

REGULAR PAPER

Volatile profiling of fruits of 17 mango cultivars by HS-SPME-GC/MS combined with principal component analysis

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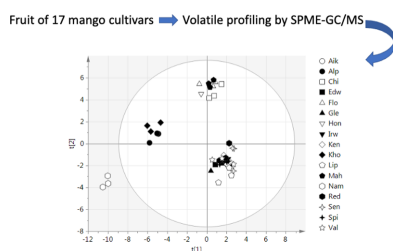
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ABSTRACT

Headspace solid-phase microextraction combined with gas chromatography/mass spectrometry is one of the strongest tools for comprehensive analysis of volatile compounds and has been used to analyze aromatic components of mango and investigate its varietal characteristics. In this study, profiling of aroma compounds in 17 mango cultivars, grown in the same green house to exclude the effect of environmental factors, was conducted and the patterns were subjected to principal component analysis (PCA) to identify the relationship between the aroma components and cultivars. Fifty-nine different volatile constituents were detected from the blends of these 17 mango cultivars. The cultivars were divided into 4 clusters using PCA based on the volatile components determined in the study. Aiko was found to mainly contain δ -3-carene and showed a composition more similar to its pollen parent, Irwin, than to its seed parent, Chien Hwang No. 1.

Graphical Abstract



Volatile composition of fruit of 17 mango cultivars was divided into 4 clusters by HS-SPME GC/MS combined with primary component analysis.

Keywords: Aiko, gas chromatography-mass spectrometry (GC-MS), mango cultivars, *Mangifera indica*, solid-phase microextraction (SPME)

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The fruit of *Mangifera indica* L. (mango) is considered “the king of fruits,” being the most popular fruit in the tropical regions (Lauricella et al. 2017). The genus *Mangifera* includes numerous species of tropical plants in Anacardiaceae (Scartezzini and Speroni 2000). Mango is native to India and Southeast Asia where it has been cultivated for at least 4000 years and over 1000 cultivars are recognized (Mukherjee 1953). It is now also grown in Central America, Africa, Asia, and Australia, and since the last few years in Europe. In some countries, this plant species has a symbolic value—mango is the national fruit of India and the Philippines, and *M. indica* is the national tree of Bangladesh (Usman, Fatima and Jaskani 2001).

Although more than 100 mango cultivars are available worldwide, only a few are grown at a commercial scale. In the late 19th and early 20th centuries, Florida became a secondary center of mango cultivation and contributed to the generation of new cultivars. The mango tree is now the most popular garden tree in this state. The cultivation and breeding of mango plants has also been conducted in Japan. A notable cultivar Aiko, grown at our experimental farm, is the result of interbreeding between the Taiwanese cultivar, Chiin Hwang No. 1 (Ueda et al. 2001), and the mainstay domestic cultivar, Irwin. Breeding and cultivation test for Aiko started in 1999 at our experimental farm and it was registered in 2008 as the first new mango cultivar in Japan (Sasaki et al. 2008).

To establish systematic breeding methods, knowledge about the fruit quality traits, such as fruit size, sugar and acid content, and aroma, is imperative. However, there have been few reports on the relationship between cultivars and their fruit aromas. Previous research in India (Idsteom and Schreier 1985), the USA (Munafo et al. 2014), Australia (San et al. 2017), Thailand (Tamura et al. 2001), and China (Liu et al. 2013) has focused on the aroma properties of mango cultivars; however, there are only a few systematic reports focusing on the characteristics of intercontinentally cultivated varieties. Breeding based on chemical analysis data of aroma components that contribute to the “taste” of mangoes has not been successful. Aroma components are usually highly complex. A problem associated with their analysis is the low concentration in samples. Moreover, there are no suitable solvents for clas-

sical distillation and solvent extraction of water-rich fruit materials.

Headspace solid-phase microextraction combined with gas chromatography/mass spectrometry (HS-SPME-GC/MS) is one of the strongest tools for comprehensive analysis of volatile compounds. It has been used in apple (Aprea et al. 2012), bayberry (Cheng et al. 2015), and mango (San et al. 2017) to analyze aromatic components and to investigate varietal characteristics. There are several difficulties associated with the evaluation of fruit aromas in mango plants. Mango flavor vary by cultivars (Pandit et al. 2009), and they change depending on the processing state and texture (Bonneau et al. 2018). In addition, the aroma components depend on the region in which the plant is grown, being affected by environmental components, such as climate and soil (Kulkarni et al. 2012). In this study, profiling of aroma compounds in 17 mango cultivars was conducted and the patterns were subjected to principal component analysis (PCA) to decipher the relationship of the aroma components and cultivars. The plants were grown in the same green house to exclude the effect of environmental factors. The phylogeny of mango has been genetically studied, and there have been reports on the classification of varieties using SSR markers (Ravishankar et al. 2011); however, the phylogeny of many of the parents of mango cultivars has not been elucidated. To evaluate the inheritance of fruit aroma in mango, a comparison of Aiko and its parents, China Hwang No. 1 and Irwin, is also presented in this study.

Materials and methods

Chemicals

Butyric acid and *cis/trans*-ocimene solution were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma and Aldrich (MO, USA), respectively. All other standards were obtained from Tokyo Chemical Industry (Tokyo, Japan).

Plant materials

The ripening mango fruits of 17 cultivars of *M. indica* (cultivars in alphabetical order, Aiko, Alphonso, Chiin Hwang No. 1,

Table 1. Material seventeen mango cultivars

Cultivar number	Cultivar name	Origin	Embryo mono or poly	Candidate parent (seed) ^a	Candidate parent (pollen) ^a	Group in Figure 2
1	Aiko	Japan	M	Chiin Hwang No. 1	Irwin	1
2	Alphonso	India	M	unknown	Unknown	3
3	Chiin Hwang No. 1	Taiwang	M	White	Kent	2
4	Edward	USA	M	Haden	Unknown	1
5	Florigon	USA	P	Haden	Unknown	2
6	Glenn	USA	M	Haden	Unknown	1
7	Irwin	USA	M	Lippens	Haden	1
8	Kent	USA	M	Brooks	Haden	1
9	Khom	Thailand	P	Unknown	Unknown	3
10	Lippens	USA	M	Haden	Unknown	1
11	Maha Chanook	Thailand	P	Unknown	Unknown	2
12	Nam Doc Mai	Thailand	P	Unknown	Unknown	4
13	Hong Long	Taiwang	M	Irwin	Unknown	2
14	Red Keitt	Taiwang	M	Keitt	Yu-Win No.6	1
15	Sensation	USA	M	Brooks	Haden	1
16	Spirit of 76	USA	M	Zill	Haden	1
17	Valencia Pride	USA	M	Haden	Unknown	1

^aParentage was described in literatures of Yamanaka et al. (2019).

Table 2. Volatile compounds in fruits of seventeen mango cultivars

Peak ID	Category	Compound name	Retention indices (RI)	RI in literature	Similarity indices
1	Acid	Butyric acid	802	CC ^a	
2		Acid-like	816		
3		Lactone-like	847		
4	Monoterpene	α -Pinene	928	CC	
5	Monoterpene	Monoterpene-like 1	931	CC	
6		Camphene	938		
7		Unidentified (aliphatic compound)	954		
8	Monoterpene	β -Pinene	959	943 ^b	90
9	Monoterpene	β -Phallandrene	967	969 ^b	90
10	Aldehyde	Monoterpene-like 2	1002	CC	
11		Octanal	1004		
12		α -Phallandrene	1006		
13	Monoterpene	δ -3-Carene	1011	CC	
14	Monoterpene	α -Terpinene	1015	CC	
15	Monoterpene	(E)-Caren-2-ol	1022	1136 ^b	92
16	Monoterpene	α -Limonene	1026	CC	
17	Monoterpene	(Z)- β -Ocimene	1031	CC, 1040 ^c , 1039 ^d	98
18	Monoterpene	Unidentified (acid-like)	1036	CC, 1050 ^c , 1050 ^d	93
19		(E)- β -Ocimene	1040		
20		Monoterpene-like 3	1046		
21	Monoterpene	γ -Terpinene	1048	CC	
22	Monoterpene	α -Terpinolene	1070	CC	
23	Monoterpene	p-Cymenene	1072	CC	
24	Aldehyde	Unidentified (aromatic compound)	1074	CC	
25		Nonanal (internal standard)	1106		
26	Monoterpene	2,6-Dimethyl-1,3,5,7-octatetraene	1116	1134 ^e	93
27	Aliphatic compound	Monoterpene-like 4	1123	1143 ^f	93
28		2,4,6-Octatriene	1127		
29		Unidentified	1130		
30		Unidentified	1137		
31		Unidentified	1139		
32	Monoterpene	Terpinen-4-ol	1170	CC, 1177 ^c	93
33	Monoterpene	p-Cymen-8-ol	1179	1183 ^c	90
34	Aromatic ester	Methyl salicylate	1205	1190 ^c , 1192 ^e	94
35	Aromatic acid	Ethyl phenylacetate	1245	1244 ^c	92
36	Sesquiterpene	Monoterpene-like 5	1256	1367 ^f , 1368 ^{g,h}	90
37		Unidentified (acid-like)	1260		
38		Unidentified (acid-like)	1284		
39		α -Ylangene	1358		
40		Unidentified (ester-like)	1377		
41	Sesquiterpene	Unidentified (ester-like)	1380	1376 ^{c,d}	92
42		α -Copaene	1385		
43		α -Gurjunene	1429	1407 ^c , 1408 ⁱ	90
44		β -Caryophyllene	1440	CC, 1418 ^c , 1420 ^d	96
45		β -Ylangene	1446	1421 ^f	90
46	Sesquiterpene	α -Guaiene	1453	1439 ^c , 1437 ⁱ ,	
1440 ^j	91				
47	Sesquiterpene	Sesquiterpene-like 1	1465	CC, 1454 ^c , 1442 ^f	96
48		Humulene	1473		
49		β -Copaene	1479	1436 ^k	
50		Alloaromadendrene	1487	1461 ^d , 1460 ⁱ ,	
1448 ^j	92				
51	Sesquiterpene	4,5-di-epi-Aristolochene	1486	1465 ^c	91
52	Sesquiterpene	Germacrene-D	1503	1480 ^{c,i}	90
53	Sesquiterpene	γ -Gurjunene	1504	1472 ^{c,i}	91
54	Sesquiterpene	Selinene	1512	1484 ^c	90
55	Sesquiterpene	Valencene	1516	1491 ^c , 1492 ⁱ	90
56	Sesquiterpene	Sesquiterpene-like 2	1519	1500 ^f	91
57		δ -Guaiene	1527		
58		γ -Murolene	1534	1498 ^f	90

Table 2. Continued.

Peak ID	Category	Compound name	Retention indices (RI)	RI in literature	Similarity indices
59	Sesquiterpene	δ -Cadinene	1542	1523 ^c , 1521 ^d	91

^aIdentified by cochromatography (CC) with authentic standards.

^bNIST Retention Index Library ver. 11.

^cPino et al. (2005).

^dAsai, Matsukawa and Kajiyama (2016).

^eFlamini, Cioni and Morelli (2002).

^fBabushok, Linstrom and Zenkevich (2011).

^gSiani et al. (2004).

^hKovats index agreed with Sibanda et al. (2004).

ⁱKovats index agreed with Babushok et al. (2011).

^jOyediji, Ekundayo and König (2003).

^kAndriamaharavo (2014).

Edward, Florigon, Glenn, Hong Long, Irwin, Kent, Khom, Lippens, Maha Chanook, Nam Doc Mai, Red Keit, Sensation, Spirit of 76, and Valencia Pride; Table 1) were collected at the Experimental Farm of Kindai University (34° 2'N, 135° 11'E, 17 m ASL), located in Wakayama Prefecture, Japan. The trees of these cultivars were grown using commercial methods in a plastic greenhouse under controlled conditions (winter: minimum 2 °C [room] and 10 °C [soil]; summer: maximum 35 °C [room] and 31 °C [soil]). Each sample was collected 3 mature fruits from 1 tree (average height: 2.5 m; age: 12-15 years, life span: 40-50 years) that were propagated by grafting. All varieties were grafted onto the uniform polyembryonic rootstock of the Taiwanese wild-type. The fruits of "Irwin" were collected in August 2019 whereas those of the other 16 cultivars were collected from late August to September 2019. The experimental farm personnel identified and collected the mature fruits. The harvested fruits were stored at -20 °C before use.

HP-SPME-GC/MS

The frozen fruits were cut and divided into flesh, peels and seeds, and peels and seeds were discarded. The flesh was cut and chopped with knives until well homogenized and a portion (0.5 g) of homogenized flesh was placed in a 20 mL glass vial. Two milliliters of saturated sodium chloride solution and 10 μ L of 10 ppb (w/w) nonanal (an internal standard, Tokyo Chemical Industry, Tokyo, Japan) in EtOH were added before capping the vial. We performed GC/MS analyses of all cultivars and checked that nonanal was not detected in all samples, and thus, we selected nonanal as the internal standard. The GC/MS analysis was performed using a GCMS-QP2010 Ultra equipped with an AOC-5000 Plus autosampler (Shimadzu, Kyoto, Japan). Headspace volatile organic compounds (VOCs) were extracted using an SPME fiber coated with 50/30 μ m of divinylbenzene/carboxen/polydimethylsiloxane at 50 °C for 60 s with continuous agitation at 600 s⁻¹. The SPME fiber was inserted into the injection port for 60 s at 230 °C to desorb the VOCs. An Rtx-5MS capillary column (30 m \times 0.25 mm [0.25 μ m], Restek, Bellefonte, PA, USA) was used, and helium was used as a carrier gas at a linear velocity of 38.1 cm/s. The oven temperature was set at 50 °C for 5 min; it was then increased to 330 °C at a rate of 15 °C min⁻¹ and maintained for 6 min. The mass spectra were obtained in electron ionization mode at 70 eV with a scanning range of m/z 85-500 and a scanning speed of 10 000 scan s⁻¹. The MS ion source and interface temperatures were 200 °C and 250 °C, respectively.

GC/MS data analysis

GC/MS chromatograms were analyzed using the GCMS solution ver. 4.11 (Shimadzu, Kyoto, Japan). The mass spectra data were compared against spectra in the NIST reference library (NIST14) of the GC/MS data system for identification of volatile compounds. Compounds were annotated under the condition of processing a similarity index of more than 90, and major components were identified by cochromatography with authentic standards. Retention indices (RIs) from the literature were used for the identification of volatiles. The threshold of RI differences was set at ± 15 for RI < 1300 and ± 40 for RI > 1300 according to the RI differences in literatures and authentic standards of (E)- β -ocimene (RI < 1300) and humulene (RI > 1300). The peak areas of all compounds relative to that of the highest peaks were used for the multivariate analysis. PCA was performed using the SIMCA ver. 13.0.3 software (Umetrics, Umeå, Sweden). Pareto scaling was applied to the data processing before PCA analysis.

Results

Mango cultivar formation and estimation of volatile components

The volatile components in the fruits of 17 mango cultivars are shown in Table 2. A total of 59 different volatile constituents were detected, 40 of which were annotated or identified based on comparison of their mass fragmentation patterns to the NIST library and retention indices in literatures or cochromatography with authentic standards. Seventeen monoterpenes were annotated, among which 13 consisted of the main components of mango fruit aroma. To assess the differences in monoterpene patterns in each variety, relative amounts of each annotated monoterpene were compared (Figure 1). The main components of Aiko, Edward, Glenn, Irwin, Kent, Lippens, Red Keit, Sensation, Spirit of 76, and Valencia Pride were δ -3-carene, whereas Chiin Hwang No. 1, Florigon, Maha Chanook, and Hong Long contained large amounts of α -terpinolene. (Z)- β -Ocimene accounted for 71% of the main components of Alphonso, whereas Khom had 60% of its isomer, (E)- β -ocimene. Nam Doc Mai had a higher proportion of α -limonene than the other cultivars.

Principal components analysis

To compare the volatile components among cultivars, the peak patterns (including unidentified peaks) of HS-SPME-GC/MS were

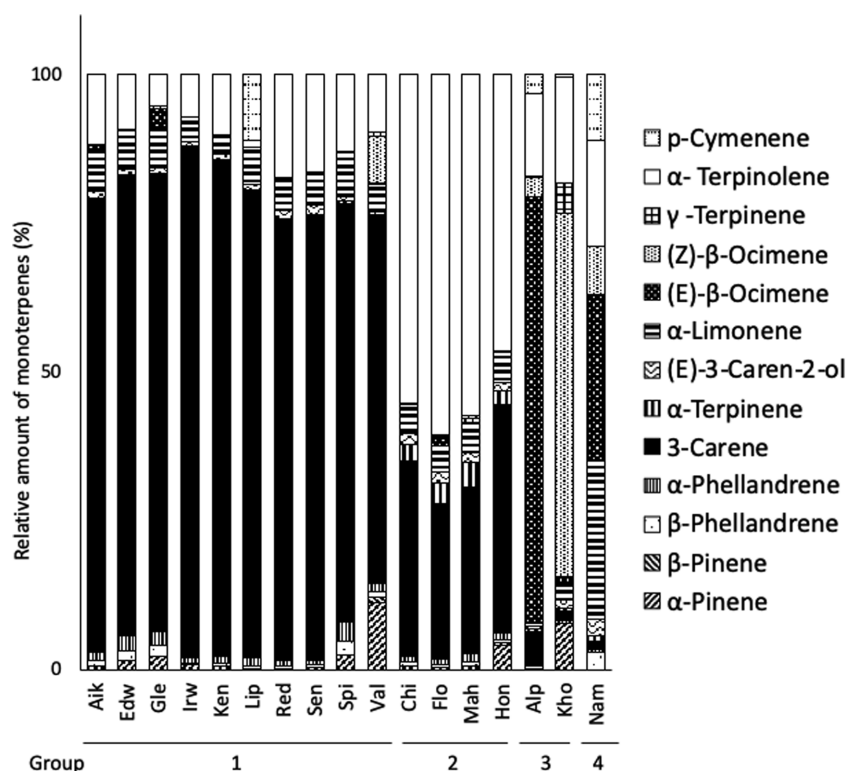


Figure 1. Comparison of the monoterpene composition of fruit in 17 mango cultivars. Cultivar names are represented by 3 letters: Aiko (Aik), Alphonso (Alp), Chiin Hwang No. 1 (Chi), Edward (Edw), Florigon (Flo), Glenn (Gle), Hong Long (Hon), Irwin (Irw), Kent (Ken), Khom (Kho), Lippens (Lip), Maha Chanook (Mah), Nam Doc Mai (Nam), Red Keit (Red), Sensation (Sen), Spirit of 76 (Spi), and Valencia Pride (Val). Different bar patterns correspond to different monoterpenes as shown in the right panel. Group represents the clusters shown in Figure 2.

subjected to PCA (Figure 2). According to the score plot, the 17 cultivars could be clearly divided into 4 groups: Group 1 contained Spirit of 76, Glenn, Valencia Pride, Sensation, Red Keit, Lippens, Edward, Aiko, Kent, and Irwin in the positive region of PC 1 and in the negative region of PC 2. Group 2 consisted of Chiin Hwang No. 1, Maha Chanook, Florigon, and Hong Long in the neutral region of PC 1 and in the positive region of PC 2. Group 3 contained Alphonso and Khom in the negative region of PC 1 and in the neutral region of PC 2 (Figure 2a). Group 4 consisted of only 1 cultivar, Nam Doc Mai. The loading plot showed that δ -3-carene strongly contributed to the clustering in the positive region of PC 1 and in the negative region of PC 2, whereas α -terpinolene significantly affected the clustering to the positive region of PC 2 (Figure 2b). Two sesquiterpenes, humulene and β -caryophyllene, and (E)- and (Z)- β -ocimene contributed to the clustering in the negative region of PC1. The largest cluster detected in the score plot was Group 1, and the cultivars in this cluster were characterized to have monotonous and refreshing scent, with δ -3-carene as the main compound (Figure 2). The second largest cluster was Group 2, and the cultivars in this cluster contain more α -terpinolene than other cultivars. The Alphonso and Khom clusters contain a lot of β -ocimene, but Alphonso mainly contains (Z)- β -ocimene, and the main component of Khom aroma is (E)- β -ocimene (Figure 1). Both the β -ocimene isomers form a characteristic mango odor, and thus, make the difference between these and other cultivars. Nam Doc Mai formed a single cultivar cluster, suggesting that the aroma pattern of this cultivar is different from that of other cultivars tested. Nam Doc Mai had a significantly low amount of δ -3-carene, the common main component in other cultivars, and contained a large amount of β -caryophyllene, a trace sesquiterpene in other cultivars.

Discussion

Monoterpenes are known to account for nearly 90% of the aroma components in the ripe fruit of Alphonso originating in India (Idsteom and Schreier 1985). They are also reported to be the main components in most of the cultivars from Florida (MacLeod and Snyder 1985) and Thailand (Tamura *et al.* 2001). However, the composition of the components differs depending on the variety. In this study, δ -3-carene was the main component of the Florida cultivars, excluding Florigon, whereas (E)- β -ocimene was the main component in the Indian variety Alphonso. Florigon and 2 Taiwanese cultivars, Chiin Hwang No. 1 and Hong Long, mainly contained α -terpinolene, but also had a lot of δ -3-carene. A part of α -terpinolene is thought to be converted to α -terpineol and terpinene-4-ol during storage (Kitao 1993). Because both the compounds have been reported to cause nonpreferable fishy and plastic odors (Kitao 1993; Elmaci and Altug 2005), storage condition of the fruit of these cultivars should be strictly controlled to avoid the production of α -terpineol and terpineneol. Alphonso and Khom were classified into the same cluster, but their main components were (Z)-ocimene and (Z)-ocimene, respectively. The basic skeleton of these compounds is the same and they are geometric isomers; both the isomers are biosynthesized via a common pathway, and thus, Alphonso and Khom have a common system for the regulation of monoterpene biosynthesis. Compared to all other cultivars, Nam Doc Mai has a high ratio of limonene and contains various volatile components at relatively higher proportions. Therefore, Nam Doc Mai shows unique fruit aroma. In general, mango cultivars belong to 2 distinct groups, ie polyembryonic types and monoembryonic types (Mukherjee 1953). Polyembryonic cultivars such as Nam Doc Mai are common in Asia, and they have been cultivated traditionally

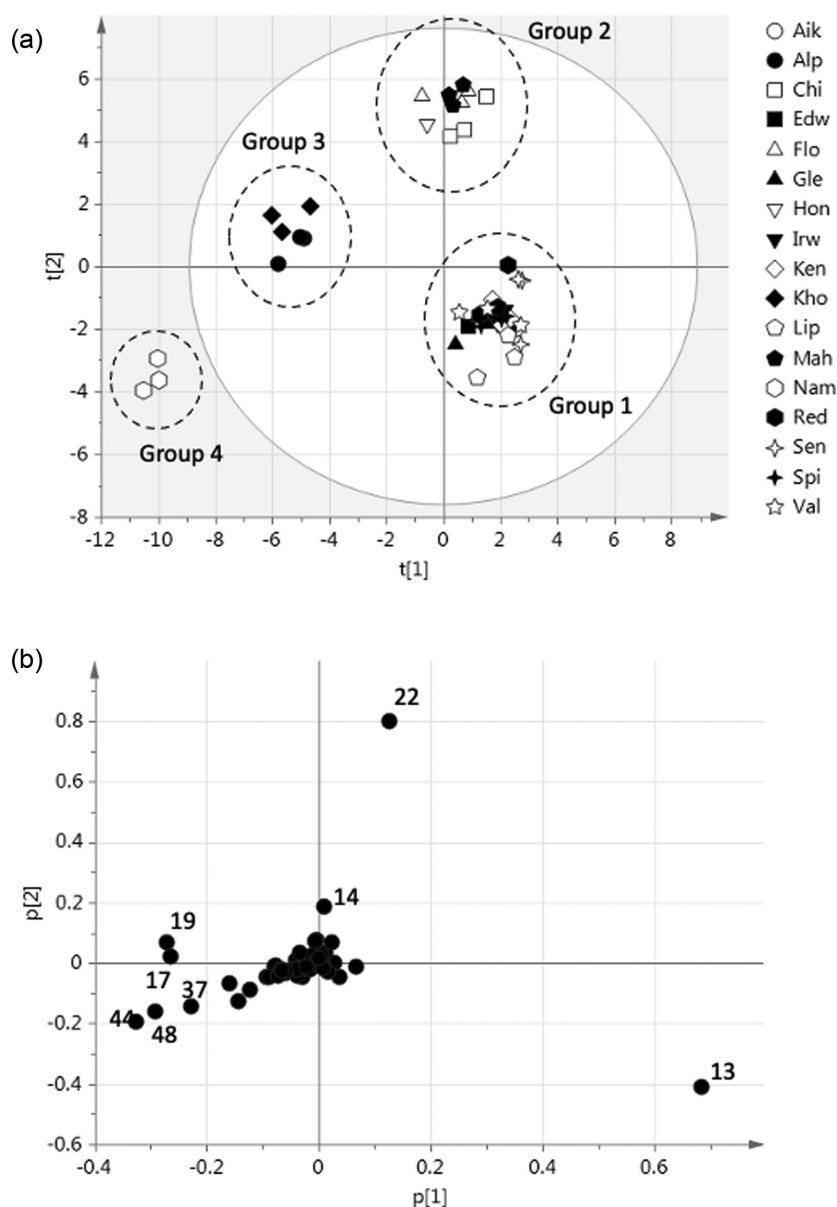


Figure 2. Principal component analysis (PCA) model of 17 mango cultivars based on HS-SPME-GC/MS analysis. (a) Score plot for principal component 1 (PC1) and PC2 ($R^2X[1] = 0.280$, $R^2X[2] = 0.203$), showing different symbols for the different cultivars: Aiko (open circles), Alphonso (closed circles), Chiin Hwang No. 1 (open boxes), Edward (closed boxes), Florigon (open triangles), Glenn (closed triangles), Hong Long (open inverted triangles), Irwin (closed inverted triangles), Kent (open diamonds), Khom (closed diamonds), Lippens (open pentagon), Maha Chanook (closed pentagon), Nam Doc Mai (open hexagon), Red Keit (closed hexagon), Sensation (open 4-pointed star), Spirit of 76 (closed 4-pointed star), and Valencia Pride (open 5-pointed star). (b) Loading plot for PC1 and PC2. Numbers are peak IDs shown in Table 2.

(Purseglove and Anacardiaceae 1974). In polyembryonic mango, each seed contains 1 sexual embryo and several somatic or nucellar ones, which share their entire genetic constitution with the mother plant (Iyer and Degani 1997). In polyembryonic cultivars the zygotic embryo may produce morphological and genetic diversity (Gálvez-López et al. 2010). However, systematic breeding is difficult because it is not always possible to obtain zygotic embryos (Ochoa et al. 2012), and thus, polyembryonic seedling selection have been carried out to exploit the diversity in polyembryonic populations generated by natural mutation (Knight and Schnell 1993). On the other hand, in the US, because of the large number of monoembryonic cultivars, general breeding has been carried out, and many varieties with fewer peculiarities have been produced (Bally, Lu and Johnson 2009). In com-

parison with commercially important cultivars, polyembryonic mangoes exhibit a stronger turpentine-like aroma and a stringy flesh characterized by many distinct tough fibers (Purseglove and Anacardiaceae 1974). Consumers in temperate countries find such flavor and texture characteristics unpleasant (Ollé et al. 1998). Therefore, most fresh mangoes and mango derived products sold on the world market originate from monoembryonic cultivars. Aiko mainly contains δ -3-carene and showed a composition more similar to that of Irwin, a pollen parent, than the seed parent Chiin Hwang No. 1. However, among the volatile components of Aiko, the proportion of sesquiterpenes was more than that in Irwin and Chiin Hwang No. 1 (Figure 3). Monoterpenes are biosynthesized via the methylerythritol phosphate (MEP) pathway in plastids, whereas sesquiterpenes are produced

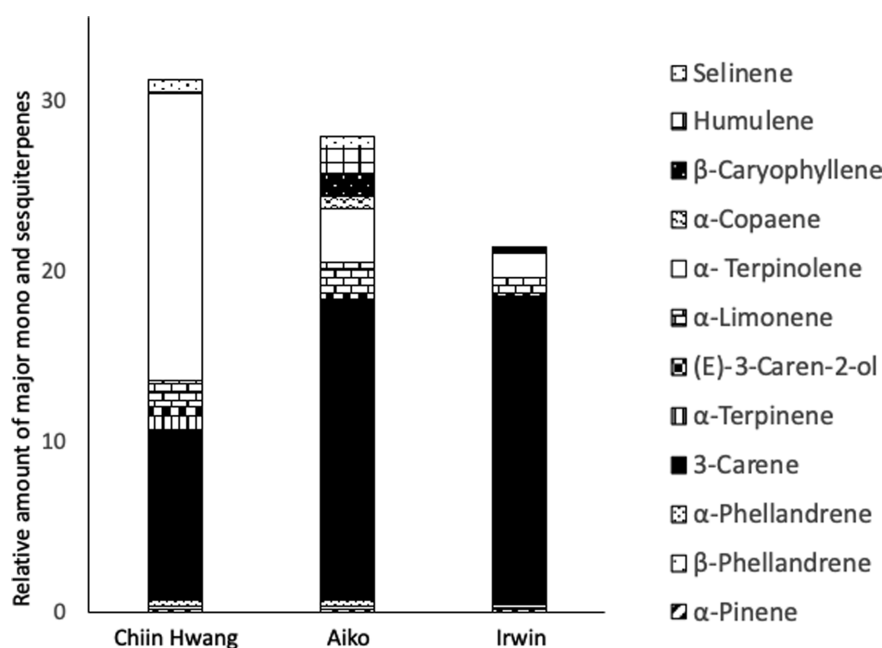


Figure 3. Comparison of mono- and sesquiterpene composition of Aiko and its parents, Chiin Hwang No. 1 and Irwin. Different bar patterns correspond to different monoterpenes as shown in the right panel.

via the mevalonate pathway in the cytosol. Our results suggest that the regulation of these 2 terpenoid pathways are independently inherited from the parents.

Recently, advances in molecular genetics have allowed the identification of pollen parents, which is difficult to be identified using conventional SSR markers (Ravishankar *et al.* 2011). It has been reported that mangoes are genetically classified into 3 clusters according to USA (Florida), India, and Asia (Yamanaka *et al.* 2019). Our results suggest that the genetic cluster is closely related to the aroma component in the fruit. Irwin, a grandchild of Haden, and most of the cultivars derived from Haden were included in the same cluster in the classification of aroma components. The volatile components of Haden have been reported to be mainly composed of 3-carene (Pino *et al.* 2005), and its closely related cultivars inherited the similar aroma components. Haden derived cultivars have been widely distributed in North America and East Asia, such as in Taiwan and Japan, suggesting that odor properties of δ -3-carene match the consumers' preferences in these areas. In contrast, cultivars originated in Thailand and India contained relatively low levels of δ -3-carene. These results imply that the cultural preference in Central and Southeast Asia is different from that in other areas.

Our results suggested that mono- and sesquiterpenes are major components of mango fruit volatiles. In addition to the terpenes, acetoin and lactones with 4 to 10 carbons were reported to be contained in the mango flavor (Chauhan, Raju and Bawa 2010). However, the report which suggested the acetoin in mango flavor used canned mango puree (Hunter *et al.* 1974). In addition, acetoin was reported to be formed by fermentation of mango juice with *Penicillium expansum* (Duarte, Delgadillo and Gil 2006). Pino *et al.* reported that acetoin was not detected in all of 20 mango cultivars tested. Considering them, acetoin may not be involved in the fresh mango flavor. In contrast to acetoin, lactones have been reported to contribute to fruit aroma as well as monoterpenes (Hadi *et al.* 2013). The previous study suggested that the SPME method showed low sensitivity than the solvent extraction method (Mahattanatawee, Goodner and

Baldwin 2005). In addition, terpenes were reported to be major and important constituents in mango flavor, while levels of lactones and other esters were extremely low (Pino *et al.* 2005). Our results may be due to the low concentrations of these compounds. However, the major mono- and sesquiterpenes were significantly detected, and the difference in cultivars were clearly characterized.

In mango, the proportion of monoterpenes decreases as the fruit matures, and the proportion of alcohols and esters increases in Alphonso (Pandit *et al.* 2009) and Irwin (Shivashankara *et al.* 2006). It has also been reported that the aroma changes depending on the processing method and texture (Bonneau *et al.* 2018). In the future, by investigating the difference of aroma components among cultivars depending on the harvest time and maturity stage, should provide an index to determine the storage characteristics and the harvest time suitable for the cultivars. Breeding of mango takes 3-10 years to confirm the fruit traits (Bally, Lu and Johnson 2009), and it is required to improve the efficiency of the individual selection method at an early stage. Therefore, it is important to collect data on the correlation between fruits and aroma components of various parts such as leaves, bark, and flowers, along with genetic information. Our results provide important information for evaluation of the mango fruit quality and establishment of efficient mango breeding systems.

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

Author contribution

T.M. and S.K. designed the study and performed statistical analysis. K.S. and R.K. performed chemical analyses and statistical analyses. K.I., S.K., and S.S. prepared and treated the plant materials. All authors have reviewed and approved the final version of the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Andriamaharavo Retention Data. NIST Mass Spectrometry Data Center. Gaithersburg, MD: NIST Mass Spectrometry Data Center, 2014
- Apra E, Corollaro ML, Betta E et al. Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. *Food Res Int* 2012;**49**:677-86.
- Asai T, Matsukawa T, Kajiyama S. Metabolic changes in citrus leaf volatiles in response to environmental stress. *J Biosci Bioeng* 2016;**121**:235-41.
- Babushok VI, Linstrom PJ, Zenkevich IG. Retention indices for frequently reported compounds of plant essential oils. *J Phys Chem Ref Data* 2011;**40**:043101.
- Bally ISE, Lu P, Johnson P. Mango breeding. In: Jain SM Priyadarshan PM (eds). *Breeding Plantation Tree Crops: Tropical Species*, 1st edn. New York: Springer, 2009, 51-82.
- Bonneau A, Boulanger R, Lebrun M et al. Impact of fruit texture on the release and perception of aroma compounds during in vivo consumption using fresh and processed mango fruits. *Food Chem* 2018;**239**:806-15.
- Chauhan OP, Raju PS, Bawa AS. Mango flavor. In: Hui YH (ed). *Handbook of Fruit and Vegetable Flavors*. Hoboken, NJ: John Wiley & Sons Inc., 2010, 319-43.
- Cheng H, Chen J, Li X et al. Differentiation of the volatile profiles of Chinese bayberry cultivars during storage by HS-SPME-GC/MS combined with principal component analysis. *Postharvest Biol Technol* 2015;**100**:59-72.
- Duarte IF, Delgadillo I, Gil AM. Study of natural mango juice spoilage and microbial contamination with *Penicillium expansum* by high resolution ¹H NMR spectroscopy. *Food Chem* 2006;**96**:313-24.
- Elmaci Y, Altug T. Flavor characterization of three mandarin cultivars (SATSUMA, BODRUM, CLEMANTINE) by using GC/MS and flavor profile analysis techniques. *J Food Qual* 2005;**28**:163-70.
- Flamini G, Cioni PL, Morelli I. Differences in the fragrances of pollen and different floral parts of male and female flowers of *Laurus nobilis*. *J Agric Food Chem* 2002;**50**:4647-52.
- Gálvez-López D, Salvador-Figueroa M, Adriano-Anaya ML et al. Morphological characterization of native mangos from Chiapas, Mexico. *Subtropical Plant Sci* 2010;**62**:18-26.
- Hadi MAME, Zhang FJ, Wu FF et al. Advances in fruit aroma volatile research. *Molecules* 2013;**18**:8200-29.
- Hunter GLK, Bucek WA, Radford T. Volatile components of canned Alphonso mango. *J Food Sci* 1974;**39**:900-3.
- Idsteom H, Schreier P. Volatile constituents of alphonso mango (*Mangifera indica*). *Phytochemistry* 1985;**24**:2313-6.
- Iyer CPA, Degani C. Classical breeding and genetics. In: Litz RE (ed). *The Mango: Botany*. Wallingford, UK: CAB International, 1997, 49-68.
- Kitao J. Quality of mango fruit (II) (in Japanese). *J Japan Soc Cold Preserva. Food* 1993;**19**:196-210.
- Knight RJ, Schnell RJ. Mango (*Mangifera indica* L.) introduction and evaluation in Florida and its impact on the world industry. *Acta Hort* 1993;**341**:125-35.
- Kulkarni RS, Chidley HG, Pujari KH et al. Geographic variation in the flavors volatiles of Alphonso mango. *Food Chem* 2012;**130**:58-66.
- Lauricella M, Emanuele S, Calvaruso G et al. Multifaceted healthy benefits of *Mangifera indica* L. (Mango): the inestimable value of an orchard recently rooted in Sicilian rural areas. *Nutrients* 2017;**9**:525.
- Liu FX, Fu SF, Bi XF et al. Physico-chemical and antioxidant properties of four mango (*Mangifera indica* L.) cultivars in China. *Food Chem* 2013;**138**:396-405.
- MacLeod AJ, Snyder CH. Volatile components of two cultivars of mango from Florida. *J Agric Food Chem* 1985;**33**:380-4.
- Mahattanatawee K, Goodner KL, Baldwin EA. Volatile constituents and character impact compounds of selected Florida's tropical fruit. *Proc Fla State Hort Soc* 2005;**118**:414-8.
- Mukherjee SK. The mango—its botany, cultivation, uses and future improvements, especially as observed in India. *Econ Bot* 1953;**7**:130-62.
- Munafo JP, Jr, Didzbalis J, Schnell RJ et al. Characterization of the major aroma-active compounds in mango (*Mangifera indica* L.) cultivars Haden, White Alfonso, Praya Sowoy, Royal Special, and Malindi by application of a comparative aroma extract dilution analysis. *J Agric Food Chem* 2014;**62**:4544-51.
- Ochoa ECM, Rodriguez MA, Andrade-Rodríguez M et al. Identification of zygotic and nucellar seedlings in polyembryonic mango cultivars. *Pesq Agropec Bras* 2012;**47**:1629-36.
- Ollé D, Baumes RL, Bayonove CL et al. Comparison of free and glycosidically linked volatile components from polyembryonic and monoembryonic mango (*Mangifera indica* L.) Cultivars. *J Agric Food Chem* 1998;**46**:1094-1100.
- Oyedede OA, Ekundayo O, König WA. Volatile leaf oil constituents of *Lantana camara* L from Nigeria. *Flavour Fragr J* 2003;**18**:384-6.
- Pandit SS, Chidley HG, Kulkarni RS et al. Cultivar relationships in mango based on fruit volatile profiles. *Food Chem* 2009;**114**:363-72.
- Pino JA, Mesa J, Muñoz Y et al. Volatile components from mango (*Mangifera indica* L.) cultivars. *J Agric Food Chem* 2005;**53**:2213-23.
- Purseglove J, Anacardiaceae W. *Tropical Crops Dicotyledons*. London: Longman Group, 1974, 18-32.
- Ravishankar KV, Mani BH, Anand L et al. Development of new microsatellite markers from mango (*Mangifera indica*) and cross-species amplification. *Am J Bot* 2011;**98**:96-9.
- San AT, Joyce DC, Hofman PJ et al. Stable isotope dilution assay (SIDA) and HS-SPME-GCMS quantification of key aroma volatiles for fruit and sap of Australian mango cultivars. *Food Chem* 2017;**221**:613-9.
- Sasaki K, Nakajima A, Shimizu K et al. Registration kind database of Intellectual Property Division, Food Industry Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries, Registration number 16162, registration date 2008/3/5, Japan, 2008. http://www.hinshu2.maff.go.jp/vips/cmm/apCMM112.aspx?TOUROKU_NO=16162&LANGUAGE=Japanese. (4 June 2021, date last accessed).

- Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J Ethnopharmacol* 2000;**71**:23-43.
- Shivashankara KS, Isobe S, Horita H et al. Volatile aromatic constituents of tree ripened and mature green 'Irwin' mango fruits during low temperature storage. *J Japan Soc Hort Sci* 2006;**75**:209-12.
- Siani AC, Garrido IS, Monteiro SS et al. *Protium icariba* as a source of volatile essences. *Biochem Syst Ecol* 2004;**32**: 477-89.
- Sibanda S, Chigwada G, Poole M et al. Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. *J Ethnopharmacol* 2004;**92**:107-11.
- Tamura H, Boonbumrung S, Yoshizawa T et al. The volatile constituents in the peel and pulp of a green Thai mango, Khieo Sawoei cultivar (*Mangifera indica* L.). *Food Sci Technol Res* 2001;**7**:72-7.
- Ueda M, Sasaki K, Utsunomiya N et al. Changes in properties during maturation and ripening of 'Chiin Hwang No. 1' mango fruit cultivated in a plastic greenhouse. *Food Sci Technol Res* 2001;**7**:207-13.
- Usman M, Fatima B, Jaskani MJ. Breeding in mango. *Int J Agric Biol* 2001;**3**:522-6.
- Yamanaka S, Hosaka F, Matsumura M et al. Genetic diversity and relatedness of mango cultivars assessed by SSR markers. *Breed Sci* 2019;**69**:332-44.