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Profiling Primary Metabolites of Governor's Plum *Flacourtia indica* (Burm.f.) Merr. at Two Different Ripe Stages

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ABSTRACT

To date, no study has investigated the variation of the primary metabolite profile of the fruit of *Flacourtia indica* (Burm.f.) Merr. (commonly known as governor's plum), an underutilised fruit in Jamaica. To fill this gap, the current study aimed to bring novel data on this fruit at two different ripe stages (light = deep wine-red colour and dark = fully darkened brown colour) and explore the variation of their metabolome profiles. The gas chromatography-mass spectrometry (GC-MS) profiling identified 10 saccharides, 4 sugar alcohols, 11 organic acids, 24 fatty acids, and 8 amino acids in the light and dark colour fruits. However, some metabolites were not shared by both fruit ripening stages. The principal component analysis (PCA) of the different classes of the primary metabolites showed that the significant difference between the light and dark colour governor's plum fruit is mainly determined by the content of sugars and organic acids, with the fully ripe (dark) stage expressing significant high levels of both. The hierarchical cluster analysis (HCA) showed that the profiled sugars, sugar alcohols, and fatty acids were grouped into two main clusters. In contrast, organic acids and amino acids were grouped into one cluster. However, some metabolites were related to the clusters observed. With these profiles, it was concluded that the

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dark colour governor's plum is in the true ripe stage, although the light colour fruit is commercially considered ripe.

Keywords: *Flacourtia indica*, primary metabolites, profiling, ripening

INTRODUCTION

Fruit science is being deeply transformed because of unprecedented and incommensurate developments and advances in analytical chemistry. As sugars and other primary metabolites play a central role in maturation and ripening processes, the use of metabolomics in quantifying these primary metabolites is undoubtedly being established as a key analytical tool. This promising approach attempts to elucidate the variations of the primary metabolite profiles through the maturation process. In fact, following the changes in primary and secondary metabolites help establish the fact that they are not merely the end product of the expression of their respective genes but that they form a part of the regulatory system of maturation and ripening in an integrated manner (Goodacre et al., 2004).

In the tropics, fruits present large biodiversity varying in structure, characteristics, and physiology (Wongs-Aree & Noichinda, 2018). Bananas, pineapples, papaya, and avocado fall within the category of major tropical fruits, while others, such as lychee, durian, rambutan, guava, passionfruit, mangosteen, and tamarind, are considered minor tropical fruits (Paull & Duarte, 2011a, 2011b). As in many parts of the world, numerous not-so-well-known, unknown, and underutilized fruits grow island-wide, as exemplified by governor's plum (*Flacourtia inidca*), rose apple (*Syzygium malaccense*), jimbilin (*Phyllanthus acidus*), and mammee apple (*Mammea americana*). These indigenous introduced and native fruits are consumed by the rural communities and people living in the island's countryside and constitute an important source of vitamin and mineral requirements in their diet.

Governor's plum (*Flacourtia indica*), also known as 'Indian plum' or 'boichi, belongs to the family of Flacourtiaceae and grows widely in dry tropical forests. It is native to India, Bangladesh, Sri Lanka, Ethiopia, and South Africa, and was introduced to Jamaica (Chatterjee et al., 2015; Eramma, 2016). The edible fruit is round, cherry-sized, fleshy with a sour or sweet taste, and astringent (Lim, 2013). The unripe fruit is green, the ripe is deep wine red, and the very ripe fruit is dark brown (Chatterjee et al., 2015).

Ripening is defined as the total changes in fruit tissue metabolism and is characterized by softening fruit tissue. It is accompanied by an increase in a unique combination of numerous volatile compounds derived from primary metabolites, which confer a delicate aroma and an attractive and appealing fruit (Adams-Phillips et al., 2004; Giovannoni, 2001; Pott et al., 2019).

The variations of the primary metabolites, i.e., sugar and sugar alcohols, organic acids, fatty acids, and amino acids, depend on the fruit type 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 the fruit develops and ripens due to the decarboxylation of organic acids and the breakdown of stored carbohydrates to produce sugars (Batista-Silva et al., 2018). Studies using omics technologies have shown evidence of a shift from the accumulation of organic acids to sugar synthesis in the final stage of fruit development in several fruit species (Etienne et al., 2013). The respiratory pathways commonly involved in the reduction of fruit sugars are glycolysis, the oxidative pentose phosphate (OPP) pathway, and the tricarboxylic acid (TCA) pathway (Tucker, 2012), triggering fruit ripening and 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 certain fruits' maturation, ripening and senescence, work is scarce on many tropical fruits (Fabi et al., 2010). There has been no study on the variation of the primary metabolites in the governor's plum.

Therefore, to bring novel data on the governor's plum fruit and explore the variation of the metabolites profile, this study aimed to perform profiling of the primary metabolites of the fruit harvested at two ripe (light colour and dark colour) stages. The importance of the study is to screen the change in the metabolites of ripe governor's plum and determine the most appropriate commercial ripening stage, which is associated with the quality of fruits. Hence, using metabolomics will provide a comprehensive and unbiased analysis of the primary metabolites of the governor's plum at two different ripe stages. In addition, comprehensive metabolite profiling of sugars, organic acids, and amino acids is lacking in the governor's plum. Therefore, the findings of this study may provide the basis for further investigation of the physiological and biochemical changes in ripe fruit.

MATERIALS AND METHODS

Fruit Samples

The fruit of *Flacourtia indica* (governor's plum) was collected freshly from trees growing in Shortwood, St. Andrew, Jamaica. Fruits were collected at two commercial and ripe stages practised by farmers and the local market retailers: light colour fruit and dark colour fruit. Immediately after harvesting, fruits were transported to the laboratory and leaves, branches, and fruit were identified by the herbarium curator (Mr Patrick Lewis, botanist) of the Department of Life Sciences, UWI Mona campus, and deposited under voucher No: UWI-Mona 35 250. Afterwards, samples were sorted, and any sample with visible defect, contamination, or injury was discarded. The fruits were separated visually into two ripening stages based on the skin colour: light = deep wine-red colour and dark = fully darkened brown colour (Figure 1). The fruits were quickly washed with distilled water, left to drain for one hour at room temperature, and then freeze-dried for 72 hours using a Labconco freeze drier (Labconco Corp., USA) and stored under a vacuum at -20 °C until use.

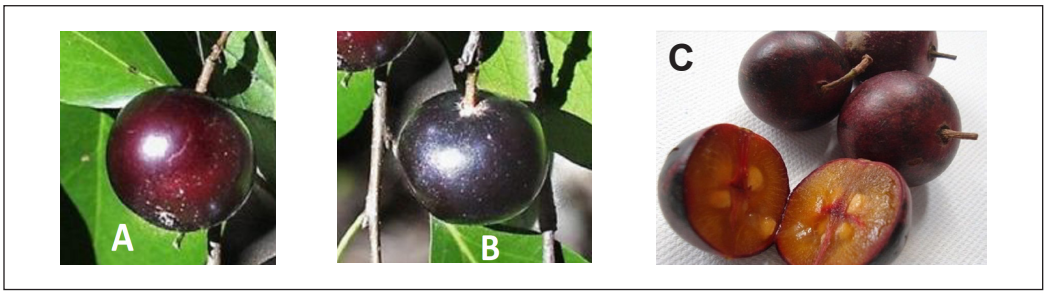

Figure 1. Governor's plum *Flacourtia indica* at the two ripe commercial stages. (A) Deep wine red (light) fruit; (B) Fully darkened brown (dark) very ripe fruit; and (C) Whole and sliced fruit

Metabolomics Profiling of *Flacourtia indica* Fruit

Sample Preparation. The metabolites of the extracts were profiled using gas chromatography-mass spectrometry (GC-MS) according to the method described by Broeckling et al. (2005) and Roessner-Tunali et al. (2003). Freeze-dried samples (300 mg) were extracted with 1 mL pure methanol (Sigma-Aldrich, USA) at 60 °C for 1 hour. Subsequently, 100 µL of ribitol (Sigma-Aldrich, USA) (0.2 mg/mL in distilled water) was added to the mixture as an internal standard. For separating polar and non-polar compounds, 1 mL of chloroform (Sigma-Aldrich, USA) was added to the mixture and then centrifuged at 1,400 × *g* for 10 min. Then the supernatant layer was separated and reduced to dryness. After dryness, the residues were redissolved in pyridine (Sigma-Aldrich, USA) (150 µL) for non-polar fractions. For polar fractions, the residues were redissolved in 150 µL of 20 mg/mL methoxyamine hydrochloride (Sigma-Aldrich, USA) in pyridine and incubated at 50 °C until the residue was completely dissolved. Subsequently, all the extracts were derivatized by the addition of 150 µL MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide) (Sigma-Aldrich, USA) + 1% TMCS (Trimethylchlorosilane) (Sigma-Aldrich, USA) and incubated at 50 °C for 1 hour. Afterwards, 200 µL of the extracts were transferred to a glass insert for GC-MS analysis.

GC-MS Analysis. GC-MS analysis was conducted using an Agilent Technologies gas chromatograph (model 6890N, USA) coupled to a mass spectrometric detector (model MSD 5973N). The operating conditions are as follows: electron impact mode, 70 eV; splitless mode (injection purge off = 0.75 min); injector temperature 250 °C; transfer line temperature 280 °C; column, DB-1701, 30 m, 0.25 mm i.d., 0.25 µm film thickness. A ramped temperature program was employed, starting at 80 °C, holding for 2 min, and increasing the temperature by 20 °C per min for 10 min and holding for 10 min. The solvent delay was 3 min, and the carrier gas helium was at a flow rate of 1.2 mL/min. All the operations were conducted in scan mode. The deconvolution of the metabolites was

done using AMDIS (Automated Mass Spectral Deconvolution and Identification System), and the metabolites were identified as TMS (trimethylsilyl) derivatives using the NIST (National Institute of Standards and Technology) database (NIST Mass Spectral Database, PC-Version 5.0, 2005, National Institute of Standardisation and Technology, USA). Other plant-specific databases, such as the Golm Metabolome Database (http://www.csbdb.mpimp-Golm.mpg.de/csbdb/gmd/home/gmd_sm.html) and RIKEN database (http://prime.psc.riken.jp/compms/msdial/main.html#MSP), based on matching mass and the highest match (probability).

Statistical Analysis. Data were treated by *t*-test to compare the means of the metabolites in the light and dark ripe fruits. Differences among means were determined by the least significant difference (LSD) test, with significance defined at $P < 0.05$. For multivariate analysis, data were transformed, and principal component (PCA) and hierarchical cluster (HCA) analyses were computed using the SPSS software package (version 25.0, IBM Corp., USA). Pearson's correlation coefficients and the furthest neighbour as the clustering method were selected to ensure that groups share a good correlation for the HCA analysis.

RESULTS

Sugars and Sugar Alcohols

The profiling of the primary sugars and sugar alcohols led to the identification of 10 saccharides and 4 sugar alcohols (Table 1). Ten (10) saccharides, were identified, including 3 pentoses, 4 hexoses, and 2 disaccharides in light colour fruit extracts, but fructose and melibiose were not detected. In dark colour fruit extracts, 11 saccharides, including 3 pentoses, 5 hexoses, and 2 disaccharides, were also identified, but sucrose was not detected. Similarly, only mannitol and galactitol were identified in light colour fruit extracts, while mannitol, arabinitol, and myo-inositol were detected in dark colour fruit extracts. In light colour fruit extracts, significantly

Table 1
Sugars and sugar alcohols profiled and identified in governor's plum Flacourtia indica *fruit at two different ripe stages*

Sugars and sugar alcohols	Light colour fruit (mg/g D.W.)	Dark colour fruit (mg/g D.W.)
Ribose	0.351[a]	76.11[b]
Galactose	2.1[a]	178.9[b]
Mannose	4.3[a]	25.4[b]
Xylose	1.3[a]	44.3[b]
Arabinose	1.1[a]	126.4[b]
Glucose	4.5[a]	32.8[b]
Fructose	(< 0.001)*[a]	0.5[b]
Sucrose	0.2[a]	(< 0.001)*[b]
Sorbose	0.2[a]	6.0[b]
Melibiose	(< 0.001)*[a]	1.0[b]
Maltose	(< 0.001)*[a]	0.2[b]
Myo-Inositol	(< 0.001)*[a]	11.8[b]
Xylitol	0.1[a]	(< 0.001)*[b]
Mannitol	(< 0.001)*[a]	(< 0.001)*[a]
Arabinitol	(< 0.001)*[a]	(< 0.001)*[b]
Galactitol	0.005[a]	(< 0.001)*[a]

Note. (< 0.001)* indicates concentrations that were not detected and below the limit of detection of the GC-MS. Values of the same row with different superscript letters are significantly different

high concentrations (> 1 mg/g D.W.) of sugars were noted, yielding 2.12, 4.31, 1.29, 1.12, and 4.48 mg/g for galactose, mannose, xylose, arabinose, and glucose, respectively totalizing 95% of the identified saccharides. In dark colour fruit extracts, significantly high concentrations (> 1 mg/g D.W.) of sugars were noted, yielding 76.10, 178.86, 25.39, 44.27, 126.36, 32.84, 6.01, and 1.02 mg/g for ribose, galactose, mannose, xylose, arabinose, glucose, sorbose, and melibiose, respectively totalizing 99% of the identified saccharides. On the other hand, sugar alcohols were found at significantly low concentrations (< 1 mg/g D.W.), except myo-inositol, which yielded 11.75 mg/g D.W. in dark colour fruit extract. These significantly low concentrations of sugar alcohols are likely due to their physiological role as they function as phloem-translocated carbohydrates, even though a few of them, such as sorbitol and mannitol, also serve as storage carbon but at low concentrations. Comparatively, the total concentration of sugars and sugar alcohols in light colour fruit extracts was significantly lower (14.06 mg/g D.W.) compared to dark colour fruit extracts (491.50 mg/g D.W.).

Organic Acids (OA)

The profiled samples led to the identification of a total of 11 different OAs, which were found in the dark colour fruit extracts. In contrast, 8 organic acids were found in the light colour extracts at a concentration higher than 1 mg/g D.W. (Table 2). Surprisingly, most OAs were found at significantly low concentrations except arabinonic and mannonic acids, which yielded 92% and 93% of the total organic acids in light and dark fruit extracts, respectively. Indeed, in ripe fruits, the amount of organic acids decreases since they are metabolized by oxidation, amino acid synthesis, or serve as precursors for synthesising secondary metabolites. It was also noted that in light colour fruit extracts, total OAs concentration was significantly lower (1.1775 mg/g D.W.) compared to dark colour fruit extracts (20.6646 mg/g D.W.).

Fatty Acids (FAs)

In the fruit samples, analysis of the fruit extracts led to identifying 24 different FAs (Table 3). However, 3 FAs, ethanimidic, pelargonic, and linoleic acids were not

Table 2
Organic acids profiled and identified in governor's plum Flacourtia indica *fruit at two different ripe stages*

Organic acids	Light colour fruit (mg/g D.W.)	Dark colour fruit (mg/g)
Malonic acid	0.001[a]	0.003[a]
Citric acid	(< 0.001)*[a]	0.495[b]
Malic acid	0.002[a]	0.005[a]
Phenylacetic acid	0.005[a]	0.363[b]
Mannonic acid	0.002[a]	19.206[b]
Gluconic acid	0.001[a]	0.099[b]
Hexahydro-benzoic acid	(< 0.001)*[a]	0.005[a]
Azelaic acid	(< 0.001)*[a]	0.010[a]
Tartaric acid	0.001[a]	0.001[a]
Arabinonic acid	1.090[a]	0.470[b]
Succinic acid	0.077[a]	0.009[b]

Note. (< 0.001)* indicates concentrations that were not detected and below the limit of detection of the GC-MS. Values of the same row with different superscript letters are significantly different

Table 3
Fatty acids profiled and identified in governor's plum Flacourtia indica *fruit at two different ripe stages*

Fatty acids	Number of carbons	Light colour fruit (mg/g D.W.)	Dark colour fruit (mg/g D.W.)
Propanoic acid	C3	0.254[a]	0.165[a]
Butanoic acid	C4	0.909[a]	4.527b
Ethanimidic acid	C4	(< 0.001)*[a]	(< 0.001)*[a]
Valeric acid	C5	0.508[a]	0.010[b]
Caproic acid	6	0.016[a]	0.099[a]
Enanthic acid	C7	0.010[a]	0.010[a]
Caprylic acid	C8	0.021[a]	0.099[b]
Pelargonic acid	C9	(< 0.001)*[a]	(< 0.001)*[a]
Capric acid	C10	(< 0.001)*[a]	0.067[b]
Sebacic acid	C10	0.010[a]	0.080[b]
Undecanoic acid	C11	(< 0.001)*[a]	(< 0.001)*[a]
Lauric acid	C12:0	0.333[a]	0.010[b]
Myristic acid	C14:0	(< 0.001)*[a]	0.007[a]
Pentadecanoic acid	C15:0	0.010[a]	0.010[a]
Palmitic acid	C16:0	0.203[a]	0.116[a]
Margaric acid	C17:0	0.010[a]	0.005[a]
Stearic acid	C18:0	0.096[a]	4.189[b]
Oleic acid	C18:1	0.057[a]	0.569[b]
Linoleic acid	C18:2	(< 0.001)*[a]	(< 0.001)*[a]
Linolenic acid	C18:3	0.032[a]	(< 0.001)*[b]
Nonadecanoic acid	C19:0	0.010[a]	0.066[a]
Arachidic acid	C20:0	0.010[a]	0.002[a]
Behenic acid	C22:0	0.002[a]	(< 0.001)*[a]
Lignoceric acid	C24:0	(< 0.001)*[a]	0.033[b]

Note. (< 0.001)* indicates concentrations that were not detected and below the limit of detection of the GC-MS. Values of the same row with different superscript letters are significantly different

detected (< 1 mg/g D.W.) in both light and dark fruit extracts. In light colour fruit extracts, 17 FAs were identified with two yielding more than 0.5 mg/g D.W., butanoic acid (0.909 mg/g D.W.), and valeric acid (0.508 mg/g D.W.). In dark colour fruit extracts, 18 FAs were identified with 3 yielding more than 0.5 mg/g D.W., butanoic acid (4.527 mg/g D.W.), stearic acid (4.189 mg/g D.W.), and oleic acid (0.569 mg/g D.W.). From the structural point of view, 7 FAs are of an odd number of carbons yielding 21.9% and 1.06% of the total FAs in light and dark colour fruit extracts, respectively. The other FAs, 13 FAs, are saturated, yielding 74% and 98.3% in light and dark fruit extract, respectively. Interestingly, 1 monosaturated (MOFA: oleic acid). Furthermore, 2 polyunsaturated (PUFA: linoleic and linolenic acids) FAs

were identified; however, only oleic, linoleic acids, and linolenic acids were significantly higher (> 1 mg/g D.W.) in light and dark colour fruit extracts, respectively. Furthermore, MOFA and PUFA yielded 2.3% and 1.3%, and 0.56% and 0.02% of the total FAs in light and dark colour fruit extracts, respectively.

Amino Acids (AAs)

Although eight different AAs were identified in the fruit extracts, only two, glycine and proline; and four, glycine, proline, leucine, and glutamine, showed concentrations significantly high than 1 mg/g D.W. in the light colour and dark colour fruit extracts, respectively (Table 4). These predominant AAs yielded 97% and 96% of the total AAs in light and dark colour fruit extracts, respectively.

Table 4
Amino acids profiled and identified in governor's plum Flacourtia indica *fruit at two different ripe stages*

Amino acids	Light colour fruit (mg/g)	Dark colour fruit (mg/g)
Methionine	(< 0.001)*[a]	(< 0.001)*[a]
Glycine	0.0172[a]	0.660[b]
Proline	0.009[a]	0.149[b]
Tryptophan	(< 0.001)*[a]	(< 0.001)*[a]
Arginine	(< 0.001)*[a]	(< 0.001)*[a]
Aspartic acid	(< 0.001)*[a]	(< 0.001)*[a]
Leucine	(< 0.001)*[a]	0.002[a]
Glutamine	(< 0.001)*[a]	0.033[b]

Note. (< 0.001)* indicates concentrations that were not detected and below the limit of detection of the GC-MS. Values of the same row with different superscript letters are significantly different

DISCUSSION

Based on the literature available, extensive research was carried out on profiling primary metabolites of numerous temperate fruits such as tomato, apple, pear, strawberry, persimmon, and other citrus species. In addition, extensive literature reported the metabolic changes during the maturation and ripening of fresh crops, such as peach (Lombardo et al., 2011), strawberry (Zhang et al., 2011), pear (Oikawa et al., 2015), and pitaya (Wu et al., 2019), while very scarce work was reported on some tropical fruits (Fabi et al., 2010).

Unfortunately, out of the seven *Flacourtia* accepted species, most of the work was conducted on the biological and therapeutic activities of some secondary metabolites, phenolic compounds mainly, while no referenced work reported on the profile of the primary metabolites of any *Flacourtia* species, including governor's plum *F. indica*. In general, fruits' organoleptic and commercial quality attributes are determined by their ripening stage (Kader, 1999). Colour and sugars content is commonly used as an index to determine the ripe stage, and the colour of the skin is one of the most important criteria for ripening in many fruits like stony fruits (Crisosto, 1994; Usenik et al., 2008), mango (Lalel et al., 2003; Malevski et al., 1977), guava (Mercado-Silva et al., 1998), and pomegranate (Manera et al., 2013). Nevertheless, many tropical fruits remain unknown, and neither appropriate harvesting time nor ripening index has been reported. Our results showed that

total sugars and OAs were significantly higher in dark colour fruit compared to light colour fruit, showing that this stage (light colour) is not the appropriate ripe stage for harvesting the governor's plum. However, the taste of the fruit is almost similar, i.e., very astringent due to the high content of phenolics and tannins (personal data).

The untargeted profiling of primary metabolites of fruits is a good approach to provide better insight into their metabolome changes. Metabolomics studies on temperate fruits revealed similar dynamic variations in the levels of sugars and organic acids, as well as other primary metabolites during ripening (Oikawa et al., 2015). Our results showing patterns of variation in sugars and sugar alcohols, organic acids, fatty acids, and amino acid levels in two ripe governor's plums provided fundamental metabolomic data that is useful for understanding this unknown fruit. The significant trend of sugars is due to photosynthates import or starch degradation, while organic acids accumulated in young fruits significantly decrease by being converted to other metabolites, including volatiles (Beauvoit et al., 2018; Carrari et al., 2006). Moreover, 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 & Tanahashi, 2008). Although barely comparable, 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 fruit physiology and biochemistry, genes related to primary metabolism showed a significantly high expression in comparison to that of the genes related to flavour production, and this agrees with our results showing a significant increase in sugars and sugar alcohols in the dark colour fruit compared to the light colour fruit.

Ripe fruit is defined as the total changes in tissue metabolism leading to increased attractiveness and organoleptic quality attributes (Adams-Phillips et al., 2004). The ripe stage is characterised by tissue softness and increased volatile compounds and pigments, such as carotenoids and flavonoids, resulting in a more appealing fruit (Giovannoni, 2001). The concentration of sugars and organic acids in fruits varies in ripe fruits. Overall, a decrease in organic acids and an increase in sugar content as fruit ripen are due to the decarboxylation of organic acids and the breakdown of stored carbohydrates to produce sugars (Batista-Silva et al., 2018). Several studies have shown evidence of a shift from the accumulation of organic acids to sugar synthesis in the ripe fruit of several species using metabolomics and other advanced omics technologies. Indeed, this shift results from the respiratory pathways commonly involved in the reduction of sugars by the glycolysis pathway, oxidative pentose phosphate (OPP) pathway, and the tricarboxylic acid (TCA) pathway (Etienne et al., 2013; Tucker, 2012). These pathways dramatically alter the complex network of primary metabolites, as well as other secondary metabolites and proteins, and

our findings showed how the profile of the primary metabolites was significantly altered, highlighting the difference between the two ripe stages of the governor's plum.

Factoring and Clustering of the Profiled Metabolites

The principal component analysis of the data sets revealed that the distribution of the metabolites seems to be governed by the ripening stage of the fruits (Figure 2). In the present analysis, the PCA of sugars and sugar alcohols showed a loading plot scoring 16% and 98% in PC1 and PC2 for both light colour and dark colour governor's plum fruit. On the contrary, the PCA of organic acids showed a different loading plot scoring less than 1% and more than 99% in PC1 and PC2 for both light colour and dark colour governor's plum fruit. The PCA of fatty acids showed that light and dark colour fruit scores were closer, with a score of 32% and 94% for both light colour and dark colour governor's plum fruit. Interestingly, The PCA of amino acids showed that light and dark colour fruit scores were very close, with a score of 60% and 80% for both light colour and dark colour governor's plum fruit. Overall, hierarchically separated sugars and sugar alcohols, as well as organic acids in the light colour and dark colour governor's plum fruit, and the 2D PCA graphs depicted this result. Thus, the loading plots of the different classes of the primary metabolites revealed that the difference observed between the light colour and dark colour governor's plum fruit is mainly due to the content of sugars and organic acids,

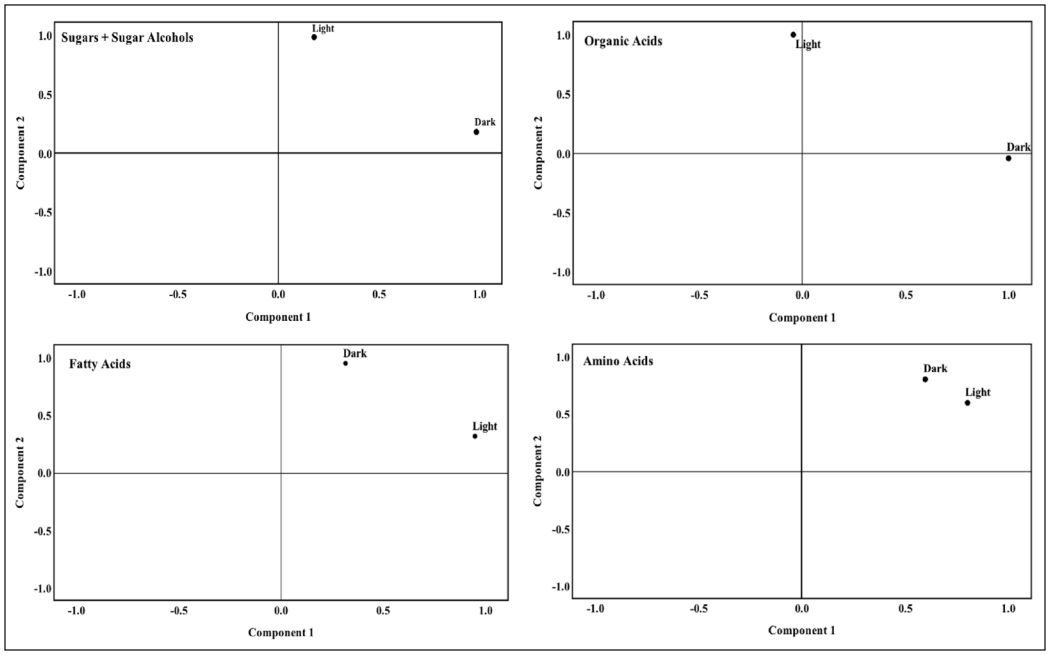

Figure 2. Score plots of the principal component analysis (PCA) of the profiled primary metabolites: sugars, organic acids, fatty acids, and amino acids of governor's plum (*Flacourtia indica*) fruit of two different ripe stages (light colour and dark colour skin)

to less extent to fatty acids. In contrast, amino acids cannot be considered markers to differentiate the governor's plum fruit's light and dark colour.

Hierarchical cluster analysis (HCA) was applied to a data set of the profiled and detected metabolites of light and dark colour governor's plum fruit (Figure 3). The dendrogram shows that the profiled sugars and sugar alcohols are distributed into different groups (clusters). Two clusters are observed at the first clustering level, while xylose, ribose, arabinose, and galactose are separated from the two main clusters. Similarly to sugars and sugar alcohols, fatty acids are grouped into two main clusters. However, the one formed by two FAs (behenic acid and stearic acid) is less related to the first cluster formed by the 22 other FAs. Interestingly, the organic acids dendrogram shows one cluster grouping all the organic acids except mannonic acid, which is separated from the unique cluster. Similarly to organic acids, the amino acids dendrogram shows one cluster grouping all AAs except proline and glycine, which

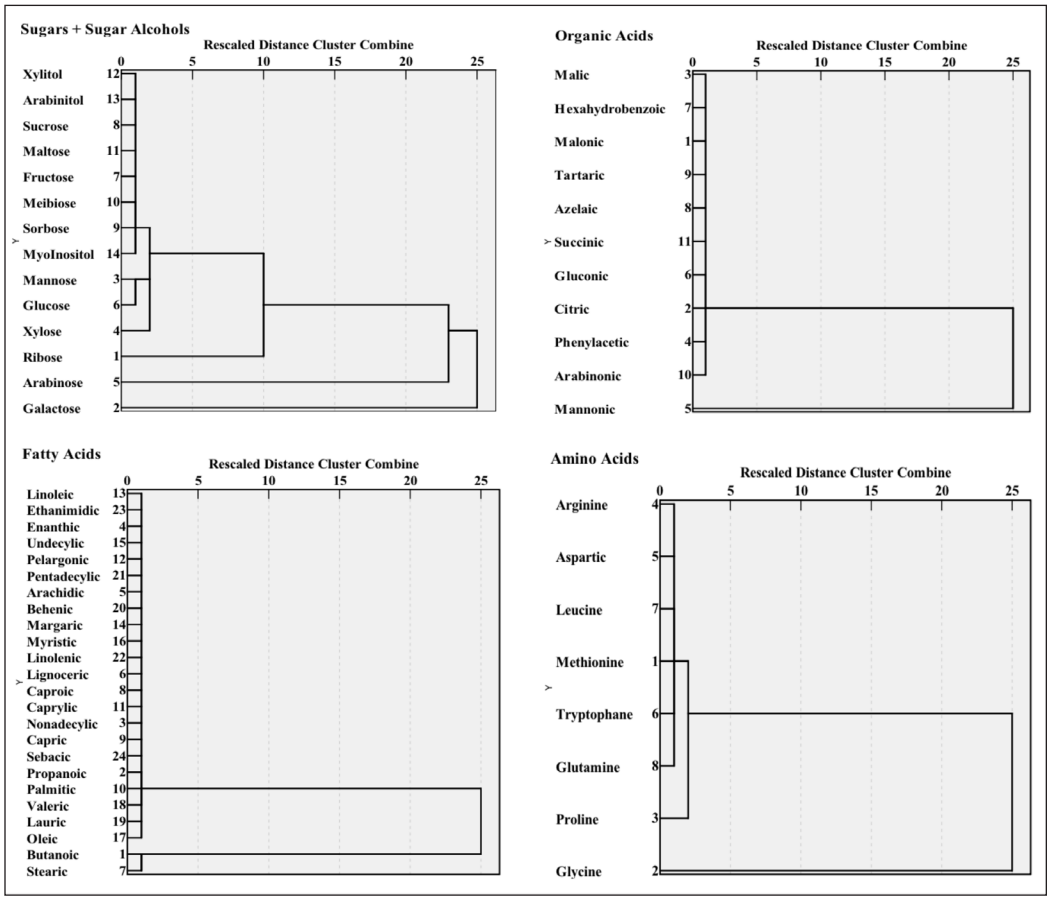

Figure 3. Dendrogram showing the hierarchical cluster analysis (HCA) of the profiled primary metabolites: sugars, organic acids, fatty acids, and amino acids of governor's plum (*Flacourtia indica*) fruit of two different ripe stages (light colour and dark colour skin)

seems unrelated to the unique cluster formed by the other AAs. Furthermore, as shown by the heatmap (Figure 4), sugars and sugar alcohols concentrate at the highest levels (higher colour intensity scale), followed by fatty acids, organic acids, and lastly, amino acids (lowest colour intensity.

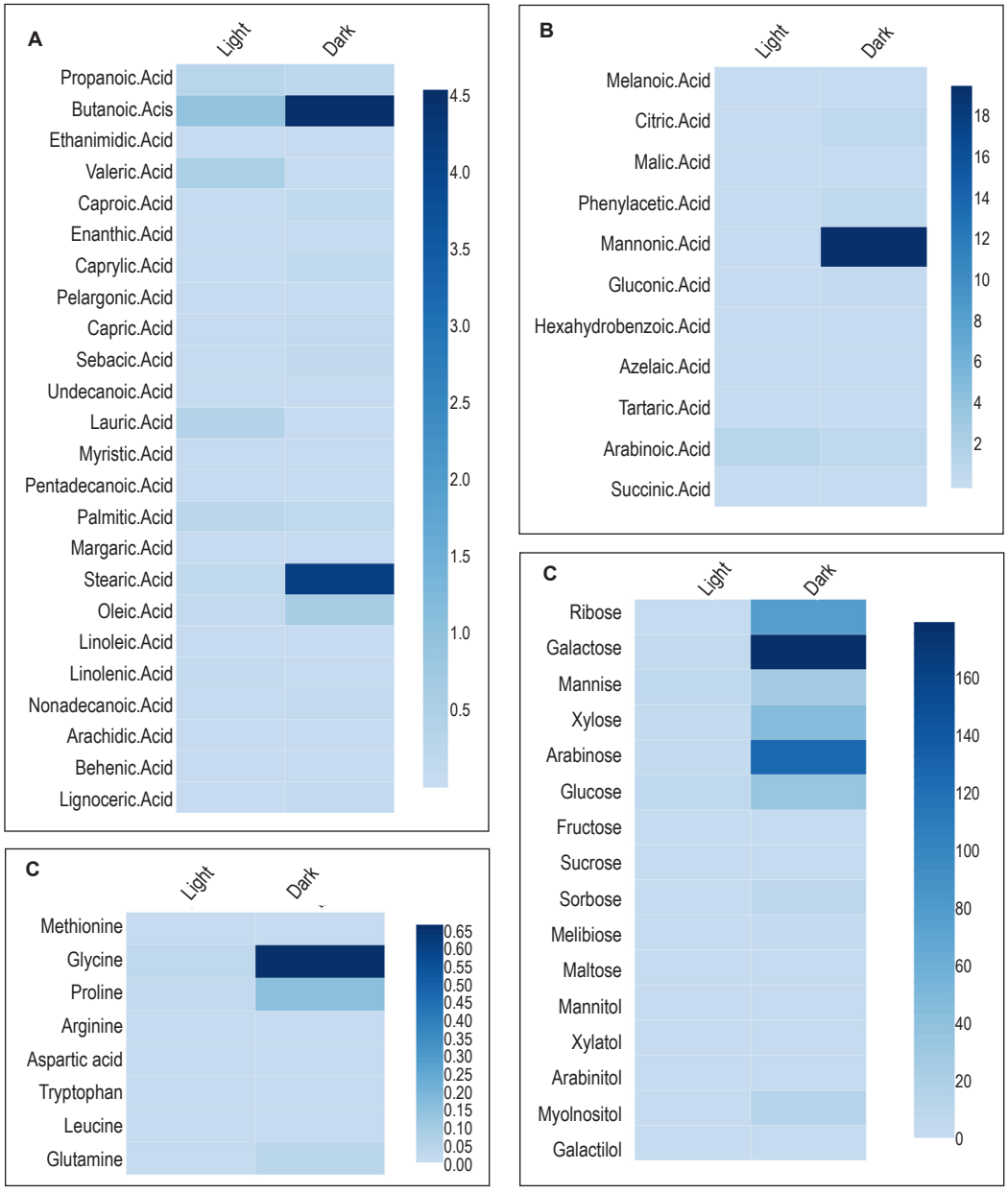

Figure 4. Heatmap of the primary metabolites: (A) sugars + sugar alcohols, (B) organic acids, (C) amino acids and (D) of governor's plum (*Flacourtia indica*) fruit of two different ripe stages (light colour and dark colour skin)

CONCLUSION

The data presented here have indicated that the profiled sugars and sugars alcohols, and organic acids varied significantly between the light and dark colours of the governor's plum fruit. However, fatty acids and amino acids did not govern the ripening stage. Multivariate analysis showed that fatty acids and amino acids of the two ripe stages fruit were much closer compared to sugars, sugar alcohols, and organic acids. Comparatively, dark colour fruit showed a significantly high content of sugars and sugar alcohols, while amino acids showed the lowest level with a significant statistical difference between the light and the dark colour fruit. The different groups of profiled primary metabolites suggest that sugars and sugar alcohols can be considered the "marker metabolites" of the ripe stage of dark colour governor's plum. In contrast, light colour fruit cannot be considered a ripe stage even though it is commercially harvested at this stage.

This study represents the first report on the profiling of the primary metabolites of governor's plum *Flacourtia indica*, and the observed variations therein are suggestive of the need to profile the secondary metabolites (mainly phenolics and volatiles) also, for a full understanding of the difference in the two ripe stages characterized by the fully darkened and red colour fruit.

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