ELUCIDATION OF PHYSICOCHEMICAL CHANGES IN FRUIT DEVELOPMENT OF 'SABARA' JABOTICABA (*Plinia cauliflora* (Mart.) Kausel)

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(Received: June 23, 2021; Accepted: November 28, 2021)

ABSTRACT

Jaboticaba is a sub-tropical fruit native to Brazil, that is cultivated in South America and Southeast Asia etc. One of the barriers for commercializing this fruit is the difficulty in distinguishing the fruit maturation stages and the fruit cannot be kept on the tree for a long time due to rapid maturation. This study aimed to understand the comprehensive maturation mechanism and make use of maturation indicators to determine the harvesting time. The primary metabolites were analyzed at three different maturation stages using high performance liquid chromatography (HPLC) and gas chromatography - mass spectrometry (GC-MS) as a data driven research. Metabolite analysis revealed the amount and the changes in individual metabolites and metabolic pathways such as the tricarboxylic acid cycle (TCA cycle), anaerobic respiration, shikimate pathway, and γ- aminobutyric acid (GABA) shunt in the process of fruit maturation. Based on the metabolome analysis, acetaldehyde and ethanol might be effective maturation indicators because these compounds changed more rapidly than sugars or organic acids. Thereafter, the physicochemical changes, including acetaldehyde and ethanol, were investigated during fruit development. The results showed that acetaldehyde and ethanol increased sharply from the ripe to overripening fruit, which could be used as the maturation indicators.

Key words: fruit firmness, fruit size, maturation indicators, metabolites composition, primary metabolism

INTRODUCTION

Jaboticaba (*Plinia cauliflora* (Mart.) Kausel), belonging to the family Myrtaceae, is native to Brazil (Shinohara et al. 2021; Wu et al. 2013b), and cultivated in tropical, subtropical, and temperate climates, such as South America, the USA, China, and Southeast Asia (Marica et al. 2018; Salomão et al. 2018). The fruit is consumed fresh (Teixeira et al. 2011b) while several processed products such as sweets, jams, juice, jelly, and wine are also known (Montes et al. 2005). It has been reported that feeding jaboticaba peel to rats prevented liver steatosis and improved insulin resistance (Lenquiste et al. 2012; Lenquiste et al. 2019). Jaboticaba fruit has the potential to contribute to human health (Wu et al. 2013a). 'Sabara' jaboticaba is cultivated mostly in Brazil (Freitas et al. 2020). The fruit is dark purple, with a thin and smooth skin, and a very sweet taste.

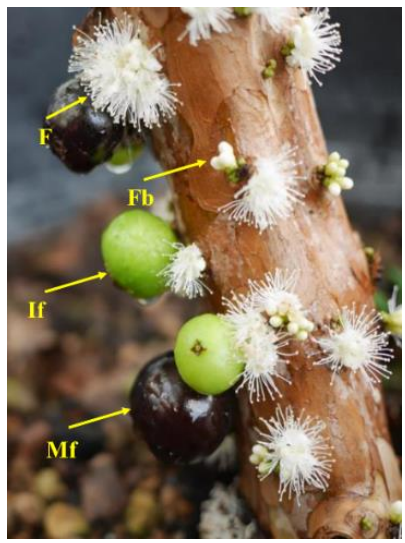

Fig 1. Jaboticaba 'Sabara' was grown in pot. Abbreviations are as follows: F, Flower; Fb, Fb, Flower bud, If, Immature fruit, Mf, Mature fruit.

However, farmers have to check each fruit visually when harvesting because the maturation stages in jaboticaba are varied. Furthermore, jaboticaba fruits mature so rapidly that the freshness of the fruit deteriorates within a few days. Therefore, it is difficult to judge the fruit maturation stages based solely on its sugars and acid content, softening, and coloration, as opposed to temperate fruit such as apple, peach, and pear, which have a long maturation period. Thus, the establishment of new maturation indicators is required.

Previous studies on fruit maturation of jaboticaba reported changes of structural and non-structural carbohydrate during fruit development (Barros et al. 1996; Magallhães et al. 1996). Some reports have studied changes of sugars, organic acids, anthocyanin, polyphenol, and ellagitannin at different maturation stages (Becker et al. 2015; Pereira et al. 2017). In addition, some reports studied comparison of organic acid, phenolic compositions, or volatile compounds among varieties (Roquim et al. 2013; Freitas et al. 2020; Jham et al. 2007). However, these studies did not propose maturation indicators. Further information such as metabolic and physicochemical changes following the fruit maturation is needed.

Metabolome analysis investigated changes of metabolites during fruit maturation for understanding the comprehensive fruit maturation, i.e., strawberry (Zhang et al. 2010), raspberry (Dincheva et al. 2013), guava (Lee et al. 2010), pitaya (Wu et al. 2019), mangosteen (Parijadi et al. 2018), peach (Lombardo et al. 2011), and pear (Oikawa et al. 2015). Data-driven research was adopted in many metabolome analyses. In other words, the theories are derived after collecting data. Data driven research in metabolome analysis is also used to search for compounds that could be indicators from metabolite data. Physicochemical changes during fruit development and maturation contribute to determine the optimal harvesting time (Candir et al. 2009; Shwartz et al. 2009) and to investigate changes in specific compounds during fruit maturation (Chapman and Horvat 1990; Ueda et al. 2000; Wu et al. 2005) in temperate, tropical, and sub-tropical fruit.

In this study, metabolome analysis was conducted to comprehensively understand fruit maturation and to search compounds as new maturation indicators. Thereafter, physicochemical changes including potential indicator compounds during fruit development were evaluated. This study

might well contribute to the comprehensive understanding of metabolism during fruit maturation and make use of maturation indicators in jaboticaba to clarify the harvesting time more clearly.

MATERIALS AND METHODS

Plant materials. Trees of 'Sabara' jaboticaba were cultivated in a greenhouse (winter minimum temperature > 10 ℃) at Tokyo University of Agriculture (35.6° N, 129.6° E) in Japan.

Metabolome analysis at the three different maturation stages. Metabolite analysis of fruit was done based on its ripening stages such that: unripe fruit, ripe fruit, and overripe fruit, as shown in Fig 1. The fruits were classified into the three different maturities, based on the subjective evaluation of the texture of the fruit, skin colour, and firmness. After harvest, jaboticaba fruits were measured of its firmness, skin colour and acetaldehyde and ethanol production. Then those fruits were frozen in liquid nitrogen for the analysis of sugars, organic acids, and amino acids by HPLC and GC-MS.

Changes in physicochemical composition during fruit development. This experiment was conducted from November to December 2017. The fruits were collected at 2 or 4 days intervals. Jaboticaba fruits were measured their length, diameter, pericarp colour, firmness, respiration rate, acetaldehyde, and ethanol production.

Fruit size, skin colour, and firmness. Fruit length and diameter were measured with a caliper according to Al-Maiman and Ahmad (2002). The measurement of length was made on the polar axis of fruit, i.e. between the apex and stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. The L, a, and b values of pericarp colour were measured three times per fruit using the Handy Colourimeter (NR- 3000, Nippon Denshoku IND. CO., LTD, Osaka, Japan) according to Jia et al (2005). The colour meter was calibrated with a dedicated calibration board before use. Fruit firmness was measured three times per fruit with a non-destructive firmness meter Multilateral Tester (Model 2519-104, INSTRON Company, Kanagawa, Japan) indicating the force (N) required for pressing the fruit skin at 1 mm/second using Φ 1 cm metal plug (Poyesh et al. 2018).

Ethylene, acetaldehyde and ethanol analysis. Ethylene, acetaldehyde, and ethanol were analyzed after 2 h of incubation under dark condition at room temperature. One ml of headspace gas was taken out using a plastic syringe, and acetaldehyde and ethanol were analyzed with GC-FID (GC-14B, Shimadzu, Japan), which was equipped with a Sunpack A column (Shinwa Kako, 2.1 m×3.2 mmφ, glass column filled with porous poly beads). The injector and FID were subjected to 180 and 200 ℃, respectively. The initial column oven temperature was set at 80 ℃ for 1 minute, then increased by 10 ℃ per minute until 160 ℃. The identification of acetaldehyde and ethanol was confirmed using standard gas.

Metabolome analysis. Metabolites were measured with GCMS-QP2010 Plus (Shimadzu, Japan), using a electron ionization, on a nonpolar phase column (DB-5, Agilent Technologies, USA) according to Yin et al (2010) and Ijima and Aoki (2009) with some modifications. Each sample (0.1 g of frozen tissue powder) was extracted with 250 µl of methanol and chloroform, one after another. After adding 50 µl of 2.0 mg/ml ribitol solution as an internal standard and 175 µl of ultrapure water, the samples were vigorously mixed. These samples were centrifuged at 12000 rpm for 10 min at room temperature. Then 80 µl of the supernatant fluid of each sample was corrected into a 1.5 ml plastic tube. These samples were evaporated to dryness for 3 h in a centrifuge evaporator (CVE-200D, TOKYO RIKAKIKAI CO, LTD, Japan). Afterwards, these samples were freeze-dried overnight using a lyophilization container (Modulyo 4K, Edwards, USA). For methylation, 40 µl of methoxylamine (20 mg/ml pyridine) was added to the samples and incubated for 90 min at 37 ℃. Trimethylsilylation was performed by adding 50 µl of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) solution for 30 min at 37 ℃. The

initial column oven temperature was set at 100 ℃ for 4 minutes, then increased by 4 ℃ per minute until 320 ℃. After that, it was kept for 10 minutes at 320 ℃. Metabolites can be identified by comparing fragment patterns and retention indices with those of standard compounds in compound databases. The identification of lactic acid, pyruvic acid, succinic acid, α-ketoglutaric acid, Shikimic acid, glutamic acid, GABA, phenylalanine was confirmed using standard solutions; a match similarity of over 70% was accepted.

Sugar and organic acid analysis. For sugars (sucrose, glucose, and fructose) and organic acids (citric acid and malic acid), each sample (0.5 g freeze-dried powder) was extracted with 1.5 ml of ultrapure water. These samples were centrifuged at 9000 rpm for 10 min at room temperature. Then the supernatant fluid of each sample was collected into another micro-tube, and this work was repeated three times. Then, these samples were filtered through filters (Sep-Pak® Plus C18, Waters, USA, 0.45μm cellulose acetate syringe filter, Membrane Solutions Limited, USA) to prepare as organic acid analysis samples of all stages and sugar analysis samples of unripe fruit. Sep-Pak cartridge filter was activated with methanol. In addition, those filters were washed three times with the extraction sample before use. Organic acid analysis samples of ripe and overripe fruit were diluted 5-fold with ultrapure water, which were used for sugar analysis.

Sucrose, fructose, and glucose were determined using an HPLC with a differential refractometer (RID-10A, Shimadzu, Japan), on a KS-801 (8.0 mmI.D.×300mm, Shodex, Japan) and KS-G (6.0 mm I.D. ×50mm) column. Ultrapure water was used as mobile phase with a flow rate of 0.7 ml/min, and the injection volume was 5 μl. The column oven temperature was set at 80 ℃. The identification of sucrose, fructose, and glucose was confirmed by using standard solutions.

Citric acid and malic acid were determined using an HPLC with a conductivity detection (CDD-10A vp, Shimadzu, Japan), equipped with two KC-811 (8.0 mmI.D. ×300mm, Shodex, Japan) and KC-G 6B (6.0mmI.D. ×50mm, Shodex, Japan) columns. $HClO_4$ aq of 3mM was used as mobile phase solution with a flow rate of 1.0 ml/min, and the injection volume was 5 μl. The column oven temperature was set at 40 ℃. The identification of citric acid and malic acid was confirmed using standard solutions.

Respiration analysis. Respiration rate, measured as CO_2 released, was analyzed after two h incubation in a glass jar under the dark conditions at room temperature. One ml of headspace gas was taken out using a plastic syringe, and the amount of CO_2 was analyzed with GC-TCD (GC-14B, Shimadzu Japan), which was equipped with a column (Shinwa Kako, 2.1 m×3.2 mmφ, glass column filled with porous poly beads). The column, injector, and TCD were set at 40, 150, and 150 ℃, respectively. The identification of CO_2 was confirmed using standard CO_2 gas.

Statistical analysis. Fruit samples were collected from 3 individual plants and 10 fruits were harvested per measuring day. In addition, if the conditions in the green house are constant, the metabolome is stable, and annual fluctuations and seasonal changes could be ignored. One-way ANOVA was conducted for statistical analysis of physicochemical components between the different maturation stages followed by Fisher's LSD ($P < 0.05$) in the open-source statistical language R environment (Version 4.0.0., R Development Core Team 2020). Principal component analysis (PCA) was used to investigate a relationship between different maturation stages and fruit colour, firmness, and metabolites with a commercial program (Pirouette version 4.5, Infometrix, USA).

RESULTS AND DISCUSSION

Metabolite analysis. Metabolite analysis of 'Sabara' fruit was done based on three maturation stages such that: unripe fruit (24~26 DAF), ripe fruit (36~38 DAF), and overripe fruit (56~58 DAF), as shown in Fig 2. The skin colour and firmness of each stage are shown in Table 1.

Fig 2. 'Sabara' jaboticaba fruit maturation used for metabolome analysis.

Table 1. Fruit color and firmness of 'Sabara' jaboticaba fruits used for metabolome analysis.

		Unripe fruit	Ripe fruit	Overripe fruit
Fruit color	L-value	50.50 ± 2.24 a	26.92 ± 0.68 b	22.86 ± 0.90 c
	a-value	1.09 ± 2.39 c	34.22 ± 3.96 a	26.75 ± 3.58 b
	b-value	20.99 ± 1.19 a	1.18 ± 0.79 b	1.40 ± 0.91 b
Fruit firmness (N)		6.67 ± 0.77 a	2.21 ± 0.40 b	0.48 ± 0.11 c

Data shown are the means of ten replicates ± standard error. The different letters indicate statistical significance of means in the different fruit maturation stages estimated by Fisher's LSD test ($P < 0.05$).

Sugars, organic acids, and amino acids determined in jaboticaba at each stage using GC-MS and HPLC are shown in Table 2.

Table 2. Metabolite levels of representatives of sugars, organic acids, amino acids, acetaldehyde, and ethanol in 'Sabara' jaboticaba unripe, ripe, and overripe fruits.

Metabolites	Unripe fruit	Ripe fruit	Overripe fruit
Fructose (mg/gFW)	15.14 ± 0.61 c	31.39 ± 1.43 b	84.34 ± 2.10 a
Glucose (mg/gFW)	7.23 ± 0.35 c	22.22 ± 0.99 b	51.77 ± 2.66 a
Sucrose (mg/gFW)	18.14 ± 1.95 c	62.34 ± 3.37 b	72.02 ± 3.13 a
Citric acid (mg/gFW)	30.52 ± 0.77 a	25.83 ± 1.04 b	18.57 ± 0.76 c
Glutamic acid (µmol/gFW)	1.56 ± 0.22 a	0.19 ± 0.04 b	-
Ketoglutaric acid (nmol/gFW)	172.14 ± 4.70 a	77.32 ± 8.15 b	216.50 ± 43.00 a
Malic acid (mg/gFW)	3.65 ± 0.17 a	0.95 ± 0.09 b	0.31 ± 0.02 c
Lactic acid (µmol/gFW)	2.94 ± 0.17 c	4.42 ± 0.31 b	12.17 ± 0.48 a
Pyruvic acid (nmol/gFW)	538.07 ± 13.46 a	200.16 ± 11.92 b	82.93 ± 6.66 c
Shikimic acid (µmol/gFW)	8.28 ± 0.76 a	2.07 ± 0.13 b	1.12 ± 0.07 b
Succinic acid (nmol/gFW)	70.51 ± 3.97 b	105.32 ± 5.55 b	278.36 ± 30.18 a
Aminobutyric acid (µmol/gFW)	0.12 ± 0.02 b	0.44 ± 0.06 b	4.18 ± 0.35 a
Phenylalanine (nmol/gFW)	9.79 ± 0.06 a	-	12.67 ± 1.95 a
Acetaldehyde (nl/g/hr)	0.52 ± 0.05 c	0.79 ± 0.03 b	29.00 ± 3.17 a
Ethanol (nl/g/hr)	1.47 ± 0.07 c	3.34 ± 0.26 b	1149.79 ± 152.92 a

Data are shown in the means of ten replicates ± standard error. The different letters indicate statistical significance of means in the different fruit maturation stages estimated by Fisher's LSD test ($P < 0.05$); - indicates not detected.

The maturation process from unripe into ripe fruit is accompanied with the increase of sugars and the decrease of organic acids. Sucrose (3.4-fold), fructose (2.1-fold), glucose (3.1-fold), acetaldehyde (1.5-fold), ethanol (2.3-fold), and lactic acid (1.5-fold) show significant increased from unripe to ripe fruit. Citric acid (0.9-fold), malic acid (0.3-fold), α-ketoglutaric acid (0.5-fold), glutamic acid (0.1-fold), pyruvic acid (0.4-fold), and shikimic acid (0.3-fold) showed significant decreased from unripe to ripe fruit. On the other hand, phenylalanine could not be detected at ripe fruit. GABA (3.5-fold) and succinic acid (1.5-fold) increased from unripe to ripe, but significant differences were not found between unripe and ripe fruit.

The maturation progress from ripe into overripe fruit showed the increase of sugars and several organic acids as well as acetaldehyde, ethanol, and lactic acid. Sucrose (1.1-fold), fructose (2.7-fold), glucose (2.3-fold), α-ketoglutaric acid (2.8-fold), succinic acid (2.6-fold), GABA (9.6-fold), lactic acid (2.8-fold), acetaldehyde (37-fold), and ethanol (340-fold) showed significant increased from ripe to overripe fruit. Citric acid (0.7-fold), malic acid (0.4-fold), and pyruvic acid (0.4-fold) showed significant decrease from ripe to overripe fruit. Shikimic acid (0.5-fold) decreased from ripe to overripe fruit, but no significant difference was found between ripe and overripe fruit. Glutamic acid could not be detected at overripe fruit.

Acetaldehyde and ethanol production increased more than sugars, organic acids, and amino acids from ripe to overripe fruit. metabolites with rapid changes during fruit maturation should be used as maturation indicators because jaboticaba fruit has an extremely short optimal harvesting time. Acetaldehyde and ethanol appear to be better than sugars or organic acids as maturation indicators.

Principal component analysis (PCA) on physicochemical parameters was conducted to compare their metabolites at three different maturation stages (Fig 3). The maturation process is divided into three stages, and two principal components could explain 98.2% of the overall variance of the metabolome analysis, with 72.5% and 25.7% for PC1 and PC2, respectively.

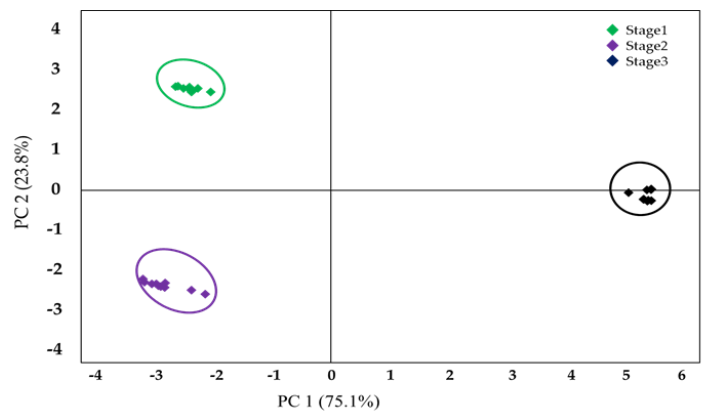

Fig 3. Score plots on principal component analysis (PCA) of physicochemical parameters derived from metabolome analysis at the different maturation stages of jaboticaba fruit. Color in green, purple, and black indicate unripe, ripe, and overripe fruit, respectively. Data of ten replicates for each stage are plotted.

The contribution of each metabolite against main principal component loading is shown in Fig 4, referring to the correlation coefficient between original variables and the PC1. The positive value indicates a positive correlation and vice versa. The strongest correlation is shown in glutamic acid (negative), followed by phenylalanine (positive), acetaldehyde (positive), ethanol (positive), and GABA (positive).

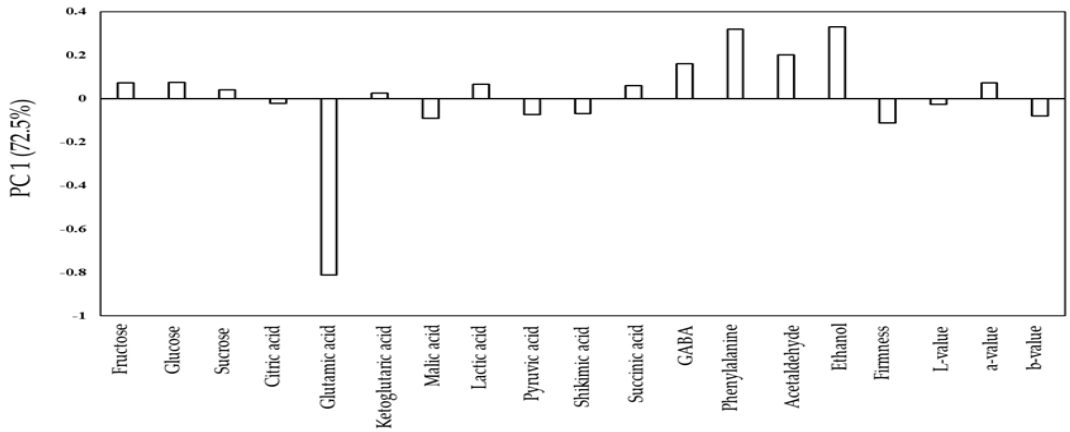

Fig 4. Contribution of each metabolite against main principal component loadings of physicochemical parameters derived from metabolome analysis at the different maturation stages of jaboticaba fruit.

A metabolic map constructed according to the results, highlighting the primary metabolic processes: glycolysis, TCA cycle, GABA shunt, shikimate pathway, and anaerobic respiration by referring to the literature (Iijima and Aoki 2009; Ji et al. 2020; Thimm et al. 2001; Tohge et al. 2013; Yin et al. 2010; Zhang et al. 2010). There observed significant metabolic changes (P <0.05) among unripe, ripe and overripe fruit as highlighted on the metabolic map (Fig 5).

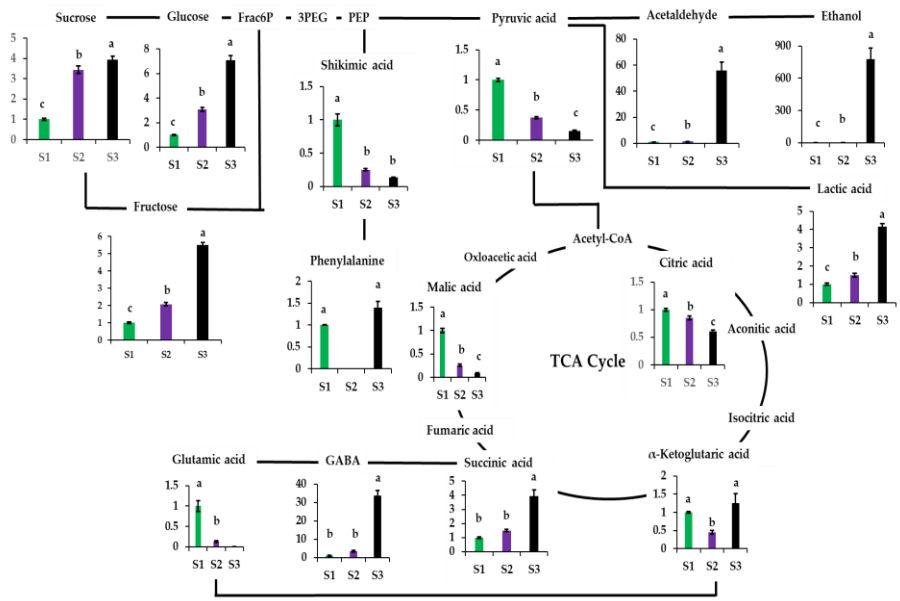

Fig 5. The changes in metabolites' level from unripe to overripe fruit are shown according to the primary metabolism such as glycolysis, TCA cycle, shikimic pathway, GABA shunt. Names of metabolites in black or grey indicate detected or not detected, respectively. The bar graphs of the three different maturation stages in each component are calculated as relative values against unripe fruit. The different letters in bar graphs indicate the statistical significance of means by Fisher's LSD test (P < 0.05). Abbreviations are as follows: S1, unripe fruit; S2, ripe fruit; S3, overripe fruit; Frac6P, fructose-6-phospate; 3PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvic acid; and GABA, γ- aminobutyric acid.

The increase in sugars and decrease in citric and malic acids were observed throughout maturation. Sucrose has the highest amount, followed by fructose and glucose in case of unripe and ripe fruit. In overripe fruit, fructose has the highest amount, followed by sucrose and glucose. Sucrose increases sharply from unripe to ripe fruit, while it is not significant between ripe and overripe fruit. Citric acid and malic acid decrease significantly throughout the maturation. The results reflect the taste changes, with increase in sweetness and decrease in sourness.

Succinic acid increased throughout maturation. There was a significant difference between ripe and overripe fruit, but not between unripe and ripe fruit. GABA increases while glutamic acid decreases from ripe to overripe fruit. It has been reported increase GABA throughout fruit maturation in mangosteen (Parijadi et al. 2018) and grape (Ali et al. 2011). From the viewpoint of human health, GABA had positive effects such as reducing stress and sleep induction (Dhakal et al. 2012; Okada et al. 2000). The amount of GABA in overripe fruit of jaboticaba is 4.18 μmol/gFW which is higher than tomato, apple, and peach (Akihiro et al. 2008; Shang et al. 2011; Trobacher et al. 2013).

The shikimate pathway links the metabolism of carbohydrates such as tyrosine, phenylalanine, and tryptophan (Schmid and Amrhein 1995). Furthermore, anthocyanins are produced via the phenylpropanoid pathway with phenylalanine as a precursor (Cheng and Breen 1991). Phenylalanine decreased from unripe to ripe fruit but increased from ripe to overripe fruit. Therefore, the changes of phenylalanine content might reflect the anthocyanins accumulation and decomposition.

Alcohol and lactic acid fermentation are regarded as a typical anaerobic metabolism (Du et al. 2018). Acetaldehyde, ethanol, and lactic acid increased sharply from ripe to overripe fruit. Ethanol is produced from pyruvic acid via acetaldehyde, involving the enzymes; pyruvate decarboxylase (PDC, EC 4.1.1.1) and alcohol dehydrogenase (ADH, EC 1.1.1.1), respectively (Ke et al. 1994; Tadege et al. 1999). In case of lactic acid fermentation, it is involved with the enzyme; lactate dehydrogenase (LDH, EC 1.1.1.27) (Pegoraro et al. 2012). In both courses, pyruvic acid is the first component in alcohol and lactic acid fermentation. In this experiment, pyruvic acid has decreased throughout maturation. Therefore, it is suggested that fermentation processes from pyruvic acid to acetaldehyde, ethanol, and lactic acid work unceasingly. They have been shown to accumulate during maturation in various fruit in temperate, tropical, and subtropical fruit tree (Beltrán et al. 2015; Chervin et al. 1999; Fuggate et al. 2010).

It was suggested that if acetaldehyde and ethanol could be established as new maturation indicators, these compounds could be applied to other fruits. In the case of some citrus fruits, the ethanol content of fruit juice is known as a maturation indicator (Pesis 2005). This might as well contribute to distinguish jaboticaba fruit that are ready to be eaten fresh. Based on such metabolic changes, future experiments should focus on acetaldehyde and ethanol production to check the possibility of their use as maturation indicators.

Changes in physicochemical composition in fruits. The changes in fruit size of jaboticaba were shown in Fig 6. The transverse and longitudinal of fruit were similarly developed. The fruit size hardly changed from 8 to 10 DAF, then increased constantly from 10 to 30 DAF. Fruit size did not change significantly until 52 DAF. The period of complete fruit growth has been reported as about 160 DAF in pear (Pei et al. 2020), 85 DAF in peach (Ishida et al. 1973), 90 DAF in blueberry (Shimura et al. 1986), 60 DAF in grape (Minemura et al. 2009), 45 DAF in strawberry (Pei et al. 2020), 40 DAF in cherry (Ren et al. 2011), and 25 DAF for pitaya (Jamaludin et al. 2011). Jaboticaba fruit reached its maximum growth at 34 DAF. Jaboticaba fruit growth was shown to be significantly faster than those fruits except for pitaya. Three types of fruit growth patterns have been reported: single sigmoid curve such as avocado, pear, and pineapple, double sigmoid curve such as blueberry, grape, and peach, and triple sigmoid curve such as kiwifruit (Coombe et al. 1976; Pratt and Reid 1974). Jaboticaba fruit grew in a way to form a single sigmoid curve during fruit development in accordance with the observation of

previous studies (Teixeira et al. 2011b).

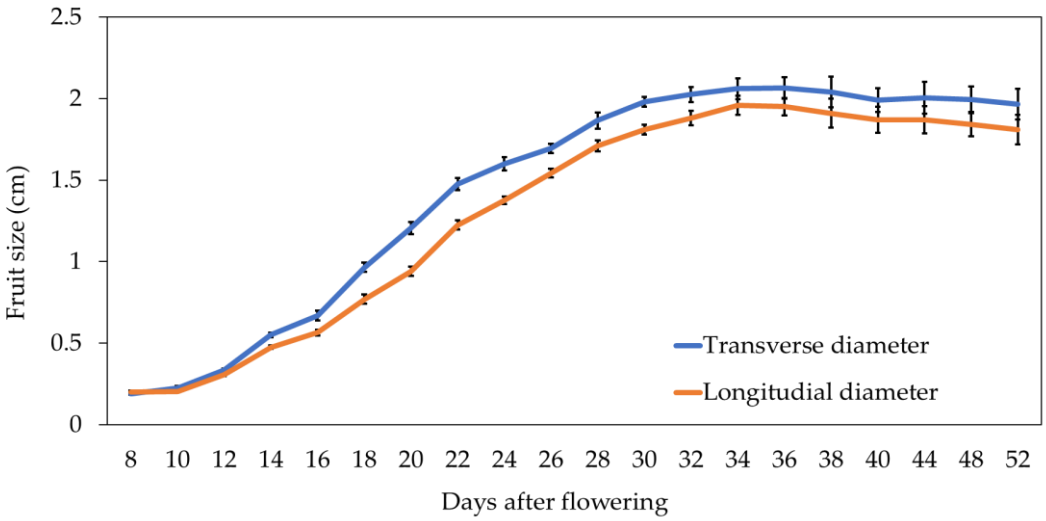

Fig 6. Changes in fruit size of 'Sabara' jaboticaba from 8 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bars represent standard errors.

The coloration parameters of the fruit skin during fruit development and maturation were shown in Fig 7. There were no significant changes in a value from 24 to 26 DAF. Then, it increased dramatically until 32 DAF. Thereafter, it decreased significantly from 40 to 52 DAF. The L and b values showed no significant changes from 24 to 30 DAF. Consequently, the values decreased quickly from 30 to 34 DAF. After that, the values gradually decreased until 52 DAF.

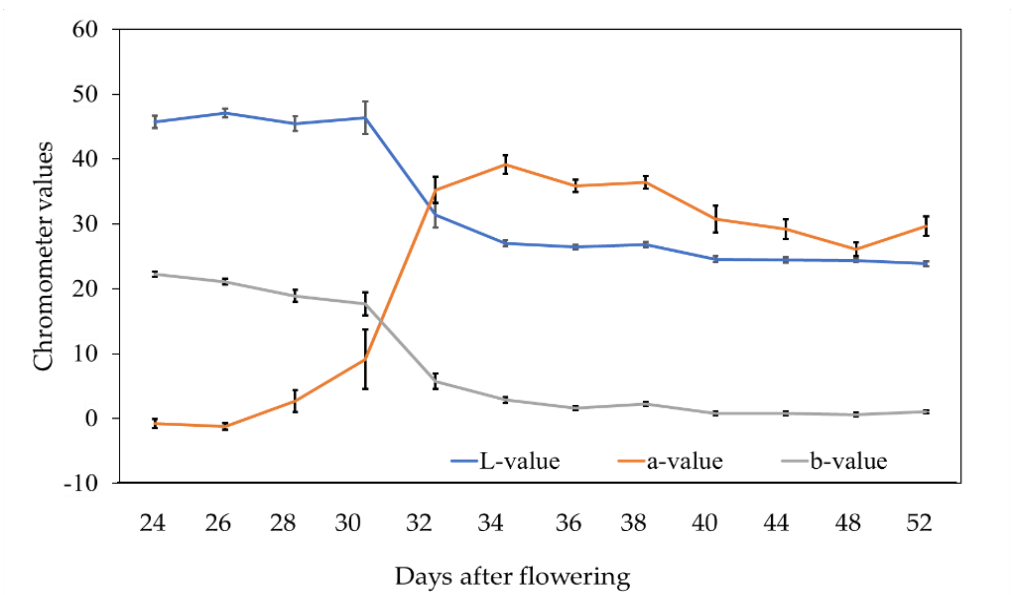

Fig 7. Changes in fruit skin color (L, a, and b value) from 24 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bar represents standard error.

The colouration period has been reported as about 45 days in grape (Minemura et al. 2009; Coombe and McCarthy 2000.), 20 days in guava (Deepthi. 2017), 15 ~ 20 days in cherry (Muskovics et al. 2006), 14 days in blueberry (Shimura et al. 1986), 6 days in strawberry (Yoshida et al. 2002), 3~5 days in pitaya (Jamaludin et al. 2011; Wu et al. 2019). The colouration period in jaboticaba fruit was about 10 days, almost the same as strawberry. It was considered that the increase of a-value and the decrease of b-value in jaboticaba might be reflective of anthocyanin accumulation and chlorophyll decomposition at the ripening stage (Becker et al. 2015). In addition, phenylalanine decreased from unripe to ripe fruit, and increased from ripe to overripe fruit in the metabolome analysis. Phenylalanine is closely related to the synthesis of anthocyanins. In particular, the decrease in a-value and b-value might be related to the decrease or restriction of anthocyanin synthesis after 40 DAF, since it is reported that anthocyanin content decreases at the late maturation stage in temperate, subtropical, and tropical fruits (Bureau et al. 2009; Faragher and Brohier 1984; Kulkarni and Aradhya.2005; Rogez et al. 2011).

The changes in respiration rate during fruit maturation in jaboticaba were shown in Fig 8. Respiration rate decreased dramatically from 24 to 28 DAF. Then, it decreased gradually from 28 to 52 DAF. Although climacteric fruits exert a peak in respiration during fruit ripening (Faragher and Brohier 1984; Rooban et al. 2016), the peak cannot be detected in jaboticaba. Therefore, it was concluded that jaboticaba is a non-climacteric fruit as in the previous studies (Duarte 2007; Teixeira et al. 2011a). Jaboticaba fruit ripening during postharvest is difficult as in the climacteric type, such as tomato and banana (Giovanelli et al. 1999; Kulkarni et al. 2011). Thus, it requires full ripening on the tree.

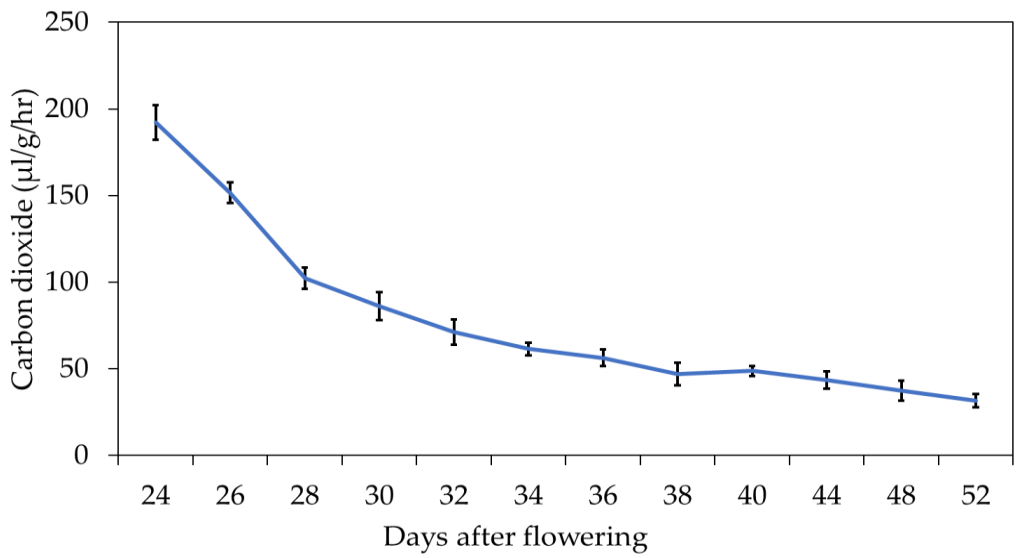

Fig 8. Change in respiration of 'Sabara' jaboticaba from 24 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bar represents standard error.

The changes in fruit firmness of jaboticaba during fruit development and maturation are presented in Fig 9. Fruit firmness remained high from 24 to 26 DAF. Then dramatic softening from 26 DAF to 32 DAF was recorded. Thereafter, a gradual decrease was observed from 32 DAF to 52 DAF. The period from the start of the decline in fruit firmness to the completion of coloration has been reported as about 30 days in cherry (Muskovics et al. 2006), 20 days in blueberry (Forney et al. 2012), 10 days in guava (Bashir and Abu-Goukh 2003), 7 days in pitaya (Wanitchang et al. 2010). Jaboticaba completed coloration 8 days after fruit firmness began to decline.

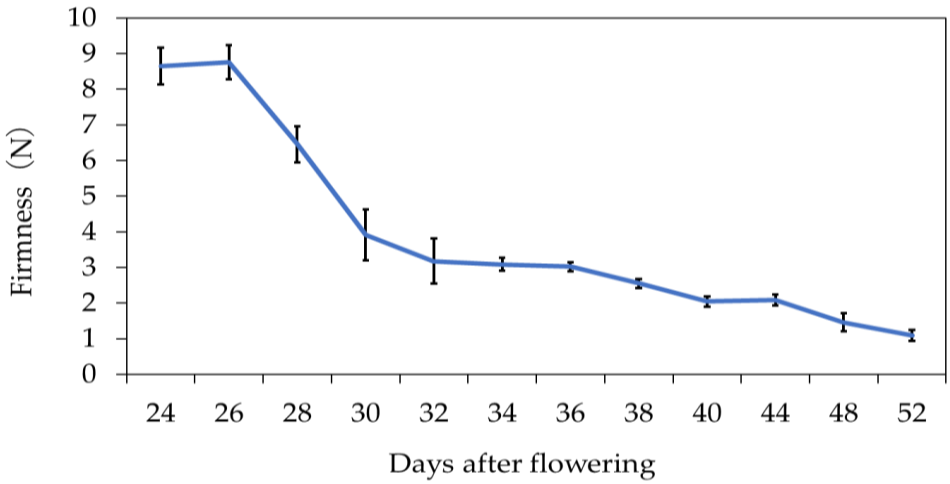

Fig 9. Changes in firmness of 'Sabara' jaboticaba fruits from 24 DAF to 52 DAF. Data are shown as the means of ten replicates and vertical bar represents standard error.

The changes in acetaldehyde and ethanol in fruits during maturation were shown in Fig 10 A and B. Acetaldehyde remained low until 34 DAF. However, it increased constantly from 34 DAF to 52 DAF. Jaboticaba fruit produces 5 nl/g/hr of acetaldehyde at 52 DAF. Ethanol remained low in concentration until 38 DAF. Then it increased rapidly from 38 DAF to 52 DAF. Jaboticaba fruit produced 85 nl/g/hr of ethanol at 52 DAF. Acetaldehyde and ethanol increased after 34 DAF and 40 DAF, respectively. The high amount of ethanol is known to affect the fruit taste significantly (Ueda et al. 2019) and the overripe fruit may not be suitable to be eaten fresh. Since the induction of acetaldehyde release precedes ethanol production by four days, acetaldehyde release would be a more accurate indicator for harvest. The maturity of jaboticaba fruit would be distinguished by the detection of acetaldehyde or ethanol using simple and non-destructive methods such as the test seal. Test seal has been reported to detect acetaldehyde and ethanol to predict abnormal fruits of melon (Agatsuma and Oshima 1981). The color of the test seal changes when fruit produces acetaldehyde or ethanol. Therefore, acetaldehyde and ethanol might be detected easily without using an analytical instrument such as GC.

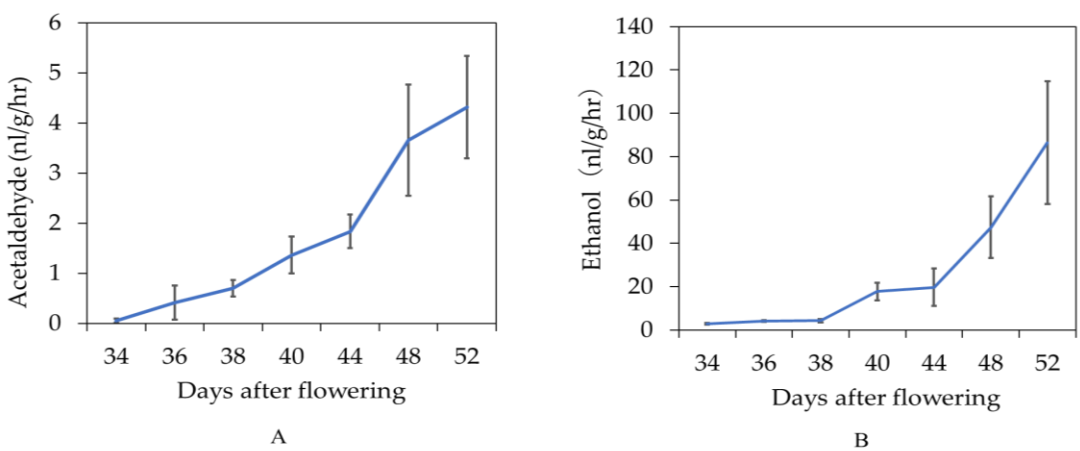

Fig. 10. Changes in acetaldehyde (A) and ethanol (B) production rate of 'Sabara' jaboticaba from 34 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bar represents standard error.

The maturation of jaboticaba fruit can be classified into 4 stages. The unripe stage is until 26 DAF, when fruit skin color is still green, and fruit firmness is high. The ripening stage is from 26 to 34 DAF, when fruit skin color changes from green to dark-purple, and fruit firmness starts to decrease. The mature stage is from 34 to 40 DAF, when fruit skin color and fruit firmness have settled down and acetaldehyde begin to increase. The overmature stage is after 40 DAF, when fruit firmness is more reduced and acetaldehyde and ethanol increase dramatically.

CONCLUSION

Metabolome analysis at three maturation stages and monitored physicochemical changes during fruit maturation in jaboticaba revealed that sugars increase, and organic acids decrease as the fruit matures. GABA shunt and anaerobic respiration such as alcohol and lactic acid fermentation can be activated from the ripe to the overripe stage. The maturity of jaboticaba fruit might be distinguished through its acetaldehyde and ethanol content. Although the overripe fruit of jaboticaba is not suitable to be eaten fresh because of the higher ethanol content, it has the potential as a healthy food due to its high amount of GABA when a suitable processing is developed. This study provides insights on how to distinguish fruit maturity and understand the comprehensive fruit maturation in jaboticaba. In order to further elucidate the metabolism of fruit maturation, enzyme activities, genetic information, and secondary metabolites should be investigated.

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