USING GCMS TO FIND OUT THE VOLATILE COMPONENTS IN THE AROMA OF THREE DIFFERENT COMMERCIAL FRUITS IN CHINA

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ABSTRACT

The agricultural sector is an essential source of income and food for humans. Fruits are important sources of nutrients, vitamins, essential elements, and as well as the source of income for humans. Volatile components make the aroma of fruit and fruit species and cultivars. However, the knowledge and information about volatile components or volatile profiles are essential, because these aroma components make fruit delicious to humans and as well as attract or repel insect pests. And the identification of volatile components from fruits is very important to a better understanding of the impact of climatic conditions. To the best authors knowledge, in this study, we reported the first time volatile profile of unidentified cultivar of mango (*Mangifera indica* L. Hanana Datai Nong Mang), guava (*Psidium guajava* Linn. China Pear), and banana (*Musa acuminata* L. Dwarf banana or Fen Jiao) fruits and these fruits are commercially selling and growing in China. The Headspace Solid-phase Microextraction (HS-SPME) and porapak Q coupled with gas chromatography-mass spectrometry analysis (GC-MS) were used to identify the volatile profiles of each fruit cultivar. A total of 10, 15, and 23 volatile components/compounds were identified from mango, guava, and banana fruits, respectively. The major constitution of volatile components obtained from mango, guava, and banana was 3-carene, caryophyllene, and cycloheptasiloxane, tetradecamethyl-.

Key words; Banana, 3-carene, caryophyllene, cycloheptasiloxane, components, tetradecamethyl-, GC-MS, guava, mango, volatile components.

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INTRODUCTION

The agricultural sector is an important source of food, e.g., fruits, vegetables, and crops, as well as income for humans (FAO, 2016; FAO, 2017). Mango (*Mangifera indica* L), guava (*Psidium guajava* Linn), and banana (*Musa* spp) are most important tropical, sub-tropical, and temperate fruits and kept most important nutrients, vitamins, essential elements that are good for humans health (Paniandy *et al.*, 2000; Joseph and Priya, 2011; Gautam and Gotame, 2020). Each fruit has its volatile components that have a great impact on making the aroma of fruits. The attraction of insect pests to volatiles that is elicited from their host, e.g., fruits have been studied (Cruz-López *et al.*, 2006; Siderhurst and Jang, 2006; Alagarmalai *et al.*, 2009; El-Sayed *et al.*, 2009; Siderhurst and Jang, 2010; Biasazin *et al.*, 2014; Jayanthi *et al.*, 2014; Jaleel *et al.*, 2019; Ali *et al.*, 2020; Jaleel *et al.*, 2020).

Mango fruits are also called “King of fruits.” Mango fruits have rich nutrient sources as well as a livelihood for millions of people (Mitra, 2014; Sial *et al.*, 2015). Volatiles components from a different cultivar of mango fruits have been identified in Africa, Australia, India, United States, Sri Lanka, and Venezuela (Rodríguez *et al.*, 2013; Souza *et al.*, 2018). Different mango cultivars still have not been used on a commercial level that exhibiting a wide range of flavors (MacLeod and Pieris, 1984; Damodaram *et al.*, 2015; Galal *et al.*, 2017). In the Coche mangoes, the predominant volatile components were 3-carene, b-selinene, terpinolene, and limonene (Malo *et al.*, 2012; Xiao-Wei *et al.*, 2016). 3-carene has been reported in the aroma of mango fruits (Tamura *et al.*, 2000; Vithana *et al.*, 2018). 3-carene, acetic, butyric, hexanoic acids, and ethyl 3-hydroxybutyrate have been reported from mango fruits (Sakho *et al.*, 1985; Kondo, 2018). Thirteen mango volatiles components were detected, e.g., acetaldehyde,

acetone, methanol, ethanol, α -pinene, caryophyllene, 3-carene, β -pinene, myrcene, limonene, terpinolene, α -copaene, and r -cymene (Baldwin *et al.*, 1999; Pino and Mesa, 2006; Shaw, 2017).

Guava fruits have been originated from tropical America (Shen *et al.*, 2008; Singh *et al.*, 2018). Guava fruits have volatile aromatic components, e.g., antioxidants, vitamins, lutein, zeaxanthine, and lycopene. Leaves, shoots, and fruits of guava are useful in medicine for the treatment of various human diseases (Zahoor *et al.*, 2017). Polysaccharides, vitamins, minerals, enzymes, essential oils, proteins (Jordán *et al.*, 2001; Deo and Shastri, 2003; Bodini *et al.*, 2019), sesquiterpenoid, triterpenoid acids, alkaloids, glycosides, steroids, tannins, and saponins are essential compounds of guava fruit (Xu *et al.*, 2017).

Bananas are very important source of human diets and income. Genus *Musa*. Linnaeus has more than 100 common names around the world (Arvanitoyannis and Mavromatis, 2009; Asif and Kaur, 2018). Jordán *et al.* (2001) have reported 43 volatile components and prominent were 1-methyl butyl isobutyrate 2,3-butanediol diacetate, 2-hydroxy-3-methylethylbutyrate, methyl butyrate, ethyl 3-hydroxyhexanoate, 2-pentanol acetate, Isoamyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methylbutanal, acetal, isobutyl acetate, hexanal, ethyl butyrate, 2-heptanol, and butyl butyrate. Banana fruits have full acceptance, among other fruits, due to nutritional value (Zhu *et al.*, 2018). More than 350 volatile components/compounds have been identified in the aroma of banana fruits, e.g., esters, alcohols, and carbonyl compounds are the most important volatile components (Girard *et al.*, 1997; Jordán *et al.*, 2001; Pino and Febles, 2013; Zhu *et al.*, 2018). Volatile components, e.g., 3-methyl butyl acetate, isoamyl butanoate, and isoamyl isovalerate, were reported as key components of banana “fruity” odor (Schwab *et al.*, 2008). Identification of phytochemicals is the most important study; because phytochemicals play an important role in attraction or repellent of insects (Forister *et al.*, 2015; Jaleel *et al.*, 2018b).

Volatile or aromatic compounds are important for the quality of fruit and can vary among fruit cultivars (Zhu *et al.*, 2018). Information about volatile components or volatile profile is very important because these aroma components are responsible for fruit quality. And the identification of volatile components from fruits is very important to a better understanding of the impact of environmental conditions. In this study, we reported first time volatile profile of unidentified cultivar of mango (*Mangifera indica* L. Hanana Datai Nong Mang), guava (*Psidium guajava* Linn. China Pear), and banana (*Musa acuminata* L. Dwarf banana or Fen Jiao) fruits and these fruits are commercially selling and growing in China.

MATERIALS AND METHODS

Fruit: Ripe mango (*M. indica*: Hanana Datai Nong Mang; guava (*P. guajava*: China Pear; and banana (*M. acuminata*: Dwarf banana or Fen Jiao) were purchased from the orchard located in Guangzhou, Guangdong, China. Fruits were washed and dried and then used for experimental purposes (Figure 1).

Fruits characteristics: Based on detailed discussion with a sailor, all fruits were bagged in the field before the stage of ripening to avoid the attack of wild insect pests. Total soluble solids (TSS or Brix) of fruits were measured using handheld pocket refractometer pal-1 (ATAGO, PR-101a, Brix 0-45%, Tokyo Tech. Japan) (Figure 2a), and the pericarp toughness or firmness of fruits were measured by a TMS-Pro texture analyzer (FTC-TV, USA) with probe (1 mm diameter) (Jaleel *et al.*, 2018c) (Figure 2b). Measurements were taken and recorded at three different locations on each fruit. Fifteen replications were done for each fruit.

Volatiles collection: Collection of volatiles from the surface of mango, guava, and banana were done by two ways as solid-phase micro-extraction (SPME) and porapak Q (80-100 mesh; Alltech, Deerfield, IL, USA) in the laboratory of Plant Protection Research Institute, Guangdong Academy of Agricultural Science, Guangzhou, China.

The solid-phase micro-extraction (SPME) fiber (assembly 100 μ m PDMS, fused silica 24 Ga, the manual holder 3 pk red) was used for the collection of fruits volatiles compounds. First, the SPME fiber was pre-conditioned in a GC injector (250 °C) for 30 min. A fruit was placed in a 500 ml clear straight-sided jar with a screw-top lid fitted with a Teflon liner. The SPME needle inserted through a small hole in the lid, and the fiber exposed till 1 h (Figure 3), the fiber was removed from the jar and then immediately inserted into the GC injection port to desorb volatiles. The whole procedure was repeated fifteen times for each fruit (mango, guava, and banana).

Collection of volatiles from mango, guava, and banana was done using porapak Q. Before collection of samples, the porapak Q tube preconditioned at 280 °C for 30 min and washed with dichloromethane, then dried under charcoal purified nitrogen. Porapak Q was connected with an air pump, that have connection with an air flow meter (AFM) (for controlling the flow of air through the system), water bottle, charcoal, plastic bag (Oven bag, Turkey size, 482 × 596 mm), and air sucker (Figure 4). In order to activate the charcoal, it was preheated at 200 °C for 3 hrs. The charcoal flask was followed by oven bag containing a specified amount (2 kg) of the sample (fruits). Air after passing through the oven bag then passed through the porapak Q, the absorbent material inside the porapak Q. The adsorption

of the volatile compounds was done after 24 hrs. Volatiles eluted from the adsorbents of porapak Q with the help of 1 ml CH₂Cl₂ and then stored at -80 °C in the refrigerator. With the help of a microsyringe (1000 microlitres) the volatile compounds/components were collected from the porapak Q into the glass tube (capacity 2 ml). The whole procedure was repeated fifteen times for each fruit. The 0.1 µl was taken from a sample of fruits and used for the analysis.

Gas chromatography–mass spectrometry (GC/MS):

The GC-MS quantitative and qualitative analysis were performed on an Agilent 7890N gas chromatograph, coupled with an Agilent 5975 C mass selective detector (GC-MS) that equipped with an HP-5 MS, capillary column (30 m × 0.25 mm ID, film thickness 0.25 µm, Agilent Technologies, USA). The temperature was programmed from 45 (held for 1 min) to 280°C at 10 °C / min. The solvent delay was kept 5 min, while the injector temperature was used at 250 °C and helium gas was used as the carrier. Electron ionization mass spectra were recorded from m / z 29 °C to 280 °C at 70 eV with ion source temperature at 230 °C

Compounds identification: Quantitative and qualitative constituents' analysis of fruits were done on the basis of their retention times (*R_t*) and mass spectra in the computer library (NIST. 11). The quantity of each fruit components was compared by measurements as using area of the peak.

RESULTS

Fruits characteristics: Characteristics like length (cm),

width (cm), thickness (cm), total soluble solids (TSS) or brix firmness/hardness (N) of and mango, guava, and banana, are shown in table 1. The pericarp toughness of mango fruit was lower than those of the other two tested fruits (banana and guava). While the Brix level of mango was higher than those of the other two tested fruits (Figure 5).

GC-MS analyses of mango: The volatile components of mango were given in table 1. Overall, 99.99 % of components were identified. The major dominating volatile/aromatic components were 3-carene, α-pinene, and butyl caprylate, which accounts (86.62 %) of total components. While other minor constitutes, which makeup the balance was filled with following components e.g. cyclopentasiloxane decamethyl-, cyclohexasiloxane dodecamethyl-, hexanoic acid, 3-hexenyl ester, (Z: 1-tridecene, tetradecane, humulene, and l-limonene (Table 1).

GC-MS analyses of guava: The GC-MS analyses of volatile components of guava are presented in table 2. Overall, 99.99 % of components were recognized. The major dominating components were caryophyllene, α-pinene, gamma. -terpinene, and α-copaene, which accounts (56.90 %) of total constitutes. While other minor constitutes, which make up the balance was filled with following components e.g. Eucalyptol, cyclohexasiloxane dodecamethyl-, aromandendrene, humulene, cycloheptasiloxane tetradecamethyl-, cis-α-bisabolene, epizonarene, phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, diisooctyl phthalate, eicosane, and octadecanal (Table 2).

Table 1. Volatile components from the mango.

Peak #	RT ^a (Paniandy <i>et al.</i>)	Relative %	Components name ^b	KI(Exp) ^c	AI(Exp) ^d
1	6.778	77.07666	3-Carene	984	986
2	8.342	4.253949	α-pinene	1003	1003
3	9.449	2.823219	Cyclopentasiloxane, decamethyl	1121	1122
4	12.142	2.687374	Cyclohexasiloxane, dodecamethyl-	1290	1291
5	12.642	0.19506	Hexanoic acid, 3-hexenyl ester, (Z)	1397	1397
6	13.232	5.314137	Butyl caprylate	1459	1460
7	13.942	1.147518	1-Tridecene	1559	1559
8	14.156	2.928431	Tetradecane	1650	1651
9	15.876	2.056999	Humulene	1656	1657
10	16.373	1.516658	l-limonene	1678	1679

^a Retention time.

^b Components are listed in order of their retention time.

^c Linear retention index on HP-5 (30 m x 0.25 mm, 0.25 µl film thickness), experimentally determined using homologous series of C₇-C₄₀ *n*-alkanes.

^d Arithmetic retention index on HP-5 (30 m x 0.25 mm, 0.25 µl film thickness), experimentally determined using homologous series of C₇-C₄₀ *n*-alkanes.

Identification methods: based on comparison with authentic compounds, NIST 08 MS databases; RI, based on comparison of calculated RI with those reported in Adams or NIST 08.

GC-MS analyses of banana: The GC-MS analyses of volatile components of banana are given in table 3. Overall, 99.99 % of volatile components were recognized. The major dominating components were cycloheptasiloxane, tetradecamethyl-, phenol, 2-ethyl-6-methyl-, tridecane, hexadecane, nonadecane, and tetradecane, which accounts (64.74 %) of total constitutes. While other minor constitutes, which make up the balance

was filled with following components e.g. menthyl acetate, cyclohexasiloxane, dodecamethyl-, 1-triethylsilyloxyheptadecane, beta.-curcumene, ethyl chrysanthemate, dodecane, 2-cyclohexyl-, pentadecane, 4-methyl-, cedrol, pentadecane, cyclooctasiloxane, hexadecamethyl-, heptadecane, pentadecane, 2,6,10,14-tetramethyl-, cyclononasiloxane, octadecamethyl-, dibutyl phthalate, lupleol, and 1-hydroxypyrene (Table 3).

Table 2. Volatile components from the guava.

Peak #	RT ^a	Relative %	Components name ^b	KI(Exp) ^c	AI(Exp) ^d
1	5.933	5.817697	α -Pinene	932	935
2	5.985	6.684064	gamma.-Terpinene	936	939
3	7.519	2.660957	Eucalyptol	1034	1037
4	11.774	1.247745	Cyclohexasiloxane, dodecamethyl-	1322	1323
5	12.651	4.521598	α -Copaene	1387	1388
6	13.255	38.08295	Caryophyllene	1434	1435
7	13.51	3.667704	Aromandendrene	1454	1455
8	13.696	2.971869	Humulene	1469	1470
9	14.016	1.32376	Cycloheptasiloxane, tetradecamethyl-	1494	1494
10	14.227	3.006783	cis- α -Bisabolene	1511	1512
11	14.503	3.332218	Epizonarene	1534	1535
			Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-		
12	23.309	3.415814	4-methyl-	2434	2434
13	24.247	17.1801	Diisooctyl phthalate	2553	2554
14	25.365	3.443879	Eicosane	2693	2693
15	29.301	2.642863	Octadecanal	3443	3444

^a Retention time.

^b Components are listed in order of their retention time.

^c Linear retention index on HP-5 (30 m x 0.25 mm, 0.25 μ l film thickness), experimentally determined using homologous series of C₇-C₄₀ *n*-alkanes.

^d Arithmetic retention index on HP-5 (30 m x 0.25 mm, 0.25 μ l film thickness), experimentally determined using homologous series of C₇-C₄₀ *n*-alkanes.

Identification methods: based on comparison with authentic compounds, NIST 08 MS databases; RI, based on comparison of calculated RI with those reported in Adams or NIST 08.

Table 3. Volatile compounds from the banana.

Peak #	RT ^a	Relative %	Components name ^b	KI(Exp) ^c	AI(Exp) ^d
1	11.155	0.846196	Menthyl acetate	1278	1279
2	11.236	3.951046	Cyclohexasiloxane, dodecamethyl-	1283	1284
3	11.312	3.218066	1-Triethylsilyloxyheptadecane	1289	1289
4	11.335	1.695342	Trimethylsilyl estrone	1290	1291
5	12.296	4.00518	Tetradecane	1361	1362
6	12.343	6.014059	Hexadecane	1364	1366
7	12.753	1.4022	beta.-curcumene	1395	1395
8	12.957	10.2603	Phenol, 2-ethyl-6-methyl-	1411	1411
9	13.04	2.58074	Ethyl chrysanthemate	1417	1418
10	13.128	6.297894	Nonadecane	1424	1425
11	13.581	29.51357	Cycloheptasiloxane, tetradecamethyl-	1460	1461
12	14.299	3.560385	Dodecane, 2-cyclohexyl-	1517	1518
13	14.374	1.760229	Pentadecane, 4-methyl-	1524	1524
14	14.809	8.604666	Tridecane	1560	1561
15	15.119	1.546458	Cedrol	1586	1586
16	15.378	2.174767	Pentadecane	1608	1608

17	15.591	3.698298	Cyclooctasiloxane, hexadecamethyl-	1626	1627
18	15.951	1.47192	Heptadecane	1658	1659
19	16.023	1.523668	Pentadecane, 2,6,10,14-tetramethyl-	1664	1665
20	17.329	1.23439	Cyclononasiloxane, octadecamethyl-	1783	1784
21	17.814	1.436389	Dibutyl phthalate	1830	1830
22	24.584	2.167665	Lupeol	2597	2597
23	24.615	1.036568	1-Hydroxypyrene	2601	2601

^a Retention time.

^b Components are listed in order of their retention time.

^c Linear retention index on HP-5 (30 m x 0.25 mm, 0.25 µl film thickness), experimentally determined using homologous series of C₇-C₄₀ *n*-alkanes.

^d Arithmetic retention index on HP-5 (30 m x 0.25 mm, 0.25 µl film thickness), experimentally determined using homologous series of C₇-C₄₀ *n*-alkanes.

Identification methods: based on comparison with authentic compounds, NIST 08 MS databases; RI, based on comparison of calculated RI with those reported in Adams or NIST 08.

Figure 1. Different fruit.
a. mango, b. guava, c. banana.

Figure 2. Handheld pocket refractometer (a) and TMS-Pro texture analyzer (b).

Figure 3. Schematic diagram for the collection of volatile components/compounds from the fruits by solid-phase micro-extraction (SPME).

Figure 4. Schematic diagram for the collection of volatile components/compounds from the fruits by using porapak Q.

Figure 5. Mean ($\pm$ SE) of physicochemical properties of mango, guava, and banana fruits (n=15).

DISCUSSION

Volatile or aromatic compounds can vary among fruit cultivars (Zhu *et al.*, 2018). Volatile components of fruit are very important for taste (Jayanthi *et al.*, 2012; Malo *et al.*, 2012; Song *et al.*, 2019). The predominant volatile components were determined in the Coche mango e.g. 3-carene, b-selinene, terpinolene, and limonene (Malo *et al.*, 2012; Jimenez *et al.*, 2019). The 3-carene is major aromatic compound in mango fruits and pulp (Tamura *et al.*, 2000; Wetungu *et al.*, 2018) and also important attractive components for insect pests (Jaleel *et al.*, 2019). The 3-carene was major constitute in mango among other constitutes e.g. acetic, butyric and hexanoic acids; and ethyl 3-hydroxybutyrate (Ghatak *et al.*, 2018). Thirteen volatiles components were reported in mango using GCMS e.g. acetaldehyde, acetone, methanol, ethanol, α -pinene, caryophyllene, 3-carene, β -pinene, myrcene, limonene, terpinolene, α -copaene, and γ -cymene (Baldwin *et al.*, 1999; Pino and Mesa, 2006; Maldonado-Celis *et al.*, 2019). In our study, 3-carene, α -pinene, and butyl caprylate were the main volatile components in mango odors. Each volatile component has a specific aroma to call or repel the insect pests (Jang and Light, 1991; Jaleel *et al.*, 2018a; Jaleel *et al.*, 2018c). Volatile components of fruits are very important to attract the female adults of insect pests (Jaleel *et al.*, 2018b). 3-carene, caryophyllene, and humulene were reported main volatile components in guava fruits (McQuate *et al.*, 2017). In our study, caryophyllene, α -pinene, γ -terpinene, and α -copaene were the main volatile components in the order of guava fruit. Schwab *et al.* (2008) reported the major portion of 3-methyl butyl acetate, isoamyl butanoate, and isoamyl isovalerate in banana fruits. And concluded that, the 3-methyl-1-methyl hexyl-2- methyl propyl or isobutyl: 3-methyl butyl or isoamyl: and hexyl esters of acetic, butanoic, and isovaleric acids are the main source of aroma in banana fruits (Wetungu *et al.*, 2018; Zhu *et al.*, 2018). Butyl acetate, isoamyl acetate, ethyl acetate, butyl butanoate, and isoamyl isobutanoate were main components in banana fruit (Zhu *et al.*, 2018; Zhang *et al.*, 2019). In our study, cycloheptasiloxane, tetradecamethyl-, Phenol, 2-ethyl-6-methyl-, Tridecane, Hexadecane, Nonadecane, and Tetradecane were the major portion of aromatic or volatile components in banana fruits. In conclusion, the main major constitution of volatile component obtained mango, guava, and banana was 3-carene, caryophyllene, and cycloheptasiloxane, tetradecamethyl- respectively.

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