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Profiling of primary metabolites of *Averrhoa carambola*, *Spondias dulcis* and *Syzygium malaccense* fruits revealed underpinning markers during "on-tree" maturation and ripening stages

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Abstract: The study aimed to profile and quantify sugars and organic acids metabolites in carambola, June plum and otaheite fruits during three different "on tree" stages: immature, green-mature and ripe stages. Metabolites were profiled and quantified by gas chromatography-mass spectrometry (GC-MS). Results showed that glucose, fructose, galactose, arabinose, and the sugar alcohol myo-inositol were detected in all fruits, while sucrose was detected in carambola and June plum only. Organic acids identified in all fruits were malic acid, citric acid, propanoic acid, and acetic acid. Comparatively, June plum showed the highest content of total sugars and carambola the lowest, while the highest total in organic acids content was found in otaheite and the lowest in carambola. On the other hand, most sugars increased during ripening of the three fruits, while organic acids decreased. Total sugars increased by 37%, 8% and 46% in ripe carambola, June plum and otaheite, respectively. Total organic acids decreased by 20% and 49% in ripe carambola and otaheite, while they slightly increased by 3% in ripe June plum. Furthermore, sugars/organic acids ratio in all fruits increased during maturation and ripening stages. Principal component analysis (PCA) showed two main groups of highly scoring metabolites, while the hierarchical cluster analysis (HCA) showed that the metabolites were grouped into three main clusters. Conclusively, results showed that glucose, fructose, malic acid and tartaric acids were the key marker metabolites of the maturation and ripening stages of the three fruits.

1. Introduction

Carambola (*Averrhoa carambola*) belongs to the family *Oxalidaceae*

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originated in Asia but has since developed a tolerance for tropical climates (Shui and Leong, 2006). Presently, carambola is cultivated extensively in India and China (Narain *et al*., 2001), but Malaysia is the largest exporter (Abdullah *et al*., 2007). Work has been done to improve carambola cultivars in the United States in the 1930s, and since then, the fruit became more popular, while some sub species of star fruit exist in the Caribbean, Central America and tropical West Africa (Neto *et al*., 2009). Unripe carambola is moderately sour, but when ripe it is very sweet and used for juice, fruit salads, chutney, stewed fruits, garnish drinks and dishes, or adding it to fruit smoothies. Physiologically, carambola does not exhibit climacteric ripening behaviour even though it continues to synthesise carotenoids and develop its yellow-orange colour (Warren, 2009).

June plum (*Spondias dulcis* Forst. Syn. *Spondias cytherea* Sonn.), a drupe belonging to the family Anacardiacea, is native to the Society Islands of the South Pacific ranging from Melanesia to Polynesia and was first introduced to Jamaica in the year 1782, and later in 1792 by Captain Bligh (Graham *et al*., 2004 a). Although the fruit is well known, there are no named cultivars, but both forms exist, one is with a thick mesocarp and a more pleasant taste, and another has long spines, a woody endocarp with a pungent and resinous taste (Daulmerie, 1994). Ripe June plum is eaten mostly raw but is also used in making refreshing drinks, jam, chutney, sauce or served with meat and seafood. When harvested green and mature, June plum ripens, and studies showed that respiratory pattern is typical of a climacteric fruit (Daulmerie, 1994; Graham *et al*., 2004 b).

Otaheite (*Syzygium malaccense*) is a berry and belongs to the family Myrtaceae. It is thought to be native to the Indo-Malay or Southeast Asian region (Whistler and Elevitch, 2006). Otaheite is, however distributed in many tropical countries throughout the world, particularly in Africa and South America (Oliveira *et al*., 2011). Other English common names for this fruit include: Malay apple, mountain apple, pomerac, and rose apple (Batista *et al*., 2017). Two colour forms exist: one which produces red flowers and fruits and another, less common variety, which produces white flowers and fruit (Whistler and Elevitch, 2006). Ripe otaheite fruit is not very sweet, and often eaten raw. However, in some tropical countries, it is stewed with sugar to make jam or wine and refreshing drinks. No study is recorded on whether otaheite is a climacteric or non-climacteric fruit except the work of Basanta (1998) who reported that otaheite is a non-climacteric fruit.

Ripening can be defined as the total changes of fruit tissue metabolism, leading to the production of an attractive fruit which can be consumed, aiding in the release and dispersal of the seed (Adams-Phillips *et al*., 2004). The ripening process is characterized by softening of fruit tissue and an increase in volatile compounds as well as pigments such as carotenoids and flavonoids which results in a more appealing fruit (Giovannoni, 2001). The concentration of sugars and organic acids in fruits varies depending on the fruit variety and the environmental conditions of the parent plant (Haruenkit, 2004). Overall, there is a general decrease in organic acids and an increase in sugar content as fruit development progresses, due to decarboxylation of organic acids and breakdown of stored carbohydrates to produce sugars (Batista-Silva *et al*., 2018). According to Etienne *et al*. (2013), using advanced technologies, i.e. proteomics, transcriptomics and metabolomics, studies have shown evidence of a shift from the accumulation of organic acids to sugar synthesis in the final stage of fruit development in several species of fruit. Thus, the respiratory pathways commonly involved in the reduction of fruit sugars are glycolysis, oxidative pentose phosphate (OPP) pathway, and the tricarboxylic acid (TCA) pathway (Tucker, 2012).

Because most fruits reach their best sensorial and commercial quality attributes when they ripen on the plants, the correct maturity for harvest of fruits impacts their postharvest shelf-life and quality attributes during storage (Thompson, 2003). Gene expression resulting from natural processes and triggering fruit ripening induces many metabolic processes leading to the formation of hundreds and even thousands of different metabolites (Pech *et al*., 2013).

Although extensive literature is readily available on the metabolic changes during the maturation, ripening and senescence of fresh crops, few researches reported on changes in metabolite profiles during postharvest ripening and senescence (Benkeblia, 2016). However, a limited work was carried out on the metabolites variation during the development and ripening of peach (Lombardo *et al*., 2011), strawberry (Zhang *et al*., 2011), pear (Oikawa *et al*., 2015), and pitaya (Wu *et al*., 2019), while scarce work was reported on some tropical fruit (Fabi

et al., 2010).

In the present work, in order to explore the variation of the metabolic profile of three tropical fruits commonly consumed in the tropics, we performed a profiling study of primary metabolites which are the main indicators of the maturation and the ripening of fruits. For this purpose, we selected three fruits namely carambola (sweet type), June plum and otaheite. One of the goals of this study was to assess how metabolically different the "on tree" maturation and ripening stages of these three fruits are and to find out if there is any particular metabolic profile which could be associated with these two stages of the fruits. On the other hand, by evaluating the metabolomic pattern at both maturation and ripening stages of the three fruits. Overall, this study is aiming to explore a part of the chemical potential of carambola (sweet type), June plum and Otaheite which will aid in the future to know the primary metabolites of these fruits that may correlate to different stages and determine which metabolites might be used as maturation and ripening markers.

2. Materials and Methods

Fruits collection

For the purpose of the present study, three physiological stages of the fruits were investigated: green-immature, mature, and ripe stages (Fig. 1). The colour and softness of the fruits were the two criteria used for discriminating the different maturation and ripening stages. A period varying from seven to ten days elapsed between each harvesting (sampling) stage. The commercial (optimal) harvesting stage of carambola is stage 3 (ripe), while for June plum and otaheite is stage 2 (mature). The fruits carambola, June plum, and otaheite of the three stages of each fruit were collected from three trees of same location. The fruit June plum was collected from a farm in St. Elizabeth. Otaheite fruits were collected from a local farm in Mona, Kingston, and carambola samples were collected from Orange River Research Station in St. Mary. The three stages differentiated and sampled based on their size and colour. For each stage, three samples were collected from three different trees, and each sample consisted of at least six fruits. Fruits collected were controlled for absent of any defect, wound or disease. Immediately after being collected, fruits were placed in plastic bags, and the bags were placed on ice in a cooler and transported to the laboratory within few hours. Then, fruits were washed with mild detergent and rinsed thoroughly, followed by seed removal, dicing or slicing and frozen for 48 hours at -20°C.

Freeze-drying

Prior to the extraction of the profiled sugars and organic acids, samples were freeze-dried in a Labconco freeze-drier (Labconco Corp., Kansas, MO, USA). After six days and complete drying, samples were sealed in plastic bags under vacuum using a MULTIVAC C100 vacuum packer (MULTIVAC, Wolfertschwenden, Germany) and stored under dryness in a desiccator until further use.

Extraction of sugars and organic acids metabolites

Sugars and organic metabolites were extracted by the method described by Broeckling *et al*. (2005) with some modifications. In an Eppendorf tube, 300 mg of freeze-dried samples were mixed with 0.75 mL HPLC grade water containing 26 µg/mL Ribitol was added as internal standard and the tubes vortexed. After equilibrating to room temperature. The tubes were incubated in a shaker for 10 min at 80˚C, fol-

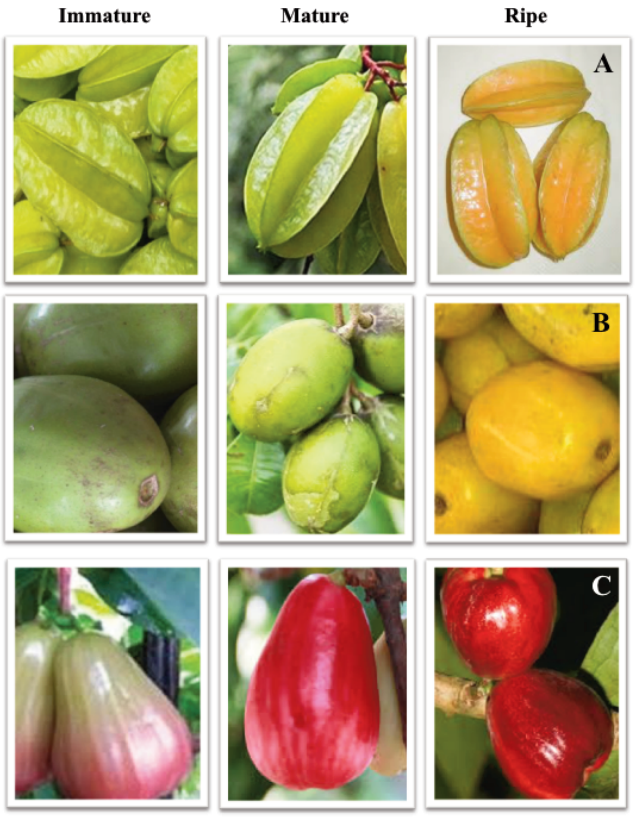

Fig. 1 - Carambola (A), June plum (B) and otaheite (C) at different development and ripening stages.

lowed by incubation at room temperature for c.a. 45 minutes. Afterwards, the tubes were cooled to 4˚C and centrifuged at 10 000 rpm for 15 minutes, the supernatant collected, and the pellet discarded. To the collected supernatants, 250 µL were mixed with 100 mL absolute EtOH and the samples dried under vacuum until dryness and stored at -20°C until GC-MS analysis.

Derivatization

Prior to GC-MS analysis, samples were derivatised as described by Broeckling *et al*. (2005) with some minor modifications. The dry residues were mixed with 80 µL of BSTFA+1% TMCS (Sigma Aldrich, St Louis, MI, USA) and 20 µL pyridine, vortexed and centrifuged for 10 seconds at 10 000 rpm. Afterwards, the mixtures were incubated for 20 minutes at 85˚C. After incubation and equilibrating to room temperature, 200 µL isooctane (2,2,4 trimethylpentane) (Sigma Aldrich, St Louis, MI, USA) were added to the mixture, vortexed and followed by a centrifugation at 10 000 rpm for 10 seconds. From the mixtures, 100 µL were transferred to a 300 µL glass insert for the GC-MS analysis.

GC-MS analysis of sugars and organic acids

The samples were analysed by GC-MS using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass spectrometer scanning in the m/z range from 40 to 550. The column used was an HP 5MS (5% Phenyl Methyl Polysiloxane, 30 m × 250 µm × 0.25 µm) with helium as the gas carrier at a constant flow rate of 1.0 mL/min. The samples were injected at a 15:1 split ratio. Initially, the inlet line was held at 260˚C and the transfer line was held at 280˚C. Separation was achieved with an initial temperature program of 40˚C for 2 min, then ramped up at 4˚C per minute to 240˚C and held for 1 minute. The temperature was then increased to 10˚C per minute to 315˚C.

In order to produce the concentration curve, a mixture containing 250 µL of 26 µg/mL of ribitol was used. To the mixture, 20 µL of pyridine and 80 µL of BSTFA containing 1% TMCS was added, vortexed and injected into the GC-MS. Prior to the injection, 50 µL of the mixture were taken and 50 µL of isooctane were added. This was repeated by adding each time 50 µL of isooctane until 6 concentration curves were produced. From the curve a scatter plot with a trend line was generated. The concentrations of the different profiled sugars and organic acids metabolites were calculated from the generated trend line. The generated MS files were extracted, and the deconvolution and identification of the metabolites was carried out by using Agilent MSD Chem Station (Version F.01.01.2017) along with NIST library (Version 11 MS Mass Spectral Library) and AMDIS (Version 2.66) software.

Statistical analyses

For the analysis of each sample (three fruits for each ripening stage and for each species), six samples were analysed, and the data were averaged. The data were analysed and compared by running analysis of variance (ANOVA), Tukey's Honestly Significant Difference (HSD) Post Hoc test using SPSS software package (version 22.0, (IBM Corp., New York, USA). The significance level of all statistical hypotheses testing procedures was predetermined at $P < 0.05$ and 0.01. For the classification, clustering, and regression, PCA (principal component analysis) and HCA (hierarchical cluster analysis) were performed using SPSS software package (version 22.0, IBM Corp., New York, USA), while the clustered heatmap was generated using ClustVis free software (https://biit.cs.ut.ee/clustvis/).

3. Results

Profile and sugar contents of carambola, June plum and otaheite fruits

The profiling of sugars showed that seven saccharides and one sugars alcohol were detected in carambola, June plum and otaheite fruits (Table 1). Glucose, fructose, sucrose, galactose, arabinose, and myo-Inositol have been detected in carambola, June plum and otaheite, however, mannose and xylose were not detected in June plum while sucrose and mannose were not detected in otaheite. Overall, fructose, sucrose, galactose, in carambola and June plum, and glucose, fructose and galactose in otaheite increased during maturation and ripening stages, while the other sugars varied differently in the three fruits.

Interestingly the highest levels of glucose, fructose and sucrose were observed in June plum, highest levels of galactose and xylose in otaheite, and the highest levels of arabinose and myo-inositol in carambola. Results also showed that glucose and fructose were the most predominant monosaccharides in carambola and June plum, while in otaheite glucose, fructose and galactoses were predominant.

Table 1 - Profiled sugars and sugar alcohols and their contents (mg/g dry weight) in carambola, June plum and Otaheite during the three development and ripening stages

Metabolites	Immature	Mature	Ripe
Carambola			
Glucose	51.40 ± 3.27 a	68.20 ± 5.18 b	56.36 ±2 .99 ab
Fructose	30.69 ± 2.71 a	28.87 ±1.23 a	57.79 ± 7.98 b
Sucrose	1.97 ± 0.08 a	13.73 ± 2.05 b	3.59a ± 0.34 a
Galactose	9.50 ± 0.45 a	14.14 ± 0.52 b	11.11 ± 0.77 a
Mannose	n.d.	n.d.	1.23 ± 0.53
Arabinose	2.18 ± 0.42 a	1.86 ± 0.01 a	1.90 ± 0.03 a
Xylose	n.d	11.62 ± 1.85 a	6.28 ± 1 .11 b
Myo-Inositol	2.16 ± 0.11 a	3.30 ± 1.28 a	2.20 ± 0.08 a
Total	97.9 a	133.2 b	134.1 b
June plum			
Glucose	124.11 ± 7.62 a	112.38 ± 12.84 a	90.63 ± 18.41 b
Fructose	79.43 ± 3.26 a	72.68 ± 14.12 a	94.38 ± 18.01 b
Sucrose	7.46 ± 2.36 a	11.33 ± 1.76 a	35.03 ± 10.06 a
Galactose	13.01 ± 0.61 a	12.36 ± 1.77 a	23.67 ± 5.75 b
Mannose	n.d.	n.d.	n.d.
Arabinose	n.d.	1.28 ± 0.09 a	1.26 ± 0.56 a
Xylose	n.d.	n.d.	n.d.
Myo-Inositol	1.88 ± 0.08 a	1.99 ± 0.11 a	1.55 ± 0.61 a
Total	225.9 a	210.8 a	245.8 b
Otaheite			
Glucose	51.88 ± 3.12 a	103.40 ± 10.2 b	111.86 ± 6.59 b
Fructose	43.97 ± 2.75 a	62.40 ± 1.24 b	68.87 ± 9.78 b
Sucrose	n.d.	n.d.	n.d.
Galactose	34.33 ± 1.66 b	12.44 ± 2.45 a	10.79 ± 2.57 a
Mannose	n.d.	n.d.	n.d.
Arabinose	1.67 ± 0.77 a	n.d.	2.13 ± 0.61 a
Xylose	6.34 ± 2.58 a	n.d.	9.31 ± 1.44 a
Myo-Inositol	1.07 ± 0.81 a	n.d.	n.d.
Total	139.3 a	178.4 ab	202.9 b

Different letters of the same row indicate significant difference at P= 0.05.
n.d. = not detected.

On the other hand, a significant increase in total sugars was noted in carambola and otaheite, while in June plum sugars content increased slightly. The total increase of sugars averaged 38% and 45% in carambola and otaheite, respectively, but in June plum increases averaged 9%.

Statistically, total sugar contents in carambola and otaheite were significantly different and their contents in immature fruits were significantly different in comparison with mature and ripe carambola. Immature carambola and otaheite had 35% and 36%, and 28% and 46% less total sugars than the mature and ripe stages, respectively. However, statistical analysis showed no significant difference in sugar contents of June plum during the three maturation and ripening stages.

Profile and organic acids contents of carambola, June plum and otaheite fruits

With ten different acids identified in carambola, June plum and otaheite fruits, eight were detected in June plum, six in carambola and four in Otaheite (Table 2). Overall, malic acid, oxalic acid, propionic acid and acetic acids were the most abundant organic

acids. In carambola and otaheite, malic and tartaric acids were the most abundant, while in June plum malic and acetic acids were predominant. Malic, citric, propionic and acetic acids have been detected in carambola, June plum and Otaheite, while tartaric acid in carambola and otaheite only. On the other hand, ascorbic acid was detected in carambola only, and succinic, oxalic, threonic and gluconic acids were detected in June plum only. Results also showed that organic acids content decreased with development and ripening of carambola and otaheite, while a steady state was observed in June plum. Total organic acids decreased by 20% and 49% in carambola and otaheite, respectively, while in June plum variation of organic acids during the three stages averaged only 3%.

Table 2 - Profiled organic acids and their contents (mg/g dry weight) in carambola, June plum and otaheite during the three development and ripening stages

Metabolites	Immature	Mature	Ripe
Carambola			
Malic Acid	22.11 ± 4.87 b	13.68 ± 2.4 ab	6.73 ± 0.88 a
Citric Acid	2.30 ± 0.2 a	2.20 ± 0.07 a	1.77 ± 0.34 a
Propionic Acid	2.23 ± 0.01 a	2.15 ± 0.04 a	2.19 ± 0.12 a
Acetic Acid	2.01 ± 0.02 a	1.78 ± 0.39 a	1.84 ± 0.24 a
Tartaric Acid	16.67 ± 3.45 a	10.24 ± 2.44 a	10.65 ± 2.65 a
Ascorbic Acid	1.74 ± 0.34 a	1.80 ± 0.3 a	2.12 ± 0.02 a
Succinic Acid	n.d.	n.d.	n.d.
Oxalic Acid	n.d.	n.d.	n.d.
Threonic Acid	n.d.	n.d.	n.d.
Gluconic Acid	n.d.	n.d.	n.d.
Total	14.73 a	14.14 ab	11.71 b
June plum			
Malic Acid	7.34 ± 0.43 a	6.33 ± 0.67 a	6.45 ± 1.45 a
Citric Acid	2.12 ± 0.09 a	2.13 ± .009 a	2.13 ± 0.12 a
Propionic Acid	1.89 ± 0.05 a	3.47 ± 0.09 b	3.45 ± 0.41 b
Acetic Acid	3.24 ± 0.36 a	3.64 ± 0.25 a	3.62 ± 0.44 a
Tartaric Acid	n.d.	n.d.	n.d.
Ascorbic Acid	n.d.	n.d.	n.d.
Succinic Acid	1.82 ± 0.03 a	1.90 ± 0.1 a	1.88 ± 0.07 a
Oxalic Acid	4.52 ± 0.3 a	4.32 ± 0.18 a	4.03 ± 0.2 a
Threonic Acid	1.97 ± 0.05 a	2.26 ± 0.02 ab	2.34 ± 0.19 b
Gluconic Acid	1.84 ± 0.03 a	1.74 ± 0.24 a	1.73 ± 0.28 a
Total	24.74 a	25.79 a	25.63 a
Otaheite			
Malic Acid	22.11 ± 4.87 b	13.68 ± 2.4 ab	6.73 ± 0.88 a
Citric Acid	2.30 ± 0.2 a	2.20 ± 0.07 a	1.77 ± 0.34 a
Propionic Acid	2.23 ± 0.01 a	2.15 ± 0.04 a	2.19 ± 0.12 a
Acetic Acid	2.01 ± 0.02 a	1.78 ± 0.39 a	1.84 ± 0.24 a
Tartaric Acid	16.67 ± 3.45 a	10.24 ± 2.44 a	10.65 ± 2.65 a
Ascorbic Acid	n.d.	n.d.	n.d.
Succinic Acid	n.d.	n.d.	n.d.
Oxalic Acid	n.d.	n.d.	n.d.
Threonic Acid	n.d.	n.d.	n.d.
Gluconic Acid	n.d.	n.d.	n.d.
Total	45.31 b	30.05 ab	23.19 a

Different letters of the same row indicate significant difference at P= 0.05.
n.d. = not detected.

On the other hand, the ratio of sugars/organic acids plays an important role that can characterise the ripe stage of fruits. During the different stages, the ratio of sugars/organic acids maintained a significant rising trend especially in carambola and otaheite. In carambola, the ratio was 6.47, 9.42 and 11.45 in immature, mature and ripe, respectively. In June plum, the ration was 9.13, 8.17 and 9.59, in immature, mature and ripe, respectively. In otaheite, the ratio was 3.07, 5.93 and 8.75, in immature, mature and ripe, respectively.

Statistical analysis showed that total organic acid contents of carambola was not significantly different between either immature and mature, or mature and ripe stages. Malic acid content varied significantly during the development and ripening of carambola and otaheite, but not significantly in June plum. Malic acid was also the main organic acid accumulating in carambola and otaheite and its level was significantly different among the three stages of the maturation and ripening of the two fruit. The statistical analysis also showed that citric acid and acetic acid contents did not show significant difference among the three developmental stages of carambola. Comparatively, total sugars and total organic acids during the three stages showed different correlations. In carambola and June plum, weak correlation (R^2 =0.18 and R^2 = 0.33, respectively) was observed between sugars and organic acids contents, however, a moderate correlation (R^2 = 0.56) was observed between sugars and organic acids contents of otaheite during the three stages.

Factoring and clustering of the profiled metabolites

The principal component analysis of the data sets revealed two individual clusters that seem to be governed by the developmental and the ripening stages of the fruits (Fig. 2). The analysis showed that in PC 1, the metabolites in carambola with the highest loading scores were glucose (0.99), galactose (0.99), xylose (0.97), sucrose (0.96), myoinositol (0.93) and mannose (0.92), while in PC 2 ascorbic acid (0.99), fructose (0.98), citrate (0.95) and acetic acid (0.98) had the highest loading scores. In June plum, the highest loading sores metabolites in PC 1 and PC 2 were oxalic acid (0.90), acetic acid (0.85), sucrose (0.84) and gluconic acid (0.80), and malic acid (0.89), fructose (0.89), arabinose (0.82, galactose (0.82) and sucrose (0.77), respectively. In otaheite, the metabolites with the highest scores were galactose (0.98), myoinositol (0.98), malic acid (0.96), tartaric acid (0.96), acetic acid (0.89), citric acid (0.79) and

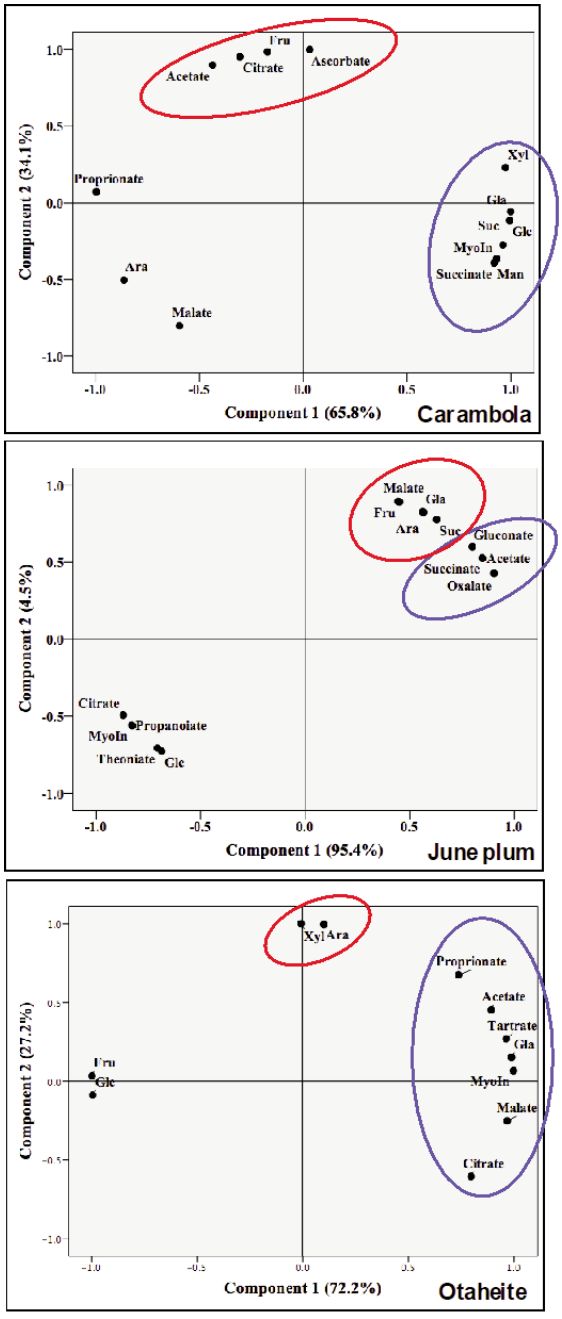

Fig. 2 - Principal component analysis (PCA) of the profiled and identified metabolites in carambola, June plum and otaheite fruits through different development and ripening stages. For PCA analysis, data sets were normalised for better comparison of the variable levels of the different metabolites.

propanoic acid (0.74), and arabinose (0.99) and xylose (0.99), respectively. Overall, principal components analysis (PCA) of samples based on the development and ripening stages revealed a difference between grouped metabolites. As suggested by the PCA in the figure 2, profiled metabolites were then divided into two classes, and loading values of sugars

and organic acids of fruits samples were found mostly in quadrant PC2+, illustrating the discriminated metabolites reflecting the development and ripening of fruits.

Hierarchical cluster analysis (HCA) was applied to a data set of the profiled and detected metabolites during the three stages of the three fruits. The dendrograms (Fig. 3) show that the profiled metabolites were quite homogeneous and tend to be distributed into three groups. According to the dendrograms of the HCA, at the distance of three, the metabolites can be grouped as shown in Table 3. Interestingly, three metabolites have been classified within the same groups of the three fruits. Myoinositol, citric acid and arabinose were classified in group 1, group 2 and group 3, respectively. The clusters of the different metabolites in the three fruits showed that the metabolites were quite clearly hierarchically separated, and these results were also clearly depicted by the PCA (Fig. 2) and the HCA (Fig. 3) which show the distribution of the metabolites into three main clusters. Furthermore, the heatmap (Fig. 4) also shows that June plum concentrates the highest levels of ten metabolites, while carambola and otaheite concentrate the highest levels of four and six other metabolites, respectively.

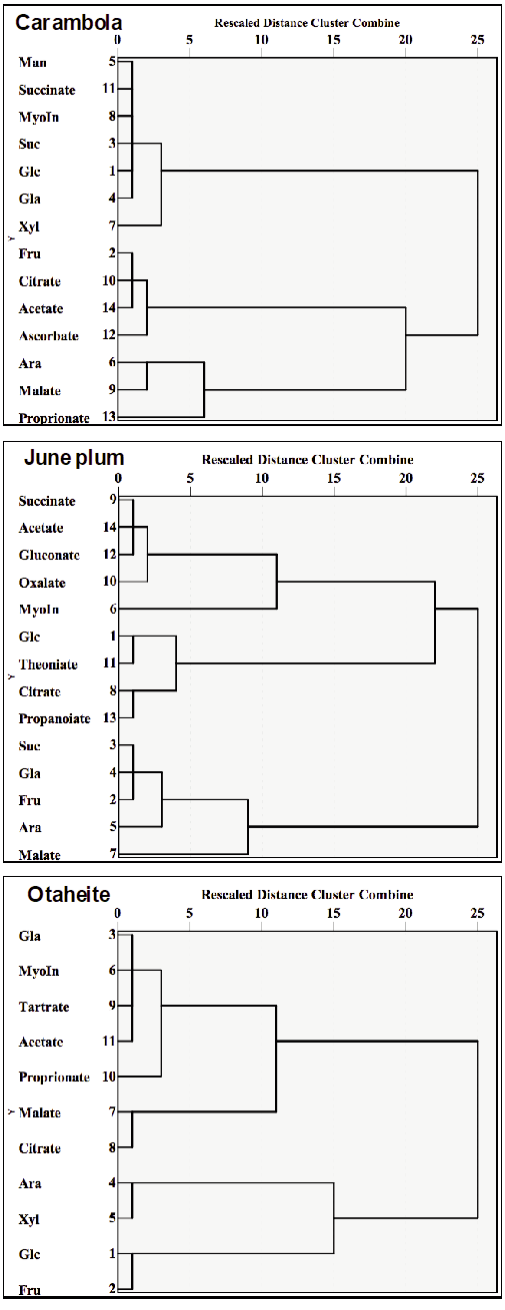

Fig. 3 - Dendrogram showing the Hierarchical Cluster Analysis (HCA) of profiled and identified metabolites in Carambola, June plum and Otaheite fruits through different development and ripening stages. To run HCA, data sets were normalised to reduce of the original data sets and hence allowing better clustering.

Table 3 - The hierarchical clusters distribution of the profiled metabolites of carambola, June plum and otaheite during the three stages (underlined metabolites are classified within the same groups of the three fruits)

	Group 1	Group 2	Group 3
Carambola	Mannose	Fructose	Arabinose
	Succinate	Citric acid	Malic acid
	Myo-inositol	Acetic acid	Propionic acid
	Glucose	Ascorbic acid	
	Galactose		
	Xylose		
June plum	Succinic acid	Glucose	Succinic acid
	Acetic acid	Threonic	Galactose
	Gluconic acid	Citric acid	Fructose
	Oxalic acid	Propionic	Arabinose
	Myo-inositol		Malic acid
Otaheite	Galactose	Malic acid	Arabinose
	Myo-inositol	Citric acid	Xylose
	Tartaric acid		Glucose
	Acetic acid		Fructose
	Propionic acid		

Indeed, primary metabolites profiling led to the identification 8, 6 and 5 sugars, and 6, 8 and 5 organic acids in carambola, June plum and otaheite, respectively. On the other hand, our results showed that the key marker metabolites of the maturation and ripening of the three fruits are glucose and fructose in carambola and otaheite, while in June plum glucose, fructose, galactose and sucrose were the key marker metabolites of the three different stages. Similarly, malic and tartaric acids were the key organic acids metabolites of the maturation and ripening

of carambola and otaheite fruits, while malic acid was the key marker metabolite of June plum maturation and ripening.

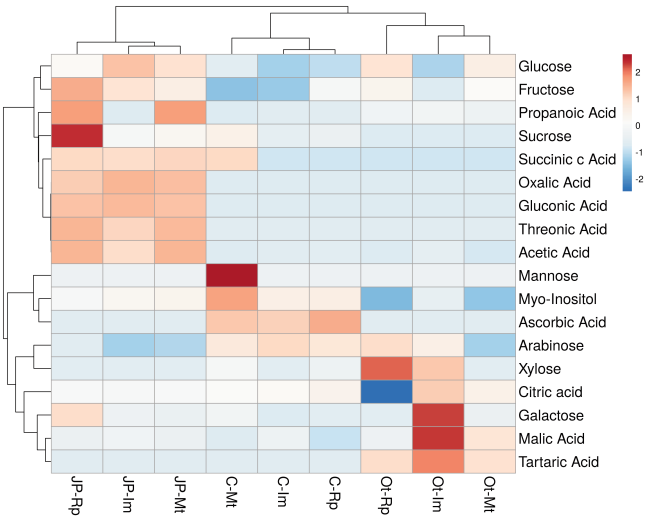

Fig. 4 - Heatmap of the profiled and identified metabolites in Carambola, June plum and Otaheite fruits through different development and ripening stages. The color scale represents the relative concentration of each metabolites.

4. Discussion and Conclusions

Although extensive literature is readily available, the variation of sugars and organic acids of many fruits including carambola and June plum at ripe stage, less and scattered work was done on the variation of the metabolites including sugars and organic acids in carambola, June plum and otaheite fruits during the development and ripening stages. On the other hand, most of the work carried out on carambola targeted the postharvest physiology and biochemistry of the fruit during storage.

In carambola, Campbell and Koch (1989) found that total soluble sugars concentration, mainly glucose and fructose, increased during ripening and varied between 22 and 27 mg/g fresh weight depending on the varieties, while Narain *et al*. (2001) investigated the variation of the chemical composition of carambola at three different ripening stages and found that total sugars increased from 2.91 to 5.60 g/100 g fresh weight. Later, Patil *et al*. (2010) reported the composition of the fruit at three stages of maturity (young, half-ripe and ripe), and they noted a tremendous increase of total sugars, oxalic acid and ascorbic acid by 100%, 89% and 65%, respectively. Similar increase by 33% and 90% of total sugars and ascorbic acid respectively were also reported by Ali and Jaafar (2012). Glucose, fructose and sucrose were reported to be the most predominant sugars in carambola (Mohd Zainudin *et al*., 2014; Benkeblia and López, 2015), however, Benkeblia and López (2015) reported an increase of glucose and fructose, but a slight decrease of sucrose in the ripe fruit compared to the green one, while Mohd Zainudin *et al*. (2014) noted an increase of the three sugars.

There is almost no work reporting on the composition of June plum fruit during maturation and ripening except from the one of Benkeblia and López (2015). The authors investigated the variation of glucose, fructose and sucrose in green and ripe June plum and found that in ripe fruit glucose and fructose increased in ripe fruit, while sucrose decreased significantly. Other scattered studies reported on the variation of sugars and organic acids in June plum fruit but at a specific stage. In a study carried out on immature green June plum, Franquin *et al*. (2005) investigated the composition at this early stage and found the concentrations of glucose, fructose, sucrose, citric acid, malic acid, oxalic acid and ascorbic acid were 1.5 (± 0.2), 1.2 (± 0.2), 3.1 (± 0.3), 0.9 (± 0.1), 0.2 (± 0.02,) 0.03 (± 0.01), and 52.0 (± 4.9) g/100 g fresh weight, respectively. In his study, Nahar *et al*. (1990) reported that 0.3% of fresh weight of the pulp is composed by free sugars, where glucose, fructose and sucrose were the most predominant (Nahar *et al*., 1990; Mahmood *et al*., 2012).

Similarly to June plum, few studies investigated the composition of otaheite during the maturation and ripening, however, few studies reported on ripe otaheite. Lu and Lin (2011) investigated the sugars in ripe otaheite and found that fructose yielded the highest content compared to glucose and sucrose which were detected at this ripe stage.

The untargeted profiling of primary metabolites during the maturation and ripening of fruits is a good approach to provide better insight into their metabolome changes during these stages. Different studies on metabolite analyses of fruits have focused on temperate and stone fruits such as tomato, peach, strawberry, and grape among many others, but scarce studies focused on tropical fruits. However, these studies revealed similar dynamic variations in the levels of sugars and organic acids, as well as many other primary and secondary metabolites during fruits maturation and ripening (Oikawa *et al*.,

2015). Our results showed patterns of variation in sugars and organic acids levels in carambola, June plum and otaheite during maturation and ripening, and provided fundamental metabolomic data that is useful for understanding fruits physiology. Our reported results are in agreement with those reported by Ramadan *et al*. (2020) who used metabolomics approach carambola fruit of different origins and at two different ripening stages. Mamat *et al*. (2019) and Parijadi *et al*. (2018) analysed the distribution of primary metabolites in mangosteen (*Garcinia mangostana* Linn.) fruit during ripening, and their results showed that fructose was the kye marker metabolite of mangosteen ripening. Similar results were reported by Ogawa *et al*. (2018) who noted that the increasing sucrose level might be a key marker metabolite of pineapple ripening.

Beside metabolomics, Pandit *et al*. (2010) used transcriptomics markers to understand the maturation and ripening programmes in mango (*Mangifera indica* L.) fruit. Among eighteen genes related to the fruit physiology and biochemistry, genes related to primary metabolism showed higher expression in comparison to that of the genes related to flavour production.

However, regardless of the origin and environmental zones, the maturation and ripening of fruits are complex and highly coordinated processes. Globally, the increase in sugar and decline in organic acids are one of the main changes associated with these processes (Giovannoni, 2001; Klee and Giovannoni, 2011; Osorio *et al*., 2013; Batista-Silva *et al*., 2018). During maturation and ripening of fruits, organic acids contents are inversely related to sugar contents. The rising trend of sugars is due to photosynthates import or starch degradation, while organic acids that accumulated in young fruits strongly decrease by being converted to other organic acids (Carrari *et al*., 2006; Beauvoit *et al*., 2018). Although environmentally different from tropical fruits, there have been a number of different studies reporting similar metabolic changes that occur in temperate fruits during maturation and ripening stages (Fait *et al*., 2008; Osorio *et al*., 2011, 2012). Sugars accumulation and organics acids decrease trend were reported in blueberries (*Vaccinium arctostaphylos* and *Vaccinium myrtillus*) (Ayaz *et al*., 2001), apple (*Malus domestica*) (Liu *et al*., 2016; Yang *et al*., 2021), apricot (*Prunus armeniaca* L.), plumcot (*Prunus armeniaca* x *Prunus salicina* L.), plum (*Prunus salicina* Lindl.), and peach (*Prunus persica* L.) (Bae *et al*., 2014), Damson plum (*Prunus domestica*) (García-Mariño *et al*., 2008), different citrus cultivars (Bermejo and Cano, 2012), grapes (Kurt *et al*., 2017; Liang *et al*., 2011; Muñoz-Robredo *et al*., 2011), loquat (*Eriobotrya japonica* Lindl.) (Amorós *et al*., 2003), mango (*Mangifera indica* L.) (Mokhtar *et al*., 2014), melon (*Cucumis melo* L.) (Wang *et al*., 1996), pomegranate (*Punica granatum* L.) (Nuncio-Jáuregui *et al*., 2014) and wolfberry (*Lycium barbarum* L.) (Zhao *et al*., 2015) among other reported fruits. Furthermore and in agreement with our finding, glucose, fructose, and sucrose were found to be the most predominant among mono and disaccharides, while malic, citric and tartaric acids were predominant organic acids (Wang *et al*., 1996; Liang *et al*., 2011; Mahmood *et al*., 2012; Kurt *et al*., 2017; Yang *et al*., 2021).

Indeed, the relative levels of sugars and organic acids in fruits are of great importance for harvesting time and are one of the determinants of the organoleptic quality attributes of fruits particularly sweetness (Itai and Tanahashi, 2008). Furthermore, the postharvest quality attributes of fruits, their shelf-life and even processed products are strongly associated to their sugars and organic acids levels (Matsumoto and Ikoma, 2012; Aprea *et al*., 2017). In order to preserve freshness and reduce economic losses, it is of great importance to understand the metabolic changes occurring during maturation and ripening which might contribute to accelerate fruits senescence and perishability after harvesting. In this sense, metabolomic profiling of key metabolites responsible for quality attributes such as sugars and organic acids can be a powerful tool for further understanding the biochemical basis of pre- and postharvest physiology and have the potential to play a critical role in the identification of the pathways affected by fruit maturation and ripening (Allwood *et al*., 2021; Pott *et al*., 2020; Tian *et al*., 2021).

The data presented here indicates that the profiled metabolites varied significantly during the maturation and ripening of the fruits. Glucose, fructose, galactose, sucrose and myoinositol were found predominantly in all the fruits and during the three stages, except sucrose in otaheite. Comparatively, June plum showed the highest content of total sugars and carambola the lowest, while the highest total in organic acids content was noted in otaheite and the lowest in carambola. On the other hand, most sugars increased during ripening of the three fruits, while organic acids decreased. Interestingly, the multi-vari-

able analysis showed than all the metabolites were clustered into three main clusters, and myoinositol, citric acid and arabinose shared group one, group two and group three, respectively. From the different profiled sugars and organic acids, our results are suggesting that glucose and fructose are the marker metabolites of the maturation and ripening of carambola and otaheite, while the ripening marker metabolites in June plum are glucose, fructose, galactose and sucrose. Because this study represents the first report on the profiling of sugars and organic acids in carambola, June plum and otaheite, it might be interesting to profile the secondary metabolites mainly phenolics and volatiles, and their variation during the maturation and ripening of these fruits.

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