

Postharvest physiological and biochemical changes in Permai 5 melons (*Cucumis melo* L. var. *cantalupensis*)

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Abstract

The Permai 5 (P5) variety is a commercially cultivated melon in Malaysia, known for its distinctive appearance. Nevertheless, its quality in terms of physiological and biochemical properties has not yet been fully evaluated. In the present work, we characterised the shelf-life duration, physiological attributes (rind colour, weight loss, firmness), climacteric behaviour (respiration, ethylene), and flavour (sweetness, acidity) of P5 melons. The P5 melons were harvested at commercial maturity, and assessed throughout the storage period. P5 melons exhibited an average shelf-life of nine days, and showed a climacteric ethylene release pattern, accompanied by significant weight loss during storage. The highest respiration rate was recorded on day 3 after harvest, reaching 1.13 ± 0.11 g CO₂/kg/h. However, other quality parameters such as rind colour, firmness, sweetness, and acidity did not show a clear association with overall fruit quality. Metabolomics analysis was performed to identify metabolites present in P5 melons. Although P5 melons displayed several non-climacteric behaviours, the observed ethylene peak confirmed their climacteric nature.

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Introduction

Melons (*Cucumis melo* L.) are a member of the Cucurbitaceae family, and cultivated worldwide for their commercial and nutritional values. Morphologically, melons vary greatly in terms of shapes (long, oblong, round), rind colours (green, yellow, white, pink), and flesh colours (orange, white, green, pink). Melons also exhibit varying rind characteristics, fruit sizes, and flesh thickness.

In general, the postharvest ripening behaviour of fruits can be categorised into climacteric and non-climacteric. Climacteric fruits are fruits that continue ripening after harvest, while non-climacteric fruits are fruits that stop ripening after harvest (Wilson, 2021). Melons are an exception, as some genotypes exhibit climacteric characteristics, while others exhibit non-climacteric characteristics. It is important to note that the production of ethylene gas governs the ripening classification. With high ethylene production, climacteric melons such as *C. melo* var. *cantalupensis*

regularly have fast ripening rates and short shelf-life. In contrast, non-climacteric melons such as *C. melo* var. *inodorus* did not produce autocatalytic ethylene, hence experiencing a slow ripening rate and long shelf-life (Pech *et al.*, 2008).

Multiple irreversible physiological and biochemical processes determine fruit quality during ripening. Fruits may exhibit loss of firmness, progressive change of fruit colour, and subtle change during ripening. As the consumer demand for melons continue to grow, preserving the melon quality has become a challenge for melon producers. Many researchers have frequently addressed the challenges in dealing with postharvest storage of melons. The Food and Agriculture Organisation (FAO) has estimated that nearly half of all fruits and vegetables are lost between harvest and consumption (Kitinoja *et al.*, 2018). Hence, establishing quality changes of the fruits during postharvest storage is critical to maintaining their quality.

Postharvest changes have been shown to

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dictate overall fruit quality, hence influencing consumer acceptability and preferences. In the present work, we focused on analysing postharvest physiological and biochemical changes of P5 melons (*C. melo* L. var. *cantalupensis*), one of the commercially established varieties in the Malaysian market.

Postharvest physiology of P5 melons is relevant beyond regional interest as it offers insight into the quality of tropical melons under warm-climate conditions, which remains underrepresented in global literature. Limited data on melon physiology has hindered producers from optimising storage conditions, with the most recent national melon production statistics dating back to 2018 (Liyana and Pebrian, 2020).

Understanding P5 melon's ripening behaviour is also valuable for breeding programs aimed at enhancing fruit quality and shelf-life, mainly for varieties adapted to high-temperature environments. Globally, melon research continues to focus on postharvest quality traits for market segmentation and consumer preference, as demonstrated by Torres *et al.* (2020) and Wang *et al.* (2024). Yet, very few studies investigate cultivars exhibiting both climacteric and non-climacteric characteristics, as observed in P5 melons. This highlights the novelty and broader applicability of the present work.

In the present work, we report both physiological and biochemical changes of P5 melons at the postharvest stage, along with metabolomics analysis. The results may provide a greater understanding of P5 melon quality and postharvest management to maintain high-quality fruits throughout storage, distribution, and marketing channels.

Materials and methods

Plant material

The P5 melons were harvested at Batu Arang Farm in Selangor, Malaysia. Melons were harvested manually in the morning at the optimal horticultural maturity based on non-destructive ripening indices (browning and drying of tendrils nearest to the melon). Melons were selected for testing following the standard grading compliance of Green World Genetics Sdn. Bhd. (uniform skin colour, free from defects including physical damage and disease infection), and immediately transported to the laboratory.

Physiological characteristics

Shelf-life determination

Melons were stored in open air at ambient temperature ($27 \pm 1^\circ\text{C}$). The physical characteristics of the melon surface were observed daily until the shelf-life was terminated. The end of melon shelf-life was determined by observing the following characteristics: (1) brown surface discolouration; (2) bruise that becomes increasingly noticeable; and (3) liquid seeping through the rind.

Rind colour measurement

Total colour differences (TCD)

Colour measurement was extracted from six equidistant points, and the results were expressed as average values using a ColorMeter mobile application (version 2.2.0/2.2.0.0; White Marten GmbH, Stuttgart, Germany). The TCD values were calculated using Eq. 1 (Ortiz-Duarte *et al.*, 2019):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (\text{Eq. 1})$$

Weight loss measurement

Melons were stored in open air at ambient temperature ($27 \pm 1^\circ\text{C}$) throughout the test period. Melon weight measurement was recorded at 24-h intervals. The percentage of fruit weight loss was calculated using Eq. 2:

$$\text{Fresh weight loss (\%)} = \frac{\text{Initial weight (kg)} - \text{Final weight (kg)}}{\text{Initial weight (kg)}} \times 100 \quad (\text{Eq. 2})$$

Biochemical characteristics

Respiration rate (carbon dioxide gas production measurement)

The melons' rate of respiration was measured according to Mangaraj and Goswami (2011). Carbon dioxide (CO_2) gas was captured in a closed system, and measured per unit time per unit weight of the produce. Melon with a known mass was placed in a hermetically sealed container with a lid for 1 h at room temperature ($25 \pm 1^\circ\text{C}$). Next, 0.1 N of NaOH aqueous solution was placed inside the container. A titration method using phenolphthalein as an indicator was conducted, and the gas production was calculated using Eq. 3, and expressed as unit g CO_2 produced/kg.h. (Saltveit, 2003):

$$\text{Respiration rate of } \text{CO}_2 = \frac{\text{Mass of } \text{CO}_2}{\text{Fresh weight loss of fruit in 1 h}} \quad (\text{Eq. 3})$$

Ethylene production rate measurement

Melons were individually placed in a closed system for 4 h at room temperature ($25 \pm 1^\circ\text{C}$), as described by Wang *et al.* (2022). The ethylene production rate in melons was measured using a portable ethylene gas detector HFP-1201 (Xi'an HuaFan Technology Co., Ltd., China), which was placed along with the melons in the system. The results were expressed in parts per million (ppm).

Soluble solid content (SSC) measurement

Melons were cut longitudinally from the stem to the calyx end using a sharp knife. Melon's mesocarp tissue from the middle of the fruit wedge was extracted, with the core and peel removed (OECD, 2018). The cut-off melon wedge was divided into three parts (top, middle, and bottom pieces), and each was subjected to Brix testing using a digital refractometer (PAL-1; Atago, Tokyo, Japan).

Firmness measurement

The melons' firmness was measured at three random points using a digital fruit harvest tester model FHT-05 (Guangzhou Lantek Instruments, China) in intervals of 24 h across the study period. The melon was held firmly with one hand before applying a steady downward pressure until the plunger penetrated its flesh, following the OECD Fruit and Vegetable Scheme (OECD, 2018). The results were expressed in kg.f/cm^2 .

Titrateable acidity (TA) measurement

The TA of melon juice was determined by the visual titration method. The test was conducted based on a previous study. The TA was expressed as the percentage of anhydrous citric acid (v/v) according to Bhat *et al.* (2011) with a slight modification.

Identification of secondary metabolites

Sample preparation was carried out at harvest (day 0), day 4, and day 8 after harvest. Five grams of pulp samples were ground in liquid nitrogen using a pestle and mortar until it was powdered. Powdered samples were transferred into 50 mL Falcon tubes before 80°C methanol was added in a 1:4 ratio. The tubes containing the mixture were placed on the rotary shaker (Orbital Shaker S01, Stuart) for 30 min at room temperature ($25 \pm 1^\circ\text{C}$), and then immediately centrifuged at 6,500 rpm for 15 min at 4°C using a refrigerated centrifuge (Beckman J2-MI

Centrifuge, California). The supernatant was carefully aspirated and evaporated to dryness in a rotatory evaporator (BUCHI Rotavap R-114 and R11, Switzerland) for 4 h at 40°C to obtain crude methanolic extract. The extract was then stored in 1.5 mL microcentrifuge tubes at -20°C until further analyses.

The Ultra-High-Performance Liquid Chromatography (UHPLC) system was coupled with a Vion IMS Quadrupole Time-of-Flight hybrid mass spectrometer from Waters, equipped with a Lock Spray ion source. The ion source was operated in negative electrospray ionisation (ESI) mode under the following specific conditions: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120°C ; desolvation gas temperature, 550°C ; desolvation gas flow, 800 L/h; and cone gas flow, 50 L/h. Nitrogen ($> 99.5\%$) was employed as desolvation and cone gas. Data were acquired in high-definition MS^E (HDMS^E) mode in the range m/z 50 – 1,500 at 0.1 s/scan. Thus, two independent scans with different collision energies (CE) were alternatively acquired during the run: a low-energy (LE) scan at a fixed CE of 4 eV, and a high-energy (HE) scan where the CE was ramped from 10 to 40 eV. Argon (99.999%) was used as collision-induced-dissociation (CID) gas.

Statistical analysis

The results of physiological and biochemical characteristics were reported as the mean of at least six biological samples. Microsoft® Excel® 2021 (Version 2312; Microsoft, Las Vegas, USA) and Minitab® (version 21.2; Minitab LLC, Pennsylvania, USA) were used to statistically analyse the results. Comparison of means was performed by One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests.

Results*Evaluation of physiological characteristics*
Shelf-life

Overall, P5 melons were acceptable in the first four days after harvest, with no visible crack or brown spot. A small brown spot appeared near the melon stem end on day 5 after harvest, but the overall fruit condition was unaffected. The brown spot became enlarged in size with time, which eventually led to the spoilage of melons. Major fruit spoilage occurred on

day 8 after harvest, where a large brown spot was present at the melons' stem and blossom end (Figure 1). Moreover, white fungal growth was observed at

the blossom end of the melons, which clearly indicated fruit quality deterioration.

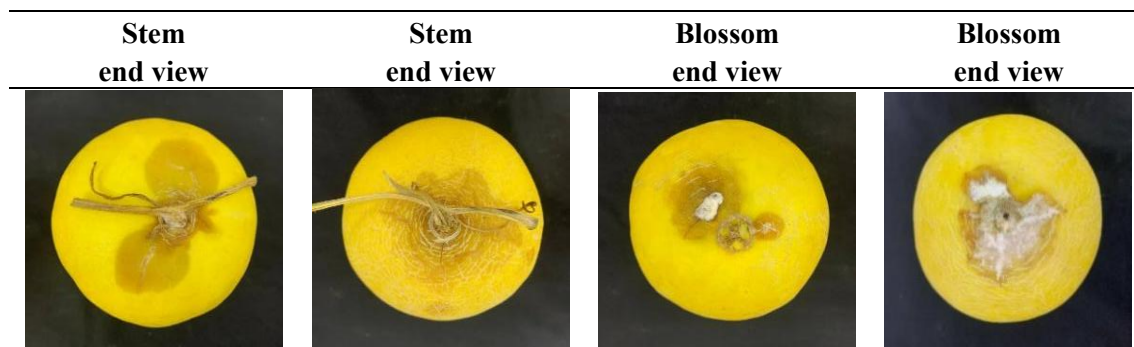


Figure 1. Spoiled melons on day 8 after harvest. Day 0 = harvesting day.

Rind colour

Based on the TCD values, the overall data fluctuated across the study period. Significant differences were detected. The lowest TCD value recorded was on day 1 after harvest (0.86 ± 0.01). The value increased to 4.77 ± 0.10 on day 2 after harvest before it decreased to 3.91 ± 0.85 and 3.65 ± 0.65 on days 3 and 4 after harvest, respectively. The rest of the TCD values showed a fluctuation.

Meanwhile, based on colour classification reported by Adekunte *et al.* (2010), the colour changes from day 1 to 2 after harvest, and from day 7 to 8 after harvest, can be classified as very distinct. Distinct colour changes were observed from day 4 to

5 after harvest, while minor colour changes were observed on the rest of the days.

Weight loss

Figure 2 illustrates the fresh weight loss of melons in three different harvesting times: (1) melon harvested at actual timing; (2) melon harvested one week earlier than actual harvest; and (3) melon harvested two weeks earlier than actual harvest. Generally, a clear upward trend was observed in all harvested melons. Actual harvested melons started to lose more than 5% of their initial weight loss on day 6 after harvest.

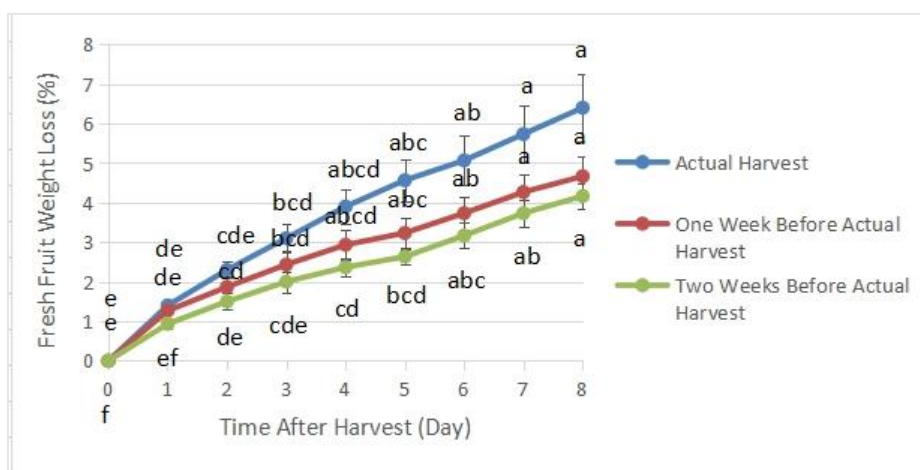


Figure 2. Fresh weight loss of melons of different harvesting times. Vertical bars indicate standard error of the mean (\pm SEM). Different lowercase letters indicate significant differences ($p \leq 0.05$) according to Tukey's Test.

Biochemical characteristics

Respiration rate (carbon dioxide gas production)

The respiration rate of melon ($n = 6$) demonstrated an upward trend from day 0 (0.43 ± 0.16 g CO₂/kg/h) until day 3 (1.13 ± 0.11 g CO₂/kg/h)

after harvest, before showing a periodical change on the following days until the end of study period (Figure 3). The respiration rate reached the highest record on day 3 after harvest (1.13 ± 0.11 g CO₂/kg/h).

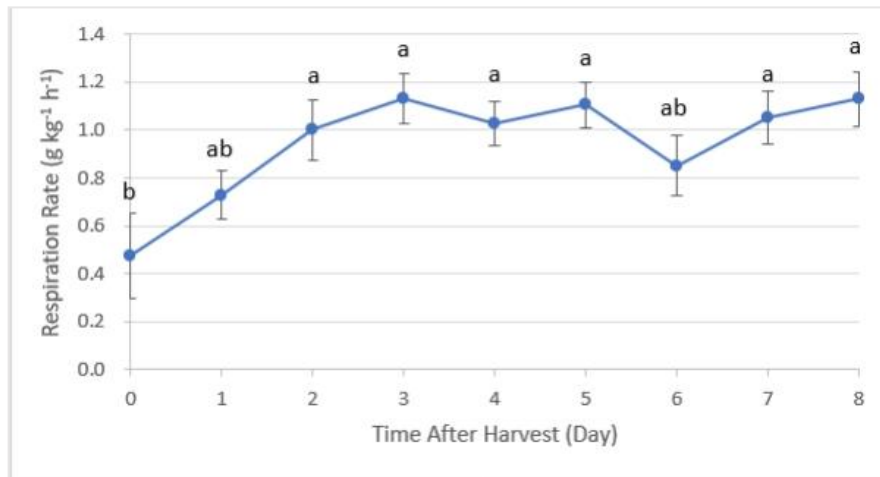


Figure 3. Changes in respiration rate of melons of different harvesting times. Vertical bars indicate standard error of the mean (\pm SEM). Different lowercase letters indicate significant differences ($p \leq 0.05$) according to Tukey's Test.

Ethylene production rate

Ethylene was not detected on the day of harvest. Ethylene was present in low quantity (0.50 ± 0.22 ppm) on day 1 after harvest. The ethylene production rate increased gradually until it reached its peak (9.00 ± 2.29 ppm) on day 4 after harvest, before it decreased steadily from day 5 after harvest until the test period was over (Figure 4).

Based on the observation, postharvest fruit

decay became more severe after the recorded ethylene climacteric peak. Brown spots started appearing on day 5 after harvest, coinciding with the day after peak ethylene release was detected. Interestingly, the postharvest decay was observed to be more severe after reaching the ethylene climacteric peak. For instance, brown spots started to be visible on the day after the ethylene climacteric peak was recorded.

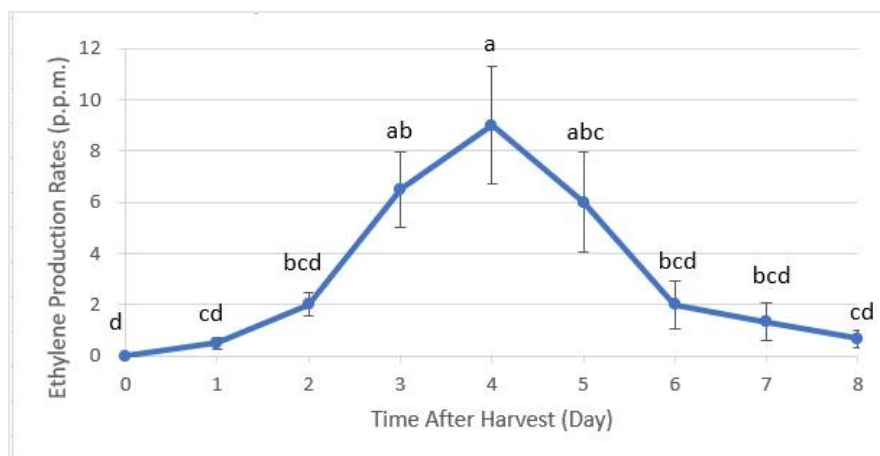


Figure 4. Changes in ethylene production rates of melons of different harvesting times. Vertical bars indicate standard error of the mean (\pm SEM). Different lowercase letters indicate significant differences ($p \leq 0.05$) according to Tukey's Test.

Soluble solid content

Brix level increased from low (less sweet) to high (sweeter) in the outer mesocarp tissue, followed by middle mesocarp tissue and inner mesocarp tissue in P5 melons. No significant difference was observed across the test period as the values were very close, showing only a minor change in the Brix levels.

Firmness

Fruit firmness is an important indicator of fruit quality, as many fruits soften during maturation. The firmness level of P5 melons was determined using a daily fruit hardness tester across the study period. There was a very minor change detected in melons' firmness level.

Titratable acidity

Organic acid accumulation is an essential criterion for melon quality determination. Melons have a minimal organic acid content, mostly restricted to malic, citric, succinic, and ascorbic acids. The melons' acidity did not change significantly across the study period. The range of TA recorded was between 0.93 ± 0.09 and $1.31 \pm 0.13\%$.

Secondary metabolites

In the present work, we used a combination of UHPLC and Traveling-Wave Ion Mobility Spectrometry with Quadrupole Time-of-Flight Mass Spectrometry (TWIMS-QTOFMS) to analyse the composition of secondary metabolites in melons' pericarp at three different time points: harvesting day (D0), day 4 after harvest (D4), and day 8 after harvest (D8) throughout the experiment.

The analyses revealed a total of 26, 24, and 32 metabolites on D0, D4, and D8, respectively. These metabolites comprised various metabolite classes including sugars and derivatives, organic acids, and amino acids. The type of secondary metabolites found in P5 melons are shown in Figure 5. 'Glycoside' and 'Others' groups dominate the chart (18.64%), followed by 'Sugar and Derivative' group (13.56%), 'Phenolic' and 'Flavonoid' groups (10.16%), 'Amino Acid' group (8.47%), 'Organic Acid' group (6.78%), 'Carboxylic Acid' group (5.08%), and 'Nucleoside' group (3.39%). The lowest percentage was recorded equally by 'Alcohol', 'Ester', 'Nitrogen Containing Compound', and 'Terpene' Groups.

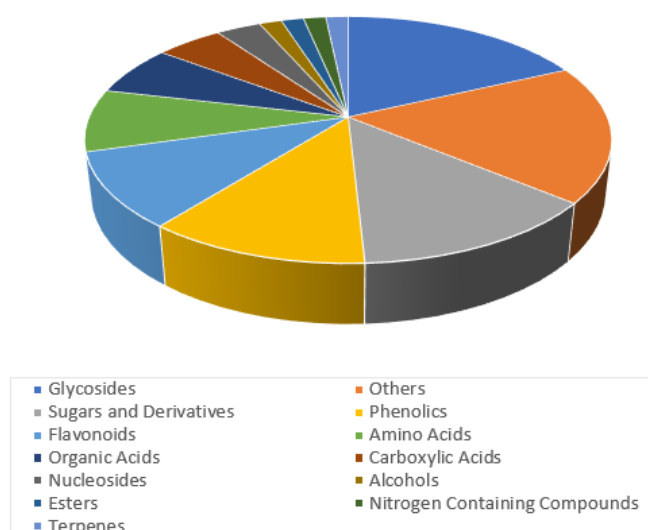


Figure 5. Types of secondary metabolites found in P5 melons.

Six metabolites were identified in all three time points across the study period. The metabolites included undulatoside A, amurenlactone A, moracin M-3'-O- β -D-glucoopyranoside, galactose, isomaltose, and 5'-Methoxy-bilobetin. Besides that, we identified the presence of five metabolites which were present in both D0 and D4 time points but were absent on D8 time point. The metabolites were taraxacoside, digupigan A, geniposide_1, methylsuccinic acid, and stachyose. On the other hand, only two compounds were present in both D4 and D8 time points but were absent on D0 time point *i.e.* raffinose and citric acid. The summary of these metabolites is tabulated in Table 1.

Discussion

Evaluation of physiological characteristics Shelf-life

Shelf-life is defined as a time when fruit remains safe for human consumption in terms of its safety, sensorial characteristics, and nutritional attributes when stored under ideal conditions. Shelf-life can be measured by sensory panels, instrumental methods, and physical, chemical, and microbiological measurements. In the present work, physical measurement was applied by observing the physical changes on the melon surface to determine the melons' shelf-life.

The shelf-life of melons varied based on the melon variety. Major spoilage of melons occurred on day 8 after harvest, suggesting that the maximum shelf-life of P5 melons was only nine days. In comparison to the other tropical melon variety named Manis Terengganu (MT) melons, P5 melons have a shorter shelf-life capacity as MT melons have a shelf-life of two weeks depending on the storage condition (Muhamad and Redzuan, 2019).

Changes in melon appearance, such as soft brown spots on the rind surface, started to occur within five to seven days after harvest, consistent with a gradual decrease of ethylene release after reaching an ethylene climacteric peak on day 4 after harvest. High ethylene production after harvest was closely associated with the deterioration of fruit quality. This was supported by previous findings which reported a correlation between ethylene production and postharvest decay after evaluating three different groups of melons respectively (cantalupensis, inodorus, and dudaim) (Zheng and Wolff, 2000).

Table 1. Compounds identified from UHPLC coupled with TWIMS-QTOFMS analyses.

No.	Compound	Presence*			Retention time (min)		
		D0	D4	D8	D0	D4	D8
Glycoside group							
1	Undulatoside A	/	↓	↑	0.76	0.76	0.76
2	Taraxacoside	/	↓		0.77	0.77	
3	Geniposide 1	/	↓		0.78	0.78	
Others							
4	Amurenlactone	/	↓	↑	0.77	0.77	0.77
5	Digupigan A	/	↓		0.78	0.78	
Sugar and derivative group							
6	Moracin M-3'-O-β-D-glucopyranoside	/	↓	↑	0.78	0.78	0.78
7	Galactose	/	↑	↓	0.84	0.97	0.92
8	Isomaltose	/	↑	↓	0.84	0.91	0.92
9	Stachyose	/	↓		1.12	1.12	
10	Raffinose		/	↑		0.97	1.17
Flavonoid group							
11	5'-Methoxy-bilobetin	/	↓	↑	1.19	1.18	1.19
Organic acid group							
12	Citric acid		/	↓		1.18	1.18
Carboxylic acid group							
13	Methylsuccinic acid	/	↓		0.97	0.97	

*Relative quantification of compound is classified based on the ion signal response; / = presence of compound; ↑ = upregulation, and ↓ = downregulation compared to day 0 or the prior time point.

Soft brown spots on the rind surface might occur due to pre-harvest injury and postharvest processes. The injury might not be visible immediately after harvest, but the spots started to enlarge over time, eventually leading to melons' spoilage (Figure 1). This was predicted as the injury rendered cells prone to fungal infection, which eventually penetrated the melons in search of nutrients to survive and proliferate. The infection might occur by direct penetration of the cuticle, entry through wounds, or abscission of scar tissue. Brown juices started seeping out of the spoiled melons, rendering the fruits unmarketable.

Comparative benchmarking can be conducted by evaluating the physical appearance of the fruits at the end of the study period. The presence of white fungus on the melon rind indicated quality deterioration, resembling the cotton-like mycelia reported on wounded muskmelons on retail shelves in Hatyai and Songkhla, Thailand (Wonglom and Sunpapao, 2020).

Rind colour

Rind colour measurement is an important factor in determining overall fruit quality as it indirectly reflects the maturity or ripening stage of the fruits. In the present work, we assessed the rind colour because some fruits showed a correlation between rind colour and fruit quality. For instance, the quality of a Malaysian papaya variety (*Carica papaya* L. cv. Sekaki) can be accurately described by the rind colour changes (Ruslan and Roslan, 2016). The portable colorimeter is convenient and easy to use in all conditions. The TCD indicates the magnitude of colour change, and is considered the most sensitive parameter for colour measurement in the harvested fruits.

In the present work, the rind colour on each testing day after harvest was compared to the rind colour on harvest. The lower the TCD value, the closer the sample colour was to the standard colour and *vice versa*. According to Adekunle *et al.* (2010), differences in colour extraction values throughout the

test period could be analytically classified as very distinct ($TCD > 3$), distinct ($1.5 < TCD < 3$), and minor differences ($TCD < 1.5$). Hence, in the present work, we interpreted that there was a minor colour difference on day 1 ($TCD = 1.06$), and a distinct colour difference on day 3 ($TCD = 2.91$) in comparison to harvesting day. A distinct colour change ($TCD > 3.23$) was detected in the remaining testing days, indicating that the rind colour changes occurred across the test period.

Weight loss

A weight management record is essential to determine the weight loss of fruit over time, which indirectly signifies the fruit's quality. Weight loss is associated with the respiration and transpiration of the fruits; hence, the loss of water and solutes seemed to be the main factor in influencing the fruit quality (Silva *et al.*, 2017). The present work found that the fresh fruit weight loss was significant across the test period. Initial water loss might have been due to reduced water, as the water supply from the parent plant was no longer available after the fruits were harvested.

According to Lufu *et al.* (2020), a 5 to 10% loss of initial fresh weight might induce fruit senescence and membrane integration, eventually rendering the fruit unmarketable. This is supported by the findings of Lester and Bruton (1986), who reported 5.7% water loss from the Magnum 45 variety of cantaloupe, rendering the fruit soft and highly perishable. Actual harvested melons in Figure 2 experienced 5% weight loss (5.07%) on day 6 after harvest. Hence, we suggest that any marketing and distribution process should be performed immediately after harvest, and advisable to be completed by day 6 after harvest (if possible) to preserve the quality of the fruits.

Biochemical characteristics

Respiration rate (carbon dioxide gas production)

Our analysis found that respiration was higher in the first few days than in the rest of the test period (Figure 3). The high initial respiration rate might have been due to the detachment of the fruits from the plants after harvest, which involved wounding at both ends of the fruit stalks. This hypothesis was in agreement with Bower *et al.*'s findings (2002) which reported that the presence of a wound might significantly increase the gas permeability of the fruits, and wound resealing of melon with wax was proven to lower the fruit respiration rate by 10%

(Burg and Burg, 1965). Other than wounding, washing and other handling processes might cause stress to the freshly harvested fruits (Ortiz-Duarte *et al.*, 2019).

Ethylene production rate

In the present work, the trend of ethylene production was consistent with Wilson's findings (2021) who stated that the climacteric fruits showed a gradual increase in ethylene production rate during maturation, increasing sharply before optimal ripening, and decreasing significantly as the fruit aged, overripen, and degraded. This was further supported by Nuñez-Paleniús *et al.*'s findings (2008), who claimed that ethylene production was low in the pre-climacteric stage, but increased dramatically during the climacteric stage. The peak of ethylene production in P5 melons was on day 4 after harvest, which was consistent with the findings of Bower *et al.* (2002), who found out that the maximum ethylene production of wild-type Charentais melon occurred on day 4 of the ethylene climacteric. In contrast, non-climacteric fruits did not experience similar patterns in fruit ripening and overripening phases, respectively. Hence, we could infer that P5 melons are climacteric type, given the presence of ethylene climacteric.

Paul *et al.* (2012) have mentioned that the distinction is fading between classical patterns of ripening behaviour as many fruits, including melons, show climacteric and non-climacteric behaviours depending on the genotype or cultivar. Shiomi *et al.* (1999) reported that Earl's Favourite melon cultivar exhibited low ethylene production during the harvesting maturity stage even though it is a climacteric melon. As previously mentioned, P5 melons are regarded as climacteric due to ethylene climacteric peak. However, this melon variety also portrayed non-climacteric characteristics on the basis of insignificant rind colour change and no aroma volatiles being produced. In short, the P5 melon variety is regarded as a climacteric fruit with some characteristics of non-climacteric fruit.

Zheng and Wolff (2000) reported a correlation between ethylene production and postharvest decay after evaluating eight market types of melons. Fernández-Trujillo *et al.* (2008) supported this finding, and mentioned that higher ethylene production after postharvest storage accelerates fruit senescence and physiological disturbance, leading to higher total weight loss. In general, it is suggested that

ethylene production in melons appears to be one of the significant factors in determining the severity of postharvest decay. This was consistent with our observation as discussed earlier.

In terms of physiology, the ethylene production trend in P5 melons was consistent with published reports. The peak ethylene production observed on day 4 after harvest matched the findings of Bower *et al.* (2022) for wild-type Charentais melons, which also reached maximum ethylene production on day 4 of the climacteric phase. This ethylene peak coincided with an accelerated weight loss in P5 melons, suggesting a coordinated ripening response of climacteric fruits.

Overall, these comparisons suggest that P5 melons follow a ripening pattern typical of climacteric fruit, further supporting its classification within this group.

Soluble solid content

SSC is an essential indicator of fruit sweetness level. From our observation, higher Brix levels were measured in inner mesocarp tissue (near seed cavity), followed by middle and outer mesocarp tissues (subpeel). This agreed with Lester's findings (2008), who reported higher SSC detected in inner mesocarp tissue compared to the outer mesocarp tissue.

Overall, the results suggested that P5 melons' sweetness did not increase in Brix level after harvest. This agreed with Bianco and Pratt (1977), who reported that the sugar content would not increase during postharvest activity. Sucrose metabolism ended when the fruit fully matured. Hence, we can deduce that Brix level measurement can be a useful predictor of consumers' acceptance.

Firmness

Fruit firmness measurement is commonly utilised as an important maturity index. In the present work, P5 melons showed no significant change in firmness throughout the storage period. This contradicted Lee *et al.* (2020), who reported a negative relationship between fruit firmness and storage time. Farcuh *et al.* (2020) reported that the differences in fruit firmness level might be associated with ethylene production rate as melons in the cantalupensis group had shown that the disassembly of the cell wall is an ethylene-dependent mechanism. However, the P5 melon variety was an exception.

Titrateable acidity

TA is often as an indicator of acidity which affects the fruit's taste. TA indicates the content of all organic acids in the fruit pulp, including citric and ascorbic acids (Manchali *et al.*, 2021). High organic acids give fruits a sour taste and vice versa. The present work found no significant change in P5 melons, suggesting that the fruit flavour did not change much across the study period. Our data showed much higher TA values than Manchali *et al.*'s (2021), which detected values in the range of 0.08 ± 0.07 (Cantaloupe 1) to 0.52 ± 0.02 (wild melon) only after studying 30 different melon cultivar samples collected from different parts of India.

Secondary metabolites

Based on the group identification, we could deduce that P5 melon flesh was dominated by 'Glycoside' group. Glycosides play an important role in living organisms including plants, where chemicals are stored in form of inactive glucosides, and can be activated for plant use by enzyme hydrolysis (Brito-Arias, 2016). Fils-Lycaon and Buret (1991) also studied the changes in the activity of glucosidases in mesocarp tissue of muskmelon (*C. melo* L. var. Alpha) during fruit development and ripening, which reflects the importance of glycosides in melons. 'Sugar and Derivative' group also ranked at the top, which agreed with the nature of P5 melons, which is known as sweet melons. Besides that, 'Ester' group ranked among the lowest compounds identified in P5 melons. This also agreed with the characteristics of P5 melons which produce little or no fruity smell.

Six metabolites present in all time points across the study period were potentially related to melons' physiological and biochemical processes. For instance, galactose and isomaltose, which were both classified under 'Sugar and Derivative' group. We could observe a similar trend of metabolite regulation; a downregulation on day 8 in comparison to day 4. This agreed with the TSS of P5 melons which showed a decrease in TSS on day 8 after harvest in comparison to day 4 after harvest. Hence, we could interpret that the downregulation of galactose and isomaltose may influence the sweetness of the harvested fruits.

Apart from that, metabolites absent on D0 but present on D4 and D8 were recognised. Taking citric acid as an example, this metabolite was present on

both day 4 and 8 with a retention time of 1.18 min, but the ion signal response showed a decrease on day 8 in comparison to day 4 (from 4,557 to 1,934). From the analysis, we could deduce a lower accumulation of metabolites present on day 8 than on day 4. These were considered normal as most phenolic-based compounds are easily oxidised. This agreed with the TA results which showed lower amount of organic acid on day 8 after harvest in comparison to day 4. It is obvious that there will be less beneficial presence of phenolic-based compounds, hence chemically, the fruits were less ideal to be consumed at the end of shelf-life duration.

Based on the screening results, we can deduce that a decrease in the level of ion signal response was mostly due to the nature of phenolic compounds. Phenolic compounds such as flavonoids and relevant classes are compounds that easily oxidise.

Although the cultivar studied was specific to P5 melons, the insight gained from gene expression profiles provided broader relevance for understanding plant physiological responses during postharvest handling. For instance, the upregulated galactose level in P5 melon flesh on D4 after harvest compared to D0 (Table 1) may indicate active galactose metabolism, in which galactose is phosphorylated by galactokinase to galactose-1-phosphate (Dai *et al.*, 2011). This pathway supports ethylene biosynthesis during ripening, hence facilitating subsequent fruit deterioration. During ripening, B-galactosidase enzymes hydrolyse galactosyl side chains from pectin, loosening the cell wall structure. High β -galactosidase activity accelerates softening, thus enhancing edibility but reducing shelf-life.

Mechanistic insight linking postharvest physiology changes to metabolic changes

Respiration begins with glycolysis, which produces pyruvate. Pyruvate is then converted into oxaloacetate, which condenses with acetyl-CoA to form citric acid in the tricarboxylic acid (TCA) cycle (Doan and Teitell, 2022). This pathway aligns with our findings, where organic acids such as citric acid and lactic acids were absent in P5 melon flesh on D0 but detectable on D4 (Table 1).

Fruit respiration primarily relies on sugars as the main energy substrate. In climacteric fruits like P5 melons, the ethylene peak stimulates ripening-related metabolic pathways, including increased respiration. As respiration rate increases during the first three

days after harvest, sugars are rapidly metabolised *via* glycolysis and the TCA cycle to produce ATP, carbon dioxide, and water. If sugar consumption exceeds the synthesis from starch reserves, overall sugar content declines, leading to reduced sweetness, which was consistent with the observed decrease in SSC during early storage.

After harvest, melons continue to respire and undergo metabolic changes, driven by postharvest physiological processes associated with fruit ripening and respiration. As P5 melons are climacteric fruits, they produce a significant amount of ethylene after harvest (Figure 4), which stimulates ripening-related biochemical pathways by upregulating enzymes such as citrate synthase, thus influencing organic acid biosynthesis.

In climacteric melons, ethylene plays a significant role in facilitating fruit ripening, including the sugar accumulation process (Pujol and Garcia-Mas, 2023). In P5 melons, the surge in ethylene levels triggers transcriptional activation of ripening genes such as ACC synthase (ACS) and ACC oxidase (ACO). These two important enzymes are responsible for converting 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene, as demonstrated by Houben and Van de Poel (2019).

The ethylene peak, observed on day 4 after harvest (Figure 4), resulted from an autocatalytic process typical of climacteric melons. Apart from that, ethylene production positively regulates sucrose accumulation. Lao *et al.* (2023) recently suggested that the ethylene hormone can inhibit the expression of *CmMYB44* *via* an Ethylene Response Factors I-2 (*CmERFI-2*) during fruit ripening, which leads to the activation of *CmSPS1* and sucrose accumulation.

The findings of Tang *et al.* (2010) revealed that organic acid metabolism in fruit is a complex physiological process, which supports our observations. At harvest, organic acid synthesis was either minimal or below detection limits because the melon's metabolism was primarily focused on growth and sugar accumulation. However, during storage, the melon's metabolism shifted towards ripening and increased respiration, resulting in organic acid accumulation.

Organic acids, such as citric and malic acids, also serve as respiratory substrates. They are being utilised in the TCA cycle to supply intermediates for ATP production, contributing to changes in acidity. Ethylene upregulates enzymes such as citrate synthase, enhancing organic acid turnover. In the

present work, the significant change in sweetness but relatively stable acidity suggested that sugar metabolism was more strongly affected than organic acid catabolism during early postharvest storage.

Research implications

The implications of these findings for postharvest management are presented below:

- i. Since P5 melons have a short shelf-life, more efforts may be needed, *e.g.* more manpower and machine utilisation to accelerate the postharvest processes (harvesting, sorting, and grading of fruits) in order to reach the consumer end as soon as possible. From an industry perspective, the findings can guide melon producers in refining marketing timelines. Lester (2006) suggested storing commercial melon fruits for less than one week, while our results indicated that P5 melons generally maintain good quality up to day 4 after harvest. This provides a cultivar-specific benchmark that can be compared with other varieties in future studies.
- ii. Discard melons with fungal spot(s) during the sorting process on harvest day. The fungi can spread fast; we fear it might appear more visible and larger when reaching the distribution places.
- iii. Melons on day 5 after harvest were proven to deteriorate faster after experiencing ethylene peak release due to the climacteric nature. This influences postharvest management, and the attempts to market the fruits by that timing might help maintain their good quality.

Limitations and potential avenues for deeper analysis

The authors acknowledge that the present work was limited by its focus on a single cultivar (P5) without direct benchmarking against other melon varieties. The absence of extended storage trials and advanced molecular analyses also constrained the depth of mechanistic interpretation.

Future studies could address these gaps by evaluating innovative storage solutions and advanced preservation techniques to extend the shelf-life of P5 melons. Investigating the genetic basis of the

cultivar's unique combination of climacteric and non-climacteric behaviours could provide valuable insights for breeding programs aimed at improving fruit quality and storage characteristics.

Additionally, the authors will strongly consider conducting a data comparison between the ColorMeter app and the calibrated chromameter in future work. Exploring the potential health benefits associated with the identified metabolites could enhance understanding of the nutritional value of P5 melons. As mentioned previously, there is also a need for future comparative studies to establish cultivar-specific postharvest benchmarks in Malaysia.

In short, addressing these limitations, and exploring potential avenues will advance postharvest biotechnology and melon breeding strategies.

Conclusion

The P5 melon variety exhibited characteristics consistent with climacteric fruit, while also showing some non-climacteric traits. Monitoring melons' physiological and biochemical changes at harvest and during postharvest storage provided a clearer indication of fruit quality. Shelf-life duration, ethylene release pattern, and weight loss were associated with quality changes, whereas other characteristics, such as rind colour, sweetness, firmness, and titratable acidity did not show a clear association. P5 melons were best consumed by day 5 after harvest under the tested conditions; therefore, appropriate postharvest handling, including timely processing, quality checks, and proper storage may preserve fruit quality before reaching the end user. Metabolomics profiling using UHPLC coupled with TWIMS-QTOFMS provided additional insight into metabolic shifts in melon pulp following harvest, indicating links between metabolite changes and physiological processes. These findings contribute to the descriptive understanding of P5 melon postharvest physiology, and provide a basis for further research.

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