Changes in the biochemical characteristics and volatile fingerprints of atemoya during postharvest ripening at room temperature

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Abstract

In order to determine the best shelf life of the atemoya, the changes in the biochemical characteristics in five different stages of senescence were investigated. During postharvest ripening at room temperature, the firmness decreased rapidly after harvest and the fruit weight loss and browning degree increased from the earliest green-colored stage onward. The total soluble solid concentrations (TSSC) and titratable acidity (TA) increased continuously throughout maturation, and the peak respiratory and ethylene production rates occurred on the 3d and 5th day of postharvest, respectively. Phenylalanine ammonia-lyase (PAL) activity steadily increased, catalase and polyphenol oxidase (PPO) activities decreased significantly on the 1st day and then gradually increased, and peroxidase activities increased during the initial 3 days, and then decreased at later stages. The volatile fingerprints of flesh samples from the five senescence stages were successfully established using gas chromatography-ion mobility spectrometry (GC-IMS) combined with principal component analysis, and 32 typical target compounds and 35 indeterminate compounds were obtained. The results provide a theoretical basis for the development of innovative preservation methods for atemoya.

Keywords: biochemical changes; headspace-gas chromatography-ion mobility spectrometry; postharvest characteristics; volatile organic compounds

Introduction

Annona includes one of the world's most famous fruit, atemoya, which has creamy white pulp, and low acidity. The genus, which belongs to the family Annonaceae, is composed of over 100 species; however, only five (*Annona squamosa*, *A. cherimola*, *A. muricata*, *A. reticulata*, and *A. atemoya)* are of significant commercial importance (Pareek *et al*., 2011). Because of their taste and high nutritional value, including carbohydrates, amino acids, lipids, vitamins (A, B, and C), fiber, and minerals (Fe, Ca, Mg, and P), *Annona* spp. are considered valuable for table purposes, and have been commercially cultivated in subtropical and tropical regions of the world in recent decades (Hiwale, 2015; Pareek *et al*., 2011).

Atemoya is a hybrid of *A. squamosa* and *A. cherimola*, and is normally heart-shaped or round, weighs approximately 300–500 g, and has pale green, bumpy skin. The majority of the skin is as bumpy as that of the sugar

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apple, but it becomes smoother, like the cherimoya, at its base. The flesh is not segmented like that of the sugar apple, bearing more similarity to that of the cherimoya. It is very juicy and smooth, tasting slightly sweet with good flavor (Horticulture Innovation, 2019; Wade, 2009). To date, it has been widely cultivated, including in India, Cuba, Venezuela, Israel, Lebanon, Australia, and Taiwan. In 2002, the fruit was successfully introduced to Mainland China from Taitung City in Taiwan, and started cultivation at large scale in tropical China (Fang *et al*., 2015). However, atemoya is a typical climacteric fruit with the following characteristics: it is highly perishable, usually softens very rapidly, and browns and cracks during ripening. Thus, reducing its commercial and edible value and limiting its cold chain distribution and circulation. The Food and Agriculture Organization of the United Nations (FAO) has reported vegetable and fruit losses in developing countries of up to 40–50% annually. Approximately, 16% of which is due to poor postharvest handling and storage, and in particular, cooling chain limitations in agricultural production storage and distribution activities (Gustavsson *et al*., 2011).

Information on the biochemical properties and aroma profiles of atemoya during postharvest ripening at room temperature is very limited. The objective of this work was to investigate changes in the biochemical properties and volatile compounds of atemoya fruit during postharvest ripening. The expected results are to provide a theoretical basis for determining consumer acceptance and developing innovative preservation methods for atemoya fruit.

Materials and Methods

Materials and reagents

"Gefner" atemoya (*A. cherimola* × *A. squamosa*) fruit with skin firmness of 30–40 N were harvested at commercial maturity from Chongguan ridge (22°21′N and 107°33′E) in Guangxi, China in September 2018. They were hand-picked and packed in cooler boxes, and transported to the laboratory within 4 h of harvest. The atemoya variety was identified by Prof. Yiji Kong from the South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Sciences. Fruit that were uniformly shaped (350 ± 50 g each) and free of defects and cracks were selected for analysis. A total of 120 fruit were divided into two lots and stored at ambient conditions for natural maturity. The maturity stage was judged by the surface color and softness, and five stages were selected, as shown in Figure 1.

Firmness determination

Firmness was measured on two opposing sides of each atemoya (n = 6) using a GY-4 Digital Fruit Hardness Tester (Zhiqu Co. Ltd., Dongguan, China) with a 5-mm diameter probe, at a speed of 1 mm s^{-1} and a load range of 200 N. The compression force was recorded at the maximum transverse diameter of the fruit and the probe was directed perpendicularly to the fruit. The results are expressed in N.

Fruit mass loss (FML) and browning degree (BD) measurements

The fruit mass loss (FML) was calculated as the percent reduction from the initial weight. And the browning degree (BD) was assessed by evaluating the extent of the browned area on the fruit surface, as previously reported by Ali *et al*. (2016).

Respiratory and ethylene production rate measurements

The respiratory rate of atemoya fruit was determined using a breath analyzer (SY-1022, Liaoning Andreas Technology Co., Liaoning, China), and the results are expressed in mg CO_2 kg^{-1} s^{-1}. Ethylene production was measured as described by Huan *et al*. (2018) with some modifications. Briefly, at each time point, three fruit per group were randomly selected and enclosed in 5.0 L

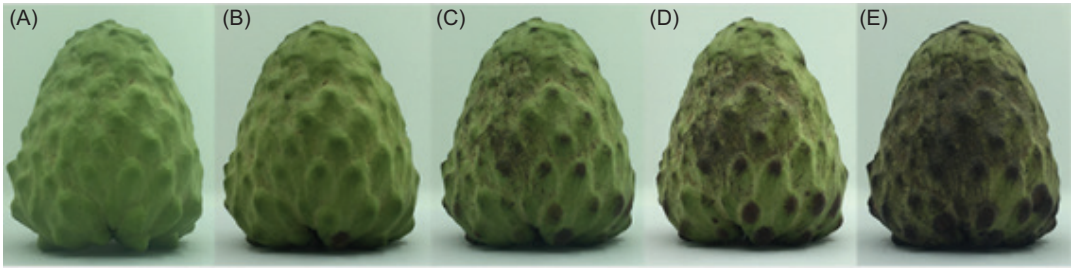

Figure 1. Five stages of postharvest atemoya fruit senescence. (A) at harvest, (B) 1d, (C) 3d, (D) 5d, (E) 7d

glass jars at 25°C for 1 h, and 1 mL of headspace gas was injected into a gas chromatographer (GC7890A, Agilent Technologies, Chandler, AZ, USA) coupled to an FID detector. The injector, column, and detector temperatures were 50, 50, and 150°C, respectively. Ethylene values are expressed as ng C_2H_4 kg^{-1} s^{-1}.

Total soluble solid concentrations (TSSC) and titratable acidity (TA) measurements

Each atemoya (n = 3) was sliced and rapidly frozen in liquid nitrogen vapor, and then stored at –40°C prior to analysis. Frozen tissues (50 g) were thawed at 4°C for 3 h, and then homogenized in a blender (Thermo Fisher Scientific, MA, USA). The soluble solid concentrations (TSSC) values were determined using an Atago PAL-1 digital refractometer (Atago Co. Ltd., Tokyo, Japan). The titratable acidity (TA) was measured by diluting 10 g of pulp in 100 mL of double-distilled H_2O and titrating to pH 7.0 with 0.01 mol L^{-1} NaOH, and is expressed as the percentage of citric acid.

Total phenol and lignin content measurements

Total phenols were measured with Folin-Ciocalteu reagent according to the method described by Chu *et al*. (2018), and the results are expressed as OD_{280} g^{-1} sample. Lignin was extracted and determined as reported by Bruce and West (1989). Approximately 1 g of pulp was homogenized in 4 mL of 95% ethanol precooled to 0°C, and centrifuged at 3800 × *g* for 5 min at 4°C. The residue was washed twice with cold ethanol and a mixture of ethanol:n-hexane (1:2). The precipitate was dried for 30 min at 60°C and was dissolved in 2 mL of 25% acetyl bromide-glacial acetic acid solution. The sample was incubated at 70°C in a shaking water bath for 30 min and then 1 mL of 2 mol L^{-1} NaOH, 1 mL of acetic acid, and 0.1 mL of hydroxylamine hydrochloride were added successively. The volume was brought to 5 mL with acetic acid and the OD_{280} was measured.

Enzyme activity assays

To determine enzyme activities, atemoya tissue (2 g) was ground with 5 mL phosphate buffer (pH 7.8) containing 40 g L^{-1} crosslinked polyvinylpyrrolidone (PVPP), 2 mmol L^{-1} ethylene diamine tetraacetic acid (EDTA), and 5 mmol L^{-1} β-mercaptoethanol in an ice-water bath. The homogenate was centrifuged at 10,000 × *g* and 4°C for 20 min. The obtained supernatant was used as the crude enzyme extract. The phenylalanine ammonia-lyase (PAL) activity was measured according to Koukol and Conn (1961). One unit of PAL activity was defined as the amount of enzyme that caused the increase in absorbance of 0.01 per minute at 290 nm, and the result was expressed as U g^{-1} fresh weight. The catalase (CAT) activity was determined as described by Aebi (1984) with slight modifications. 0.1 mL of enzyme extract was mixed with 1 mL of 0.1 mol L^{-1} H_2O_2 and 2.5 mL of 0.05 mol L^{-1} phosphate buffer (pH 7.8). The reaction mixture was determined at 240 nm at an interval of 30 s for 3 min. One unit of CAT activity was defined as the amount of enzyme that decreased in absorbance of 0.01 at 240 nm per minute. The results were expressed in U kg^{-1} fresh weight. The polyphenol oxidase (PPO) activity was assayed according to Galeazzi *et al*. (1981) with some modifications and were extracted from 5 g frozen tissue with 25 mL of 0.2 mol L^{-1} sodium phosphate buffer (pH 6.5) containing 1% polyvinylpyrrolidone. One unit of PPO activity was defined as the amount of enzyme that caused an increase in the absorbance of 0.01 per minute at 420 nm. The POD activity was determined according to Hammerschmidt and Kuć (1982) with slight modifications. The reaction mixture consisted of 50 mmol L^{-1} sodium phosphate buffer (pH 6.0), 0.5 mol L^{-1} H_2O_2, 25 mmol L^{-1} guaiacol, and 0.1 mL crude enzyme extract. One unit of peroxidase (POD) activity was defined as the amount of enzyme that caused the increase in absorbance of 0.01 per minute at 470 nm and the data are expressed in U mg^{-1} protein.

Quantification of volatile compounds

Samples of atemoya fruit were analyzed on a gas chromatography-ion mobility spectrometry (GC-IMS) instrument (FlavourSpec, GAS, Dortmund, Germany) equipped with an autosampler (Hanon Auto SPE 100, Shandong, China) for headspace analysis. Samples (2.0 g) were weighed into 20 mL headspace vials (CNW Technologies, Germany), and then incubated in headspace volume at 60°C for 10 min. Headspace (500 µL) was automatically loaded into the injector in splitless mode through a syringe heated to 65°C.

The analytes were separated on a FS-SE-54-CB-1 capillary column (15 m × 0.53 mm) at a constant temperature of 40°C and then ionized in the IMS instrument (FlavourSpec®, Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany) at 45°C. High purity nitrogen gas (99.999%) was used as the carrier and drift gas, with an initial flow rate of 150 mL min^{-1} and the following program was: 2 mL min^{-1} for the first 2 min, then a linear increase to 150 mL min^{-1} over 18 min, then 150 mL min^{-1} for 10 min. Ketones C_4–C_9 (Sigma Aldrich, St. Louis, MO) were used as an external standard to determine the retention index (RI) of volatile organic compounds. Analyte identification was performed using the Laboratory Analytical Viewer (LAV) 2.2.1 (GAS, Dortmund, Germany) by comparing RI and the drift time of standard in the GC-IMS Library.

Statistical data analysis

All samples were prepared in duplicate and tested at least six times, and the result was expressed as mean ± standard deviation and the level of statistical significance ($P < 0.05$) was analyzed by using Tukey's range test of SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The principal component analysis (PCA) was performed using the LAV software in-built "Dynamic PCA" plug-in to model patter of aroma volatile.

Results and Discussion

Firmness changes

Fruit softening is a complex physiological process, which is closely related to postharvest ripening and senescence and plays an important role in determining fruit-flavor quality Chen *et al*., (2019). In practice, most consumers subjectively estimate fruit firmness by applying a compression force with their fingers. Brown *et al*. (1988) found that when ripened at 20°C, atemoya fruit reached acceptable edible quality after the first detectable softening changes. As shown in Table 1, the firmness decreased rapidly from 10.95 N to 3.26 N after 1 day of storage in ambient conditions, and then decreased slowly from 3.26 N to 1.35 N within the next 6 days in storage. The firmness change corresponded to the softening characteristics of atemoya fruit, and was consistent with a previous report (Zainal *et al*., 2019). However, details on how the mechanical properties of atemoya fruit change during the ripening process are limited, and how they are affected by postharvest processing is unknown. It has been inferred that the softening of atemoya fruit is not only dependent on the expression of xyloglucan endotransglycosylase and expansion genes, but is also affected by the cultivar, production area, harvest timing, and other factors (Pareek *et al*., 2011; Shen *et al*., 2009). Pulp softening and apparent color change during ripening and senescence are two major characteristics affecting the shelf life and market value of postharvest fruit (Lin *et al*., 2018).

Fruit mass loss and browning degree

After harvest, respiration is the cause of FML in most horticultural products. For most fruit, FML ≥ 8% results in a wilted or shriveled appearance, which makes the fruit more perishable and begins to significantly affect its acceptability to consumers (Cruz-Bravo *et al*., 2019). The FML of postharvest atemoya steadily increased, reaching 8.46% on the 7th day under room temperature (25 ± 3°C and 70 ± 5% relative humidity). For this reason, we measured the effects of atemoya fruit storage for 7 days. Importantly, atemoya fruit decay is also associated with genotype, maturity, and postharvest handling (Pareek *et al*., 2011).

The intensity of color development determines the harvest time and ripening duration (Pareek *et al*., 2011). As shown in Figure 1, atemoya skin color changed from dark green to light green or greenish-yellow through the postharvest ripening process, and visible brown spots were observed on the 3rd day, with 1.41% BD and acceptable marketability. By the 5th day, more obvious browning occurred, with 15.67% BD and limited marketability. By the 7th day, obvious browning was observed, with >45.49% BD and poor sensory acceptability. In practice, the harvesting time and eating quality is commonly determined by the fruit skin color, which changes with physiological maturity.

Table 1. Changes of FML, BD, skin firmness, TSSC, TA, total phenols and respiratory rate, ethylene production, and lignin content of atemoya fruit during postharvest storage.

Factor	At harvest	1 day	3 days	5 days	7 days
	Storage time (day)				
Firmness (N)	10.95 ± 0.31^{e}	3.26 ± 0.40^{d}	2.63 ± 0.32^{c}	1.97 ± 0.25^{b}	1.35 ± 0.19^{a}
FML (%)	0^{a}	1.27 ± 0.23^{b}	4.39 ± 0.18^{c}	6.66 ± 1.02^{d}	8.46 ± 0.91^{e}
BD (%)	0^{a}	0.54 ± 0.17^{b}	1.41 ± 0.40^{c}	15.67 ± 1.71^{d}	45.49 ± 1.72^{e}
TSSC (%)	13.91 ± 0.56^{a}	18.93 ± 0.79^{b}	19.40 ± 0.90^{c}	19.65 ± 1.10^{c}	20.05 ± 0.79^{d}
TA (% citric acids)	0.04 ± 0.02^{a}	0.05 ± 0.03^{ab}	0.06 ± 0.04^{b}	0.08 ± 0.05^{c}	0.06 ± 0.04^{d}
Respiratory rate (mL CO_2 kg^{-1} h^{-1})	317.43 ± 15.42^{c}	551.95 ± 25.23^{d}	575.22 ± 22.01^{e}	307.85 ± 19.98^{b}	259.91 ± 32.56^{a}
Ethylene production rate (μL C_2H_4 kg^{-1} h^{-1})	0.23 ± 0.01^{a}	3.98 ± 0.14^{b}	15.67 ± 1.85^{c}	34.39 ± 5.17^{d}	22.38 ± 3.20^{e}
Total phenols ($OD_{280\ nm}$ g^{-1})	0.64 ± 0.03^{ac}	0.93 ± 0.12^{c}	1.34 ± 0.08^{d}	0.77 ± 0.02^{b}	0.77 ± 0.06^{b}
Lignin (A_{280} g^{-1})	73.50 ± 0.13^{a}	74.56 ± 0.25^{b}	95.05 ± 0.62^{c}	98.56 ± 0.70^{d}	124.92 ± 1.40^{e}

For each factor, the result was expressed as mean ± standard error (SE), and within the same row followed by different letters are significantly different by Tukey's multiple range test at $P \leq 0.05$.
FML: Fruit mass loss; TSSC: total soluble solid concentrations; BD: browning degree; TA: titratable acidity.

Respiratory rate and ethylene production

Atemoya displays typical climacteric maturation, characterized by an increased respiration rate (Morena and De La Plaza, 1993). Fruit respiration involves the oxidation of organic molecules (e.g., carbohydrates, amino acids, and organic acids) in cells into small molecule metabolites with concurrent energy production, and is closely related to fruit maturity and senescence (Liu *et al*., 2019). The respiration rates of postharvest atemoya fruit at different senescence stages are shown in Table 1. The respiration rate initially increased rapidly postharvest and then decreased, and the peak respiration rate (0.23 mg CO_2 kg^{-1} s^{-1}) occurred on the 3rd day, consistent with the results of Alique and Zamorano (2000). Ethylene production in atemoya fruit increased after harvest and the peak rate (approximately 0.12 mg CO_2 kg^{-1} s^{-1}) was detected on the 5th day of storage, after the onset of climacteric respiration, similar to the results of Brown *et al*. (1988). Moreover, ethylene emission rates were significantly higher in the mature stage (1.33–2.30 ng C_2H_4 kg^{-1} s^{-1}) than in the immature stage (0.02–0.34 ng C_2H_4 kg^{-1} s^{-1}) ($P < 0.05$). Fruit softening during storage is usually triggered by ethylene (Krongyut *et al*., 2011), and the respiration and ethylene emission rates both depend on the cultivar and maturity stage, and are affected by the temperature and humidity of the harvesting area, as well as other factors (Pareek *et al*., 2011). For climacteric fruit, delaying the peak respiration rate can help to extend the shelf life.

Total soluble solid concentrations (TSSC) and titratable acidity (TA)

The total soluble solid concentrations (TSSC), mostly composed of sugars and acids together with small amounts of dissolved compounds, is the most important quality parameter used by the industry to indicate the sweetness of fresh horticultural products and determine marketing standards (Magwaza and Tesfay, 2015). Tietz (1988) found that the flavor development of ripe cherimoya was closely correlated with the TSSC. The TSSC of atemoya fruit increased from approximately 13.91 to 20.05% during 7 days of postharvest ripening (Table 1). Similar to the TSSC, the TA content is also associated with atemoya taste and sensory properties, and increased acidity can be ascribed to organic acid production during ripening (Gutierrez *et al*., 1994). Along with increased fruit maturity, we observed a slight increase in acidity during the initial stages of atemoya fruit ripening, followed by a significant decrease ($P < 0.05$) compared to the initial value (0.04% citric acid). This result is consistent with a previous report (Bolivar-Fernandez *et al*., 2009). Changes in TSSC and TA could be used to measure the process of commercial maturation.

Total phenol and lignin contents

Phenols, secondary metabolites found in fruit and vegetables, are associated with quality and flavor formation, are precursors for lignin synthesis, and are natural substrates in enzymatic browning reactions (Li *et al*., 2009). As shown in Table 1, the total phenol content gradually increased from 0.64 OD_{280} g^{-1} at harvest to 1.34 OD_{280} g^{-1} on the 3rd day, and then decreased during the pulp lignification process, falling to 0.77 OD_{280} g^{-1} by the 7th day.

Lignin is a secondary metabolite in plant tissues, and its accumulation is the key reason for postharvest deterioration in fruit quality. After 7 days of storage, obvious lignin synthesis was observed in atemoya flesh compared with at harvest, and the lignin content increased significantly, from 73.50 OD_{280} g^{-1} at harvest to 124.92 OD_{280} g^{-1} at day 7 ($P < 0.05$). Storage at 4°C significantly delayed the lignification process by inhibiting lignin-related enzymes such as PAL and PPO activities (data not shown), resulting in less lignin content. Lignification is among the most important physiological disorders in a wide variety of horticultural products, and is correlated with cell wall metabolism abnormality generated by pectin metabolism, manifesting as a woolly texture with a lack of juiciness, which decreases consumer acceptance (Li *et al*., 2009; Lin *et al*., 2018; Suo *et al*., 2018; Ubi *et al*., 2016).

Enzyme activity assays

Figure 2 shows the activity changes in PAL, catalase and polyphenol oxidase, and peroxidase activities during the postharvest ripening of atemoya fruit in ambient conditions. The change in PAL activity correlated with lignin accumulation, and was consistent with results in kiwifruit (Suo *et al*., 2018). Catalase and polyphenol oxidase activities displayed similar trends, decreasing significantly on the 1st day compared to at harvest, and then gradually increasing during the remaining period. Peroxidase activity oscillated throughout the ripening period. In most cases, enzymatic browning catalyzed by PPO is considered to be one of the most important oxidative reactions in fruit, leading to loss of sensory and nutritional qualities (Ali *et al*., 2016; Pareek *et al*., 2011; Zainal *et al*., 2019).

Volatile compound identification and principal component analysis

Characteristic volatile fingerprints were obtained by comparing ion mobility spectrometry drift times and relative RIs with those of authentic reference compounds. The changes in volatile compounds in postharvest atemoya fruit at five different senescence stages are presented

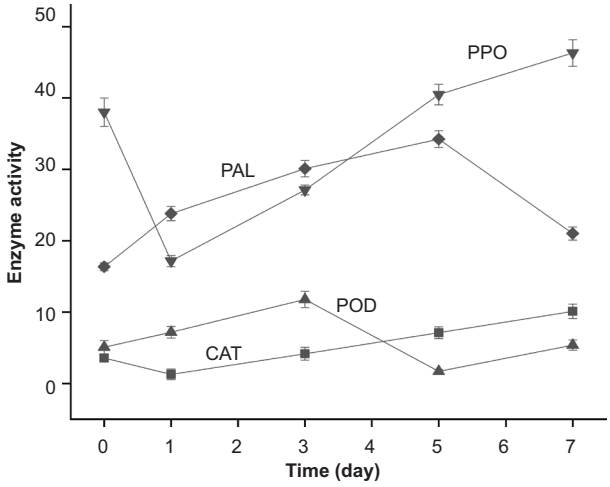

Figure 2. Changes in atemoya fruit enzyme activities during postharvest ripening in ambient conditions (the result was expressed as mean ± standard deviation, n = 6).

as a 3D topographical visualization in Figure 3A. The ion migration time and the position of the reactive ion peak (RIP) were normalized, and 3D topographical plots at different stages are shown in Figure 3B. A total of 32 typical target compounds were identified by the GC × IMS Library (Table 2) and 35 indeterminate compounds were also obtained. Due to their different concentrations, some single compounds may have produced multiple signals (dimers and multimers).

As shown in Figure 4, the volatile compounds detected in atemoya fruit at the five stages of senescence were similar, and trans-beta-ocimene, beta-pinene, gamma-terpinene, limonene, myrcene, alpha-pinene, alpha-terpineol, linalool, acetone, and ethanol were detected at all stages, with slight differences in signal intensity. Volatile aroma compound composition and content in atemoya fruit changed significantly during postharvest storage. The signal intensities of trans-2-hexenol, 1-pentanol,

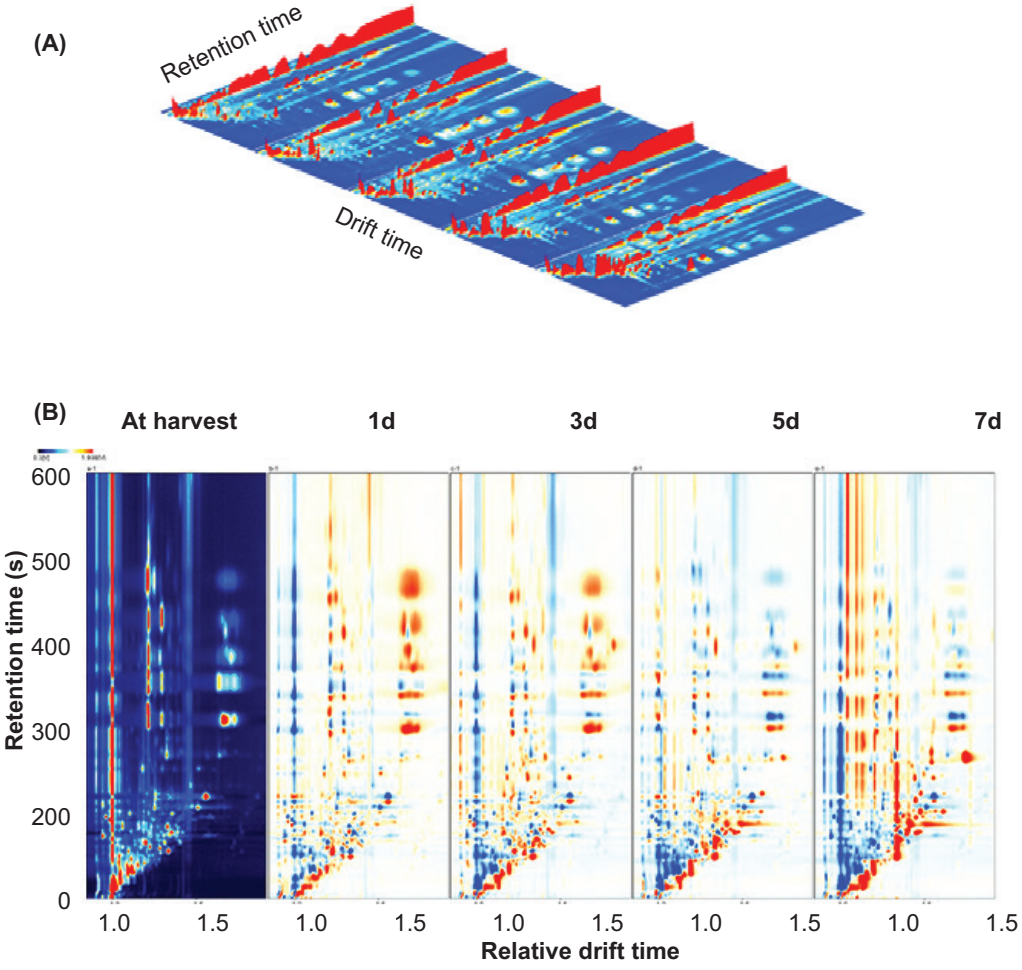

Figure 3. 3D topography of flesh samples of atemoya fruit at five stages of senescence. (A) the y-axis represents the GC retention time, the x-axis represents the ion migration time for identification, and the z-axis represents the peak height for quantification. (B) 3D topographical plots at different stages.

Table 2. GC-IMS integration parameters of volatile compounds of postharvest atemoya fruit.

No.	Compound	MW	RI[a]	RT [s][b]	MT [RIPrel][c]	Identification approach
1	Alpha-terpineol	154.3	1194.8	881.652	1.2184	RI, DT
2	Limonene	136.2	1020.6	472.29	1.2183	RI, DT
3	Ethyl hexanoate dimer	144.2	1004.3	441.021	1.8229	RI, DT
4	Ethyl hexanoate	144.2	1004.3	441.021	1.3429	RI, DT
5	Trans-beta-ocimene	136.2	1045.5	525.124	1.2143	RI, DT
6	Myrcene	136.2	991.3	418.245	1.217	RI, DT
7	Beta-pinene	136.2	968.2	382.971	1.217	RI, DT
8	Alpha-pinene	136.2	928.6	334.924	1.2155	RI, DT
9	3-Methylbutyl acetate dimer	130.2	879.3	289.918	1.7444	RI, DT
10	3-Methylbutyl acetate	130.2	880.1	290.527	1.2984	RI, DT
11	1-Hexanol	102.2	874.2	285.871	1.3295	RI, DT
12	1-Hexanol dimer	102.2	873.5	285.309	1.6405	RI, DT
13	Butyric acid	88.1	805	238.076	1.1636	RI, DT
14	Ethyl butanoate	116.2	793.4	231.047	1.2082	RI, DT
15	Dimethyl disulfide	94.2	734.4	198.715	0.9879	RI, DT
16	1-Pentanol	88.1	766	215.302	1.2556	RI, DT
17	1-Pentanol dimer	88.1	765	214.74	1.5122	RI, DT
18	Acetoin	88.1	711.9	188.312	1.3309	RI, DT
19	Hydroxyacetone	74.1	636.7	162.727	1.2278	RI, DT
20	Ethyl acetate	88.1	607.3	155.136	1.3379	RI, DT
21	Butanal	72.1	631.4	161.321	1.2877	RI, DT
22	Acetone	58.1	502.8	129.551	1.119	RI, DT
23	Ethanol	46.1	448.9	116.337	1.0493	RI, DT
24	2-Pentanone	86.1	687.2	178.472	1.3714	RI, DT
25	3-Methyl-3-buten-1-ol	86.1	736.7	199.839	1.4969	RI, DT
26	Butyl acetate dimer	116.2	809.1	240.606	1.6196	RI, DT
27	Butyl acetate	116.2	810	241.168	1.2389	RI, DT
28	Linalool	154.3	1103	660.627	1.22	RI, DT
29	Hexanal dimer	100.2	791.2	229.747	1.5584	RI, DT
30	Hexanal	100.2	790.2	229.155	1.2582	RI, DT
31	3-Methylbutanal	86.1	663.8	170.588	1.4034	RI, DT
32	Propanoic acid	74.1	750.1	206.675	1.3583	RI, DT
33	Trans-2-hexenol	100.2	849.8	267.574	1.1794	RI, DT
34	Trans-2-hexenol dimer	100.2	850.2	267.88	1.5165	RI, DT
35	3-Pentanone	86.1	695.8	181.713	1.3528	RI, DT
36	2,3-Pentanedione	100.1	685	177.683	1.3102	RI, DT
37	2-Methylpropyl acetate	116.2	767.3	215.986	1.6155	RI, DT
38	Ethyl propanoate	102.1	716.9	190.482	1.4538	RI, DT
39	Gamma-terpinene	136.2	1062.6	564.372	1.217	RI, DT

[a]Represents the RI calculated using *n*-ketones C_4–C_9 as external standard on FS-SE-54-CB-1 column.
[b]Represents the retention time in the capillary GC column.
[c]Represents the migration time in the drift tube.

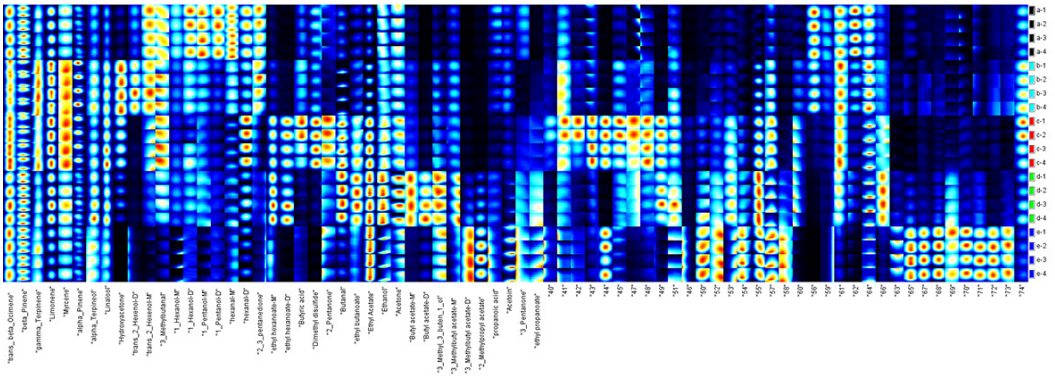

Figure 4. Gallery plot of selected signal peak areas obtained from atemoya fruit flesh at five stages of senescence (a–e, at harvest, 1, 3, 5, and 7 days, respectively).

3-methylbutanal, hexanol, and 2,3-pentanedione were much higher in fresh fruit at harvest than in other stages, and gradually decreased during the ripening process, such that only very weak signals were detected at the final storage stage. Increasing volatile compounds were identified as postharvest ripening progressed, alpha-terpineol and linalool contents increased, and acetone and ethanol contents were decreased. Hydroxyacetone, ethyl hexanoate, butyl acetate, butyric acid, and dimethyl disulfide compounds contributed to flavor metabolism during mid-stage storage. As the fruit completely matured and before quality deteriorated, butanal, ethyl butanoate, ethyl acetate, 3-methylbutyl acetate, 2-methylpropyl acetate, propanoic acid, acetoin, 3-pentanone, 3-methyl-3-buten-1-ol, and ethyl propanoate were identified, contributing to the typical aroma of the fruit. In addition, many dimers of volatile compounds were observed at different storage stages, with significantly different compositions and levels.

The principal component analysis (PCA) was applied to evaluate the contribution rates of characteristic volatile compounds based on signal intensities. As presented in Figure 5, samples of postharvest atemoya fruit from different ripening stages existed in fully independent spaces and were well distinguished in the score map, demonstrating that the volatile compounds changed markedly through the postharvest storage process. The PCA described 75% of the accumulative variance contribution rate, with PC1 and PC2 describing 47 and 28%, respectively. These volatile fingerprints of atemoya samples at different senescence stages will contribute to the sensory evaluation of this fruit.

Conclusions

In this work, the peak respiratory and ethylene production rates of atemoya fruit in ambient conditions were observed before physiological maturity on the 5th day, accompanied by a skin color change from dark green to light green or greenish-yellow. The characteristic flavor developed after climacteric respiration, and the fruit was at its best edible condition on the 5th day postharvest.

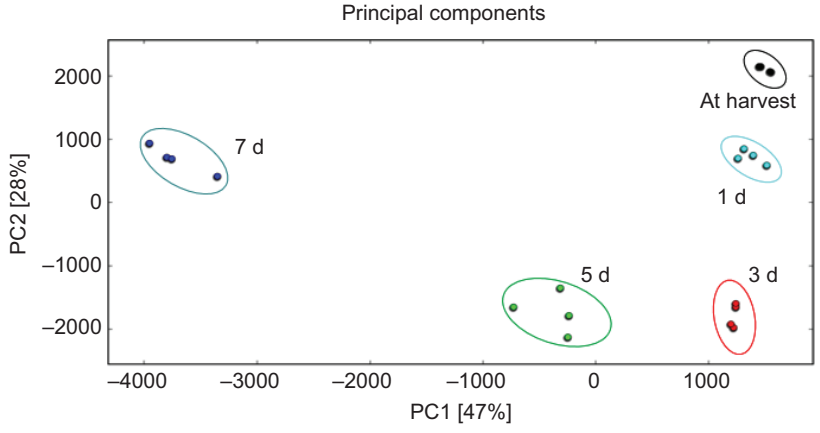

Figure 5. Principal component analysis of postharvest atemoya fruit at five stages of senescence. Results are based on the spectral region at a drift time of 8 ms and retention time from 90 to 920 s.

Generally, after 7 days of storage, the fruit had lost almost all commercial value because of softening and skin browning. Based on GC-IMS, the volatile fingerprints from five stages of senescence were successfully established and were successfully distinguished using PCA. The results are expected to provide a theoretical basis for the development of strategies to extend the shelf life of atemoya fruit.

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