyanin, which is associated with red, purple, and blue fruit colors. Therefore, we expected that anthocyanin levels would also vary with ripening of mangosteen.

Mangosteen samples were collected from mature fruit that had reached maximum weight and volume, but were at different ripening stages. A color-change index was used for sample classification: stage 0 and 1 are yellow-green, contain yellow gamboge, and have flesh inseparable from the rest of the peels. Stage 2–4 exhibit a gradual color change to purple-black, decreasing yellow gamboge, and eventual separation of the flesh from the other peels portions. Finally, stages 5 and 6 are fully purple-black, considered ready for consumption and distributed to domestic markets.

Mangosteen color changes (including depth, vividness, and hue variation) per stages were measured with a colorimeter, then calculated using the non-destructive CIELAB method, which generates a dLAB value useful as a quantitative phenotype for describing fruit ripening stages (17,18,20). CIELAB has been applied to other fruits (e.g., strawberry, mango, and apple) to ascertain ripeness, typically after an edible coating treatment to prolong the shelf-life of the fruits (20–22). We observed increasing dLAB as fruit color shifted from green to purple-black (Table 1). We also noted a significant decrease in hue as the fruit ripened, corroborating previous studies (8).

Elucidation of metabolic changes during mangosteen ripening stages Metabolic changes during mangosteen ripening stages were assessed with GC/MS for the first time. Three fruits per ripening stage were used and the analysis was performed separately for each part of mangosteen. We tentatively identified 70 metabolites from flesh, 62 metabolites from peels, and 69 metabolites from seeds using the National Institute of Standards and Technology and our laboratory in-house library (Table S1).

Principal component analysis (PCA) of the GC–MS derived dataset revealed two principal component explaining 71.35%, 52.2% and 44.6% of metabolite variation in flesh, peels, and seeds, respectively (Fig. 2). The results clustered mangosteen ripening stages into three phases (early, middle, and late) based on metabolite distributions. Metabolite quantification was normalized with

TABLE 1. Postharvest physiological quality indices of mangosteen fruit during ripening process.

Ripening stages	Lightness (ΔL)	Vivid color (Δa)	Hue color (Δb)	ΔLab value
I	3.59a	3.16a	2.59a	10.76a
II	−14.25b	15.49b	−15.69b	26.56b
III	−26.79bc	17.25b	−25.19c	41.37c
IV	−27.67c	17.71b	−28.66c	43.80c
V	−32.58c	12.82b	−30.34c	46.42c
VI	−35.29c	7.38ab	−32.59d	48.78c

Mangosteen ripening process was classified using color determination as described by previous study (13). CIELAB method of color changes measurement during mangosteen ripening process. Stage 0 was used as a target during CIELAB calculation during mangosteen ripening stages.

FIG. 1. Mangosteen ripening stages. A photograph of typical mangosteen fruits during ripening stages as described by previous study (13) and CENTROFS, IPB.

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