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Aroma profile of fruit juice and wine prepared from Cavendish banana (*Musa sp.*, Group AAA) cv. Robusta

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ABSTRACT

A comparative study of the aroma profile of an alcoholic beverage (wine) and natural juice from banana cv. Robusta was performed. The study showed disappearance and synthesis of many aroma compounds during the fermentation process. Relative abundance of carbonyl compounds was high in the juice, and carboxylic acid content was higher in the wine. Aroma signature compounds of banana juice, isoamyl acetate, butyl isovalerate, isopentyl isovalerate, trans-2- hexenal and butanoates were present only in a low proportion in the wine, while decanoic, dodecanoic and hexa decanoic acids (as well as their esters) were abundant in the banana wine. Synthesis compounds like methyl nonyl ketone, isoeuginol and 2-methoxy 4-vinyl phenol was greater during fermentation. Elemicin was present in high quantity in both the juice and the wine.

Key words: Banana wine, head-space volatiles, esters, fermentation-derived aroma, SPME method

INTRODUCTION

Banana is an important tropical fruit crop used mainly for dessert and culinary purposes. Ripe fruits are ideal for beverage preparation owing to high Total Soluble Solids (T.S.S.) content (21-24°B), medium acidity (0.4-0.6%) and pleasant flavour (Pe´rez *et al*, 1997). Fermented banana beverages with 5-7% alcohol are popular conventional products in the African countries and, further, quality enhancement of the beverage is reported in scientific literature (Iwuoha and Eke 1996; Akubor *et al*, 2003; Aurore *et al*, 2009; Carvalho *et al*, 2009). Fermented fruit beverages are distinct from unfermented fruit juices as these possess a unique flavour due to synthesis and molecular rearrangement of esters, carbonyl compounds, alcohols and fatty acids during fermentation, clarification and subsequent storage (Rapp and Mandery, 1986). Extensive research has been done on flavour profile of the banana fruit, and scientists have documented banana flavour as a varietal character. Major flavour principles in the banana are: esters, carbonyl compounds, free alcohols and short-chain organic acids (Mattei, 1973; Marriott, 1980; Macku and Jennings, 1987). However, there is no information published to date on aroma profile of the banana wine. This study describes aroma profile of a high-quality fermented beverage compared with unfermented juice of the banana in a commercially important Cavendish cultivar, Robusta.

MATERIAL AND METHODS

Preparation of the substrate for fermentation

Pulp obtained from the ripe banana of cv. Robusta was mixed with 0.5% Pectinase CCM plus (a cocktail of enzymes obtained from Biocon Ltd., Bangalore), incubated for 90 min. at 50°C and the juice extracted by straining through a muslin cloth. The juice was then diluted with water in the ratio 2:1, TSS and acidity were adjusted to 22°Brix and 0.6%, respectively, followed by addition of 200ppm potassium metabisulphite and 0.03% diammonium phosphate.

Fermentation

Log phase culture of *Saccharomyces cerevisiae* UCD522 was inoculated @ 2% v/v to the above-mentioned substrate material, and fermentation was carried out at 18°C in 3 litre conical flasks fitted with loose cotton plugs. Progress in the fermentation process was measured using a Brix hydrometer, and, completely fermented juice was racked and clarified using Bentonite and stored at 10°C for two months.

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Biochemical and sensory analysis of banana wine

Banana wine was analyzed for pH, acidity, phenolics, residual sugar and alcohol (Amerine and Ough, 1982). Sensory properties of the wine were evaluated using a nine-point hedonic scale.

Head-space volatile analysis of banana juice and wine using GC-FID and GC-MS

Headspace aroma of banana juice and wine was extracted by solid phase micro-extraction (SPME) technique and analyzed using GC-FID and GC-MS/MS. Highly crossed-linked 50/30μm Divenylbenzene/Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco Inc. Bellefonte, PA, USA) was used for extraction of volatile compounds from banana juice and wine. Sodium chloride was added to the sample prior to head-space sampling to improve extraction efficiency, thus increasing the amount of analytes adsorbed onto the fibre (Pawliszyn, 1997). Extraction process followed for estimating head-space volatiles in banana fruit juice and wine was as described earlier by (Vermeir *et al*, 2009). Ten ml of the fruit juice diluted with an equal quantity of water, and, 20ml wine samples plus 1g NaCl were transferred to two 50 ml vials with screw-caps silicon rubber septum and a small magnetic bar. Vials were sealed with the rubber septum immediately after transfer of the sample, and were kept at 37±1°C for 15 min. with continuous stirring to facilitate transfer of analytes and equilibration to the head-space. Sampling was done by inserting the pre-conditioned fibre in the head-space for 90 min. at 37±1°C with continuous stirring. Subsequently, the SPME device was introduced into the injector port for gas chromatographic analysis, and was kept in the inlet for 10 min. for desorption. GC-FID analysis was performed on a Varian-3800 gas chromatograph (VarianBV, Harculesweg B,4338 Pl Middelburg, The Netherlands) system equipped with 30m X 0.25mm ID with 0.25μm film-thickness VF-5 fused silica capillary column (Varian Inc., 25200 Commercentre Drive, Lake Forest, CA 92630-8810, USA). The detector and injector were net at temperatures 270 and 250°C, respectively, and the column oven temperature program was: 50°C for 2 min. followed by increment of 3°C/min up to 200°C held for 3 min., and then, with increment rate of 10°C/min. up to 220°C and held for 8 min. at the same temperature. The carrier gas was helium, with a flow rate of 1.0ml/min. with 1:5 split ratio. For qualitative identification of volatile substances and for comparative variation of retention time and index, standards such as ethyl acetate, propanol, butanol, amyl alcohol, isoamyl acetate, pentanol, hexanol, 1-octene-3-ol, eugenol were co-chromatographed.

Varian-3800 Gas Chromatograph coupled to a Varian-4000, Ion-trap mass spectra detector (VarianBV, Harculesweg B,4338 Pl Middelburg, The Netherlands) equipped with a fused-silica capillary column VF-5MS with 30m x 0.25mm id, 0.25μm film-thickness from Varian (Varian Inc., 25200 Commercentre Drive, Lake Forest, CA 92630-8810, USA) was used for GC-MS analysis of volatile constituents. Helium, with a flow rate of 1ml/min, was used as the carrier gas. The mass spectrometer was operated in the external electron ionization mode at 70eV, with full mass scan-range 45–450 amu. The ion trap, transfer line and ion source temperatures were maintained at 200°C, 240°C and 210°C, respectively. Temperature was programmed as described earlier. Head-space volatiles were quantified as relative per-cent area in GC-FID chromatogram, and were identified by comparing retention index as determined using homologous series of n-alkanes (C_5 to C_{32}) as the standard (Kovats, 1965) and comparing the spectra available with two spectral libraries, Wiley-2005 and NIST-2007.

RESULTS AND DISCUSSION

In the present experiment, an alcoholic beverage was prepared from ripe fruits of the popular banana cultivar, Robusta. The beverage possessed biochemical characteristics typical of dry table wines (Table 1). It possessed a pleasant aroma, distinct from unfermented juice, and was scored as “like very much” by a panel of trained judges (scoring 8±0.6 in the nine-point hedonic scale). Analysis of the head-space volatiles revealed a clear difference between aroma principles of the fresh juice and the wine.

The principal groups of aroma compounds found in the head-space volatiles of juice and wine were esters,

Table 1. Biochemical composition of alcoholic beverage prepared from banana cv. Robusta (AAA Group)

Parameter	Value
pH	4.00 ± 0.15
Total acidity (% Citric acid)	0.50 ± 0.012
Alcohol (%v/v)	10.98 ± 0.56
Volatile acidity (% Acetic acid)	0.03 ± 0.00
Phenolics (mg/l)	606 ± 32
Residual sugars (mg/l)	700 ± 25
*Total antioxidant potential (mg AEAC/l)	326 ± 1.20
**Sensory score (in 9 point Hedonic scale)	8 ± 0.6

Values given are a mean of three replicates ± std deviation
* AEAC= Ascorbic acid equivalent antioxidant capacity
** Mean of 10 replications

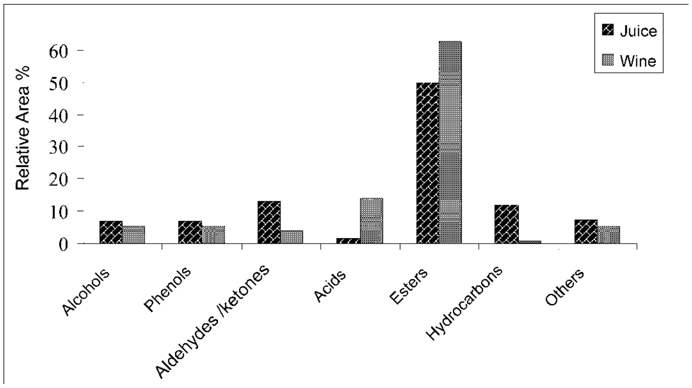

Table 2. Head-space volatile compounds identified in juice and wine from banana cv. Robusta (AAA Group)

alcohols, phenols, carboxylic acids, carbonyl compounds, hydrocarbons, and a few others like phenyl-methoxy compounds (Fig. 1). Esters were the most prominent aromatic principles in juice (50%) and wine (62.9%). Levels of carbonyl compounds and hydrocarbons were high in fruit juice (13.1% and 11.86%, respectively), and the proportion of alcohols in head-space was similar in both the products. Carboxylic acid fraction was very negligible (1.4%) in banana juice, but its proportion increased significantly in wine (14.1%). The above observation points to disappearance or modification of a few aroma compounds, and simultaneous production of another set of flavor principles during wine-making. Research on production of flavor compounds in grape wine is rather advanced, and studies have shown that during alcoholic fermentation of the fruit, yeast produces ethanol, carbon dioxide and a number of by-products, including esters (Mateo *et al*, 1992). Aromatic profile pattern of a wine is a function of the fermentative strain, vinification temperature and glycosylated aroma precursors present in the fruit (Lilly *et al,* 2000). Occurrence of glycosylated compounds like fatty acids and shikimic acid derivatives have been reported earlier in banana fruit (Perez *et al,* 1997).

Relative abundance of individual aroma compounds is presented in Table 1. A total of 19 alcoholic compounds were present in the banana juice, while the fermented beverage possessed only 15 compounds belonging to the same functional group. It was found that amyl alcohol, (E,E)-dodeca-8,10-dien-1-ol, 11-tridecyne-1-ol were the major hydroxyl compounds in banana juice, while, amyl alcohol, α-methylphenethyl alcohol, (Z,Z)- dodeca -3, 6-dien-1-ol, etc. were prominent in banana wine. Phenols such as methyl euginol, euginol, isoeuginol and methoxy euginol were present in both banana juice and wine, while, 2-methoxy-4 vinylphenol was detected only in the banana juice. These are low aroma-threshold compounds and have been earlier reported to contribute the floral note to banana aroma (Shiota, 1991). The compound, 2-phenyl ethyl alcohol, was identified as one of the major aglycon moieties in the ripe banana fruit (Perez *et al,* 1997). The glycosidases present in the banana juice would have helped release this bound flavour from its precursor molecule. Phenyl ethyl alcohol imparts a sweet, floral, rosy note to the product (Ribereau-Gayon *et al,* 2006).

The flavour profile of banana juice was characterized by approximately 21 types of carbonyl compounds, of which (Z,Z)-oxacyclo trideca-4-7-dien-2-one, trans-2- hexenal, β-ionone, isoamyl aldehyde, etc. were present in higher quantities. But, wine possessed very low levels of carbonyl compounds, which were predominated by isoamyl aldehyde, methyl nonyl ketone and cycloisolongifolene. This observation leads to the inference that even though most carbonyl compounds are incapable of surviving vinification, some carbonyls like isoamyl aldehyde and methyl nonyl ketone are synthesized during banana wine making. The carbonyl fraction, along with the alcohols, contributes to the woody or musty flavor, among which, trans-2-hexenal and pentan-2-one contribute to the herbal note in banana juice (Shiota, 1991). Decrease in levels of hexanal and trans-2-hexenal are reported earlier in grape fermentation (Kotseridis and Baumes, 2000). The above observation suggests that radical differences in the levels of highly odorous carbonyl compounds also contribute to the distinctness of banana wine aroma.

There were 11 kinds of acid in the head-space of banana juice, and this number were 12 in the banana wine. The banana juice had higher quantity of short-chain carboxylic acids, while, the wine was predominated by long-chain acids, of which n-decanoic acid was the most predominant. Fatty acids in wines result from auto-oxidation of saturated lipids in the fruit and the cell membrane component of yeast. The most abundant fatty acid in the banana wine, n-decanoic acid (capric acid), is an important component of yeast cell membrane, and autolysis of the yeast cell gives way to its release in the wine (Rapp, 1998; Torija *et al,* 2003).

Esters were, by far, the predominant compounds in banana juice and wine. Banana juice contained 38 esteric compounds, of which, short-chain organic acid esters such as that of acetic, propanoic, butyric, isovaleric acid, etc., were the most abundant. Banana wine had 37 ester compounds in the head space, with large amounts of longer-chain acid esters like that of decanoic, dodecanoic, octanoic, octadecanoic acids etc. In the present study, the most

Table 2. Head-space volatile compounds identified in juice and wine from banana cv. Robusta (AAA Group)

Name of the compound	Kovat's index	Relative area percentage	
		Juice	Wine
Alcohols			
Amyl alcohol	765	1.33	1.61
1-Hexyne-3-ol	779	0.06	—
2,3-Butanedithiol	898	0.02	0.02
4,4-Dimethyl-2-pentanol	801	0.32	—
Butanediol	910	—	0.03
1-Methyl-2-cyclohexen-1-ol	918	0.14	—
1,2-Pentanediol	923	—	0.17
(3E)-2-Ethyl-3-hexen-1-ol	1010	0.05	—
α-Methylphenethyl alcohol	1155	—	0.82
2-(4-Methylcyclohexyl)-2-propanol	1159	0.33	0.18
(E,Z)-3,6-Nonadien-1-ol	1175	0.20	0.45
2-(1,1-dimethylethyl)-Cyclohexanol	1191	0.19	—
Citronellol	1234	0.09	—
1-Cyclohexyl-1-butanol	1245	0.04	—
(Z,Z)-Dodeca-3,6-dien-1-ol	1418	0.39	—
(E,E)-Dodeca-8,10-dien-1-ol	1482	1.07	
7-Tridecanol	1492	0.09	0.33
1,10-Decanediol	1514	0.09	—
Nerolidol	1564	—	0.10
11-Tridecyn-1-ol	1582	1.45	—
(Z,Z)-6,9-Pentadecadien-1-ol	1783	0.03	0.21
Phytol	2122	—	0.06
Phenols			
2-Methoxy-4-vinylphenol	1191	—	1.26
Methyleugenol	1338	0.55	0.10
Eugenol	1384	0.95	0.16
Isoeugenol	1428	0.08	1.72
Methoxyeugenol	1609	1.16	1.52
Aldehydes and Ketones			
Isoamylaldehyde	653	0.42	1.89
trans-2-Hexenal	859	3.63	—
2,4-Hexadienal	911	0.22	—
1-(1-Methyl-2-cyclopenten-1-yl) ethanone	962	0.44	—
2-Octanone	994	0.42	0.01
2,2-Dimethylocta-3,4-dienal	1109	0.04	—
Pulegone	1175	0.31	—
Citronellal hydrate	1248	0.10	0.02
5,6-Decanedione	1288	0.07	—
Methyl nonyl ketone (2 Undecan2-one)	1292	0.50	1.44
(E,E)-2,4-Decanedienal	1294	0.03	—
2,4-Decadienal	1312	0.02	—
1-(2,6,6-Trimethyl-2-cyclohexen-1-yl) acetone	1326	0.04	—
Dodecanal	1371	0.28	—
2-Butyl-2-octenal	1385	0.03	—
(Z,Z)-Oxacyclotrideca-4,7-dien-2-one	1445	4.75	—
8,9-dehydro-9-formyl-Cycloisolongifolene	1469	—	0.44
β-Ionone	1493	0.77	—

Table 2. Contd.

Name of the compound	Kovat's index	Relative area percentage	
		Juice	Wine
Tridecan-2-one	1498	0.30	—
Tridecanal	1522	0.09	—
Tetradecanal	1584	—	0.13
2-Methylhexadecanal	1835	0.32	—
(Z)-9,17-Octadecadienal	1988	0.33	—
Acids			
2-Hydroxy-2-methylbutanoic acid	966	—	0.06
4-Hexenoic acid	984	0.35	—
2,3,3-Trimethyl-4-pentenoic acid	1019	0.01	—
Heptanoic acid	1083	0.09	0.07
Benzoic acid	1178	—	0.61
4-Butoxybutanoic acid	1249	0.39	—
8-Nonenoic acid	1274	0.21	—
3-Ethyl-3-methylpentanedioic acid	1451	0.23	—
Tridecanoic acid	1324	—	0.03
3-Ethyl-3-methylpentanedioic acid	1454	—	0.16
Undecanoic acid	1458	—	0.43
n-Decanoic acid	1573	—	11.88
Dodecanoic acid	1592	—	0.15
Pentadecanoic acid	1843	—	0.06
Hexadecanoic acid	1961	0.21	0.63
cis-9-Octadecenoic Acid	2095	—	0.04
Esters			
Methyl 2-propenoate	578	—	0.82
Ethyl 2-butynoate	794	0.02	—
trans-2-Hexenyl formate	803	0.24	—
Ethyl butanoate	806	0.15	—
Butyl acetate	812	0.12	0.03
Ethyl isovalerate	858	—	0.01
Isoamyl acetate	872	6.60	2.92
Propyl butanoate	912	0.54	—
Propyl isovalerate	956	0.48	—
Propyl-2-methyl butanoate	978	0.15	—
Ethyl 3-hexenoate	992	—	0.16
Butyl butanoate	994	2.35	—
Ethyl hexanoate	996	—	0.41
Isoamyl butanoate	1008	2.11	—
Hexyl acetate	1010	0.07	—
Isopentyl 2-methyl propanoate	1013	1.17	—
Butyl isovalerate	1072	10.02	—
6-Heptenyl acetate	1073	—	0.03
Ethyl sorbate	1089	—	0.20
Ethyl heptanoate	1099	—	0.06
Isopentyl isovalerate	1105	19.45	0.38
Ethyl benzoate	1172	—	2.43
Isoamyl iso valerate	1183	0.02	—
Butyl hexanoate	1186	0.05	—
Hexyl butanoate	1190	1.48	0.38
trans-2-Hexenyl Butyrate	1191	1.21	0.04
1-Methylhexyl butyrate	1198	0.01	0.04
1-Methylheptyl butyrate	1217	0.06	—
Butyl sorbate	1222	0.29	—
β-Phenylethyl acetate	1226	—	0.62

Table 2. Contd.

Name of the compound	Kovat's index	Relative area percentage Juice	Wine
Hexyl iso-valerate	1240	1.32	—
Butyl (E)-2-hexenoate	1257	0.05	—
n-Hexyl iso-valerate	1259	0.04	—
Propyl octanoate	1277	—	0.05
Isomenthyl acetate	1298	0.16	—
Isobutyl benzoate	1302	—	0.02
1-Octen-3-ol butyrate	1322	0.36	—
Butyl octanoate	1387	—	0.39
1-Ethylpropyl octanoate	1417	—	0.28
Amyl octanoate	1478	—	1.36
Propyl decanoate	1493	—	20.51
Eugenyl acetate	1526	0.03	—
Methyl dodecanoate	1528	—	0.06
Isobutyl decanoate	1545	—	0.20
Ethyl dodecanoate	1562	—	11.77
Phenylethyl valerate	1565	0.58	—
E-2-Hexenyl benzoate	1583	—	0.11
Iso-amyl decanoate	1647	0.06	2.88
Ethyl trans-4-decenoate	1760	—	1.46
Ethyl tetradecanoate	1793	—	1.84
3-Methylbutyl dodecanoate	1858	0.10	—
Methyl -9-hexadecenoate	1877	0.02	0.93
Methyl hexadecanoate	1889	—	0.48
(E)-4-Tridecenyl acetate	1892	0.24	—
Methyl (E)-7-hexadecenoate	1898	0.06	4.69
Butyl hexadecanoate	1978	0.01	—
Ethyl hexa decanoate	1991	0.22	5.12
Isopropyl hexadecanoate	2021	0.03	—
Ethyl heptadecanoate	2098	—	0.12
Methyl octadecanoate	2128	—	0.22
Ethyl -cis,cis-9,12-octadecadienoate	2185		0.37
Ethyl cis-9-octadecenoate	2189	0.05	0.78
Ethyl octadecanoate	2209	0.02	0.38
Hydrocarbons			
Naphthalene	1175	0.33	—
(4E,8Z)-1,4,8-Dodecatriene	1225	0.67	—
(E,Z)-5,7-Dodecadiene	1239	0.36	—
(E,Z)-5,7-Dodecadiene	1246	3.53	—
3-Dodecyne	1252	1.57	—
Azulene	1311	—	0.51
Caryophyllene	1435	0.27	—
(Z)-5-Pentadecen-7-yne	1552	0.61	—
(E)-7-Pentadecen-5-yne	1556	0.19	—
(Z)-4-Hexadecen-6-yne	1641	4.33	—
1,E-8,Z-10-Pentadecatriene	1518	—	0.17
Others		—	—
2-Ethylfuran	705	0.12	—
1-Nitro-2,2-dimethylpropane	794	0.12	—
2,3-Butanedithiol	901	0.35	—
2-Propyloctahydro-1-benzothiophene	1394	0.09	—
3,4,5-Trimethoxyallylbenzene	1560	6.64	5.58
(Z)-5-Propenyl-1,2,4-trimethoxybenzene	1621	0.14	—

predominant ester in banana juice was found to be butyl isovalerate (10.6%). Butyl isovalerate (3-methyl butyl butanoate) was identified as the major constituent of head-space volatiles in all the banana cultivars from Madeira Island (Nogueira *et al*, 2003). Acetate esters alone contributed 10.6% of the total head-space volatiles in banana juice, while, their share in the wine dropped to 4.63%. Isoamyl acetate was the major acetic acid ester present in banana juice (6.6%). Butanoates alone contributed 10.38% to the total head-space volatiles in banana juice, while, their share in the wine aroma profile was reduced to 0.79%. Butyl and isopentyl alcohol esters of isovaleric acid constituted 29.47% of the total juice head-space volatiles. A rise in production of isopentyl isovaleric acid during the ripening of banana was observed by Macku and Jennings (1987). Banana owes its fruity aroma to acetates and butanoates of butanol, isoamyl alcohol, pentan-2-ol and hexyl acetate (Shiota, 1991). Abundance of decanoic acid and dodecanoic esters in the wine was 23.59% and 23.39%, respectively, while their share in the juice was almost negligible. In order of abundance, esters found in the juice were: isoamyl acetate> butyl acetate> butyl butanoate >hexyl isovalerate. The wine contained propyl decanoate, methyl-(E)-7-hexadecenoate, ethyl benzoate, isoamyl decanoate, amyl octanoate, etc., in high quantities. Decanoic acid and dodecanoic acids are components of the yeast cell membrane, and are abundant at low-temperature growth of the yeast (Torija *et al*, 2003). Ethyl dodecanoate is reported to have a typical wine-yeast background aroma and propyl decanoate has a waxy, sweet aroma with low odour-strength. These two compounds are reported in fermented and distilled beverages like wine, brandy and whisky (Comuzzo *et al*, 2006). The esters are synthesized by alcohol acetyl transferases, using higher alcohols and acetyl co-A as substrates. Esterases present in the yeast can significantly synthesize or hydrolyze the esters, based on physico-chemical conditions of the wine, a fact which supports discovery of new esters in wine, as compared to the juice (Lilly *et al*, 2006).

A few hydrocarbons were also identified in the present study. (Z)-4-hexadecen-6-yne, (E,Z)-5,7-dodecadiene and 3-dodecyne were the important hydrocarbons present in banana juice, but were absent in the banana wine. Instead, new compounds such as azulene and 1, E-8, Z-10-pentadecatriene were present, though in very small levels, in banana wine. A variety of hydrocarbons have already been detected in banana volatiles (Shiota, 1991). Nevertheless, their contribution to banana juice aroma may be negligible, as, the alkanes, alkenes, alkynes, naphthalenes, etc. have high aroma-thresholds. Among other flavouring compounds, 3,4,5-trimethoxy allyl benzene (elemicin), a natural phenyl propene, and a constituent of the essential oil in nutmeg, was also present in a high proportion in both banana juice and the wine. Euginol and elemicin give a pleasant, mellow aroma to the ripe banana fruit (Wang *et al*, 2007). Another significant volatile was 2-methoxy 4-vinyl phenol, present in the wine but not in the juice. This compound is a major odour compound in many white wines, and aroma of the pure compound is described as wine-like (Comuzzo *et al*, 2006). This suggests that origin of this compound in banana wine lies in the fermentation process.

CONCLUSION

There is a clear difference in head-space volatile profiles of banana juice and wine. Aroma signature compounds of banana juice, viz., isoamyl acetate, butyl isovalerate, isopentyl isovalerate, trans-2-hexenal, butanoates, etc. were present only in a low proportion in banana wine. At same time, the decanoic, dodecanoic acid, hexa decanoic acid esters, and, highly odorous compounds like methyl nonyl ketone and isoeuginol, 2 methoxy 4-vinyl phenol were synthesized during fermentation, and were retained in the finished wine in relatively higher quantities. These facts justify distinctness of the banana wine as perceived by judged by the sensory evaluation panel.

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