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Effect of 1-methylcyclopropene (1-MCP) on volatile compound production in papaya (*Carica papaya* L.) fruit

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Abstract
Volatile production in papaya fruit (Var. CO 8.) treated with 1-methylcyclopropene compared to untreated fruit under ambient condition were evaluated in the present study. The volatile production evaluation provide evidence that after the treatment of papaya fruit with 1-MCP the synthesis of aliphatic esters, saturated and unsaturated, derived from fatty acids metabolism are reduced as these are ethylene dependent processes.

Keywords: Volatile compound production, *Carica papaya* L.

Introduction
Fruits produce volatile compounds as indicators of fruit ripening. The volatile profiles of fruit are complex and vary depending on the cultivar, harvest stage, ripeness, pre-and post-harvest environmental conditions, fruit sample (either intact fruit, slices, or homogenized samples), and analytical methods utilized [1,2].
Fruit volatile compounds are mainly comprised of diverse classes of chemicals, including esters, alcohols, aldehydes, ketones, lactones, and terpenoids. However, some sulfur compounds, such as S-methyl thiobutanoate, 3-(methylthio) propanal, 2-(methylthio) ethyl acetate, 3-(methylthio) ethyl propanoate, and 3-(methylthio) propyl acetate, also contribute to the flavor of fruit such as melons (*Cucumis melo* L.) [3].
The volatile compounds emitted by the fruit are cultivar specific [4]. Although the volatile aroma compounds has been extensively investigated and identified in papaya fruit and in different varieties [5], the literature is lacking in quantitative data related to specific cultivars.
1-MCP blocks ethylene action and in practice delays ripening of climacteric fruit [6]. In addition, 1-MCP inhibits the production of aroma volatile compounds, particularly esters, in bananas, plums and apples [7; 8; 9; 10].
Hence, the present study was undertaken to evaluate the volatile production in papaya fruit (TNAU Var. CO.8.) treated with 1-methylcyclopropene compared to untreated fruit under ambient condition.

Experimental methods
1-MCP application and GCMS analysis
Papaya (TNAU var. CO.8) fruit harvested at color break stage were selected for uniformity, devoid of injury or diseases and separated into two batches for administration of the 1-MCP treatments. One batch was treated with 1-MCP at 900 ppb, while the other batch was left untreated. The fruit were pre-treated with 100 ppm ethylene to trigger the ethylene receptor in order to avoid rubbery texture as reported in previous studies [11]. 1-MCP (obtained from Agrofresh Inc., Mumbai) at 900 ppb concentration was applied to papaya fruit by vapourization in a closed container for a duration of 14 hours @ 14 °C, RH 90-95 per cent.
After 1-MCP vaporization, half of the fruit from each treatment box were loaded into crates and kept at ambient temperature of 27±2 °C and rest of the fruit were kept at cold storage 14 °C. Thus, the treated and untreated fruits were allowed to undergo normal ripening at the respective storage conditions. Volatile compounds were estimated in 1-MCP treated and control papaya fruit stored under ambient condition at 4th day of storage when significant morphological changes were observed. Since, the shelf life of ambient stored fruit could be extended only up to 7days, to study the significant difference between fruit at different storage conditions; the analysis was carried out on day 4.

100 g of papaya puree was extracted in 25 mL of diethyl ether until the pulp turns white. After centrifugation at 5000 rpm, the combined ether extracts were dried with anhydrous sodium sulfate and the solvent was removed by evaporation at 30 °C under reduced pressure in a rotary evaporator. The condenser was kept at 0-10 °C during this operation. The extracts obtained were concentrated to a final volume of 1 mL.

From this 20µL sample was siphoned and loaded into GCMS (Agilent GC/MSD). Capillary GC separation of volatile compounds was achieved by injecting 1µl of each sample into a KONIK 4000A gas chromatograph operated with a DB-1 (Chrompack) capillary column (30 m x0.25 mm x0.25 µm). Helium was used as a carrier gas at a flow rate of 1 ml/min. Injector temperature was 250 °C, detector temperature, 250 °C and the oven temperature programme featured an initial temperature of 65 °C (2 min hold), rising to 250 °C at 4 °C/min and held isothermal for 20 min. Quantitative data were calculated as area ratios of each compound/standard, from the areas measured electronically. Mass spectra were obtained using a Hewlett–Packard 5972 gas chromatograph-mass spectrometer. Identification of peaks was achieved by matching electron impact spectra (70 eV) to those of library spectra.

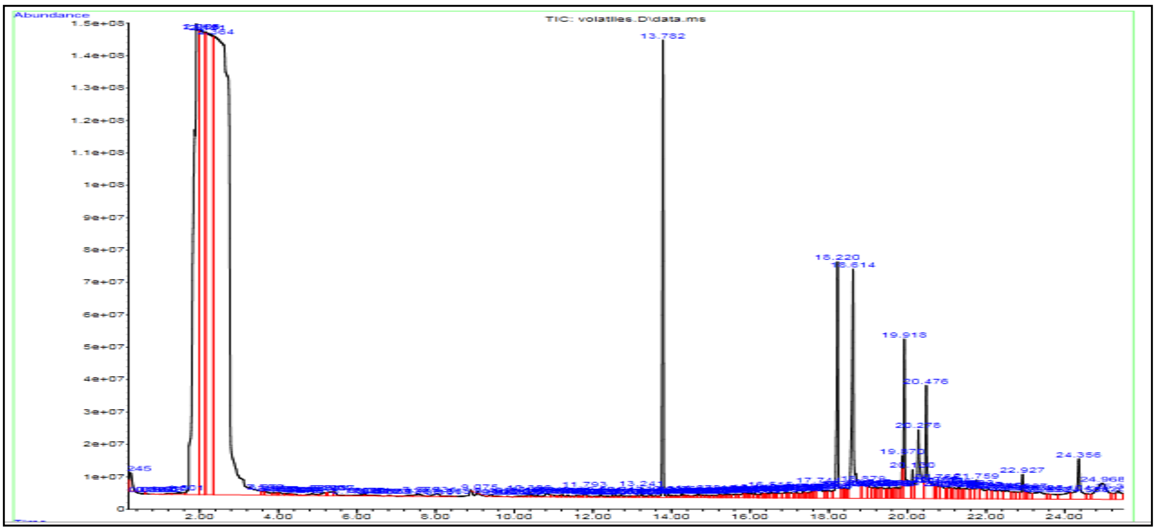
Results and Discussion

Major volatile components typically present in the extract obtained by simultaneous distillation/solvent extraction from a puree of papaya fruit are listed in Table 1. The GC-MS chromatogram (Fig a, b) for treated and untreated fruits revealed, a total of 56 peaks of significant importance (≥0.1% of the plot area) were identified. Of these 56, 9 peaks were in common in both samples,*viz* 2,2-diethoxy Ethanol, 2,2-Dimethoxyethylamine, Decanoic acid, Hexadecane, di-Phenylephrine, 4-Fluorohistamine, 15-methyl-, methyl ester-Hexadecanoic acid, Methylpent-4-enylamine and 1-chloro-Heptacosane were identified in common. To avoid cumbersome data the *m/z* of the individual compounds are not mentioned as they can be easily obtained over chemical databases.

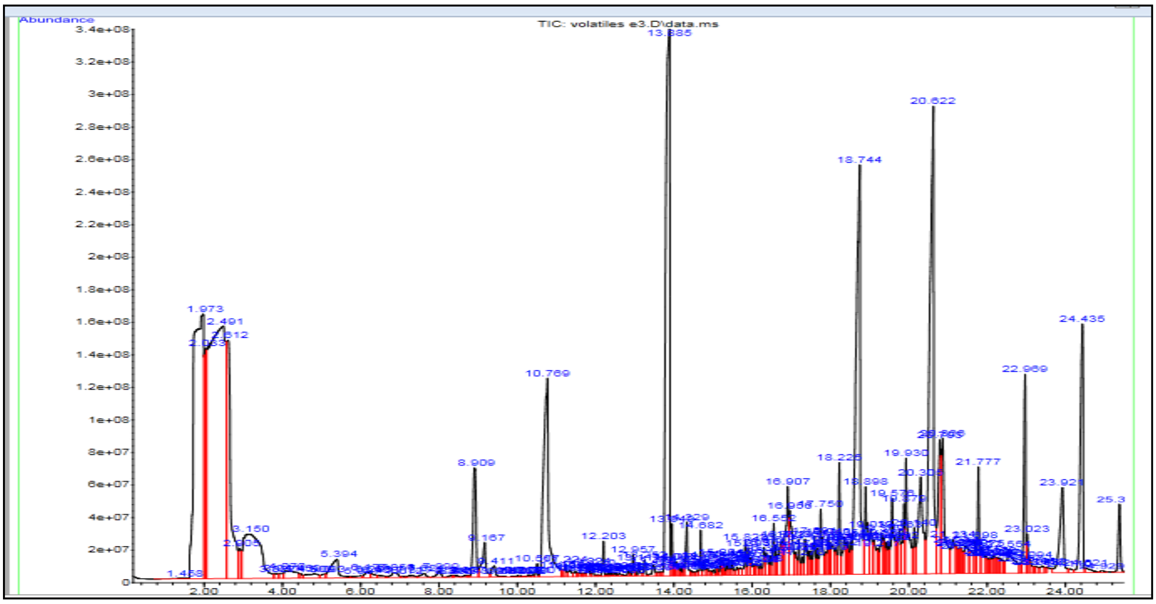
A mixture of small to moderately large compounds (C8-C43) was identified in the chromatography. Fewer compounds were identified in control compared to treated samples. As hypothesized, the results obtained are in coherent with the treatment induced. 2,2-diethoxy Ethanol a simple alcohol associated with uneven ripening is found in highest levels in control (50.02%) compared to control (19.87%). Compounds associated with ripening, aroma and flavour development have been observed in both samples. But a relatively higher proportion was observed in control samples. The ripening associated compounds viz. 2,4-Dimethyl-2-thiazoline; 2,2-Dimethoxyethylamine; n-Heptadecanol-1; Methylpent-4-enylamine; 2-amino-Imidazole-5-carboxylic acid; 1,4-Dibromo-2,3-butanediol was found in much higher concentration in control sample. This observation confirms the effectiveness of 1-MCP in regulating the ripening process. [12] Observed similar odour active compounds from headspace of ripening papaya fruits cv. Red Maradol. The presence of ripening associated volatile esters can be understood as the samples were taken lately. Thus it can be realized that the effect of 1-MCP are diminishing and can be subsidized leading to initiation of ripening process.

Further a number of natural and induced antimicrobial compounds (volatile and non-volatile) have been observed in the chromatogram. 2-methyltetracosane; Hexacosane; 2-hexyl-1-Decanol; Octatriacontyl pentafluoropropionate; 1-Octadecanesulphonyl chloride; Benzoic acid and 2-propyl tetradecyl ester sulfurous acid were the antimicrobial compounds found exclusively in 1-MCP treated fruits. Such an observation is significant to confirm that 1-MCP not only regulates ripening but also ripening induced microbial contaminants. The treatment of the fruit with 1-MCP strongly inhibited the production of esters.

Samples were prepared from the fruit in a crude method as described by [13] hence only few compounds were detected in comparison with [12]. [12] Performed the analysis of volatiles emitted from ripening papaya fruits using SPME fibre inserted in the headspace. This technique resulted in identification of almost 137 compounds of which 93 positively contributed to ripening volatiles. Methyl butanoate, 1-hexen-3-one, hexanal, ethyl butanoate, (Z)-3-hexen-1-ol, phenylacetaldehyde, linalool, benzyl isothiocyanate, and (E)-β-ionone were the most-odour active compounds in the SPME extract. In the present study, interestingly most of these compounds were identified either in small quantity or as analogous forms. This reduced identification of compounds in treated samples can be attributed due to effective blockage of ethylene receptors and hence supressed ripening phenomenon in fruits leading to no release of any volatiles.



A. Chromatogram of untreated sample



B. Chromatogram of 1-MCP treated sample

Fig 1

Table 1: GC-MS profiling from treated and untreated samples of papaya fruit

Sl. No.	Compound	RT (min)		Area (%)		Significance
		C	T	C	T	
1	2,2-diethoxy Ethanol	2.37	2.49	50.02	19.87	simple alcohol associated with ethylene & subsequent uneven ripening
2	2,4-Dimethyl-2-thiazoline	1.97	-	12.93	-	Volatile organic sweet smelling
3	2,2-Dimethoxyethylamine	2.01	1.97	11.82	9.65	major substance used for preventing cell wall degradation by aiding for accumulation of chitosan
4	Decanoic acid	18.61	14.33	2.56	0.38	volatiles produced by microbial load
5	1-Ethenyl-1,1,3,3-tetramethyl-Disiloxane	13.78	-	2.44	-	Si containing small C8 organic non-volatile compound
6	methyl ester Decanoic acid	18.22	-	1.72	-	fungal volatile that destroy the waxy cuticle of the plant
7	nonanoic acid	20.48	-	1.28	-	fungal volatile that destroy the waxy cuticle of the plant
8	n-Heptadecanol-1	19.92	-	2.36	-	straight chain, flavouring agent fruity flavor
9	Hexadecane	24.36	14.68	0.75	0.19	straight chain, flavouring agent, aromatic compound
10	di-Phenylephrine	17.74	16.35	0.79	0.22	straight chain non-volatile-compounds
11	4-Fluorohistamine	18.88	13.11	0.34	0.1	substrate for several enzymes and inhibitor for hisitidine ammonia lyase
12	6-Dodecenol	19.87	-	0.3	-	Alcohol, unknown significance
13	l-Guanidinosuccinimide	20.87	-	0.3	-	Unknown function
14	4-amino-1-Pentanol	24.10	-	0.3	-	N containing alcohol
15	N-methyl-1,3-Propanediamine	21.76	-	0.28	-	aliphatic ammonia based compound
16	15-methyl-, methyl ester-Hexadecanoic acid	20.13	18.23	0.57	0.27	aliphatic ester compounds characterized by fungal and bacterial growth
17	Methylpent-4-enylamine	22.51	6.86	0.27	0.14	flavor indicating volatiles characterized by ripening [14]
18	2-amino-Imidazole-5-carboxylic acid	22.34	-	0.2	-	aromatic ester
19	1,4-Dibromo-2,3-butanediol	20.7	-	0.14	-	fatty acid present in peels of ripened fruits
20	cis-2-Methyl-.beta.-methyl-beta-nitrostyrene	-	13.89	8.29	-	a key volatile phenolic compound in confirming the presence of bacterial decomposition and increased flavour of fruit
21	Octacosyl acetate	-	20.62	-	6.93	a major waxy fatty acids esters
22	1-Tetradecyl acetate	-	18.74	6.57	-	16 C, long chain volatile ester compound associated with attraction for fruit flies
23	1,3-Dihydroxybenzene	-	10.77	-	3.87	a strong reducing phenolic compound associated with un-ripeness
24	1-methoxy-Pentane	-	3.15	-	3.01	odourless short chain 6C alcohol with non-significant function
25	Cyclododecane	-	24.44	-	2.61	3 molecules of 1-MCP fuse to form this, leading to waxy lustre on fruit surface
26	2-methyltetracosane	-	20.87	-	1.84	acyclic 25 alkane found in raw fruits for antibacterial properties
27	2-(2-hydroxyethoxy)-	-	2.033	-	1.8	unknown importance

	Acetamide					
28	1-Hexacosanol	-	20.31	1.77	-	a volatile aromatic alcohol
29	2-Methyl-1-dodecanol	-	22.97	1.38	-	straight chain C13 alcohol produced from fungal and bacterial growth
30	Hexacosane	-	20.79	-	1.45	a long chain C21 volatile alkane found mostly in antimicrobial oils [15]
31	Octadecane	-	23.92	-	1.3	C18 long chain alkane
32	2-ethyl-Benzenamine	-	8.91	-	1.26	ethyl derivative of aniline; non-significant
33	nonyl-Cyclopropane	-	19.93	1.19	-	a medium C14 straight chain volatile alkane
34	2-hexyl-1-Decanol	-	18.98		1.77	a C10 guerbet alcohol identified by reduced microbial load and increased life of fruits [16]
35	Heneicosane	-	16.91	-	1.7	a long chain C21 volatile alkane found mostly in - antimicrobial oils [15]
36	Octatriacontyl pentafluoropropionate			-	0.89	a secondary metabolite with antimicrobial activity in unripe fruits
37	10-Methylnonadecane	-	21.78	-	0.65	a straight chain C20 oleic constituent found in fresh fruits
38	p-Benzoquinone	-	5.39		0.59	effective phenolic antioxidants used to improve the freshness of fruits
39	1-Octadecanesulphonyl chloride		19.88	-	0.99	Phenolic natural antifungal and antimicrobial S containing compound
40	2-ethoxyethyl ethyl ester Phthalic acid	-	25.38	-	0.85	Unknown significance
41	Benzoic acid	-	9.17	-	0.49	a phenolic ester present to prevent microbial growth in fresh fruits
42	Cyclobutyl heptadecyl ester Oxalic acid	-	19.76	-	0.41	Not much significant
43	Ethyl ether	-	2.91	-	0.3	associated with ripening and aroma development of fruits [17]
44	3,5,24-trimethyl-Tetracontane	-	19.48	-	0.24	a long chain C43 alkane
45	Ethylparaben	-	13.95	-	0.22	an aromatic single ring antimicrobial compound
46	2-Amino-1-(o-methoxyphenyl)propane	-	18.13	-	0.21	aromatic amide nonsignificant
47	Cyanuric acid	-	23.02	-	0.21	non-significant
48	Undecane	-	12.20	-	0.18	straight chain, flavouring agent, aromatic compound
49	2-propyl tetradecyl ester Sulfurous acid	-	19.41	-	0.18	S containing antimicrobial ingredient especially for postharvest contaminants like botrytis
50	1-Bromodocosane	-	19.69	-	0.18	a C24 medium chained bromide alkane
51	Tritetracontane	-	17.88	-	0.16	straight chain, flavouring agent, aromatic compound
52	Cyclobutanol	-	8.00	-	0.11	non-significant
53	2,2'-Dipyridylamine	-	12.97	-	0.11	non-significant
54	3-Methoxyamphetamine	-	24.52	-	0.11	non-significant
55	Oxalic acid, allyl tridecyl ester	-	21.39	-	0.10	non-significant
56	3-Hydroxy-N-methylphenethylamine	-	23.41	-	0.10	non-significant

Conclusion

The volatile production evaluation provide evidence that after the treatment of papaya fruit with 1-MCP the synthesis of aliphatic esters, saturated and unsaturated, derived from fatty acids metabolism are reduced as these are ethylene dependent processes.

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