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Research article

First characterization of aroma-active compounds in ripe Okrong mango (*Mangifera indica* L.)

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Abstract

Importance of the work: Okrong mango has a sweet taste and unique aroma; however, the active compounds contributing to such distinctive aroma remained uncharacterized.

Objectives: To identify aroma-active compounds of ripe Okrong mangoes using a sensomics approach.

Materials and Methods: An acceptability test and the olfactory profile of Okrong mango were evaluated. Okrong mangoes were separately extracted using diethyl ether or dichloromethane, followed by solvent-assisted flavor evaporation to remove nonvolatile components. The aroma isolates were concentrated using a microdistillator and analyzed using gas chromatography-olfactometry/flame ionization detection (GC-O/FID). Characterization of the aroma-active compounds was performed based on Comparative analysis of Aroma Extract Dilution Analysis (cAEDA), comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) and comparisons with authenticated samples.

Results: Of the mangoes evaluated, Okrong mango ranked the top in overall aroma and flavor. Of the eight Okrong mango attributes (caramel/sweet-like, fruity, sulfury, floral, terpeny, sweaty, coconut water and green in olfactory profile), the first three received the highest scores. The mango extracts using diethyl ether had an olfactory profile nearly identical to that of the freshly cut mango samples. In total, 43 aroma-active compounds were identified, with flavor dilution (FD) factor values ranging from 4 to ≥2,048, after the diethyl ether extract had been analyzed using GC-O/FID and GC×GC-TOFMS.

Main finding: In total, 43 aroma-active compounds with FD factor values ranging from 4 to ≥2,048 were discovered. The caramel-like aroma (a distinguishing character of ripe Okrong mango) was potentially associated with two aroma-active compounds with FD factor values ≥2,048, namely 4-methoxy-2,5-dimethyl-3(2H)-furanone and 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

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Introduction

Mango (*Mangifera indica* L.) is a popular fruit in many countries and is a highly valued tropical fruit (Exporting Thai fruits to the world market, 2020). From the data on Thai fruit exports in 2018 reported by the Bank of Thailand (2019), it was found that the market value of Thai fruit exports reached 2,475 million US dollars. Mangos are an export product that makes up 3 percent of all Thai fruits, accounting for an export value in the market of 74.25 million US dollars are mainly exported to South Korea, America and Japan (Exporting Thai fruits to the world market, 2020). However, mangoes are fragile, with substantial post-harvest losses occurring during the processing chain for export due to inadequate handling during transportation, storage and ripening (Medlicott et al., 1986).

Each mango cultivar has its unique flavor, as evidenced by Munafo (2014) in which German and USA researchers applied a sensomics approach to explore the aroma-active compounds from five different mango cultivars—Haden, White Alfonso, Praya Sowoy, Royal Special and (Munafo et al., 2014). They reported that based on the aroma profiles of fresh fruit flesh obtained from these five cultivars, each cultivar had a distinctive aroma, with 54 aroma-active compounds identified in at least one of the cultivars with flavor dilution (FD) facor values in the range 4 to ≥2,048. Although ripe mangoes may contain many aroma-active compounds, few of them are key odorants; for example, the key odorants in Haden mangoes were investigated based on the 34 aroma-active compounds that had been identified (Munafo et al., 2016). Based on omission testing, 15 aroma-active compounds were successfully verified as key odorants:, ethyl 2-methylbutanoate, (3E,5Z)-undeca-1,3,5-triene, ethyl 3-methylbutanoate, ethyl butanoate, (2E,6Z)-nona-2,6-dienal, ethyl 2-methylpropanoate, (*E*)-β-damascenone, ethyl hexanoate, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF), 3-methylbut-2-ene-1-thiol, γ-decalactone, β-myrcene, (3Z)-hex-3-enal, 4-methyl-4-sulfanylpentan-2-one and ethyl octanoate.

Numerous studies of the volatile aroma-active compounds have been reported in mango from the pulp (Munafo et al., 2014; Ma et al., 2018), the leaf and peel (Damić et al., 2010), puree and mango juice (Morlock et al., 2021) or even in the sap (Syed Ghulam et al., 2016). The volatile components were extracted using various techniques: headspace solid phase microextraction, simultaneous distillation extraction and steam distillation, followed by identification using gas chromatography-mass spectrometry (Zhang and Li, 2007; Munafo et al., 2014; Munafo et al., 2016).

Among the mango cultivars popular in Thailand, Okrong has been recognized as a premium mango due to its unique sweet aroma and taste, with its flavor impressing consumers, particularly when served with sticky rice (Karlaand Kocha, 2025). However, Okrong is relatively small in size and its peel turns black more easily during the ripening stage; therefore, it is less cultivated and promoted. Consequently, the current research aimed to identify the aroma-active compounds in Okrong mango (*Mangifera indica* L.) in the ripening stage, as such information could be used to control the quality of mangoes to better suit and meet the needs of the international market.

Materials and Methods

Reference aroma-active compounds

Reference aroma-active compounds were purchased from Sigma-Aldrich, USA supplied: methyl acetate, 2,3-butanedione, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, hexanal, 3-methylbutyl acetate, myrcene, 1,8-cineole (eucalyptol), limonene, 3-methyl-1-butanol, p-cymene, acetoin (hydroxy-2-butanone), (*E*)-2-heptenal, dimethyl trisulfide, p-mentha-1,3,8-triene, ethyl octanoate, acetic acid, 3-(methylthio) propanal (methional), β-caryophyllene, 4-methoxy-2,5-dimethyl-3(2H)-furanone(MDMF), butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, pentanoic acid, methyl salicylate, hexanoic acid, 2-methoxyphenol, benzyl alcohol, (*E*)-β-ionone, δ-octalactone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone(HDMF),2-ethyl-4-hydroxy-5-methylfurane-3(2H)-one (ethylfuraneol), γ-decalactone, nonanoic acid, 2'-aminoacetophenone, dodecanoic acid, 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-acetyl-2-methoxyphenol. In addition, ethyl acetate was purchased from CARLO ERBA Reagents S.A.S., Italy.

Miscellaneous chemicals and reagents

Diethyl ether and *n*-alkane C_7-C_{30} for analysis were purchased from Sigma-Aldrich, USA. Calcium chloride ($CaCl_2$), sodium sulfate anhydrous (Na_2SO_4) for extraction, hexane, and pentane for analysis and silica gel 60 (0.040−0.063 mm) for column chromatography were purchased from Merck, Germany. Liquid nitrogen for extraction was purchased from Biologix, Thailand.

Acceptability testing of Okrong, Kaew and Khiew Sawoey mango cultivars

Three mango cultivars (Okrong, Kaew and Khiew Sawoey) which were the most available commercially, were used in these experiments. Acceptability testing was applied to these native Thai mango cultivars to study their overall aroma and flavor. The study involved Okrong, Kaew and Khiew Sawoey mangoes harvested in January 2024. The Okrong mangoes that had been picked at 120 d after flowering (DAF) were purchased from a plantation in Ratchaburi province, Thailand. The Khiew Sawoey and Kaew mangoes that had been picked at 120 DAF were purchased from a plantation in Chachoengsao province in Thailand. Samples of all three cultivars were left to ripen naturally and were delivered (after about a 2 hr drive) from the orchards to Chulalongkorn University, Bangkok, Thailand. The state of ripeness of each mango was estimated by experienced personnel based on sensory evaluation of the color, total soluble solids and aroma. Afterward, some of each of the cultivars mangoes frozen at -20°C to be stored for further extraction. For the acceptability test, ripe mango samples were peeled and cut into 10 g pieces, placed in 85 g plastic cups with a plastic lid and labeled using three-digit random numbers. Samples were analyzed immediately after preparation in a temperature-controlled room (20−25°C) to reduce the possibility of some aroma-active compounds disappearing.

The sensory panelists consisted of 55 untrained Thais, who had declared that they regularly consumed mangoes. All sensory tests were carried out during the same sampling period based on a multiple comparison test design with Okrong mangoes set as the benchmark.

In these multiple comparison tests, each panelist evaluated each sample for overall liking, overall appearance, overall taste, overall aroma and flavor by rating each sample using a seven-point hedonic scale 1 to 7 (1 = dislike extremely, 4 = neither like nor dislike and 7 = like extremely) for all three types of mangoes with sticky rice. Data were subjected to two-way mixed-effect analysis of variance at the 95% confidence level ($p < 0.05$) and mean scores were compared using Tukey's b *post-hoc* comparisons test. The model comprised two main effects (consumer and sample) without an interaction term. The consumer was treated as a random effect and the sample was treated as a fixed effect. All statistical analyses were performed using the SPSS v.23 software (IBM Corp.; USA).

Olfactory profiling

Fresh ripe Okrong, Khiew Sawoey and Kaew mango samples (each 10 g) were peeled and cut into sections and placed into 85 g plastic portion cups with lids. The mango samples (Fig. 1) were orthogonally evaluated based on free-choice profiling and olfactory profiling by nine trained sensory panelists (3 males, 6 females, age range 23-45 yr) in a standardized panel test room with separate booths for each trained sensory panelist. The descriptors used in the olfactory profiling were defined based on the aroma of a reference compound dissolved in water at a concentration of each aroma-active compound is shown in Table 1.

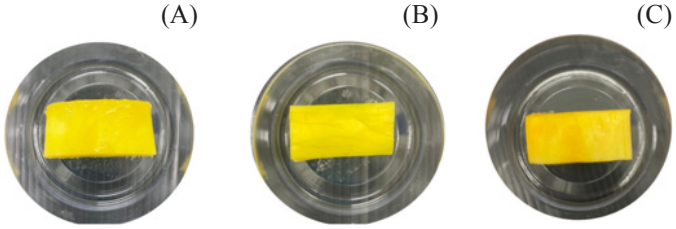

Fig. 1 Mango samples used in olfactory profiling: (A) ripe Okrong, (B) Khiew Sawoey; (C) Kaew

Table 1 Aroma-active compounds, their characteristic odor description and the corresponding concentrations associated with aroma intensity scores of 1, 3 and 4.5.

Aroma-active compound	Character	Score		
		1	3	4.5
Ethyl butanoate	Fruity	10 ppm	30 ppm	100 ppm
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel, sweet	50 ppm	100 ppm	500 ppm
Butanoic acid	Sweaty	100 ppm	500 ppm	1000 ppm
Linalool	Floral, citrus	10 ppm	50 ppm	200 ppm
Myrcene	Terpeny	100 ppm	300 ppm	500 ppm
2-(Methyl sulfanyl)butane	Sulfury	500 ppm	1000 ppm	-
Acetaldehyde	Coconut water	10 ppm	100 ppm	500 ppm
Hexanal	Green	10 ppm	30 ppm	60 ppm

ppm = parts per million.

Reference aroma-active compounds used in the sensory experiments were ethyl butanoate (fruity), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF; caramel, sweet), butanoic acid (sweaty), linalool (floral, citrus), myrcene (terpeny), 2-(methyl sulfanyl)butane (sulfury), acetaldehyde (coconut water) and hexenal (green). Panelists rated the descriptor for each sample on an eleven-point scale in 0.5 increments from 0 to 5 (0 = not detectable, 1 = weak, 3 = moderate and 5 = strong). The relationship between the strength of each aroma-active compound and the concentration of each aroma-active compound is shown in Table 1.

Preparation of isolates for aromatic testing

Three frozen ripe Okrong mangoes picked at 120 DAF were peeled, the seed stone was removed and the flesh was cut into small pieces. A portion of cut mango (approximately 100 g) was added to saturated calcium chloride solution and the mixture was blended using a hand mixer (Philips; NL9206AD-4; the Netherlands). The mixture in a beaker was stirred sequentially using diethyl ether (once with 200 mL and then twice with 150 mL) at 7°C for 15 min per round. The mixture was passed through filter paper into a separatory funnel. The diethyl ether layer was collected and dried over sodium sulfate anhydrous, while the volatile substances from the diethyl ether were extracted using solvent-assisted flavor evaporation (SAFE). The temperature of the apparatus controlled using a thermostat at 40°C and kept under a high vacuum (1×10^{-3} mbar). The sample was added dropwise during 40 min to an evaporation flask containing the solvent-assisted flavor evaporation device. After an additional 30 min, the vacuum was broken, and the distillate was thawed at room temperature. Then, the sample was concentrated to approximately 5 mL using a Vigreux column (60 cm × 3 cm; Pfaudler Normag Systems; Germany) and finally to 1 mL using a micro-distillation column (13 cm × 2 cm; Glasblasecrei Bahr; Germany). Preparation of aroma-active isolates was carried out separately using dichloromethane. Then, the aroma profiles of the isolates extracted using diethyl ether or dichloromethane were evaluated to determine which isolates most closely matched with the fresh ripe Okrong mango profile.

Screening aroma-active compounds using gas chromatography–olfactometry/flame ionization detection

A Shimadzu® Model GC-O 60-2030 AF with a Shimadzu® AOC-20i Plus auto-injection unit using the Shimadzu® LabSolutions software (all from Shimadzu Corporation; Japan) was equipped with a DB-5 analytical column (nonpolar and 5%-phenyl-methylpolysiloxane) or a DB-FFAP (polar and nitroterephthalic acid modified polyethylene glycol) capillary (each 30 m × 0.32 mm, 0.25 µm film thickness; Agilent; USA. Each sample (1 µL) was injected using the cold-on-column technique at 30°C with helium as the carrier gas into uncoated, deactivated, fused silica capillaries (2 m × 0.32 mm; Agilent; USA) and connected with an I-type splitter to an analytical column. The linear velocity was set to 36.0 cm/s. After 3 min at 30°C, the oven temperature increased at 4°C/min to 250°C and held for 12 min. The effluent was split equally by volume at the end of the capillary using a Y-type splitter into two 50 cm uncoated, deactivated, fused-silica capillaries. One section was directed to flame ionization detection (FID) at 250°C and the other part to a heated sniffing port at 230°C. The sniffing port was mounted on the front of the FID instrument base and consisted of a custom-machined aluminum cylindrical cone (80 mm length × 25 mm base internal diameter) housing the capillary. During the GC-O/FID analysis, the aroma of the effluent from the sniffing port was evaluated by a panelist. Once the aroma had been detected, the retention time, odor quality and odor strength were recorded. Linear retention indices (RIs) were calculated from the retention times of the aromas detected and adjacent *n*-alkane C_6-C_{30} based on y linear interpolation using the formula from Van den Dool and Dec Kratz (1963).

Comparative analysis of aroma extract dilution

The aroma-active isolates were diluted to obtain serial dilutions of 1:2, 1:4, 1:8,...$1:2^n$ and each dilution was analyzed using GC-O/FID with the DB-FFAP column and the conditions described above. Each aroma-active region was assigned an FD factor corresponding to the dilution factor of the highest diluted sample in which the aroma was detectable.

Fractionation of the mango aroma-active isolates

The aroma-active isolates prepared as described above were concentrated to 1 mL in diethyl ether using a micro-distillation column. First, the isolates were pretreated by adding *n*-hexane

(1 mL) and the mixture was distilled at 60°C to remove any diethyl ether. The distillation process was performed until no drop of diethyl ether was observed and the volume of isolates dissolved in *n*-hexane remained at 1 mL in a heart-shaped flask. The silica gel used for this fractionation was pretreated to remove residual ferric oxide. Briefly, 10 g of silica gel 60 (0.040−0.063 mm) were soaked in fuming HCl for 2 hr. The slurry silica gel in HCl was decanted and subsequently washed with distilled water until the silica gel was neutral to litmus paper. The treated silica gel was dried in the oven at 200°C and kept in a desiccator until use. The aroma-active isolates in hexane were loaded onto a glass column (20 cm length by 1 cm diameter) packed with the pretreated silica gel. Elution was performed using pentane (50 mL; fraction A) followed by a series of pentane-to-diethyl ether ratios (90:10, fraction B; 70:30, fraction C; and 50:50, fraction D; each 50 mL) and finally diethyl ether (fraction E; 50 mL). Then, each fraction was concentrated to a final volume of 1 mL using a microdistillator (Glasblasecrei Bahr; Germany). The aroma-active compounds detected during elution using Comparative analysis of Aroma Extract Dilution Analysis (cAEDA) were localized into fractions A–E using GC-O/FID and the mass spectra were recorded using GC×GC-TOFMS.

Comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry

GC×GC-TOFMS (LECO Pegasus® 4D; Germany) was used, with the nonpolar × polar column combination consisting of the 1st column being a fused silica Rxi-5Sil MS capillary (30m length and 0.25 mm diameter, with 0.25 µM film thickness; Restek; USA), with the 2nd column being an Rxi-17Sil MS capillary (10 m length and 0.15 mm diameter, with 0.15 µM film thickness; Restek; USA). Each sample (1 µL) was injected into the split mode with a split ratio of 101:1. The conditions were as for GC-O/FID. The volatile compounds were determined based on comparison with the mass spectrum from the NIST MS Search library (National Institute of Standard and Technology; USA).

Identification of aroma-active compounds based on confirmation with reference compounds of matching linear retention index, odor quality and flavor dilution factor

The candidate aroma-active compounds were tentatively screened by matching RI, odor quality (based on GC-O/FID) and the mass spectral data (based on GC×GC-TOFMS) using the Leibniz-LSB@TUM Odorant Database, Version 1.2 (Kreissl et al., 2022). The potential compounds with FD factors ≥4 were identified by comparing the retention time and odor quality with those of reference compounds. The identity of aroma-active compounds was verified using the following procedure. Briefly, initially, the aroma-active isolates were injected for analysis using GC-O/FID equipped with the DB-FFAP and DB-5 columns, followed by the injection of reference compounds diluted in diethyl ether (three concentrations: 10 ppm, 100 ppm and 1,000 ppm). The isolates and reference compounds with very similar retention times and odor quality values were considered identical compounds.

Ethics statement

This study was approved by The Research Ethics Review Committee for Research Involving Human Research Participants Group I of Chulalongkorn University, Thailand (Approval no.050/66).

Results and Discussion

Acceptability testing of Okrong, Khiew Sawoey and Kaew mango cultivar samples

Although Thailand has more than 100 native mango cultivars, the three tested cultivars (Okrong, Kaew and Khiew Sawoey) are currently the most popular and are readily available in markets (De la Cruz Medina and Garcia, 2003). An acceptability test was first conducted to evaluate the three mango cultivars for overall aroma and overall flavor (Fig. 2). Clearly, Okrong received the highest acceptability scores (5.44 and 5.58) for overall aroma and overall flavor, respectively. The acceptability score of Okrong in terms of overall aroma was significantly different from those of Kaew and Khiew Sawoey. These observations prompted the subsequent investigation of the attributes of mango aroma based on their olfactory profiles.

In addition, Okrong and Khiew Sawoey ranked top in overall taste. However, Okrong received lower acceptability scores (4.36 and 4.29) in terms of overall liking and overall appearance, respectively (Fig. 10S).

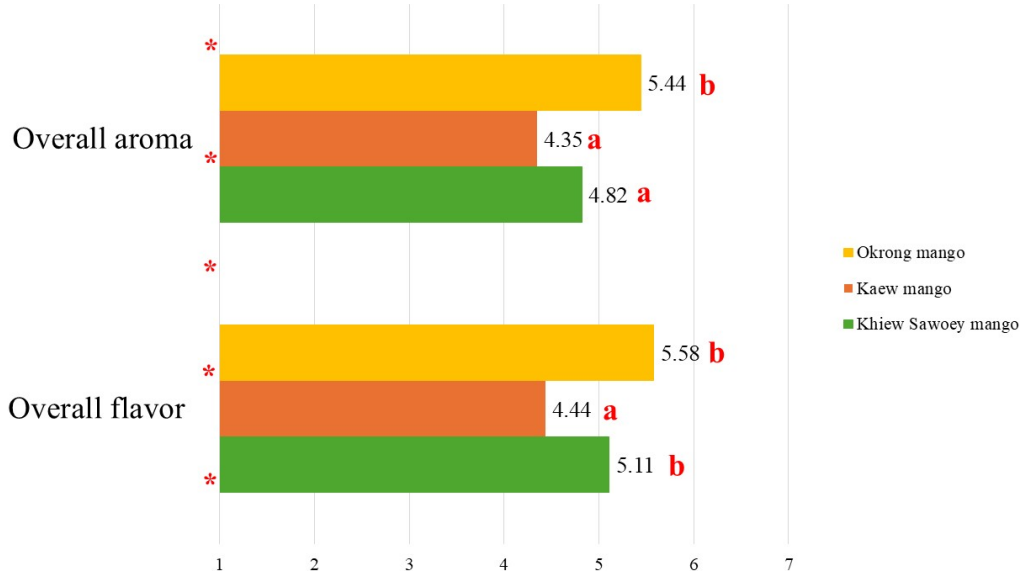

Fig. 2 Acceptability scores for overall aroma and overall flavor based on rating descriptors using a seven-point hedonic scale (1 = dislike extremely, 4 = neither like nor dislike and 7 = like extremely). Significant differences between cultivars ($p < 0.05$) are indicated by different lowercase letters, with * denoting significance according to analysis of variance at $p < 0.05$.

Olfactory profiling

Freshly cut pieces of the ripened fruits of Okrong, Kaew and Khiew Sawoey mangoes were evaluated by nine trained sensory panelists (3 males, 6 females, age range 23-45 yr), using free-choice and olfactory profiling, respectively (Fig. 3). Eight attributes (caramel/sweet-like, fruity, sulfury, floral, terpeny, sweaty, coconut water and green) were recognized by the panelists as contributing to the aroma of each of the three mango cultivars.

The olfactory profile of Okrong was dominated by a strong caramel/sweet-like, fruity and sulfury aroma with intensity scores of 4.91, 4.00 and 3.78, respectively. A hint of floral, terpeny, sweaty, coconut water and green aroma characteristics were also detected, although to a lesser extent. The olfactory profile of Khiew Sawoey mangoes was dominated by moderate levels of coconut water and green aroma. A hint of caramel/sweet-like, fruity, sulfury, floral, terpeny and sweaty aroma characteristics were also detected, although to a lesser extent. The olfactory profile of Kaew mangoes was dominated by moderate levels of terpeny and sweaty aroma. A hint of caramel/sweet-like, fruity, sulfury, floral, coconut water and green aroma characteristics were also detected, although to a lesser extent.

According to the acceptability test and olfactory profile, the aroma of Okrong was strikingly dominant by three attributes, caramel/sweet-like, fruity and sulfury aroma together with five hint attributes. To further identify aroma-active compounds contributing to Okrong, the sensomics approach was applied.

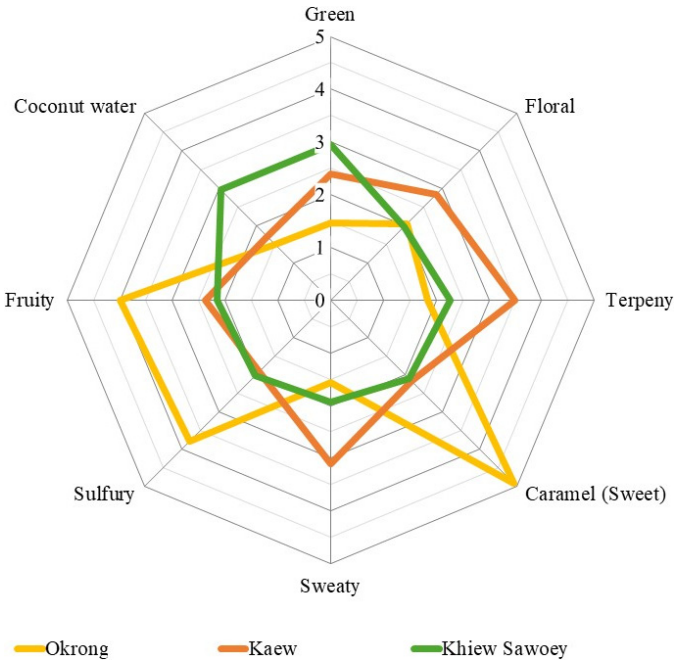

Fig. 3 Olfactory profiles of fresh ripe Okrong, Khiew Sawoey and Kaew mango fruit, where panelists rated intensity of each attribute on an eleven-point scale in 0.5 increments from 0 to 5, with 0 = not detectable, 1 = weak, 3 = moderate and 5 = strong.

Screening of Okrong aroma-active compounds and their identification

A portion of cut mango (approximately 100 g) was added to saturated calcium chloride solution (75 mL) and immediately extracted using diethyl ether. After the water had been removed, approximately 380 mL of diethyl ether extract was obtained. Nonvolatile compounds were removed from the extracts using SAFE and the distillates were concentrated. The complete experiment was conducted throughout in a chilling bath (7°C) to avoid artifact formation and compound degradation during extraction. After that, the volatile components had been collected using SAFE (approximately 330 mL) and concentrated using the Vigreux column (approximately 210 min to produce 5 mL of concentrates) and the micro-distillation column (approximately 15 min to produce 1 mL of final volume). The aroma-active isolates extracted using dichloromethane were prepared using the protocol mentioned above. A comparison of the typical aroma characteristics of ripened Okrong with aroma-active isolates separately extracted using diethyl ether or dichloromethane suggested that the isolates prepared using diethyl ether represented the characteristic aroma of Okrong. Therefore, the isolates extracted based on diethyl ether were further analyzed using GC-O/FID.

In total, 82 aroma-active compounds were detected together with different retention indexes and odor quality. After applying the cAEDA methodology, 43 aroma-active compounds were identified, with FD factors in the range from 4 to ≥2,048 (Table 2). Their RIs, odor characteristics and mass spectra were compared to those obtained from reference compounds to classify them. To separate aroma-active compounds from coeluted components and to aid in their identification using GC×GC-TOFMS, the aroma-active isolates were fractionated over silica gel to produce five fractions (A-E). Subsequently, all fractions were analyzed using GC-O/FID to localize the aroma-active compounds and then using GC×GC-TOFMS to obtain their mass spectra. This procedure allowed for the structural assignment of 43 aroma-active compounds (Fig. 4). However, odorants 4, 5 and 26 were only tentatively identified, even though they produced a single peak on the GC chromatogram. Comparison with reference compounds could not resolve this ambiguous identification due to their nearly identical characteristics based on their RI, odor quality and MS data.

Table 2 Aroma-active compounds with flavor dilution (FD; factor ≥4) in solvent-assisted flavor evaporation distillates obtained from Okrong mango cultivar.

No.*	Aroma-active compound†	Odor quality‡	RI§		FD factor	Fraction¶	Ref#
			FFAP	DB-5			
1	Methyl acetate	Fruity	639	603	256	B	Heinz and Peter (1985)
2	Ethyl acetate	Fruity	907	631	1024	C	Hunter et al. (1974)
3	2,3-Butanedione	Buttery	958	614	16	A, B	Ackerman and Torline (1984)
4	Ethyl 2-methylbutanoate††	Fruity	1032	849	64	E	Heinz and Peter (1985)
	Ethyl 3-methylbutanoate††		1042	852			Engel and Tressl (1983)
5	β-Pinene**,††	Terpeny	1106	973	32	D	Engel and Tressl (1983)
	Sabinene**,††						Heinz and Peter (1985)
6	3-Methylbutyl acetate	Sweaty	1114	878	32	B, C	Engel and Tressl (1983)
7	Myrcene	Terpeny	1142	991	8	B	Hunter et al. (1974)
8	1,8-cineole (eucalyptol)	eucalyptus-like	1172	1036	8	A	Farag et al. (2022)
9	Limonene	Citrus	1178	1030	4	B, C	Hunter et al. (1974)
10	3-Methyl-1-butanol	Fatty	1196	739	32	D	Hunter et al. (1974)
11	p-cymene	Terpeny	1237	1026	8	C, E	MacLeod and de Troconis (1982)
12	(E)-β-ocimene**	Fruity	1253	1132	8	B, C	Hunter et al. (1974)
13	Acetoin (hydroxy-2-butanone)	Buttery	1260	710	64	C	Hunter et al. (1974)
14	(E)-2-heptenal	Terpeny	1296	958	8	E	RI, odor quality, ms
15	1-octen-3-oneh	Musty/honey	1304	979	4	C	Ansari et al. (1999)
16	Dimethyl trisulfide	Onion	1340	969	512	B	Yukawa et al. (1994)
17	p-Mentha-1,3,8-triene**	Terpeny	1379	1132	16	A	Quijano et al. (2007)
18	Ethyl octanoate	Fruity/pungent	1414	1200	16	B, C	Engel and Tressl (1983)
19	Acetic acid	Vinegar-like	1417	645	32	A	Hunter et al. (1974)

Table 2 Continued

No.*	Aroma-active compound†	Odor quality‡	RI§		FD factor	Fraction¶	Ref#
			FFAP	DB-5			
20	3-(Methylthio)propanal (methional)	Seasoning	1425	904	256	A, B	Munafo et al. (2014)
21	Isocaryophyllene**	Sweet	1551	1397	1024	E	Koulibaly et al. (1992)
22	β-Caryophyllene	Cucumber	1550	1433	32	A, B	MacLeod and de Troconis (1982)
23	4-Methoxy-2,5-dimethyl-3(2H)-furanone (MDMF)	Caramel-like	1565	1067	≥2048	C	Hunter et al. (1974)
24	Butanoic acid	Sweaty	1603	830	32	C	Idstein et al. (1985)
25	γ-Butyrolactone**	Sweaty	1631	894	16	C	Hunter et al. (1974)
26	2-Methylbutanoic acid††	Sweaty	1643	889	4	C	Idstein et al. (1985)
	3-Methylbutanoic acid††		1637	883			Idstein et al. (1985)
27	Pentanoic acid	Sweaty, fruity	1706	933	4	B, C	Idstein et al. (1985)
28	Methyl salicylate**	Petroleum	1777	1195	8	B	Wang (2006)
29	2-Methoxyphenol	Smoky, gammon-like	1834	1092	8	B	Adedeji et al. (1992)
30	Hexanoic acid	Sweaty	1847	1011	32	A	Idstein et al. (1985)
31	Benzyl alcohol	Coconut	1855	1038	128	C	Adedeji et al. (1992)
32	(E)-β-ionone	Fruity	1935	1497	4	C, E	Hunter et al. (1974)
33	δ-Octalactone	Coconut	1937	1284	4	B	Hunter et al. (1974)
34	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)	Caramel-like	2024	1074	≥2048	C	Hunter et al. (1974)
35	2-Ethyl-4-hydroxy-5-methylfurane-3(2H)-one (ethylfuraneol)	Sweet	2066	1168	16	C	RI, odor quality, ms
36	γ-Decalactone	Waxy	2165	1488	128	B	Hunter et al. (1974)
37	2'-Aminoacetophenone	Cocoa	2192	1308	32	C, D	Munafo et al. (2014)
38	Dodecanoic acid	Fruity	2471	-	8	D	Idstein et al. (1985)
39	4-Hydroxy-3-methoxybenzaldehyde (vanillin)	Vanilla	2559	1407	4	C	Munafo et al. (2014)
40	4-Acetyl-2-methoxyphenol	Roasted-nutty	2628	-	4	B, C	RI, odor quality, ms

FD = flavor dilution factor; RI = linear retention index

* = aroma-active compounds numbered in first column according to retention time on the polar and nitroterephthalic acid modified polyethylene glycol capillary (FFAP) column.

† = identified by comparing retention indices on FFAP and analytical column (nonpolar and 5%-phenyl-methylpolysiloxane) DB-5 columns and mass spectra, as well as aroma quality and intensity, with data obtained from authentic reference standards analyzed in parallel.

‡ = odor quality, as perceived during gas chromatography–olfactometry/flame ionization detection (GC-O/FID).

¶ = fraction(s) in which aroma-active compounds were detected using GC-O/FID after fractionation.

= reference providing first report of compound as a mango volatile.

** = reference compound was not available, so structure was tentatively assigned by comparing retention indices, mass spectra and aroma characteristics with literature data.

†† = Aroma-active compounds not separated on either GC column.

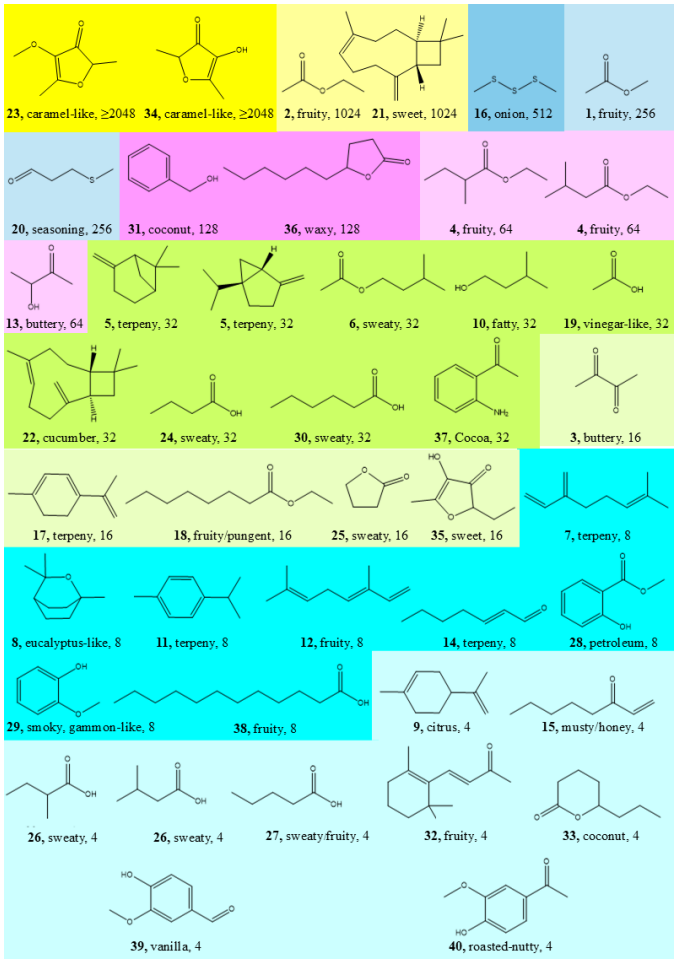

Fig. 4 Structures of aroma-active compounds with flavor dilution (FD) factor ≥4 from Okrong mango analysis, where bold number refers to order of compound (Table 2) followed by odor quality and finally FD factor with different background colors

Aroma-active compounds contributing to 8 attributes detected in Okrong mango were grouped and discussed.

Caramel and sweet aroma: This attribute played a critical role in Okrong aroma since it had the highest intensity score (4.91) in the olfactory profile. Aroma-active compounds categorized in this attribute were: 4-methoxy-2,5-dimethyl-3(2H)-furanone (MDMF, 23, FD≥2,048), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF, 34, FD≥2,048), isocaryophyllene (21, FD 1,024), 2-ethyl-4-hydroxy-5-methylfurane-3(2H)-one (ethylfuraneol, 35, FD 16) and 4-hydroxy-3-methoxybenzaldehyde (vanillin, 39, FD 4). MDMF (23) and HDMF (34) have been reported in several mango cultivars, in which the latter was detected with very high FD values (≥2,048). Subsequent investigations of the Haden mango indicated that HDMF (34) is one of the key odorants and is an inevitable biomarker for the quality control of mangoes (Munafo et al., 2016). Notably, MDMF (23) was detected in Okrong with a high FD factor≥2,048 while it was detected with lower FD factors (4-128) in the other cultivars. The occurrence of both MDMF (23) and HDMF (34) with high FD factors possibly resulted in a more striking sweet attribute in the mango cultivars reported so far. Isocaryophyllene (21) was a potential aroma-active compound found not only in Okrong mango but also in seven different Indian mangoes, being particularly abundant in the Amrapali cultivar collected in Kolkata during April–May 2013 (Ipsita and Bratati, 2019). Further study in metabolomics suggested that isocaryophyllene (21) ranked as a top influential biomarker to distinguish Indian mango cultivars (Ipsita and Bratati, 2019). The abundance of isocaryophyllene (21) in Okrong and Indian mangoes may also be applied as a biomarker to distinguish mango cultivars collected from tropical habitats in South and Southeast Asia (Pradhan and De, 2019) and the Florida (Munafo et al., 2014). In addition to MDMF (23) and HDMF (34), ethylfuraneol (35) was a closely related furanone derivative, with the current being the first to report it in mango. Despite the relatively lower odor threshold of ethylfuraneol (35), the much lower FD factor suggested that its natural concentration was approximately 64-times diluted than compounds 23 and 34. In addition, vanillin (39) was identified with a low FD factor (4). Notably, vanillin (39) was also detect in Florida mangoes, whose extraction method involved SAFE.

Fruity aroma: This attribute was the second strongest aroma, with an intensity score of 4.00. It mainly comprised esters consisting of ethyl acetate (2, FD 1,024), methyl acetate (1, FD 256), ethyl 2-methylbutanoate (4, FD 64), ethyl 3-methylbutanoate (4, FD 64) and ethyl octanoate (18, FD 16), along with the terpenes, (*E*)-β-ocimene (12, FD 8) and (*E*)-β-ionone (32, FD 4) and the carboxylic acid, dodecanoic acid (38, FD 8). Okrong contained smaller esters with lower FD factors than those of Haden mango, whose fruity attribute was more dominant than caramel (Munafo et al., 2014).

Sulfury aroma: This attribute ranked as the third most intense aroma (intensity score 3.78) in Okrong, with aroma-active compounds consisting of dimethyl trisulfide (16, FD 512) and 3-(methylthio)propanal—trivially known as methional (20, FD 256). Although only two sulfur-containing compounds were detected, the intensity score for sulfury aroma was comparable to that of fruity aroma. This finding was possibly due to their very low odor threshold compared with those of fruity esters.

Other minor attributes with intensity scores in the range 1.47−2.04 were floral, terpeny, sweaty, coconut water and

green aroma. The aroma-active compounds responsible for these attributes are listed in Table 2. In addition, there were 12 aroma-active compounds with FD factors of 4−128, such as 3-methyl-1-butanol, that were not categorized into the above-mentioned attributes.

In conclusion, the current study successfully produced the first published characterization of aroma-active compounds in Okrong mango using a sensomics approach. The olfactory profile provided the initial vital guide in this exploration that deduced a caramel/sweet-like aroma as the most characteristic attribute. The combination of GC-O/FID, GC×GC-TOFMS and the cAEDA methodology assisted in identifying MDMF (23) and HDMF (34) as potential aroma-active compounds. However, further study should focus on applying static headspace GC-O/FID as a complementary technique to cover up highly volatile odorants contributing to a coconut water aroma detected during olfactory evaluation. More importantly, exploring key odorants from the aroma-active compound pool using stable isotope dilution assay, odor activity value, aroma reconstitution and omission testing would lead to a more complete construction of the Okrong aroma model.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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