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Temporal variation of volatile compounds from Sri Lankan mango (Mangifera indica L.) fruit during ripening

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1. Introduction

Volatile compounds found in mango (Mangifera indica L.) fruit are responsible for its aroma and contribute to overall flavour [\[1\]](#page-8-0), which differs markedly among mango cultivars grown globally [\[2\]](#page-8-1). Lebrun et al. reported that a wide range of volatile compounds viz. monoterpenes, sesquiterpenes, esters, lactones and furanones (up to 435) have been identified in mango fruit [\[3](#page-8-2)]. Among these, 3-carene is the predominant compound found in most cultivars and is principally responsible for the typical mango aroma whilst limonene, β-ocimene, myrcene and α-terpinolene are prominent in other cultivars [\[4](#page-8-3)–[6\]](#page-8-3).

Mango fruit undergo many physiological and biochemical changes during ripening [[7](#page-8-4)–[10](#page-8-4)]. The levels and composition of volatile compounds in mango fruit depend on various factors such as genotype, pre-harvest factors [[11](#page-8-5)], harvest maturity [[12\]](#page-8-6), handling [\[13](#page-8-7)], postharvest storage conditions [\[14](#page-8-8)], postharvest treatments and processing [[8](#page-8-9),[13](#page-8-7)], and chilling injury [[15\]](#page-8-10). Most of the volatile compounds (terpene alcohols, nor-isoprenoid derivatives and aromatic alcohols) are glycosidically bound and are only liberated during ripening [\[3\]](#page-8-2).

Volatile compounds that contribute to the aroma of mango fruit have been studied extensively in several cultivars; however there is a paucity of information on volatiles of mango cultivars endemic to Sri Lanka. MacLeod and Pieris [\[4\]](#page-8-3) reported on volatile profile of Sri Lankan mango cvs. Willard, Karutha Colomban and Parrot; the volatiles were analysed using a gas chromatography-electron ionization mass spectrometry (GC-EIMS) and GC-chemical ionization mass spectrometry (GC-CIMS) techniques. This previous work did not detail how volatile compounds change according to postharvest storage. Therefore, the aim of this study was to quantify the most important volatile compounds of prominent Sri Lankan mango cultivars over two harvests using a GC-FID (GC- flame ionization detector) technique facilitated with Headspace–Solid Phase Microextraction (HS-SPME) and assess for the first time how postharvest temperature regimes affects volatile profile.

The following statements were tested in this experiment;

- Sri Lankan mango fruit has a significant quantity of key volatile compounds responsible for their characteristic aroma.
- The identity and concentration of mango fruit volatiles vary according to tissue type (skin/flesh) and pre- and postharvest factors.

2. Materials and methods

2.1. Preparation of calibration standards

Volatile calibration standards viz. α-pinene, β-pinene, 3-carene, terpinolene, β-caryophyllene, β-ocimene, myrcene, limonene, α-terpinene,

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α-humulene and toluene were obtained (Sigma-Aldrich, Dorset, UK). Twenty microliter of individual standards were separately mixed with 5 mL of methanol into 20 mL glass vials (Borosilicate, Waters) with Polytetrafluoroethylene (PTFE)-silicon septum screw caps at the concentration of 4 mL/L and extracted using a 100 μm polydimethylsiloxane coated HS-SPME fibre for 30 min at room temperature. Upon removal from the HS, the SPME fibre was thermally desorbed in the injector of a GC-FID (Agilent Technologies, 6890 N Network GC system, USA) coupled with HB5 capillary column $(30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \text{ µm}$, Agilent Technologies). A temperature program was set in the method development in order to get better peak separation within a relatively short run time. Briefly, oven temperature was maintained at 40 \degree C for 10 min and then ramped at 12.5 °C/min to 65 °C followed by 2.5 °C/min to 110 °C and 10 °C/min to 150 °C and then maintained for 5 min. Detector and injector temperatures were set as 270° C and 220° C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min. Retention time of individual volatile standards was recorded.

The mixed calibration standards were made by mixing a 30 μL of each standard together into a 10 mL of methanol; the stock standard (3 mL/L) was further diluted into 1.5, 0.75, 0.3, 0.15, 0.03 and 0.015 mL/L since mango had different volatile compounds at a wide range of concentrations. Then mixed standards were extracted and analysed as previously described. The peaks of mixed calibration standards were confirmed with peaks of individual standards according to their retention time. The calibration curve of each mixed standard was used for the quantification of volatile compounds in mango samples.

2.2. Experiment 1

Preclimacteric mango fruit from the cvs. Willard and Karutha Colomban were picked at harvest maturity from the Eastern Region of Sri Lanka (Batticaloa) and air-freighted to the UK. Mangoes reached the Plant Science Laboratory, Cranfield University within four days from harvest date. Upon arrival (fruit remained at mature green stage which is recognised as a defined maturity stage in mango production), fruit were ripened at 30 °C or 20 °C for 6 days. Furthermore, fruit were transferred from 20 \degree C to 30 \degree C and 30 \degree C–20 \degree C after day 3 of ripening in order to understand how temperature variation impacts biochemical composition. Sampling (out-turns) was carried out at day 0, 3 and 6 during ripening. Whole mango fruit were weighed using a precision electronic balance (Sartorius, Switzerland) before ripening trials began and at each out-turn. Peel (15 g) and pulp (20 g) samples were taken from each fruit and immediately snap-frozen in liquid nitrogen, and stored at -40° C. Ethylene levels and respiration rates were not measured as the focus of the work was to quantify and profile the temporal change in volatiles in different genotypes as affected by storage temperature.

2.3. Experiment 2

Mango fruit (15 per cultivar) from the cvs. Willard, Karutha Colomban and Malgova were picked from the Eastern Region of Sri Lanka (Batticaloa) and air-freighted to the UK. Upon arrival at the Plant Science Laboratory, Cranfield University, fruit were ripened at 32° C for 4 days and the peel and pulp samples were taken at day 0, 3 and 4 as described in Experiment 1.

2.4. Sample preparation

Sample preparation was based on work by Engel and Tressl, Lalel et al. and Dang et al. [[8](#page-8-9)[,16](#page-8-11)[,17](#page-8-12)]. Briefly, 5 g of frozen pulp and peel samples were thawed for 10 min and then separately homogenized in 17.5 mL of saturated sodium chloride solution in a 40 mL glass vial (VGA-090-030 N, Borosilicate, Waters) with PTFE-silicon septum screw caps at room temperature. Peel samples were treated differently since they were crushed into small particles using a pestle and mortar whilst frozen in liquid nitrogen before being adding to a saturated sodium

chloride solution. The slurry was left on the bench for 30 min in order to stabilise the temperature of the slurry before being extracted using a HS-SPME fibre.

2.5. Extraction and quantification of volatiles

The extraction of volatiles followed the methods of Engel and Tressl, Lalel et al. and Dang et al. with modification $[8,16,17]$ $[8,16,17]$ $[8,16,17]$ $[8,16,17]$ $[8,16,17]$ $[8,16,17]$. The SPME fibre was inserted in the head space of the homogenized samples and volatiles were extracted for 30 min at ambient temperature $(23 \pm 2 \degree C)$. Upon removal, the SPME was thermally desorbed into GC as described in section [2.1](#page-0-1). Volatiles were detected using FID and quantified using calibration standards.

2.6. Statistical and chemometric analysis

Data were subjected to analysis of variance using Genstat for Windows Version 10 (VSN International Ltd. Herts., UK). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation. Tests for correlations between mean values for concentrations were made using Spearman's Rank Correlation. Correlations are presented with the Spearman's Correlation Coefficient (r) and P value based on a two-tailed test. The Principle Component Analysis (PCA) was carried out using Unscrambler X version 10.0.1 (Oslo, Norway) in order to understand the chemometric profile of spatial and temporal variation.

3. Results

Up to 435 volatile compounds including monoterpenes, sequiterpenes, esters, lactones and furanones have been identified in various mango fruit using different extraction and quantification methods with the major contribution being from 3-carene, limonene, β-ocimene, myrcene and α -terpinolene [[3](#page-8-2)–[6,](#page-8-2)[18\]](#page-8-13). However, only the 10 most important volatiles were targeted and quantified in cultivars tested in this study using the described newly developed method.

3.1. Experiment1

Terpinolene was the prominent volatile compound measured in both peel (1849.0 μg/kg) and pulp (588.0 μg/kg) of mango cv. Willard followed by α-pinene (peel 1:1.8 and pulp 1:2), β-caryophyllene (peel 1:4.1 and pulp 1:5.4), 3-carene (peel 1:7.7 and pulp 1:8.8) and α -humulene (peel 1:7.9 and pulp 1:10). However, other targeted volatile compounds were also found in considerable quantities. Generally, peel had three-to ten-fold higher concentrations of volatile compounds than pulp. Ocimene was the main volatile in peel (9194.0 μg/kg) and pulp (1749.0 μg/ kg) of cv. Karutha Colomban followed by β-caryophyllene (peel 1:8.3 and pulp 1:56.4) and α-humulene (peel 1:15.3 and pulp 1:92) whilst other target volatiles were measured at much lower concentrations. The concentration of 3-carene (important volatile compound responsible for the characteristic aroma of many commercial mango fruit) was generally lower in cultivars tested, but cv. Willard contained several fold higher levels of 3-carene than cv. Karutha Colomban (Table 1a and b).

As expected, the concentration of volatile compounds increased significantly in cv. Willard pulp during ripening, but the increase was higher in fruit ripened at 20 °C rather than at 30 °C. Meanwhile, volatile concentrations decreased in peel during ripening; however the decrease was higher in fruit ripened at 20° C than 30° C. In contrast, volatile content of cv. Karutha Colomban pulp decreased in fruit ripened at 20 \degree C, but increased by more than two-fold in fruit ripened at 30 $^{\circ}$ C. Mango cv. Karutha Colomban peel also showed a similar variation of volatiles during ripening, but the concentration was about three-to five-fold higher than that of cv. Willard peel. The temperature change during ripening had a significant impact on volatile concentration of both peel and pulp of the cultivars tested as it decreased the concentration markedly at the end of day 6 (Table 1a and b).

Variation in volatile compounds (μg/kg) in peel and pulp of mango cv. Willard fruit during ripening at 20 °C and 30 °C. Mango (n = 3) ripened at 20 °C was transferred to 30 °C whilst fruit (n = 3) ripened at 30 °C was transferred to 20 °C at day 3. Samples were taken (n = 3) before the start of ripening trial (day 0), day 3 (Temperature swap only) and day 6 from peel and pulp.

NS: not significant.

Table 1 (b)

Variation in volatile compounds (μg/kg) in peel and pulp of mango cv. Karutha Colomban fruit during ripening at 20 °C and 30 °C. Mango (n = 3) ripened at 20 °C was transferred to 30 °C at day 3. Samples were taken $(n = 3)$ before the start of ripening trial (day 0), day 3 (Temperature swap only) and day 6 from peel and pulp.

NS: not significant.

3.2. Experiment 2

Ripening of mango fruit at \pm 30 °C resulted in significantly higher concentrations of volatiles in the cultivars tested, than that observed in many mainstrean commercial cultivars [\[13](#page-8-7)]. As per Experiment 1, terpinolene and ocimene were dominant in peel and pulp of mango cvs. Willard ([Fig. 1](#page-3-0)) and Karutha Colomban ([Fig. 2\)](#page-4-0), respectively. Yet, the concentration of ocimene was about two-fold lower than that previously described [\(Table 2](#page-5-0)). However, generally, the concentration of volatile compounds reduced in both peel and pulp of cv. Karutha Colomban whilst they increased in peel and reduced in pulp of cv. Willard during ripening. Myrcene was the major volatile compound in peel (1437.0 μ g/kg) and pulp (2644.0 μ g/kg) of cv. Malgova ([Fig. 3](#page-6-0)), but the concentration increased about five-fold in peel during ripening whilst it

Fig. 1. Examples of GC-FID chromatogram of volatile compounds from Sri Lankan mango (A-peel, B-pulp) cv. Willard. 5 g sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME at ambient temperature (23 \pm 2 °C) for 30 minutes.

Fig. 2. Examples of GC-FID chromatogram of volatile compounds from Sri Lankan mango (A-peel, B-pulp) cv. Karutha Colomban. 5 g sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME at ambient temperature (23 ± 2 °C) for 30 minutes.

reduced in pulp. Ocimene (peel 1:1.8 and pulp 1:2.1), β-caryophyllene (peel 1:1 and pulp 1:11), α -pinene (peel 1:1.4 and pulp 1:1.7) and α-humulene (peel 1:1.8 and pulp 1:9.4) were also found in relatively higher concentrations in both peel and pulp of cv. Malgova than other cultivars tested and followed a similar pattern to that observed in cv. Willard during ripening ([Table 2\)](#page-5-0). Even though, β-caryophyllene and α-humulene were not the principal volatile compounds in the cultivars tested, they were found in relatively higher concentrations with similar

variation. In general, cv. Malgova showed higher temporal variation of volatile compounds than other cultivars tested whilst cv. Karutha Colomban demonstrated lowest temporal variation of volatiles during ripening. The variance was most marked in pulp samples of cv. Willard than other cultivars tested. This revealed that volatile characteristics of cv. Malgova may be more susceptible to various factors that influence ripening i.e. temperature variation and enzyme production.

able 2

3.3. Chemometric analysis

Volatile composition was the major discriminatory factor between cultivars followed by variation between peel and pulp ([Figs. 4 and 5\)](#page-7-0). According to PCA score plots, genotypic variation was distinct for each cultivar tested as each contained speci fic volatile compounds. PC 1 differentiated Malgova from cvs. Willard and Karutha Columban; PC 2 differentiated between Willard and Karutha Columban [\(Fig. 4\)](#page-7-0). Peel had a higher variance than pulp, since it contained signi ficantly higher concentration of volatile compounds with the greater variation occurring during ripening at different temperatures tested. PC 1 tended to show trends of difference between peel and pulp [\(Figs. 4 and 5](#page-7-0)). Therefore, PCA demonstrated genotypic and spatial variations in volatile concentration during ripening. Baseline peel samples of cv. Karutha Colomban had very high concentration of ocimene compared to other samples, and was clustered away from other samples (out of con fidence ellipse. [Fig. 5\)](#page-7-1).

4. Discussion

4.1. Experiment1

It was clearly demonstrated that peel samples of cultivars tested contained similar volatile composition, but the concentration was generally higher than in pulp. However, there were some de finable differences among compounds observed in both peel and pulp. Presence of higher unbound (free) volatile compounds (aldehydes, fatty acids and terpenes) in peel than pulp may be a cause for this variation. Lalel et al. [[8](#page-8-9)] reported that most of the glycosidically-bound volatile compounds of cv. Kensington Pride are higher in peel than pulp at the mature and half-ripe stage compared to the fully ripe stage.

Ocimene has a very strong aroma, which is the principle volatile compound in Indian mango cv. Alphonso [[19\]](#page-9-0), whilst 3-carene is abundant in mango cultivars grown in Florida (Keitt, Kent and Tommy Atkins), Brazil and Venezuela. 3-carene was comparatively low in Sri Lankan mango cultivars and looked to be more sensitive to ripening temperature since it decreased drastically during higher temperature ripening (30 C) yet increased during lower temperature (20 C) ripening (two-fold). Terpinolene content increased in cv. Willard pulp during lower temperature ripening by two-to three-fold compared to higher temperature ripening, which is in agreement with that found in cv. Kensington Pride pulp [[17](#page-8-12)], even though terpinolene content (966.7 μg/kg) increased two-to three-fold (2517.2 μg/kg) after 3 weeks during ripening at 13 C. In addition to terpinolene, 3-carene content of cv. Kensington Pride was also in line with cv. Willard and followed a similar variation during lower temperature ripening [\[17](#page-8-12) [,20](#page-9-1)]. In contrast, ocimene concentration of cv. Karutha Colomban pulp increased during higher temperature ripening whilst it reduced in lower temperature ripening.

Factors related to genotype, variety and treatment also in fluence production of volatiles compounds. Amino acid, lipid depolymerisation, shikimic acid and isoprenoid pathways play important roles in the synthesis of aroma compounds. Whereas, aldehydes and alcohols are produced through the lipid depolymerisation pathway, isoprenoids are synthesised due to the degradation of β -carotene and lycopene [\[13](#page-8-7),[14](#page-8-8), [21\]](#page-9-2). Production of volatile compounds reduced in the peel at ripe stage (the trend was vice versa in pulp) with some of the glycosidically bound volatiles being probably migrating from peel to pulp as the integrity of the cell walls declined [[22\]](#page-9-3). The choice of ripening temperature is cultivar-oriented as far as volatiles are concerned. Acids, esters and lactones are usually developed via lipid metabolism during fruit ripening; however lipid metabolism is further enhanced by the ripening temperature. This was previously supported in that mango fruit (cv. Kensington Pride) ripened at 20 C showed higher terpene production than fruit ripened at 15, 25, 30 and 35 °C [\[12](#page-8-6)]. Variation in volatiles during higher and lower temperature ripening may be due to the inhibition of enzymes involved in several metabolic pathways (conversion of acetyl CoA to

Fig. 3. Examples of GC-FID chromatogram of volatile compounds from Sri Lankan mango (A-peel, B-pulp cv. Malgova. 5 g sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME at ambient temperature (23 \pm 2 °C) for 30 minutes.

volatile compounds) and the suppression of ethylene biosynthesis [\[23](#page-9-4)]. Since all other volatile compounds except ocimene were found at very low concentration in cv. Karutha Colomban, ocimene could be responsible for the characteristic aroma, which is synonymous with this endemic Sri Lankan cultivar.

4.2. Experiment 2

Terpinolene has been reported to have floral, sweet and pine-like aroma properties, whilst ocimene and myrcene are responsible for the green aroma of mango fruit [\[16](#page-8-11)]. The 3-carene (ripe mango flavour) is

Fig. 4. PCA score plot of mango cvs. Willard (right) and Karutha Colomban (left) fruit peel (upper) and pulp (lower) ripened at 20 or 30 °C for 6 days with a temperature shock from 20 to 30 °C at day 3 (Experiment 1) was performed in Genstat. Hotelling ellipse shows 95% confidence. W: Willard; K.C: Karutha Columban; 0, 3 and 6: Days after ripening; 20-20, 20–30 and 30-30: Ripening temperatures; P: Peel; F: Pulp (flesh).

Fig. 5. PCA score plot of mango cvs. Willard, Karutha Colomban and Malgova fruit (peel and pulp) ripened at 32 °C for 4 days (Experiment 2). Hotelling ellipse shows 95% confidence. W: Willard; K.C: Karutha Columban; M: Malgova; 0, 3 and 4: Days after ripening; P: Peel; F: Pulp (flesh).

the most abundant free terpene found in many mango cultivars, but it may be converted into other compounds during ripening. Caryophyllene and humulene are responsible for the woody-spicy odour of some mango fruit [[2](#page-8-1)]. Therefore, higher terpinolene content of cv. Willard may enhance its flavour and appeal to some consumers. MacLeod and Pieris [[4](#page-8-3)] reported that cv. Karutha Colomban produces mainly (38%) cis-β-ocimene (95.1 μg/kg) whilst cvs. Willard (135.5 μg/kg) and Parrot (219.8 ^μg/kg) contains mainly terpinolene (32–35%). However, these concentrations were several fold lower than the respective cultivars tested in this study, and thus differences may be due to extraction and quantification methods, location and mango harvesting season.

The presence, abundance and the relative changes of volatile profile during ripening can determine the marketability of mango fruit [\[24](#page-9-5)]. The type and concentration of volatile compounds varied greatly among cultivars tested. The overall concentration of volatiles was higher in Sri Lankan cultivars than for more mainstream commercial cultivars such as Kensington Pride and Delta R2E2. Volatile concentration also varied significantly within the same cultivar in the experiments conducted in this study. These differences might have been due to the varied growing location of fruit and transport conditions. Though fruit were picked during the major harvesting season (April to July) of Dry zone (Eastern region) of Sri Lanka for both experiments, the source of fruit were from different orchards in the Eastern region. Different locations may have differences in age of orchards, soil nutrients, source of plant stock (budded/grafted or seedling) and fruit size. Therefore, these factors may influence the type and concentration of volatile compounds. These findings are in agreement with other authors, where volatiles of mango cultivars vary according to location [\[25](#page-9-6)–[27](#page-9-6)]. The distinct differences among cultivars can be attributed to volatile compounds unique to each cultivar and this is likely to impact on flavour and perhaps perceived likeability. However, mango flavour cannot be attributed specifically to any single component [[4](#page-8-3)[,28](#page-9-7)].

Diversity of both soil and tropical ecology have contributed to mango varieties having a unique appearance, taste and aroma in different geographical regions [\[29](#page-9-8)]. A number of mango cultivars have been cultivated in Sri Lanka. Since the country has vast climatic variation, cultivars differ among agro-ecological regions. Cultivars such as Willard, Karutha Colomban, Vellai Colomban, Chempattan, Malgova and Ampalavi are commonly grown in the low country (Dry zone) that experiences more or less a tropical climate whilst cvs. Karutha Colomban, Vellai Colomban, Willard and Betti Amba are grown in the mid country (Intermediate zone). Cultivars such as Vellai Colomban, Peterpassand, Dambara and Gira Amba are grown in the up country (Wet zone) which has a temperate climate [\[30\]](#page-9-9). However, mango cvs. Willard, Karutha Colomban, Malgova, Ampalavi and Vellai Colomban are very popular in Sri Lanka since they are widely appreciated for their aroma and taste. Authors report a high-density genetic map based on large-scale marker development in mango using specific-locus amplified fragment sequencing, and this might form the basis for fine QTL mapping of mango [[31\]](#page-9-10) and could help elucidate the underlying genetic factors influencing development of mango aroma in the future.

4.3. Chemometric analysis

GC-FID coupled with HS-SPME was utilized to analyse volatiles from peel and pulp of mango fruit ripened at different ripening temperatures (32 °C and 20 °C/30 °C). The temporal variation and spatial distribution of volatile compounds were measured and optimum ripening period defined according to the concentration and composition of volatiles at different temperatures. The type and concentration of volatile compounds responsible for the characteristic aroma of mango fruit varied significantly among Sri Lankan mango cultivars tested. The levels and composition of volatile compounds in mango fruit depend on various factors i.e genotypes, pre-harvest factors [\[11](#page-8-5)], harvest maturity and harvesting [[12\]](#page-8-6), handling, metabolic pathway during ripening [\[13](#page-8-7)], postharvest storage conditions (temperature, gas composition, etc.) [\[14](#page-8-8)], postharvest treatments and processing [[8](#page-8-9),[13\]](#page-8-7) and chilling injury [[15\]](#page-8-10).

5. Conclusions

The extraction of volatiles using HS-SPME for 30 min at ambient temperature resulted in higher volatile concentrations in the cultivars tested. However, the identity and concentration of volatiles varied significantly according to genotype. Terpinolene was the main volatile compound in cv. Willard whilst ocimene and myrcene were predominant in cvs. Karutha Colomban and Malgova, respectively. Peel samples contained higher content of volatiles than pulp samples. The influence of ripening temperature on the volatile content of mango fruit varied according to the genotypic and spatial differences. Since each volatile compound has its own characteristic aroma, it may determine the consumer acceptance and marketability of mango fruit.

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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