

Short Communication

Volatile flavour compounds of mangosteen juice and wine fermented with *Saccharomyces cerevisiae*

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Abstract

Mangosteen (*Garcinia mangostana* L.) is a popular tropical fruit known as “the queen of fruits”. This study investigated the volatile components of mangosteen before and after fermentation with *Saccharomyces cerevisiae* using HS-SPME-GC-MS/FID. Mangosteen wine was subjected to GC-olfactometry (GC-O) analysis. In total, 5 acids (C2-C10), 6 alcohols, 6 carbonyls (5 aldehydes, plus acetone), 10 esters and 5 terpenes were detected in the juice. After fermentation, while the respective number of acids and alcohols remained unchanged, only 2 carbonyls (aldehydes), 1 terpene (α -copaene) and now 20 esters were present in wine. (Z)-3-Hexen-1-ol, hexan-1-ol, hexanal and (E)-2-hexenal were the main volatiles in the juice. Besides ethanol, the compounds isoamyl alcohol, 2-phenylethyl alcohol, ethyl decanoate and ethyl octanoate were the major volatiles in the wine. GC-O CharmAnalysisTM revealed the key aroma-active compounds in the wine as isoamyl alcohol (flavour dilution value, FDV, 729), 2-phenylethyl alcohol (FDV, 81) and isoamyl acetate (FDV, 27), together with first-time reported longifolene (FDV, 9).

Keywords

Mangosteen

Wine

Fermentation

Flavour, Volatiles,

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Introduction

Mangosteen (*Garcinia mangostana* L.), which is often referred to as “the queen of fruits”, is generally regarded as one of the most appealing tropical fruits. It is also classified as a “superfruit” due to its high content of anti-oxidants especially xanthenes in the pericarp (Gutierrez and Failla, 2013; Suttirak and Manurakchinakorn, 2014). The fruit is native to Malaysia, but is widely grown in and around the Indian sub-continent (Macleod and Pieris, 1982). Mangosteen is usually consumed fresh but can also be canned, frozen or processed into juice, jam, preserve, syrup and candy (Bin Osman and Rahman Milan, 2006). Production of mangosteen wine is another way to add value to the fruit and meet the needs of consumers looking for wines with novel flavours.

The literature on the aroma compounds of mangosteen is limited. The first research on volatile compounds of mangosteen juice was conducted by Macleod and Pieris (1982). They were able to detect 52 volatiles, of which 28 were identified by GC-MS. These volatiles included alcohols, esters, hydrocarbons, terpenes, aldehydes, ketones and miscellaneous compounds. The authors concluded

that the main contributors to mangosteen aroma were hexyl acetate, cis-hex-3-enyl-acetate and cis-hex-3-en-1-ol. Laohakunjit *et al.* (2007) studied the volatile compounds of mangosteen fruit. A total of 25 compounds were detected and 21 were identified using HS-SPME-GC-MS. The major volatiles were 2,2-dimethyl-4-octanal, (E)-2-hexenal, benzaldehyde, (Z)-3-hexen-1-ol, hexyl n-valerate, 1,4-pentadiene, and 2-methyl-1,3-buten-2-ol.

Information on the volatile composition of mangosteen wine is even more scarce. In fact, there is only one report on the volatile components of mangosteen wine. Yu *et al.* (2013) detected 18 volatile compounds in mangosteen wine, mostly alcohols and esters. The major volatiles of mangosteen wine included ethanol, isoamyl alcohol, active amyl alcohol and ethyl acetate, but aroma-active volatiles were not identified in this study. No comparison between the volatiles before and after fermentation was made.

There have been strong recent interests in tropical fruits and wines such as mango, pineapple, durian, lychee and soursop (Cheong *et al.*, 2011; Lee *et al.*, 2013; Li *et al.*, 2013; Thepkaw and Chomsri, 2013; Chen and Liu, 2014; Wasnin *et al.*, 2014).

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The objective of this study was to assess the volatile composition of mangosteen juice and wine fermented with *Saccharomyces cerevisiae* MERIT.ferm as well as to identify the major aroma-active compounds of mangosteen wine.

Materials and Methods

Yeast and culture media

The freeze-dried commercial yeast *S. cerevisiae* MERIT.ferm (Chr.-Han., Horsholm, Denmark) was propagated in a sterile synthetic medium (2% w/v glucose, 0.25% w/v yeast extract, 0.25% w/v bacteriological peptone and 0.25% w/v malt extract, pH 5.0) for 48 h at 25°C. The pure culture was stored at -80°C until the preparation of fresh juice pre-cultures described below.

Mangosteen juice pretreatment

Mangosteen fruits imported from Malaysia were purchased from a local supermarket in Singapore. The fruits were washed, pulps extracted manually and the parts of pulp which mainly consisted of seeds were excluded. The remaining fruit pulp was blended to obtain mangosteen juice (0.6 litre), which was then used for triplicate laboratory-scale fermentations. Blended mangosteen juice had an initial °Brix of 16.07% and initial pH of 3.25, which was then adjusted to pH 3.50 with 1M NaOH. The adjusted mangosteen juice was centrifuged at $16,873 \times g$ (Beckman Centrifuge, Palo Alto, CA, USA) for 20 min at 4°C. After centrifugation, the juice was filter-sterilised through 0.65 µm and 0.45 µm polyethersulfone filter membranes (Sartorius Stedium Biotech, Goettingen, Germany).

Mangosteen juice fermentation

Prior to fermentation, a preculture was prepared from sterilised mangosteen juice (20 ml). The preculture was prepared by thawing a pure yeast culture (previously stored at -80°C), followed by inoculating the sterilised juice with the yeast culture (10% v/v), then incubating the inoculated mangosteen juice at 25°C for 48 h under static conditions. The fermentation was conducted in triplicate in sterile Erlenmeyer flasks. Each flask contained 200 ml of pretreated juice and was inoculated with 1% v/v of a juice preculture. The fermentations were conducted under static conditions at 20°C for 10 days.

Standard analyses

Yeast growth, pH and °Brix were measured on the first and final days of fermentation. Yeast growth was determined by the spread-plating method using potato

dextrose agar (Oxoid, Basingstoke, Hampshire, UK). The pH of samples was measured using a pH meter (Metrohm, Switzerland). °Brix values were obtained using a refractometer (ATAGO, Tokyo, Japan).

Analysis of sugars by HPLC

Sugars in mangosteen juice and wine were analysed in triplicate by HPLC (Shimadzu HPLC, Class-VP software version 6.1, Kyoto, Japan) using a Zorbax carbohydrate column (150 x 4.6 mm; Agilent, Santa Clara, CA, USA) with the method of Lee *et al.* (2013). Identification and quantification of sugars was carried out using retention times and standard curves of pure sugars (sucrose, glucose and fructose) (Sigma-Aldrich, Oakville, ON, Canada).

Volatiles analysis by HS-SPME-GC-MS/FID

Volatiles were analysed in triplicate using the headspace (HS) solid-phase microextraction (SPME) coupled with gas chromatography (GC)-mass spectrometer (MS) and flame ionisation detector (FID) as described elsewhere (Lee *et al.*, 2013). Volatiles extraction from a 5-ml sample was performed at 60°C for 40 min under 250 rpm agitation, using a carboxen/polydimethylsiloxane fibre (Supelco, Barcelona, Spain). GC-MS/FID was performed using an Agilent 7890A gas chromatograph (Santa Clara, CA, USA) equipped with a DB-FFAP capillary column (60 m × 0.25 mm, 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). Volatiles were identified by comparison of retention times and spectra in the database of Wiley MS library. Linear retention indices (LRI) were determined using alkanes standards (C5-C40) run under the same HS-SPME-GC-MS/FID condition.

Volatiles analysis by GC-Olfactometry (GC-O) and CharmAnalysis™

Four mL of mangosteen wine sample, 0.8 g of salt and a small magnet stirrer were transferred into a screw-capped headspace vial (Agilent Technologies, CA, USA) and incubated for 10 min (45°C, 250 rpm), followed by 20-min headspace extraction performed using a 50/30µm DVB/CX/PDMS fibre with a manual holder (Supelco Co., Bellefonte, PA, USA). Desorption of volatiles was performed by injecting the fibre in the injection port of the GC for 10 min at 270°C.

GC-O analysis was conducted using the method of SEOW and co-workers (2010). An Agilent GC 7890A (CA, USA) was equipped with a DB-5 column (30 m × 0.25 mm, film thickness 0.25 µm) which was connected directly to the ion source of Agilent 5975C Series MSD for mass spectrometry. Volatiles

were identified by comparison of the spectra with the database in Wiley Registry 8th Edition NIST 05 MS Spectra software.

CharmAnalysis™ of mangosteen wine was conducted by two trained panellists. GC-O dilution samples were achieved by diluting mangosteen wine with water by a dilution factor of 3. GC-O analysis was repeated up to the last dilution-fold of the sample which was the sample where the panellists were no longer able to detect any volatiles. Response data from all the dilutions of each sample were then combined to produce the charm response chromatograms and reports for the samples using GC-O Stationware™ Software (Datu Inc., Geneva, NY, USA).

Statistical analysis

Mean values and standard deviations were calculated from data obtained from triplicate fermentations and statistically analysed using SPSS.

Results and Discussion

Yeast growth and physicochemical properties

The initial cell count of 2.32×10^5 cfu/ml increased to 8.27×10^7 cfu/ml by the end of 10-day fermentation. The initial pH 3.56 decreased to 3.50; the °Brix value decreased from 15.50 to 5.33, which translates into a potential ethanol level of approximately 6.00 % v/v. The initial total sugar concentration in the mangosteen juice was 16.43 g/100 ml (sucrose, 12.67 g/100 ml; glucose, 2.10 g/100 ml; fructose, 1.66 g/100 ml). Most of the sugars were consumed during fermentation and only trace quantities of sucrose (0.37 g/100 ml), glucose (0.13 g/100 ml) and fructose (0.28 g/100 ml) remained in the resultant mangosteen wine.

Volatiles in mangosteen juice and wine determined by HS-SPME-GC-MS/FID

Volatiles found in mangosteen juice and wine are shown in Table 1. Twenty compounds are reported for the first time either in mangosteen juice, wine or both. These compounds were 6 acids: acetic, hexanoic, octanoic, decanoic, 9-decenoic and dodecanoic acid. Additionally, one aldehyde (4-methylbenzaldehyde) and 13 esters were reported for the first time. Previous studies have not reported the following esters: isoamyl acetate, isobutyl octanoate, isoamyl octanoate, ethyl 9-decenoate, isobutyl decanoate, 2-phenylethyl acetate, ethyl dodecanoate, isoamyl decanoate, ethyl myristate, 2-methylbutyl decanoate, ethyl palmitate, ethyl 9-hexadecanoate and ethyl octadecanoate.

The alcohol group was the biggest class in terms

of its relative peak area (RPA) which made up almost half of the juice volatiles RPA (49.39%) and more than three quarters of wine volatiles RPA (78.43%) (Table 1). (Z)-3-Hexen-1-ol, hexan-1-ol and ethanol were the main alcohols by RPA in mangosteen juice; the former two volatiles, which impart green, grassy and slightly fruity odours, were detected as the main volatiles in mangosteen juice before (Macleod and Pieris, 1982; Laohakunjit *et al.*, 2007). However, almost all of the (Z)-3-hexen-1-ol and hexan-1-ol were depleted after fermentation; some of them could have been converted into esters.

Ethanol had the highest RPA (75.63%) in mangosteen wine, followed by isoamyl alcohol (1.35%) and 2-phenylethyl alcohol (1.25%). This was consistent with the result of Yu *et al.* (2013). Ethanol contributes to the alcoholic flavour, whereas isoamyl alcohol and 2-phenylethyl alcohol impart malty, fruity and rose flavour, respectively (Guth, 1997). 2-Methyl-3-buten-2-ol was only present in the juice but isobutyl alcohol was only detected in the wine, suggesting that the former was consumed and the latter was produced by yeast (Table 1).

The second largest group of compounds in the juice was the aldehyde and ketone group (25.68%). (E)-2-Hexenal (fruity, grassy) and hexanal (green, diacetyl-like) had the biggest RPA (10.33% and 10.99%, respectively) in this group, being similar to findings reported elsewhere (Macleod and Pieris, 1982). Like the C6 alcohols discussed above, most of the aldehydes were exhausted, possibly being reduced to their corresponding alcohols with only trace quantities of benzaldehyde (0.01%) and 4-methylbenzaldehyde (0.01%) remaining in mangosteen wine (Table 1).

Esters were the group with the second largest RPA (19.69%) in the mangosteen wine. Most of the esters were produced during fermentation, in which ethyl decanoate (12.35%) and ethyl octanoate (2.85%) were the two main esters (Table 1). These two esters contributed fruity and sweet flavour to the wine (Guth, 1997). Ethyl hexanoate might also contribute to the apple-like fruity flavour to wine because of its low threshold (0.005 mg/L) (Ong and Acree, 1999). (Z)-3-Hexenyl acetate was the only compound with decreased RPA.

Acids were the third largest group of volatiles in the juice (4.04%) and wine (1.49%). Five volatiles were common for both mangosteen juice and wine. The volatile acid with the biggest RPA in the juice was hexanoic acid (1.75%), which was the only acid not detected in the wine (Table 1). The main acid in mangosteen wine was decanoic acid (0.74%). 9-Decenoic acid was the only volatile acid not

Table 1. Volatile compounds (GC-FID peak area x 10⁶) and their relative peak areas (RPA%) in mangosteen juice and wine

Compound	LRI		Juice		Wine	
	Ref ⁶	Calculated	Peak area	RPA (%)	Peak area	RPA (%)
<i>Acids</i>						
Acetic	1454	1460	1.30 ± 0.15	1.03	3.47 ± 0.48	0.06
Hexanoic	1828	1849	2.36 ± 0.29	1.75	N.D. ⁵	N.D.
Octanoic	2062	2100	0.59 ± 0.32	0.33	29.66 ± 1.58	0.55
Decanoic	2275	2276	0.63 ± 0.20	0.41	39.76 ± 1.06	0.74
9-Decenoic	-	2340	N.D.	N.D.	2.34 ± 0.34	0.02
Dodecanoic	2487	2487	0.23 ± 0.08	0.18	4.77 ± 0.18	0.06
<i>Alcohols</i>						
Ethanol ⁴	936	945	10.79 ± 1.21	8.60	4051.8 ± 375.6	75.63
2-Methyl-3-buten-2-ol ³	1036	1037	2.38 ± 0.02	1.91	N.D.	N.D.
Isobutyl alcohol ⁴	1084	1102	N.D.	N.D.	8.80 ± 1.65	0.15
Isoamyl alcohol ^{1,4}	1222	1212	4.61 ± 0.11	3.67	72.29 ± 8.11	1.35
Hexan-1-ol ^{1,2}	1350	1353	21.84 ± 0.37	17.40	0.61 ± 0.08	0.01
(Z)-3-Hexen-1-ol ^{1,2,3}	1400	1385	22.31 ± 0.57	17.78	0.76 ± 0.08	0.01
2-Phenylethyl alcohol ^{1,4}	1938	1927	0.49 ± 0.09	0.36	67.09 ± 2.69	1.25
<i>Aldehydes and Ketones</i>						
Acetone ²	845	862	0.53 ± 0.05	0.42	N.D.	N.D.
Hexanal ²	1107	1082	13.79 ± 1.33	10.99	N.D.	N.D.
(E)-2-Hexenal ^{2,3}	1224	1223	12.97 ± 1.40	10.33	N.D.	N.D.
2-Furaldehyde ²	1457	1478	0.27 ± 0.03	0.21	N.D.	N.D.
Benzaldehyde ^{2,3}	1539	1540	2.03 ± 0.20	1.62	0.33 ± 0.07	0.01
4-Methylbenzaldehyde	1642	1668	2.86 ± 0.65	1.98	0.41 ± 0.01	0.01
<i>Esters</i>						
Ethyl acetate ⁴	926	905	N.D.	N.D.	10.87 ± 0.34	0.16
Ethyl butyrate ⁴	1032	1036	N.D.	N.D.	3.42 ± 0.12	0.05
Isoamyl acetate ¹	1117	1117	0.59 ± 0.13	0.47	6.68 ± 0.38	0.12
Ethyl hexanoate ^{1,4}	1226	1224	N.D.	N.D.	14.06 ± 1.48	0.25
Hexyl acetate ^{2,3}	1268	1268	0.67 ± 0.03	0.53	0.85 ± 0.06	0.01
(Z)-3-Hexenyl acetate ²	1328	1315	0.49 ± 0.12	0.39	0.30 ± 0.05	0.01
Ethyl octanoate ⁴	1432	1432	0.28 ± 0.14	0.22	152.79 ± 7.11	2.85
Isobutyl octanoate	1552	1549	N.D.	N.D.	1.01 ± 0.16	0.02
Ethyl decanoate ⁴	1638	1637	0.53 ± 0.28	0.42	661.75 ± 7.97	12.35
Isoamyl octanoate ¹	1652	1660	N.D.	N.D.	13.19 ± 1.78	0.25
Ethyl 9-decenoate ¹	1694	1691	0.11 ± 0.04	0.09	57.38 ± 10.32	1.07
Isobutyl decanoate	1755	1756	N.D.	N.D.	2.66 ± 0.36	0.05
2-Phenethyl acetate ¹	1827	1829	0.16 ± 0.02	0.12	7.83 ± 0.09	0.12
Ethyl dodecanoate	1844	1844	0.25 ± 0.08	0.16	81.97 ± 2.11	1.53
Isoamyl decanoate	1863	1864	N.D.	N.D.	16.67 ± 2.68	0.26
Ethyl myristate	2047	2100	N.D.	N.D.	2.64 ± 0.45	0.04
2-Methylbutyl decanoate	-	2100	N.D.	N.D.	1.06 ± 0.20	0.02
Ethyl palmitate	2229	2257	0.22 ± 0.04	0.16	13.14 ± 2.54	0.20
Ethyl 9-hexadecanoate	2292	2285	0.15 ± 0.04	0.13	5.66 ± 0.46	0.09
Ethyl octadecanoate	-	2461	N.D.	N.D.	1.03 ± 0.18	0.02
<i>Terpenes</i>						
α-Copaene ^{2,3}	1488	1488	1.04 ± 0.11	0.83	0.46 ± 0.02	0.01
α-Terpineol ²	1695	1701	0.05 ± 0.02	0.04	N.D.	N.D.
δ-Guaiene ²	1729	1720	0.09 ± 0.01	0.07	N.D.	N.D.
α-Muurolene ³	1730	1729	0.18 ± 0.05	0.15	N.D.	N.D.
δ-Cadinene ²	1761	1762	0.47 ± 0.01	0.37	N.D.	N.D.

¹compounds detected in mangosteen wine by GC-O.

²compounds present in the study conducted by Macleod and Pieris (1982).

³compounds present in the study conducted by Laohakunjit *et al.* (2007).

⁴compounds present in the study conducted by Yu *et al.* (2013).

⁵ N.D., not detected.

⁶Ref: literature LRI obtained from <http://webbook.nist.gov/>, www.pherobase.com, references.

detected in the juice.

Five terpenes were detected with the buttery odour compound α-copaene being a major terpene in the juice, agreeing with the report of Macleod and Pieris (1982). After fermentation, only α-copaene was present in trace amounts in the mangosteen wine (Table 1).

Volatiles in mangosteen wine obtained by HS-SPME-GC-O and CharmAnalysis™

A total of 10 volatile compounds were tentatively identified in the mangosteen wine (Table 2). Five esters, four alcohols and one terpene were found to be the odour-active compounds in the wine. Esters usually possess a pleasant fruity or floral aroma, which was confirmed by the results obtained. The alcohols detected had either green, grassy odour (hexan-1-ol,

Table 2. Odour-active volatiles identified by GC-O and CharmAnalysis™ in mangosteen wine

Compound	Flavour dilution value	Panel description	Literature description ¹
Isoamyl alcohol	729	Chocolate	Whiskey, malt
2-Phenylethyl alcohol	81	Floral	Honey, rose
(Z)-3-Hexen-1-ol	0	Floral	Grass
Hexan-1-ol	0	Musty	Green, resin
Isoamyl acetate	27	Fruity	Banana, pear
Isoamyl octanoate	9	Woody	Green, waxy
2-Phenylethyl acetate	3	Fruity	Rose, honey
Ethyl 9-decenoate	3	Floral	Fruity, fatty
Ethyl hexanoate	0	Grape	Apple
Longifolene	9	Green	Sweet, woody, rose, pine like

¹Literature descriptions obtained from www.thegoodscentscompany.com.

(Z)-3-hexene-1-ol) or malty, floral aroma (isoamyl alcohol, 2-phenylethyl alcohol).

Terpenes are one of the primary constituents of essential oils of many types of plants and flowers and can be described as floral, green. One previously unrecognised volatile compound sesquiterpene, longifolene (1,4-methanoazulene, decahydro-4,8,8-trimethyl-9-methylene), was identified as one of the 10 odour-active compounds determined by GC-O (Table 2) but could not be detected in the mangosteen juice or wine by HS-SPME-GC-MS/FID analysis.

Longifolene is reported to be one of the two most abundant aroma constituents of lapsang souchong tea smoked over pine fires, because this woody, pine-like terpene is primarily found in certain pine resins (Yao *et al.*, 2005) and is rarely reported in alcoholic beverages. This is the first time that longifolene was found in mangosteen wine and this terpene could have originated from the mangosteen juice, although the juice was not subjected to GC-O analysis. Our hypothesis is supported by the report of Macleod and Pieris (1982) who also found an unidentified volatile with a pine-like odour in mangosteen fruit using GC-MS coupled with olfactometry. Longifolene could also have been released from a glycosidically bound precursor by yeast glycosidases during fermentation. Indeed, longifolene has been found in wines fermented with *Schizosaccharomyces* yeasts (Delfini and Formica, 2001), although it has not been reported in wines fermented with *S. cerevisiae* yeasts. Further research is required to confirm the presence of

longifolene in mangosteen fruit.

To further analyse the 10 odour-active compounds, CharmAnalysis™ was performed for mangosteen wine by two trained panellists. A dilution factor of three was used (1:3, v/v). The results show that the volatile with the highest flavour dilution value (FDV) of 729 was isoamyl alcohol (Table 2), followed by 2-phenylethyl alcohol (FDV, 81) and isoamyl acetate (FDV, 27). Longifolene and isoamyl octanoate had the same FDV of 9. Two esters, 2-phenylethyl acetate and ethyl 9-decenoate, were present in the first dilution (FDV, 3) only. Three volatiles, (Z)-3-hexenol, hexan-1-ol and ethyl hexanoate, were only present in the original (undiluted) mangosteen wine (FDV, 0), indicating that they were not major aroma-active compounds in the wine.

Conclusion

The volatile components of mangosteen juice and wine were investigated. (Z)-3-hexen-1-ol, hexan-1-ol, hexanal and (E)-2-hexenal were the main volatiles in mangosteen juice. Only trace quantities of the three volatiles in the juice remained in the wine, resulting in a diminished mangosteen character of the wine. Besides ethanol, isoamyl alcohol, 2-phenylethyl alcohol, ethyl decanoate and ethyl octanoate dramatically increased after fermentation, which contributed to the alcoholic, floral and fruity flavour of the wine. Odour-active volatiles in the wine were isoamyl alcohol, 2-phenylethyl alcohol and isoamyl acetate. Longifolene was tentatively identified to be an odour-active volatile in mangosteen wine for the first time.

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