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# American Journal of Essential Oils and Natural Products

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Journal of  
Essential  
Oils and  
Natural  
Products

ISSN: 2321-9114  
AJEONP 2018; 6(2): 29-36  
© 2018 AkiNik Publications  
Received: 20-02-2018  
Accepted: 26-03-2018

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## Volatile aroma chemical constituents of fruit pulp of some Kenyan varieties of mango (*Mangifera indica* L.)

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### Abstract

Volatiles are key constituents of mango fruit aroma and vital in defining fruit quality besides influencing consumer preferences. In this paper, volatiles of eight Kenyan mango varieties: Ngowe, Apple, Keitt, Boribo, Tommy Atkins, Van Dyke, Sabre and Kent were collected on porapak Q adsorbent filters (mesh 40-80 $\mu$ m) using air entrainment kit and analyzed by Gas Chromatography (GC) and GC coupled to a Mass Spectrometer. Fifty-eight compounds, representing monoterpenes (66.1%), esters (19.7%), sesquiterpenes (4.8%), non-terpenoid hydrocarbons (3.1%), and oxygenated compounds (ethers, alcohols, acids and carbonyls) (6.3%), were identified. Some quantitative differences were observed in the distribution of monoterpenes in the mangoes with three distinguishable aroma groups being noted. The first was rich in  $\alpha$ -pinene and present in all the eight varieties, with significant amounts in Ngowe (10.93%), Apple (34.92%) and Van Dyke (7.42%). The second group, rich in myrcene, included Ngowe (22.74), Apple (24.36%) and Boribo (57.04%). The third was rich in  $\delta$ -3-carene and comprised Keitt (25.76%), Tommy Atkins (27.07%), Van Dyke (25.6%), Sabre (30.31%) and Kent (35.0%). (*E*)-Caryophyllene was identified in all varieties while ethyl butanoate was present in all except Sabre and Kent. Ethyl butanoate,  $\alpha$ -pinene, myrcene, and  $\delta$ -3-carene have also been found as dominant aroma volatiles in different mango varieties globally. Moreover,  $\delta$ -3-carene, which is a major compound in Keitt, Tommy Atkins and Kent in this study, also dominates the same varieties in other parts of the world. The qualitative and quantitative variations in aroma volatile composition of the mango varieties explain the differences in their flavors.

**Keywords:** Mango, fruit, volatiles, aroma

### 1. Introduction

Mango (*Mangifera indica* L.) is considered as one of the most important commercial crops worldwide in terms of production, marketing and consumption<sup>[1]</sup>. Ripe mango fruit is one of the most popular and widely sold tropical fruits in the world. It is good source of dietary antioxidants, such as ascorbic acid, carotenoids and phenolic compounds<sup>[2, 3]</sup>, which are beneficial to health due to their antioxidant capacity<sup>[4]</sup>. Its applications as fruit include stewed fruits, fruit jams, juices and cakes. In medicine the aqueous bark extract of mango, "Vimang" has been as a nutraceutical in Cuba<sup>[5, 6]</sup>. In addition, mango leaves are used to manage and treat a number of medical conditions including diabetes mellitus, cough, diarrhea and hemorrhoids<sup>[7]</sup>.

Chemical analysis of the aroma volatiles of several mango fruit cultivars reveal the presence of a wide range of compounds including esters, lactones, monoterpenes and sesquiterpenes. Monoterpene hydrocarbons such as  $\delta$ -3-carene, *cis*-ocimene,  $\alpha$ -pinene and  $\beta$ -pinene, myrcene and limonene have been reported as key contributors to the aroma and flavor of the fresh mango fruit, depending on the variety<sup>[8-15]</sup>.

The aroma volatile composition is one of the significant and decisive parameters of quality in the customer selection of a product. Aroma compounds are present in raw foods in free volatile form and also as non-volatile precursors such as substituted cysteine sulfoxides, thioglycosides, glycosides, carotenoids and cinnamic acid derivatives<sup>[16]</sup>. The different proportions of volatile components and the presence or absence of trace components often determine aroma properties<sup>[17]</sup>. The volatile compounds that are involved in fruit flavor are produced through metabolic pathways during ripening, harvesting and post-harvest storage, and depend on many factors related to the species, variety and type of technological treatments<sup>[18]</sup>.

In this work, we present the aroma volatile composition of the mango fruit juice extracts from eight mango varieties; Ngowe, Apple, Keit, Boribo, Tommy Atkins, Van Dyke, Sabre and

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Kent grown in Kenya. Prior to this work, there have been no phytochemical investigations of aroma volatile composition of the juice extracts of these varieties of Mango plant in Kenya, and this is the first report on their volatile aroma chemistry.

## 2. Materials and methods

### 2.1 Fruits and other materials

Fresh ripe mango fruits were obtained from mango farms at Cheptebo, Kerio Valley in Kenya. The fruits at ripe stage, which were classified as those possessing totally orange–yellow color, were transported to the laboratory in small cardboard boxes. Fruits free from any apparent skin damage were selected for volatile collection, extraction and analysis.

### 2.2 Volatiles Collection

The fruits were washed with distilled water and wiped with a dry cotton cloth to dry them. Their skin and kernel were separated manually by using a stainless steel knife, and 200 g of each macerated fruit pulp was placed into a glass container (20 × 10 cm) and sealed with a Teflon tape. The collection of volatile components was undertaken on different Soxhlet cleaned adsorbent glass tube filters using an air entrainment kit for one hour<sup>[19]</sup>. The glass filter tubes contained porapak Q polymer (15mg), which was used to adsorb the aroma volatile constituents of the mango fruit pulp<sup>[20]</sup>. After collection, the tubes were heat-sealed in glass ampoules and stored under refrigeration conditions until when required for analysis. The volatiles were extracted from the porapak filters by elution with GC grade dichloromethane whereby 100- $\mu$ m samples were collected and kept under ice-cold conditions for chemical characterization.

### 2.3 Chemical analysis

Qualitative and quantitative analysis of the volatiles was performed using a gas chromatograph with a flame ionization detector (GC-FID) and gas chromatograph coupled to an organic mass spectrometer (GC-MS). The GC-FID analysis was carried out on a GC HP-5890 II apparatus, equipped with splitless injector, attached to HP-5MS column (25 m × 0.32 mm, 0.52 mm film thickness) and fitted to FID. Carrier gas flow rate (He) was 1 mL/min, split ratio 1:30, injector temperature was 250 °C, detector temperature 270°C, while column temperature was linearly programmed from 40–240°C (at rate of 5°C/min). The same analytical conditions were employed for GC/MS analysis, where HP G1800C Series II GCD system equipped with HP-5MS column (30 m × 0.25 mm, 0.25 mm film thickness) was used. Transfer line was heated at 270°C. Mass spectra were acquired in EI mode (70 eV), in *m/z* range 40–400 a.m.u, scan time 1.5 s with the filament solvent delay time set at 3 min.

### 2.4 Chemical identification of the volatiles

Identification of volatile compounds was carried out by matching compounds' mass spectra and linear retention indexes (LRIs) with those of authentic standards in addition to NIST library and Kovats index databases,<sup>[21, 22]</sup> using a computer search and literature.<sup>[23, 25]</sup> For the purpose of quantitative analysis, area percent data obtained by GC-FID were used as the base together with the area of the internal standard (*n*-heptanol) of known concentration, which got added to the volatiles eluted from the glass filter tubes.

## 3. Results and discussion

The results of chemical analyses of *M. indica* fruit volatiles, is shown in the GC-MS total ion chromatograms (Figures 1-8) and Table 1.

**Table 1:** Fruit volatile chemical constituents of the mango varieties

Peak	RI	Volatile Compound	Ngowe	Apple	Keitt	Boribo	Tommy Atkins	Van Dyke	Sabre	Kent
1	713	3-Hydroxy-2-butanone	0.98	1.94	—	—	4.82	1.17	—	—
2	745	2,4,5-Trimethyl-1,3-dioxolane	—	—	—	—	0.34	—	—	—
3	782	Toluene	5.51	—	—	—	—	—	—	—
4	785	2,3-Butanediol	0.30	—	—	—	3.74	—	—	—
5	800	Ethyl butanoate	15.10	0.39	4.44	0.82	6.68	3.37	—	—
6	835	Ethyl ( <i>E</i> )-2-butenate	5.89	1.05	3.42	2.36	2.17	4.88	—	—
7	871	Ethylbenzene	—	—	—	—	0.23	—	0.85	1.10
8	874	<i>p</i> -Xylene	—	—	—	—	—	—	0.74	1.28
9	888	1,3-Dimethylbenzene	—	—	—	—	—	—	—	0.30
10	926	Methyl hexanoate	8.66	0.09	0.72	0.49	1.56	1.26	—	—
11	932	$\alpha$ -Pinene	10.93	34.92	0.52	0.27	7.42	3.95	7.83	0.78
12	936	5-Methyl-3-heptanone	—	5.30	—	—	—	—	—	—
13	946	Camphene	0.76	1.23	—	—	0.50	—	0.34	0.38
14	958	Isobutyl butanoate	—	—	—	—	0.73	—	—	0.29
15	974	$\beta$ -Pinene	1.00	—	10.16	0.08	0.73	0.64	1.07	—
16	988	Myrcene	22.74	24.36	—	57.04	1.11	1.41	1.41	1.35
17	993	Butyl butanoate	—	—	—	—	1.90	2.03	—	—
18	997	Ethyl hexanoate	0.23	—	4.12	—	—	—	—	—
19	999	Unknown	—	—	—	—	—	—	0.33	0.55
20	1002	$\alpha$ -Phellandrene	—	0.51	—	—	0.22	—	0.28	0.31
21	1008	$\delta$ -3-Carene	0.40	—	25.76	—	27.07	25.60	30.31	35.00
22	1020	<i>o</i> -Cymene	0.16	—	—	—	0.73	0.49	0.47	0.52
23	1022	1-Methyl-3-(1-methylethyl)-benzene	—	0.94	—	—	—	—	—	—
24	1025	$\beta$ -Phellandrene	1.39	4.03	2.08	0.26	2.11	1.76	1.82	1.91
25	1032	( <i>Z</i> )- $\beta$ -Ocimene	—	—	—	0.77	—	—	—	—
26	1044	( <i>E</i> )- $\beta$ -Ocimene	0.24	—	—	0.47	—	—	—	—
27	1062	$\gamma$ -terpinene	—	0.26	—	—	0.43	—	—	—
28	1086	Terpinolene	—	—	—	—	1.00	0.63	1.21	0.68

29	1090	6,7-Epoxy myrcene	—	0.51	—	0.80	—	—	—	—
30	1130	<i>allo</i> -Ocimene	—	—	—	0.45	0.16	—	—	—
31	1141	Camphor	0.28	—	—	—	—	0.29	—	0.35
32	1164	4-Ethylbenzaldehyde	—	0.29	—	—	0.42	—	0.79	0.54
33	1166	<i>p</i> -Mentha-1,5-dien-8-ol	—	—	—	0.25	0.35	—	—	—
34	1166	Coahuilensol	—	—	—	—	—	—	1.43	—
35	1179	<i>p</i> -Cymen-8-ol	—	—	—	—	0.29	—	—	—
36	1190	Methyl salicylate	—	—	—	—	2.94	—	3.78±0.12	9.29
37	1196	Ethyl octanoate	0.31	0.29	13.42	0.35	—	—	—	—
38	1242	<i>p</i> -Ethylacetophenone	—	0.30	—	—	1.00	—	6.57	0.73
39	1260	5-Butyldihydro-2(3 <i>H</i> )-furanone,	—	0.30	—	—	—	—	—	—
40	1295	Methyl 2,4-dimethylbenzoate	—	—	—	—	—	—	0.77	—
41	1316	1-(2-Hydroxy-5-methylphenyl)-ethanone	—	—	—	—	—	—	1.27	—
42	1341	4'-Ethylpropiofenone	—	—	—	—	—	—	0.22	—
43	1345	$\alpha$ -Cubebene	—	0.64	—	—	—	—	—	—
44	1374	$\alpha$ -Copaene	0.41	—	—	0.84	0.36	0.53	0.40	—
45	1409	$\alpha$ -Gurjunene	—	—	—	0.58	—	—	—	—
46	1417	( <i>E</i> )-Caryophyllene	0.77	0.54	0.58	1.56	0.50	0.62	0.70	1.30
47	1423	Linalyl butanoate	—	0.30	—	—	—	—	—	—
48	1437	$\alpha$ -Guaiene	0.29	—	—	0.92	—	—	—	—
49	1444	<i>p</i> -Acetylacetophenone	—	—	—	—	0.36	—	1.06	—
50	1452	$\alpha$ -Humulene	0.42	—	—	0.90	0.35	0.37	0.71	0.67
51	1475	$\gamma$ -Gurjunene	—	—	—	1.62	—	0.56	—	—
52	1484	Germacrene D	—	—	0.37	3.13	—	—	0.48	—
53	1509	$\alpha$ -Bulnesene	—	—	—	1.13	—	—	0.43	0.43
54	1513	$\gamma$ -Cadinene	—	—	—	0.27	—	—	—	—
55	1528	<i>cis</i> -Calamenene	0.31	—	—	1.16	—	—	0.42	—
56	1577	Spathulenol	—	—	—	0.84	—	—	—	—
57	1582	Caryophyllene oxide	0.20	0.27	0.65	0.34	—	—	—	—
58	1688	$\alpha$ -Germacre-4(15),5,10(14)-trien-1 $\alpha$ -ol	—	—	—	0.34	—	—	—	—
59	2344	Butyl dodecyl succinate	—	—	—	—	0.51	—	—	—

A total of 58 volatile compounds were identified in the fruits of the eight mango varieties. One compound (peak 19) was unidentified. The main class of compounds (Table 2) was the monoterpenes which represented 66.1% of the total volatile components. The other compounds were esters (19.7%) sesquiterpenes (4.8%), non-terpenoid hydrocarbons (3.1%) and other oxygenated compounds (ethers, alcohols, and

carbonyls including carboxylic acids) (6.3%). Monoterpenes and esters were dominant in Ngowe, Keitt, Tommy Atkins, VanDyke and Kent varieties. Apple was dominated by monoterpenes and hydrocarbons while Boribo comprised mostly of monoterpenes and sesquiterpene, Sabre was dominated by monoterpenes and oxygenated compounds.

**Table 2:** Summary of Volatile Components

	NG	AP	KEI	BO	TA	VD	SB	KEN	TOTAL	%TOTAL
Monoterpenes Hydrocarbons	37.7	65.1	38.6	59.4	41	34.4	44.7	41	361.5	66.1
Sesquiterpene Hydrocarbons	2.2	1.4	1.7	12.1	1.2	2.1	3.1	2.4	26.2	4.8
Alcohols, Ethers epoxides	0.5	0.8	0.7	2.6	4.6	0	1.4	0	10.6	1.9
Aldehydes and Ketones	1.3	3	0	0	6.4	1.5	10	1.6	23.8	4.4
Esters	30.2	1.9	26.6	4	16.5	13.1	6	9.6	107.9	19.7
Non-terpenoid Hydrocarbons	5.5	6.5	0	0	0.6	0	1.6	2.7	16.9	3.1
Total									546.9	100

**Key:** NG-Ngowe; AP-Apple; KEI-Keitt; BO-Boribo; TA-Tommy Atkins; VD-Van Dyke; SB-Sabre; KEN-Kent

The variations in aroma volatile compounds of the mango depend on cultivars, the maturity stage of the fruit storage and conditions, and processing [26-30]. There were differences in quantitative distributions of monoterpenes in the eight varieties with three major aroma groups being identified.

The first group was rich in  $\alpha$ -pinene (peak 11) and was present in the volatiles of all the eight varieties, with Ngowe (10.93%), Apple (34.92%) and Van Dyke (7.42%) having significant amounts of the compound (Figures 1-3 & Table 1).

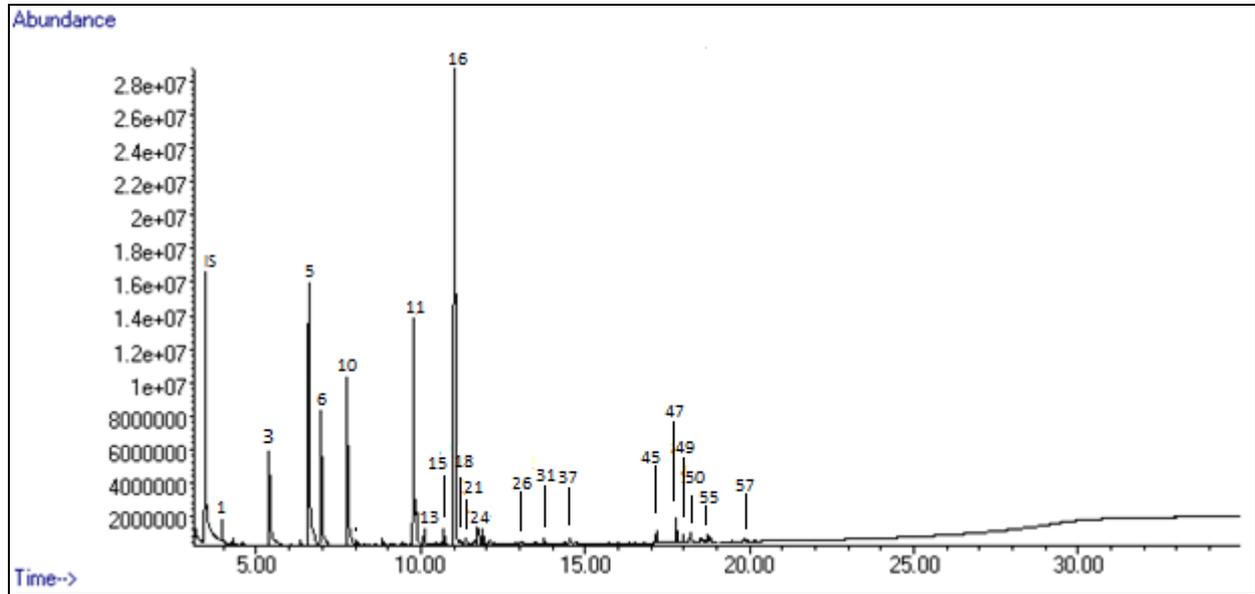


Fig 1: Representative total ion chromatogram of Ngowe variety

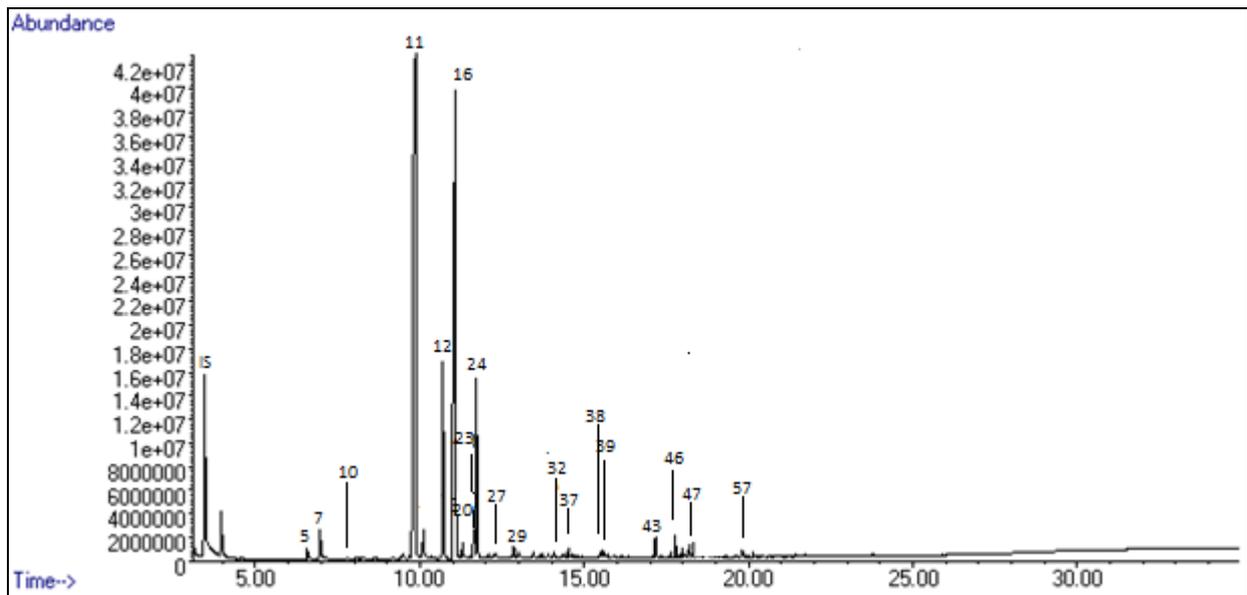


Fig 2: Representative total ion chromatogram of Apple variety

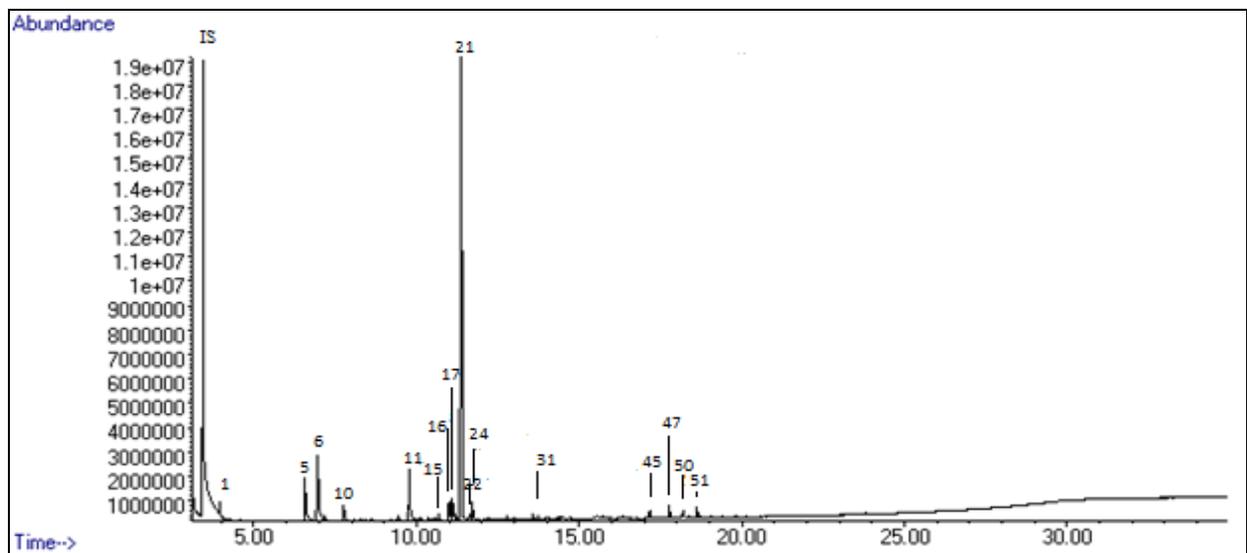


Fig 3: Representative total ion chromatogram of Van Dyke variety

The second group, rich in myrcene (peak 16), was found in Ngowe (22.74%), Apple (24.36%) and Boribo (57.04%) (Figures 1,2& 4; Table 1).

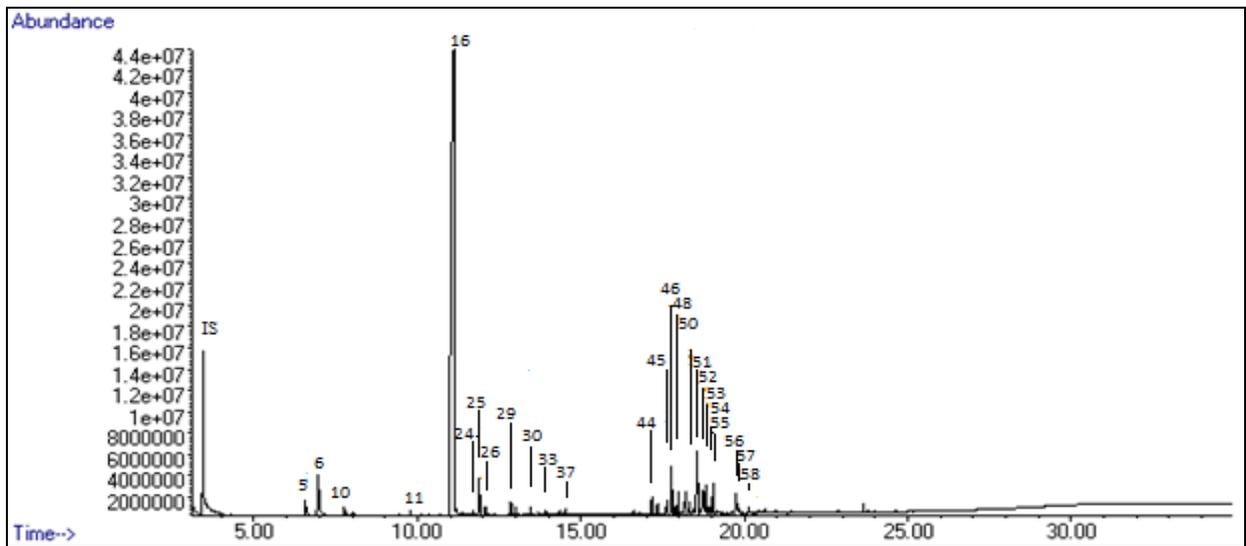


Fig 4: Representative total ion chromatogram of Boribo variety

The third group was rich  $\delta$ -3-carene (peak 21) and was observed in Keit (25.76%), Tommy Atkins (27.07%), Van Dyke (25.6%), Sabre (30.31%) and Kent (35.0%) (Figures 3, 5-8; Table 1).

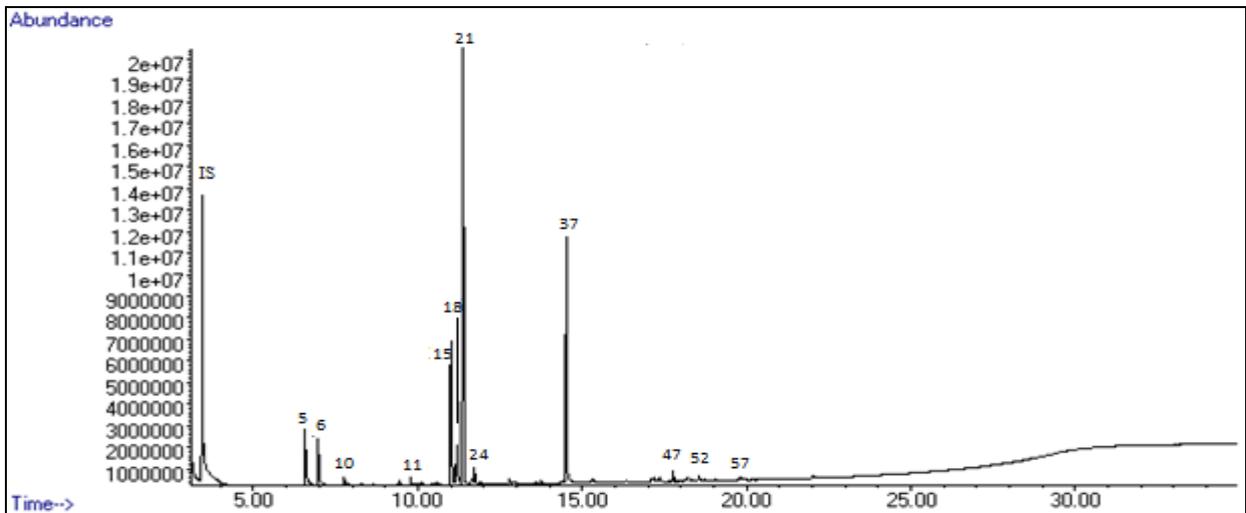


Fig 5: Representative total ion chromatogram of Keit variety

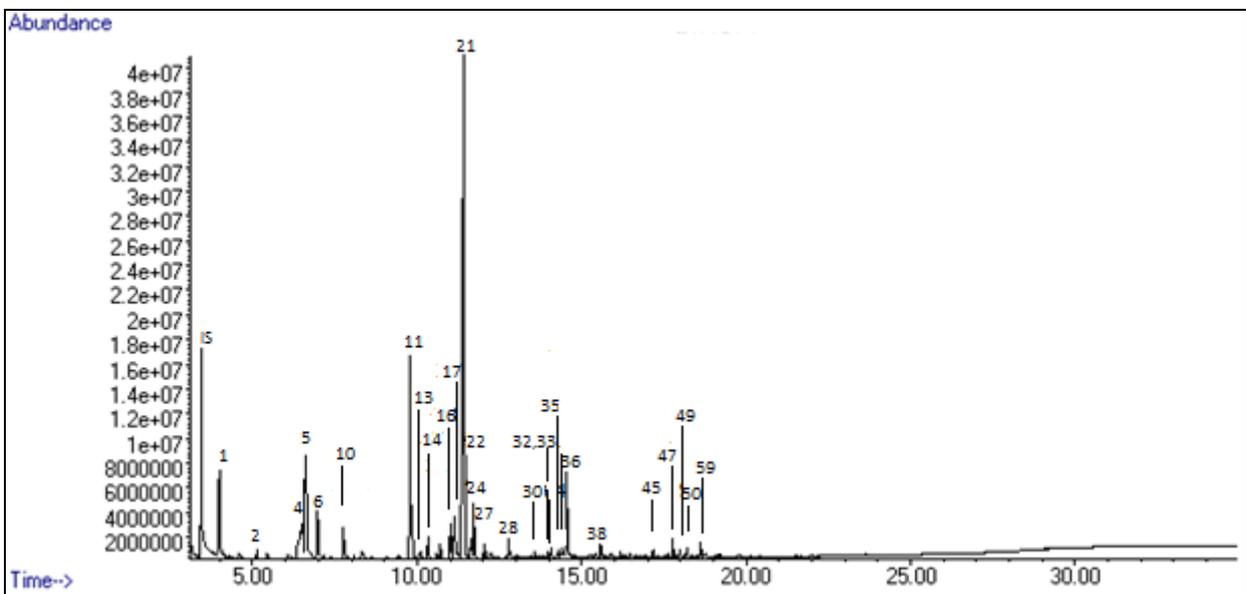


Fig 6: Representative total ion chromatogram of Tommy Atkins variety

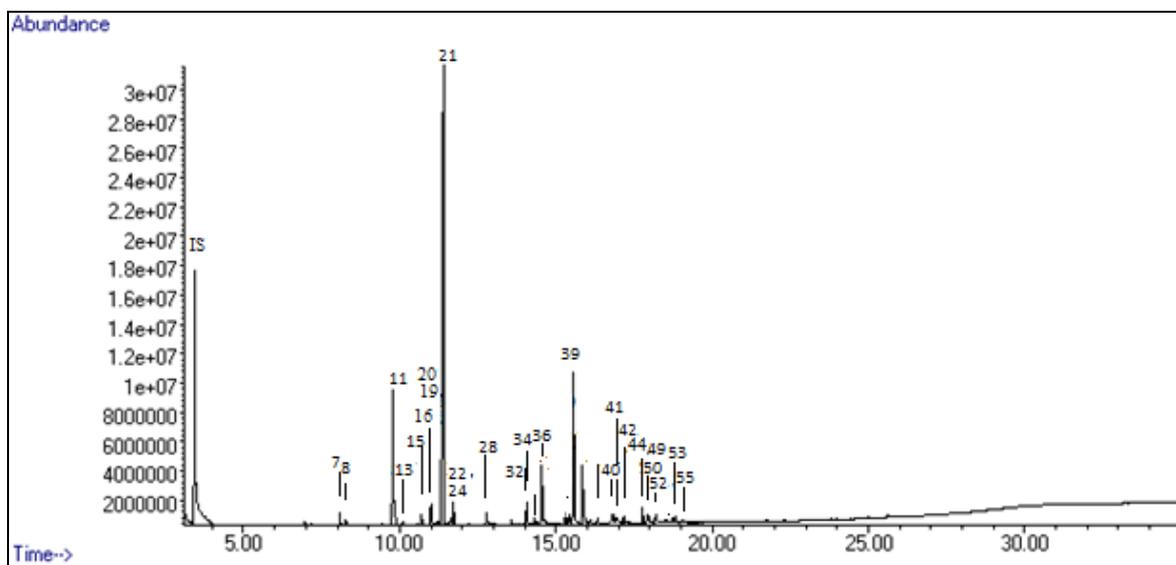


Fig 7: Representative total ion chromatogram of Sabre variety

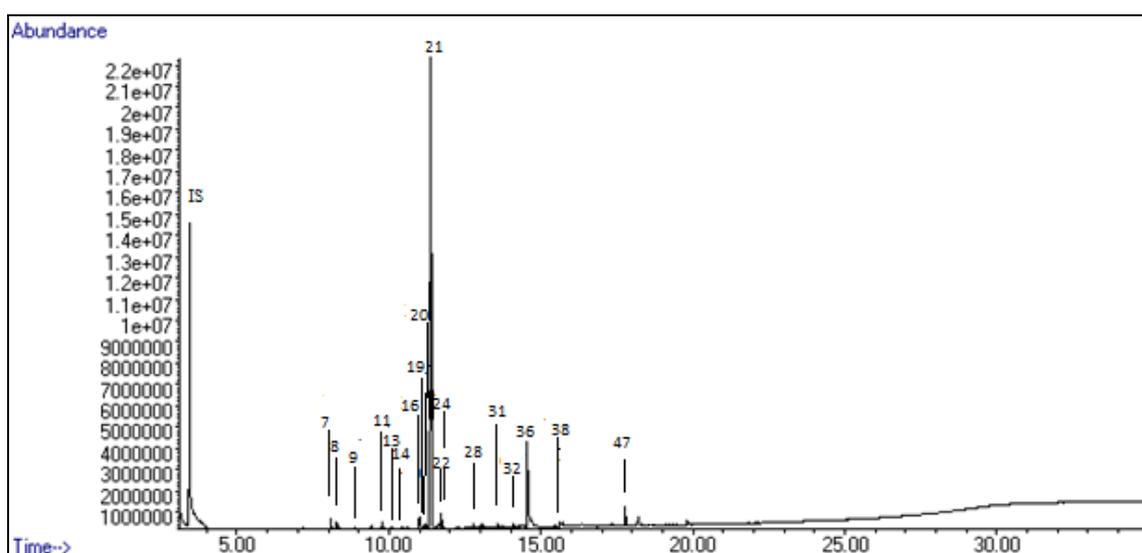


Fig 8: Representative total ion chromatogram of Kent variety

The three monoterpenes;  $\alpha$ -pinene, myrcene and  $\delta$ -3-carene have also been reported as dominant aroma volatiles in different mango varieties from other parts of the world. The Colombian varieties, Hilacha and Vallenato, were found to be rich in  $\alpha$ -pinene [31]. Myrcene was identified in Brazilian varieties namely, Cavalo, Rosa, Espada and Paulista; [32] and also in the Alphonso and Jalna varieties from India and Sri Lanka, respectively. [12, 33]. The  $\delta$ -3-carene was dominant in Haden, Irwin, Manila and Tommy Atkins varieties from Colombian [31] and a Venezuelan mango fruit. [34] Others were Tommy Atkins and Keitt varieties from Florida [35], M'Bingue, Tête de Chat and Palmer mangoes grown in Africa, [36] Haden, Rubi, Tommy Atkins and Keitt varieties from Brazil [32, 37] and 10 Cuban varieties, Haden, Manga amarilla, Macho, Manga blanca, San Diego, Manzano, Smith, Florida, Keitt and Kent [38].

These findings also show great similarities between the Kenyan Keitt, Tommy Atkins and Kent and the same varieties from the other parts of the world with respect to  $\delta$ -3-carene as a dominant compound. The Kenyan Keitt variety agrees with the Florida, Brazillian and Cuban Varieties while Tommy Atkins is in agreement with the same variety from Colombia, Florida and Brazil and Kent with the Cuban variety in terms of the aroma volatile components of the major monoterpenoid

compounds. The  $\delta$ -3-carene has a sweet, limonene-reminiscent odor [39] and considered as the most important aroma constituent, due to the high percentage in some volatile fractions and it is a major contributor to the ripe mango flavor [12, 32, 34].

Most of the dominant chemical compounds identified in this work have been previously reported to have activity against various species of insects. The  $\alpha$ -pinene is one of the compounds that have been reported to possess repellent properties against *Kilifia acuminata* (Signoret) (Hemiptera: Coccidae) [40], but attractive to *Temnochilachlorodia* [41]. A trap baited with a blend of myrcene,  $\alpha$ -pinene, and (*E*)- $\beta$ -ocimene has been used to capture male and female. *Anastrephaobliqua* (Macquart) (Diptera: Tephritidae). [42] Likewise  $\delta$ -3-carene has been reported to be attractive to *Epitragussallaei* (Champion), a beetle that feeds on the flower of *MangiferaindicavarAtaulfo*. [43]

Terpinolene, which is present in the Kenyan varieties Tommy Atkins, Van Dyke Sabre and Kent in amounts ranging between 0.6-1.2% (peak 28); has been reported in considerable quantity in Willard and Parrot varieties from Sri Lanka [12], Bowen, Kensington Pride and Florigon varieties from Australia [42] and in Brazilian Espada mango [37].

The terpene hydrocarbons are considered to be important

contributors to the flavor of Brazilian and Venezuelan mango varieties, <sup>[45]</sup> as well as Florida mango varieties, such as Keitt, Kent and Tommy Atkins <sup>[46]</sup> and 20 varieties of Cuban mangoes <sup>[9, 38]</sup>. The green aroma typical of mango was reported to be correlated to monoterpenes <sup>[37]</sup>. The hydrocarbons (*Z*)- and (*E*)- $\beta$ -ocimene have a warm, herbaceous and floral odor while myrcene and (*Z*)- $\beta$ -ocimene are major contributors to green mango flavor <sup>[34]</sup>.

Among the sesquiterpenes, (*E*)-caryophyllene (peak 46) was identified in all the varieties (0.5-1.6%), while germacrene D (peak 52) was present in Keit (0.37%), Boribo (3.13%) and Sabre (0.48%), and  $\alpha$ -humulene (peak 50) was present in Ngowe (0.42%), Boribo, Tommy Atkins, Van Dyke, Sabire and Kent (0.37-0.9%). (*E*)-Caryophyllene and  $\alpha$ -humulene have a wood-spicy odor and make a minor contribution to the odor of some mango cultivars <sup>[47]</sup>. Hilacha, Haden, Irwin, Manila, Tommy Atkins and Yulima varieties in Springfield mango have been reported to contain *trans*- $\alpha$ -bergamotene as a major compound while the Vallenato variety which was rich in  $\gamma$ -gurjunene <sup>[32]</sup>.

Esters, which formed the second dominant class after the monoterpenes, had a total of 10 compounds in the studied varieties, and formed 19.7% of the total volatile compounds. Among them, ethyl butanoate (peak 5) was present in all varieties except Apple, Sabre and Kent, with Ngowe (15.1%) having the highest amount. Large amounts of ethyl butanoate were observed in Colombian varieties<sup>[31]</sup>, Baladi mango from Egypt<sup>[36]</sup>, Kensington Pride grown in Australia <sup>[44]</sup> and Cuban varieties<sup>[9]</sup>. Ethyl butanoate was reported to be responsible for the fruity flavor of mango <sup>[38, 44]</sup>, and has been reported as among the compounds that attract females *Anastrepha striata* Schiner (Diptera: Tephritidae) in guava <sup>[48]</sup>.

Among the other oxygenated compounds, eight carbonyls were identified with a total contribution of 4.4% to the total volatile compounds (Table 2). 3-Hydroxy-2-butanone (peak 1) was identified in Ngowe, Apple, Tommy Atkins and Van Dyke in varying amounts of between 1% - 4.8%, while *p*-ethyl acetophenone ( peak 39) was in Apple, Tommy Atkins Sabre and Kent (0.3-6.6%). *p*-Acetylacetophenone (Peak 49) was found in Tommy Atkins (0.36%) and Sabre (1.06%). The rest of the carbonyls had less than 1% of the volatiles in all of the mango varieties in this study. Six alcohols were identified with 2,3-butanediol (3.74%) being the highest amount in Tommy Atkins. Considerable amounts of oxygenated volatile compounds; such as esters, furanones and lactones have also been identified in some Australian varieties <sup>[38]</sup>. The other differences between the volatile components are summarized in Table 1. Other than bringing out the qualitative and quantitative differences in chemical composition among the eight Kenyan mango varieties, this study has also been able to establish the Kenyan mango varieties that are closely related to the varieties from the other parts of the world that contain the same dominant volatile chemical compounds.

#### 4. Conclusions

There are significant variations in the aroma volatile compositions amongst ripe fruit pulps of the eight Kenyan mango varieties studied. The major compounds that dominate the volatile chemical composition of the eight mango varieties include the monoterpenes;  $\alpha$ -pinene, myrcene,  $\delta$ -3-carene and the ester, ethyl butanoate. The qualitative and quantitative differences in aroma chemical composition of the eight mango varieties in this study explains the differences in their flavor and general appeal to consumers in terms of preferences.

#### 5. Acknowledgement

We are grateful to National Commission for Science, Technology and Innovation (NACOSTI), Kenya, for funding this research. We also thank Mr. Francis K. Kiplagat, a mango farmer, for providing us with the samples and for his contribution in the identification of the mango varieties. The work was done at the Center for African Medicinal and Nutritional Flora and Fauna (CAMNNF) at Masinde Muliro University of Science and Technology (MMUST).

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