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Varietal variation of hexane soluble chemical components of Indian mango (*Mangifera indica* L.) fruit pulp

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Abstract
Hexane soluble non-polar chemical components of fruit pulp of seven Indian *Mangifera indica* L. varieties were investigated and analyzed by GC-MS. The varieties were 'Amrapali', 'Himsagar', 'Fazli', 'Langra', 'Goplapkhas', 'Gopalbhog', 'Mohanbhog'. Twenty one aromatic components were identified and some were unidentified components. Multivariate analysis of the identified components indicated that the mango varieties were different on the basis of these non-polar metabolites. The ten most important contributory metabolites for varietal variation were delta-3-carene, beta-myrcene, 1R-alpha-pinene, beta-pinene, humulene, isocaryophyllene, longifolene, undecane, tetradecane, and alpha pinene.

Keywords: Mango, aromatic components, GC-MS

Introduction
Mangifera indica L. is one of the most popular tropical fruits cultivated and consumed worldwide. The fruits are very well known for its taste and distinctive flavors [1]. The fruit has nutritional benefits due to its high fibre, vitamine C and β-carotene content [2]. Several hundreds of cultivars are grown in various parts of the world and are known to vary markedly in their flavour characteristics [1]. This fruit is with green skin which turns yellow or lightly reddish yellow when ripe. The skin is not consumed and underneath is a luscious, edible (flesh) mesocarp which surrounds a central seed. The fruits, which are fairly sweet, are mainly eaten fresh but to extend the shelf life and meet consumer demand, mangoes are processed into juice, puree, jam and dried fruits [3, 2]. Although the aromatic compounds of exotic mangoes have been studied extensively [4] but there is very little data on the most popular Indigenous varieties of India. The aroma of each individual variety are extremely complex due to presence of many components, each may have different polarities and volatilities. Most of the aromatic constituents consists of oxygenated compounds which include terpene hydrocarbons, esters, furanones, lactones, ketones, alcohols aldehydes, acid and other groups [5]. The type of aromatic components of mango depends on cultivars [6], maturity level of the fruit [7], the part of the fruit [8], area of production [9], processing method and solvent used [10]. Most fruits produce significant number of aromatic compounds as indicators of fruit ripening. Many of these compounds are produced in trace amounts, which are below the detection level of the analytical instruments [11]. The aim of the current work was to analyze and identify aroma causing non-polar components of seven indigenous varieties, e.g. 'Amrapali', 'Himsagar', 'Fazli', 'Langra', 'Golapkhas', 'Gopalbhog', 'Mohanbhog' grown in Kolkata, West Bengal using GC-MS, and to find out varietal variations through chemometric analysis.

Materials and methods
Plant materials
Seven indigenous varieties of mango were 'Amrapali', 'Himsagar', 'Fazli', 'Langra', 'Golapkhas', 'Gopalbhog', 'Mohanbhog', they were collected in ripe, ready to consume stage, from University of Calcutta Agricultural field, Baruipur, Kolkata, West Bengal, in the month of April- May 2013.

Chemical reagents
HPLC grade hexane and a mixture of n-alkanes, [nonene (C_9), decane (C_{10}), undecane (C_{11}), dodecane (C_{12}),tridecane (C_{13}), tetradecane (C_{14}), pentadecane (C_{15}), hexadecane (C_{16}), heptadecane (C_{17}), octadecane (C_{18}), nonadecane (C_{19}), eicosane (C_{20})] were purchased from Sigma-Aldrich.

Extraction of nonpolar aromatic components

The ripened mango of every variety was surface cleaned with distilled water, and then they were peeled properly. The extraction of the mesocarp was done modifying the previously published methods [12, 13]. The yellowish orange pulp portion of the fruits was separated, crushed to powder with liquid nitrogen. The pulp powder (150 ± 10 mg each) was then transferred in to micro centrifuge tubes, as soon as possible and n-hexane (500 µl) was added. The tubes were capped airtight. They were kept at 25˚C for 30 minutes with frequent shaking. The tubes were centrifuged for 10 minutes at 10000 rpm and the supernatants were collected in separate tubes. Five biological replicas of each variety were prepared in the same way. Each extract was treated with Na_2SO_4 to remove moisture. The whole process was done minimizing direct exposure to air as much as possible to avoid loss of volatile components. The supernatants were then analyzed by GC-MS directly.

GC-MS analysis

GC-MS analysis was carried out using (Agilent gas chromatography system) 7890 A GC and 5975C MSD with triple axis detector. The GC-MS was operated on EI mode (70V). HP-5MS capillary column (Agilent J&W GC columns, USA) (length 30 m, diameter o.25 mm narrow bore, 0.25 µm) was used. The analysis was performed as detailed earlier [14] under the oven temperature program: injection at 60 ºC (5 min), temperature increasing at the rate 4 ºC/min to 220 ºC, 10 minutes hold time before cooling. The injection temperature was set at 230 ºC. The MSD transfer line was set at 280 º C and ion source at 250 º C. Helium was used as carrier gas at a constant flow rate of 1ml/minute, carrier liner velocity being 36.623 cm/sec. Sample (1 µl)was injected via the split mode onto the GC column. Mass spectra ranging from 30 to 500m/z were recorded. Metabolites were identified by comparing the fragmentation patterns of the mass spectra with entries of mass spectra library G1033A NIST. The Arithmetic Indices (AI) were calculated for each of the component identified and matched with those reported by Adams, (2009) [15]. Arithmetic index was calculated from the equation: AI (x)=100 Pz+100 [(RT (x)-RT (Pz))/RT (Pz+1)-RT (Pz))] where x: compound; RT: retention time; Pz: alkane before x; Pz+1: alkane after x. n-alkane mixture C_8-C_{20} was used. Other scientific journals were also studied for the identification of components [16, 17, 18].

Statistical analysis

The data were analyzed by different statistical and multivariate analysis such as Principal Component Analysis (PCA), Partial Least Squares - Discriminant Analysis (PLS-DA) obtained using METABOANALYST 4 software.

Result and discussion

In this analysis 27 aromatic components were identified from the hexane extract of the seven varieties of mango pulp of by comparing mass spectral fragmentation pattern and AI, RI (Retention Index) [15] and by mass spectral fragmentation pattern and R match value from NIST library (Table 1).

The statistical analysis was done with the most confirmed 27 aromatic metabolites. Heat map of the identified metabolites indicated that these seven varieties were different from each other on the basis of non-polar metabolite profile (Fig 1). The data were also analyzed by PCA (Fig. 2), PLS-DA (Fig.3). All the models segregated the varieties distinctly from each other. Also in Ten most influential aromatic compounds responsible for the differences were selected (Fig. 4) from the VIP score. Here delta-3-carene, beta-myrcene, 1R-alpha-pinene, (-)-beta-pine, are the first four most important non polar aromatic compounds which are responsible for the varietal separation on the basis of PLS-DA. The result clearly shows that the different varieties of mango fruit pulps were aromatically different. Delta-3-Carene, the most important contributory metabolite for distinction of the varieties could be detected in 'Langra', 'Himsagar' and 'Mohagbog'. The next important compound beta-myrcene could be detected in 'Gopalbhog', 'Amrapali' and 'Fazli'. 1R-alpha-pinene was detected in 'Amrapali' in noticeable amount; beta-pinene in 'Amrapali'.

Attempts are being made to characterize different polar and non-polar metabolites of mango fruits. Different ripening stages of mango fruits were distinguished on the basis of volatile organic compounds in variety "Tommy Atkins" [19] and different organic acids, sugars, polyphenolic compounds [20]. Varietal variation on the basis of different polar metabolites e.g. sugars, organic acids, amino acids, phenols including mangiferin has been reported [21]. In this work attempt was made to distinguish some Indian varieties of mangoes based on their hexane soluble non-polar metabolites which were identified to be mainly aromatic compounds. Two most important metabolites for varietal variation were delta-3-carene and beta-myrcene. The study may be important for rapid identification of mango varieties.

Table 1: Identified non-polar components from the pulp of the seven varieties of *M. indica* fruit pulp

Metabolites	Formula	MW	R match	AI	AI	Reference	MS Fragmentation
α-Pinene	C10H16	136	984	933	932	*(15)	136, 121, 105, 94, 93 (100), 92, 91, 81, 79, 77, 69, 67, 65,
Beta-Phellandrene	C10H16	136	867	1013	1025	*(15)	136, 121, 93(100), 77, 65, 53, 41
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-/(-)- beta pinene	C10H16	136	962	989	986	*(15)	136, 121, 107, 93, 79, 69, 53
alpha Phellandrene	C10H16	136	892	1004	1002	*(15)	136, 93(100), 77(40), 65, 51, 41
1R-α-Pinene	C10H16	136	925	932	936	*(17)	136, 121, 115, 105, 94, 93(100), 91, 89, 81, 79, 77, 69, 67, 65, 63, 57, 55, 53, 51
β-Myrcene	C10H17	136	932	991	986	*(15)	136, 121, 107, 93(100), 79, 69, 53
delta 3-Carene	C10H16	136	952	1009	1008	*(15)	136, 121, 115, 105, 94, 93, 91, 81, 79, 77, 69, 67, 65
γ-Terpinene	C10H16	136	892	1040	1055	*(15)	136, 121, 115, 105, 94, 93, 92, 91, 89, 81, 79, 77, 67
Limonene	C10H16	136	922	1029	1024	*(15)	136, 121, 107, 93, 91, 86, 79, 68, 65, 58, 53
Terpinolene	C10H16	136	880	1088	1089	*(15)	136, 121, 105, 93, 91, 80, 79, 77, 67, 65, 63, 55, 53, 51

β-Caryophyllene	C15H24	204	808	1420	1417	*(15)	204, 189, 175, 161, 147, 133, 120, 105, 93, 91, 81, 79, 69, 67, 55
Isocaryophyllene	C15H24	204	828	1406	1407	*(16)	204, 189, 175, 161, 147, 133, 115, 119, 105, 98, 93, 91, 85, 79, 77, 69, 67, 55
gamma Patchoulene	C15H24	204	840	1495	1502	*(15)	204, 189, 186, 175, 161, 147, 133, 121, 105, 91, 81, 67, 55
Germacrene D	C15H24	204	925	1484	1482	*(15)	204, 161, 147, 133, 119, 107, 105, 95, 91, 81, 79, 77, 67, 55
Humulene	C15H24	204	992	1456	1455	*(15)	204, 189, 161, 147, 121, 105,
γ-Elemene	C15H24	204	965	1441	1434	*(15)	204, 189, 175, 161, 147, 133, 121, 107, 93, 79, 67, 53
β-Copaene	C15H25	204	982	1435	1430	*(15)	204, 161, 147, 139, 133, 119, 105, 95, 91, 81, 79, 71, 67, 59, 55
Longifolene	C15H24	204	935	1406	1407	*(15)	204, 189, 175, 161, 147, 133, 119, 107, 105, 91, 79, 67, 57, 55
Longicyclene (L)	C15H24	204	956	1372	1373	*(15)	204, 189, 161, 147, 133, 119, 105, 94, 91, 79, 69, 65, 59, 55
Undecane	C11H24	156	865	1102	1100	*•	156, 127, 113, 85, 71, 57(100), 43
Tetradecane	C13H28	184	912	1401	1400	*•	141,127,85,71,57(100),43
Dodecane, 2,6,11-trimethyl-	C15H32	212	836	1280	1275	*	212, 196, 182, 169, 154, 140, 126, 112, 99, 85, 71, 69, 57
Tridecane, 3-methyl-	C14H30	198	843	1371	1371	(18)	198,169,113,99,85,71,57(100),56(50),55(50)
2,6-Dimethyl-6-trifluoroacetoxyoctane	C12H21F3O2	254	765	1079	1067	*	140, 125, 111, 97, 85, 83, 71, 70, 69, 67, 58, 57(100), 55
2-Butyloctanol	C12H26O	186	807	1274	1277	*	186, 154, 140, 125, 111, 97, 85, 72, 69, 67, 58, 57
1-Chlorotetradecane		232	717	1646	1659	*	232, 204, 189, 119, 105, 91, 71(50), 57(100), 55
1-Iodo-2-methylundecane	C12H25I	296	841	1555	1564	*	296, 113, 99, 97, 85, 83, 71, 69, 57, 55, 53

• Authentic; * NIST Library; MW: Molecular weight

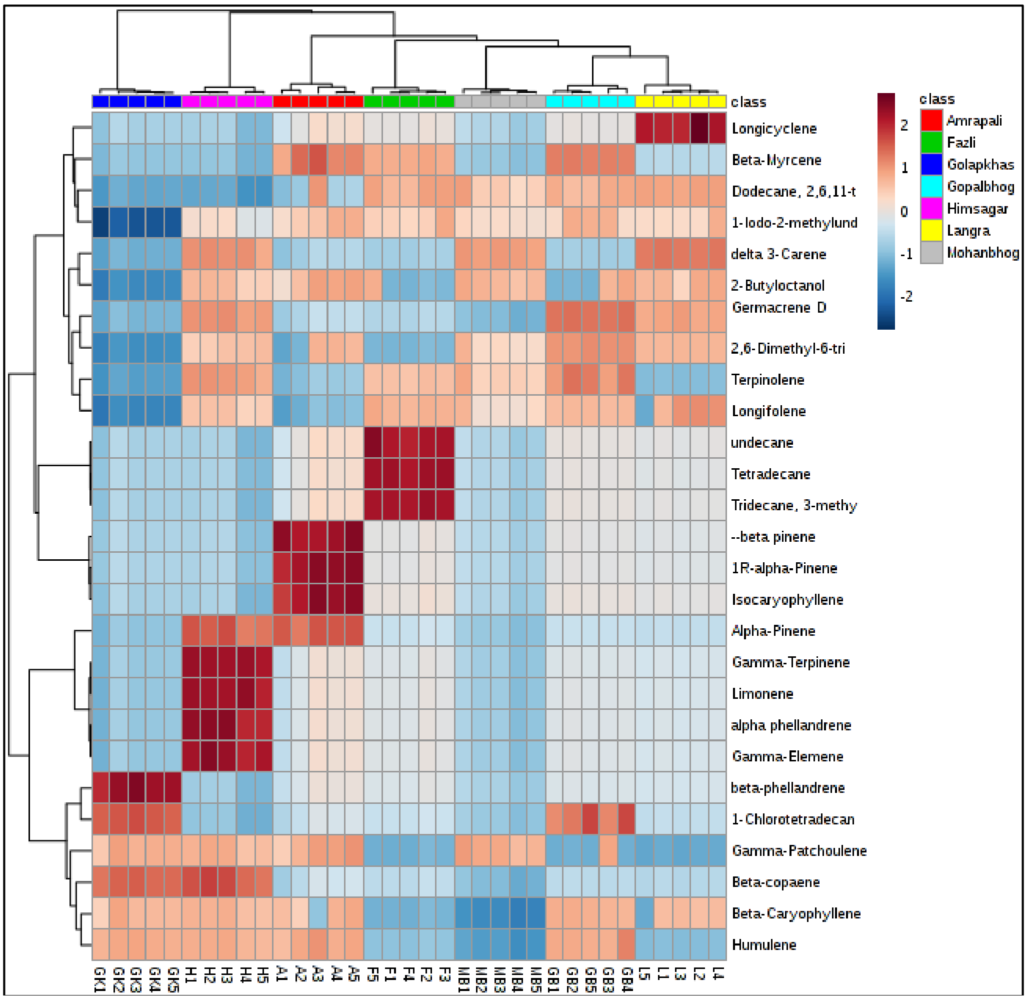


Fig 1: Heatmap showing differences in relative response ratios of identified non-polar components from the pulp of the seven varieties of *M. indica*

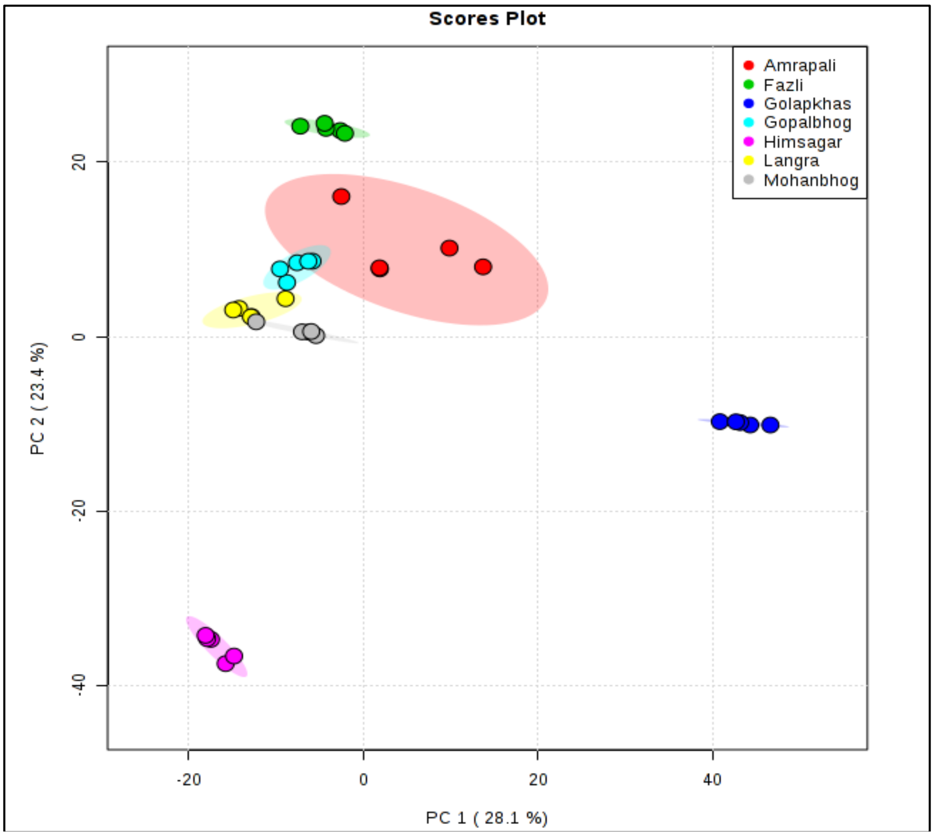
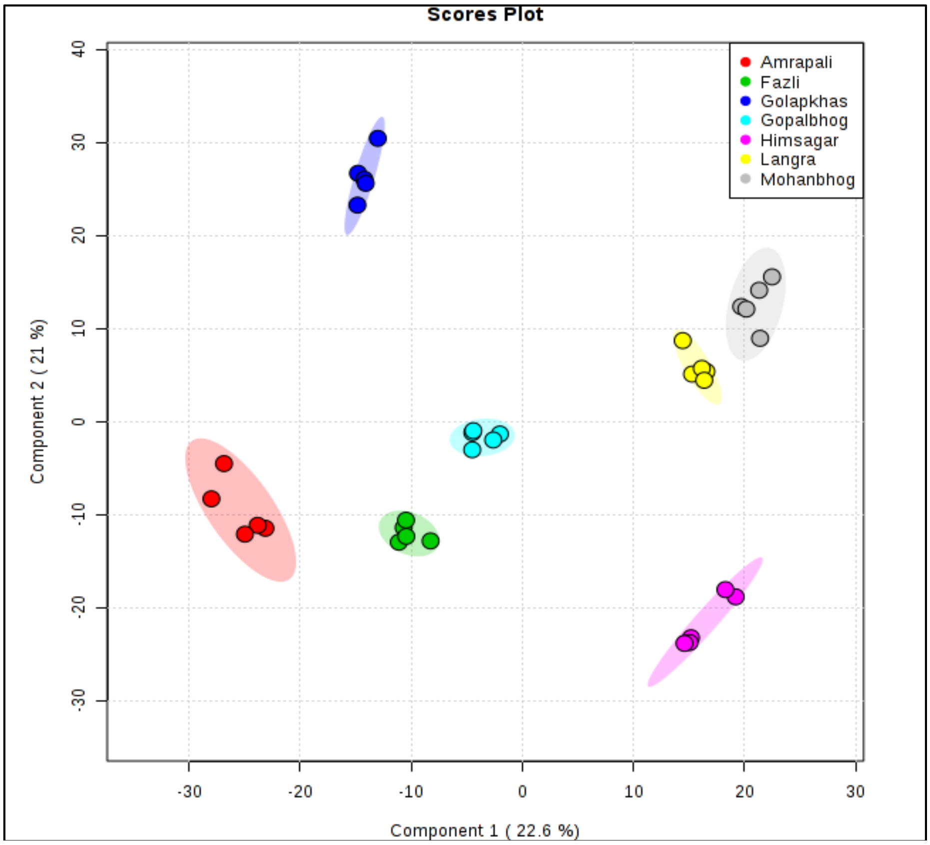


Fig 2: Multivariate analysis of identified metabolites in PCA score plot



PLS-DA cross validation details

Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.0	0.14286	0.48571	0.42857	0.94286
R2	0.90773	0.97246	0.98945	0.99115	0.99208
Q2	0.87679	0.95636	0.98247	0.98488	0.98527

Fig 3: Multivariate analysis of identified metabolites in PLS-DA score plot

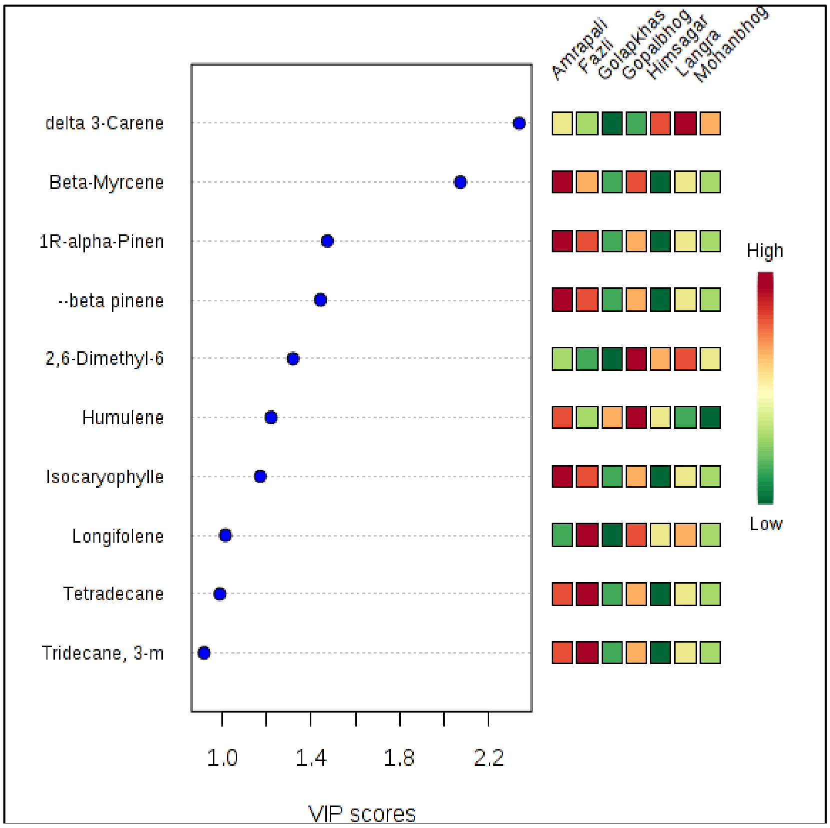


Fig 4: 10 most influential metabolites for varietal separation represented in VIP plot

Conclusion

Hexane soluble non-polar metabolites of seven varieties of Indian mango pulp were analyzed by GC-MS. On the basis of multivariate statistical analysis of the identified metabolites, the varieties could be distinctly differentiated from each other on the basis of four most important aromatic components such as delta-3-carene, beta-myrcene, 1R-alpha-pinene, beta-pinene.

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