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Volatile and Non-Volatile Compounds Profiling of Brazilian Pitanga (*Eugenia uniflora* L.) Varieties During Ripening using Gas Chromatography-Mass Spectrometry Approach

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Brazil is home to a rich biodiversity of native edible fruits renowned for their health-promoting compounds and flavors. Among these, *Eugenia uniflora* L. (Brazilian pitanga) is particularly notable. The fruit varies in color from yellow to purple and is prized for its intense aroma and versatile taste profile. However, information about the compounds responsible for their nutritional and sensory potential is still limited. This study analyzed volatile compounds and metabolites in yellow, red and purple pitanga fruits (*Eugenia uniflora*) at various ripening stages, exploiting a metabolomics approach. Yellow pitanga had 30 volatile compounds, with esters (40%), terpenes (26.67%) and aldehydes (16.67%) constituting 83.34% of identified volatile compounds. Red pitanga featured 26 volatile compounds, primarily terpenes and esters (each 30.77%) and aldehydes (15.38%), making up 76.92%. Purple pitanga contained 39 volatile compounds, with terpenes and esters (each 35.90%) and aldehydes (17.95%), totaling 89.75% of identified volatile compounds. Across all pitanga types, 124 metabolites were identified, including saturated fatty acids (17.74%), terpenes (17.74%) and sugars (16.93%), constituting 52.41% of identified metabolites. Future studies should provide nutritional information, consumption methods and industrial applications for pitanga fruits, focusing on sensory analysis, health benefits, preservation and breeding to enhance bioactive compounds and flavor.

Keywords: aroma, *Eugenia uniflora* L., GC-MS analysis, ripening stages, volatile compounds, non-volatile compounds

Introduction

Brazil hosts an extensive array of native edible vegetables that offer health-promoting bioactive compounds and exhibit distinctive flavors and aromas that captivate individuals. The diverse wildlife of the country spans across its biomes.[1,2] *Eugenia uniflora* L., commonly known as "Brazilian pitanga," belongs to the Myrtaceae family and stands out among many native fruit-bearing trees in Brazil. It thrives as a shrub or tree, well-suited to tropical and subtropical regions of South America and is prevalent in various Brazilian biomes, particularly the Cerrado and Atlantic Rainforest.[2-4] Brazilian pitanga is a globoid fruit featuring eight to ten longitudinal grooves and varies in color from yellow to purple, depending on the biotype.

The exploitation of pitanga fruits in various regions of Brazil continues to be extractive, primarily aimed at commercialization in street markets. Pitanga fruits are predominantly consumed fresh or processed into products

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such as pulps, juices, ice creams, alcoholic beverages like liqueur and wine and jam. They are characterized by their intense aroma and offer a taste profile that ranges from sweet to sour, depending on their stage of maturity.[2,5] This wild fruit displays significant genetic variability, resulting in fruits of various biotypes, notably yellow, red and purple. These biotypes exhibit distinct levels of several compounds, including primary metabolites such as sugars, fatty acids and amino acids, as well as volatile compounds like esters, terpenes and aldehydes. These compounds vary with ripening stages and play crucial roles in defining key sensory characteristics such as flavor and aroma.[5]

The ripening process of pitanga fruit significantly influences the abundance and composition of volatile compounds and metabolites. Volatile compounds are crucial for the formation of aroma and flavor in fruits and are derived from various sources: primary metabolites (sugars, fatty acids and amino acids) and secondary metabolites (phenolic compounds, carotenoids and terpenoids). These compounds are considered precursor substrates and must be available for the metabolic pathways that form volatile compounds. Therefore, the accumulation of primary and secondary metabolites in fruits during ripening is essential for regulating the formation of volatile compounds.[6] In this context, pitanga fruit provides essential nutrients such as bioactive compounds including phenolic compounds and carotenoids, along with dietary fiber, supporting overall health.[2,7]

Research on *Eugenia uniflora* has predominantly focused on its leaves and essential oils[8] and, so far, no study has been found that evaluated simultaneously the profile of volatile compounds and metabolites in three biotypes (yellow, red and purple) of *Eugenia uniflora*, assessing changes in abundance, types of substances and their impacts on metabolic pathways during different ripening stages.

In this context, this study aimed to assess the metabolites and volatile compounds in three biotypes of pitanga fruit at various ripening stages, aiming to identify the key compounds involved in ripening.

Experimental

Sampling

Pitanga fruits (*Eugenia uniflora* L.) from three biotypes (yellow, red and purple) were sourced from the Brazilian Agricultural Research Corporation (EMBRAPA), Temperate Climate Unit, in Pelotas, Rio Grande do Sul (RS), Brazil (latitude: 31°46'19" S; longitude: 52°20'33"W). The voucher of the plants has been deposited in the Herbarium of EMBRAPA, Temperate Climate Unit, Pelotas, RS, Brazil and has been identified by D. R. Vhal.

The fruits were harvested at three ripening stages: green, intermediate and ripe, determined by the color of the skin. It was provided 150 g of each fruit biotype (50 fruits *per* biotype and ripeness stage). The fruits were sanitized, the seeds removed and the edible part (pulp and skin) was frozen in liquid nitrogen and stored at –80 °C for subsequent analyses. Each pitanga biotype (yellow, red and purple) at each ripeness stage (green, intermediate and ripe) was divided into four biological replicates (n = 4).

Material and equipment

The laboratory reagents used in this work were D-glucose, D-fructose, maltose, sucrose, D-galactose, myo-inositol, citric acid, malic acid, tartaric acid, L-alanine, L-serine, L-proline, L-aspartate and L-glutamate, methyl laurate, methyl tetradecanoate, methyl palmitate, methyl octadecanoate, methyl arachidate, methyl docosanoate, methyl lignocerate, methyl linoleate, (*Z*)-9-oleyl methyl ester, methyl linolenate, methyl palmitoleate, sodium hydroxide, metaphosphoric acid, phosphoric acid, methanol, sodium nitrite, ribitol, chloroform, methoxyamine hydrochloride, pyridine, *n*-tridecane, sodium sulfate, hexane, toluene, hydrochloric acid and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide. All standards, reagents and solvents used were from Sigma-Aldrich, Saint Louis, MO, USA.

The equipment used in this work to analyze volatile compounds was a gas chromatograph (Agilent Technologies, HP 6890, Santa Clara, CA, USA) coupled with mass spectrometer (Agilent Technologies, HP 5973, Santa Clara, CA, USA) and to analyze metabolites was used a gas chromatography coupled with a mass spectrometry (Agilent Technologies, HP 5977, Santa Clara, CA, USA).

Methods

For this research, we identified volatile compounds and non-volatile compounds that can be classified as polar metabolites or non-polar metabolites. For polar metabolites we identified sugars, organic acids and amino acids; for non-polar metabolites we identified some compounds as: fatty acids, phytosterol, tocopherol, etc., as described below.

Volatile compounds: analysis and identification

The volatile compounds of sample were extracted by using the solid-phase microextraction (SPME) technique. Three grams (3 g) of ground and frozen pitanga fruits were homogenized with saturated sodium chloride solution (30% m/v). It was frozen at –20 °C

until the volatile compounds analyses were conducted. Briefly, the sample was thawed for 30 min at room temperature and placed under agitation at 40 °C for 10 min to achieve headspace equilibrium. The SPME fiber (PDMS/DVB/CAR 50 µm, Supelco, USA) was inserted into the vial for adsorption during 50 min at room temperature. Subsequently, the SPME fiber was directly injected into the gas chromatograph (Agilent Technologies, HP 6890, Santa Clara, CA, USA) coupled with a quadrupole mass spectrometer (Agilent Technologies, HP 5973, Santa Clara, CA, USA) and held for 5 min for desorption of volatile compounds. The volatile compounds were separated using a Supelcowax 10 column (30 m × 0.25 mm) with a thickness of 0.25 µm (Supelco, USA). The sample was injected with the injector temperature set at 200 °C in splitless mode, using helium gas as the carrier at a flow rate of 1 mL min^{-1}. The column temperature was set at 40 °C and the temperature ramp was 2 °C min^{-1} up to 170 °C, totaling 65 min of the run. Mass spectrometer parameters were as follows: ion source (230 °C), interface (240 °C), configured for full scan, mass range between 40-550 *m/z* scanned at 2.9 scans *per* second. The raw chromatograms of volatile compounds were initially deconvoluted using the Mass Hunter Analysis software[9] (Agilent, Santa Clara, CA, USA) which automatically used mass spectral libraries for volatiles identification. Confirmation of the volatile compounds was achieved by Kovats index (retention index) using saturated alkanes (C7-C30) as a reference standard 1000 µg mL^{-1} in hexane (Sigma-Aldrich, 49451-U Supelco, USA). All aromatic notes identification of the volatile compounds were obtained by using databases available on the internet.[10,11]

Non-volatile compounds (polar and non-polar metabolites): analysis and identification

The analyses of polar and non-polar metabolites were conducted as described by Meza *et al*.[12] Briefly, methanol, ribitol (internal polar standard), chloroform and deionized water were used to extract polar metabolites. The supernatant (polar phase) was derivatized with methoximation reagent (methoximation solution in pyridine) and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA). For quality control, a mixture of standards of polar metabolites, methyl esters of saturated and unsaturated fatty acids and alkanes were used. The following standards of polar metabolites were employed: glucose, fructose, maltose, sucrose, galactose, myo-inositol, citric acid, L-alanine, L-serine, L-proline, L-aspartate, L-glutamate. The following standards of non-polar metabolites were employed: a mixture of methyl esters of saturated fatty acids (C12-C24) and a mixture of methyl esters of unsaturated fatty acids (C16:1, C18:3, C18:1 and C18:2). For determination of the retention index, a mixture of *n*-alkanes (from C7 to C30) was used (description: C7-C30 Saturated Alkanes Std., 1 × 1.0 mL, 1000 µg mL^{-1} in hexane, Supelco, USA). Chloroform, methanol and tridecanoic acid (C13, internal standard) were used to extract non-polar metabolites. Chloroform and sodium sulfate were added to the extracts. For the first derivatization, the samples were resuspended in hexane, toluene, methanol and HCl in methanol. After the reaction, hexane and ultrapure water were used. For the second derivatization, hexane, pyridine and MSTFA were used. A gas chromatograph coupled with a mass spectrometry (Agilent Technologies, HP 5977, Santa Clara, CA, USA) was used for polar and non-polar metabolomic analysis. Metabolites were separated on an HP 5MS UI column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). The sample was injected at 250 °C in splitless mode, using helium gas as the carrier at a flow rate of 1 mL min^{-1}. The column temperature was set at 60 °C for 1 min, followed by a temperature ramp of 5 to 325 °C for 10 min. Mass spectrometer parameters included ion source temperature (230 °C), interface temperature (290 °C), full scan, mass range of 50-600 *m/z* scanned at 2.7 scans *per* second. Data acquisition and deconvolution were performed using Mass Hunter Analysis software[9] (Agilent, Santa Clara, CA, USA) which automatically used mass spectral libraries for metabolite identification.

Statistical analysis

The MetaboAnalyst, a web-based platform, was employed to provide comprehensive support for data analysis, particularly volatile and non-volatile (polar and non-polar metabolites) compounds and data.[13,14]

Sample data were normalized by the median, transformed on a logarithmic scale and scaled by Pareto. Multivariate analysis was conducted by using principal component analysis (PCA); one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test were employed to determine the behavior of each variable, identifying statistically significant differences between groups at a significance level of 5% ($p < 0.05$).[14]

In addition, Chemical Similarity Enrichment Analysis (ChemRICH) was utilized to show the global behavior of variables at each ripening stage.[15] ChemRICH was performed to provide chemical classes (volatile and metabolites) significantly altered in three types of pitanga (yellow, red and purple) at three ripening stages. The *p*-values of the clusters were obtained by employing the Kolmogorov-Smirnov test. A *p*-value less than 0.05 indicates a statistically significant enriched compound cluster. Each circle reflects the cluster of significantly altered volatile compounds or polar or

non-polar metabolites. The size of the circles represents the total number of compounds in each cluster. The color scale of the cluster (circles) shows the proportion of significantly increased (red cluster) or decreased (blue cluster) volatile compounds or metabolites. The analysis was done in pairs of ripening stages, with the first stage as a reference, as follows: intermediate stage *versus* green stage; ripe stage *versus* intermediate stage; and ripe stage *versus* green stage. The Y-axis explains the significance of the enriched volatile compound or metabolite groups (false discovery rate (FDR) < 0.05) and the X-axis shows the lipophilicity of the volatile compound or metabolite groups.[15,16]

Results and Discussion

Global overview of volatile and non-volatile compounds (polar and non-polar metabolites) of Brazilian pitanga varieties

The present study identified 51 volatile compounds across three varieties of pitanga fruits (yellow, red and purple) at different ripening stages (green, intermediate and ripe). Among these compounds, 19 terpenes (37.25%), 17 esters (33.33%), 8 aldehydes (15.69%), 2 ketones (3.92%), 2 alcohols (3.92%), 2 aromatic hydrocarbons (3.92%) and 1 carboxylic acid (1.96%) were identified. Thus, pitanga fruits predominantly contained terpenes, esters and aldehydes, accounting for 86.27% of the identified volatile compounds. The most abundant volatile compounds identified were β-elemene (23.91%), *E*-germacrene D (15.46%) and ethyl acetate (12.87%), which together represented 52.24% of the total. Regarding biosynthetic precursors, 21 (41.18%) volatile compounds were originated from fatty acid pathways, 19 (37.25%) from isoprenoid pathways and 10 (19.61%) from amino acid pathways (Table 1).

The yellow pitanga (considering all three ripening stages) presented 30 volatile compounds. The three main classes found in yellow pitanga fruits were: 12 esters (40%), 8 terpenes (26.67%) and 5 aldehydes (16.67%), accounting for 83.34% of the volatile compounds. Esters and terpenes alone constituted 66.67% of the identified

Table 1. A global overview of the main volatile compounds identified in pitanga fruits (yellow, red and purple biotypes) during ripening and their aroma description

%	Volatile compound	Class	PubChem ID	RI calc.	RI lit.[a]	Aroma description	Biosynthetic precursor
23.91	β-elemene	(sesqui)terpenes	6918391	1557	1570	waxy, herbal, sweet	isoprenoid
15.46	(*E*)-germacrene D	(sesqui)terpenes	5373727	1680	1680	woody	isoprenoid
12.87	ethyl acetate	ester	8857	865	867	ethereal fruity sweet weedy green	fatty acids
8.53	β-pinene	(mono)terpenes	14896	1137	1137	herbal; dry woody fresh pine hay green resinous	isoprenoid
8.22	(*E*)-β-ocimene	(mono)terpenes	5281553	1204	1200	sweet herbal	isoprenoid
7.43	δ-elemene	(sesqui)terpenes	12309449	1441	1445	herbal	isoprenoid
3.42	γ-elemene	(sesqui)terpenes	6432312	1607	1641	green woody oily	isoprenoid
2.36	ethyl (*E*)-but-2-enoate	ester	429065	1146	1146	fermented; pungent chemical diffusive sweet alliaceous caramel rum	fatty acids
2.03	linalool	(mono)terpenes	6549	1539	1539	floral; citrus, orange, floral, terpy, waxy and rose	isoprenoid
2.02	α-cubebene	(sesqui)terpenes	25244677	1436	1463	herbal waxy	isoprenoid
1.58	acetic acid	carboxylic acids	176	1435	1435	acidic; sharp pungent sour vinegar	fatty acids
1.58	3-hexenal	aldehydes	643139	1124	1126	green; leafy green stem tomato melon apple	fatty acids
1.52	valencene	(sesqui)terpenes	9855795	1686	1713	sweet fresh citrus grapefruit woody orange asprin dry green oily; citrus	isoprenoid
1.24	hexanal	aldehydes	6184	1066	1066	fresh green fatty aldehydic grass leafy fruity sweaty; green	fatty acids
1.22	α-cadinene	(sesqui)terpenes	10657	1756	1720	woody	isoprenoid
0.85	aromadendrene	(sesqui)terpenes	12305243	1573	1622	woody	isoprenoid
0.72	limonene	(mono)terpenes	440917	1158	1183	citrus	isoprenoid
0.67	3-methylbutyl acetate	ester	31276	1106	1105	sweet, banana, fruity with a ripe estry nuance; fruity	amino acids
0.48	α-humulene	(sesqui)terpenes	5281520	1628	1625	woody	isoprenoid
0.42	α-guaiene	(sesqui)terpenes	5317844	1679	1651	sweet woody balsam peppery; woody	isoprenoid
0.41	toluene	aromatic hydrocarbon	1140	1022	1022	phenolic narcissus animal mimosa; phenolic	amino acids
0.34	methyl acetate	ester	6584	813	813	ether sweet fruity; ethereal	fatty acids
0.34	ethyl butanoate	ester	7762	1023	1023	fruity juicy fruit pineapple cognac; fruity	fatty acids

Table 1. A global overview of the main volatile compounds identified in pitanga fruits (yellow, red and purple biotypes) during ripening and their aroma description (cont.)

%	Volatile compound	Class	PubChem ID	RI calc.	RI lit.[a]	Aroma description	Biosynthetic precursor
0.34	methyl heptanone	ketone	9862	1317	1317	citrus green musty lemongrass apple; citrus	fatty acids
0.23	2-phenylethanol	alcohol	6054	1884	1885	sweet, floral, fresh and bready with a rosey honey nuance; floral	amino acids
0.22	isobutyl acetate	ester	8038	999	998	sweet fruity ethereal banana tropical; fruity	fatty acids
0.20	cubenol	(sesqui)terpenes	11770062	2025	2030	spicy herbal green tea; spicy	isoprenoid
0.18	α-gurjunene	(sesqui)terpenes	15560276	1498	1501	woody	isoprenoid
0.15	β-cubebene	(sesqui)terpenes	93081	1585	1589	citrus fruity radish; citrus	isoprenoid
0.14	spathulenol	(sesqui)terpenes	522266	2087	2129	earthy herbal fruity; earthy	isoprenoid
0.13	ethyl phenyl acetate	ester	7590	1761	1765	sweet floral honey rose balsam cocoa; floral	amino acids
0.13	nonanal	aldehydes	31289	1370	1372	waxy aldehydic rose fresh orris orange peel fatty peely; aldehydic	fatty acids
0.10	acetaldehyde	aldehydes	177	762	748	pungent ethereal aldehydic fruity; ethereal	amino acids
0.10	ethyl propanoate	ester	7749	937	938	sweet fruity rum juicy fruit grape pineapple; fruity	fatty acids
0.09	carotol	(sesqui)terpenes	442347	2016	2024	pleasant mild	isoprenoid
0.06	ethyl-2-methylbutanoate	ester	24020	1043	1041	sharp sweet green apple fruity; fruity	amino acids
0.06	phenethyl isobutyrate	ester	7655	1804	1850	floral fruity rose tea rose peach pastry; floral	amino acids
0.05	octanal	aldehydes	454	1265	1264	aldehydic waxy citrus orange peel green fatty; aldehydic	fatty acids
0.03	pentanal	aldehydes	8063	958	955	fermented bready fruity nutty berry; fermented	fatty acids
0.03	3-methylhexanal	aldehydes	140511	1116	1116	sweet green	fatty acids
0.03	2-ethyl-1-hexanol	alcohol	7720	1480	1492	citrus fresh floral oily sweet; citrus	amino acids
0.02	*cis*-3-hexenyl acetate	ester	5363388	1293	1295	fresh green sweet fruity banana apple grassy; green	fatty acids
0.01	propan-2-one	ketone	180	804	802	solvent ethereal apple pear	fatty acids
0.01	ethyl 3-methylbutanoate	ester	7945	1055	1055	fruity sweet apple pineapple tutti frutti; fruity	amino acids
0.01	ethyl hex-3-enoate	ester	5362622	1286	1286	sweet fruity pineapple green metallic fresh; fruity	fatty acids
0.01	ethyl isobutyrate	ester	7342	946	944	sweet ethereal fruity alcoholic fusel rummy; fruity	fatty acids
0.01	methyl (*E*)-2-butenoate	ester	12181	1090	1088	sharp green fruity; green	fatty acids
0.01	styrene	aromatic hydrocarbon	7501	1223	1261	sweet balsam floral plastic; balsamic	–
0.002	butyl acetate	ester	31272	1059	1059	ethereal solvent fruity banana; ethereal	fatty acids
0.002	ethyl 2-hexenoate	ester	5364778	1323	1328	rum fruity green sweet juicy; fruity	fatty acids
0.001	propanal	aldehydes	527	792	790	earthy alcohol wine whiskey cocoa nutty; ethereal	fatty acids

[a]Values between 10 and 50 units were considered as the maximum deviation for identification usually accepted for polar columns, as follow: $\Delta = |RI\ lit. - RI\ calc.|$.[17-19] RI calc.: retention index calculated in relation to *n*-alkanes (C7 to C30) in polar column Supelcowax 10. RI lit.: retention index obtained from literature in online databases (NIST Chemistry WebBook,[20] ChemSpider,[21] Pherobase[10] and PubChem.[22]

compounds. The red pitanga (considering all three ripening stages) presented 26 volatile compounds. The three main classes of compounds found in red pitanga fruits were: 8 terpenes (30.77%), 8 esters (30.77%) and 4 aldehydes (15.38%), accounting for 76.92% of the volatile compounds. Esters and terpenes together constituted 61.54% of the identified compounds. The purple pitanga (considering all three ripening stages) presented 39 volatile compounds. The three main classes of compounds found in purple pitanga fruits were: 14 terpenes (35.90%), 14 esters (35.90%) and 7 aldehydes (17.95%), accounting for 89.75% of the volatile compounds. Esters and terpenes together constituted 71.80% of the identified volatile compounds.

Regarding metabolites in three types of pitanga fruits (yellow, purple and red) at various ripening stages (green, intermediate and ripe), the present study identified 124 polar and non-polar metabolites. Among these compounds, the

identified metabolites included: 22 saturated fatty acids (17.74%), 22 terpenes (17.74%), 21 sugars (16.93%), 14 organic acids (11.29%), 10 amino acids (8.06%), 8 unsaturated fatty acids (6.45%), 7 phytosterols (5.64%), 6 quinones (4.84%), 4 fat-soluble vitamins (tocopherols) (3.23%) and 10 metabolites in other classes (8.08%). Saturated fatty acids, terpenes and sugars accounted for more than half of the identified metabolites (52.41%).

Figure 1 shows a significant separation among all three ripening stages (green, intermediate and mature) for each variety of pitanga fruits (yellow, red and purple) for both volatile compounds and metabolites. Each variety of

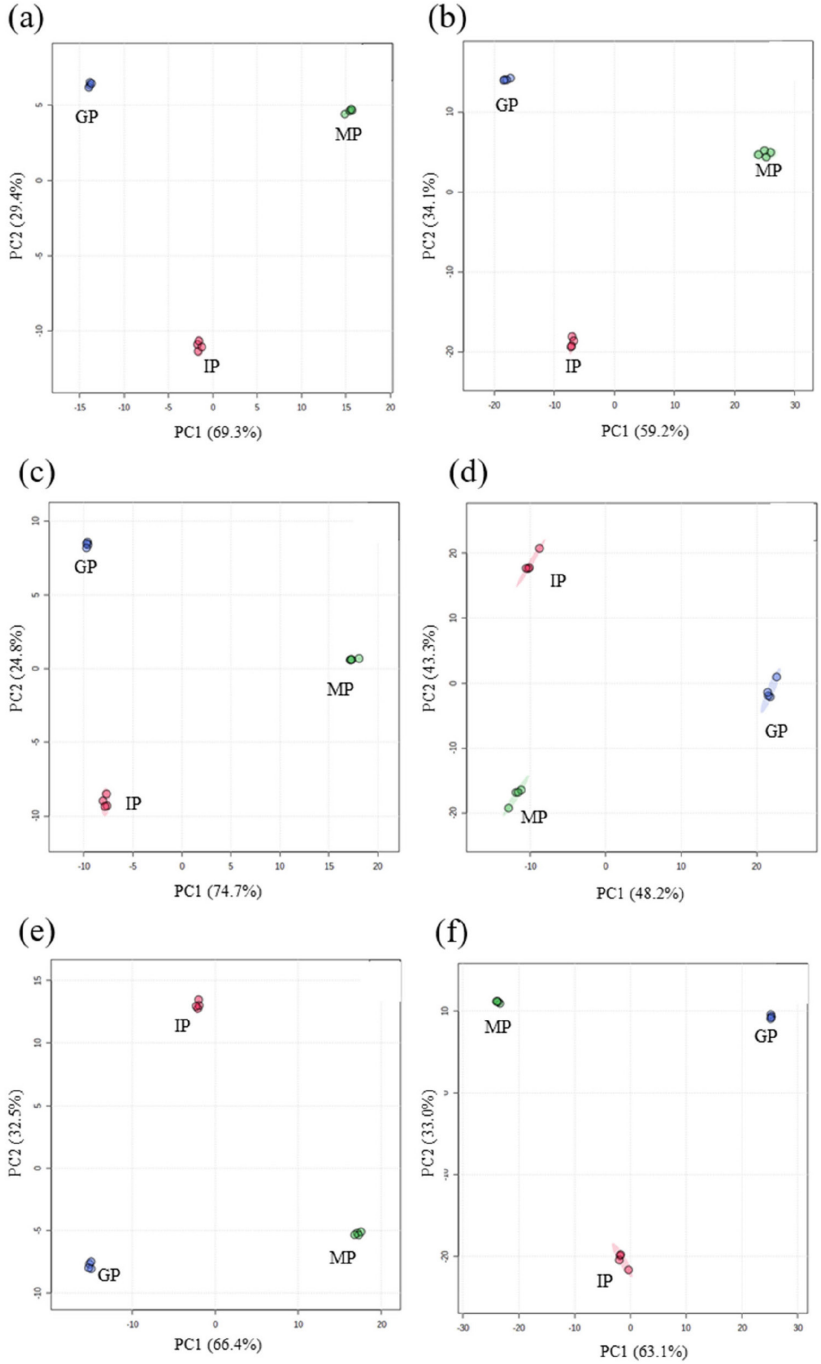

Figure 1. Principal component analysis (PCA) of volatile compounds and metabolites in pitangas fruits (yellow, red and purple biotypes) at three ripening stages. Each variety of pitanga (yellow, red and purple) were separated in three groups of ripening: green pitanga (GP), intermediate pitanga (IP) and mature pitanga (MP). (a) PCA of volatile compounds in yellow pitanga in three groups of ripening: GP, IP MP; (b) PCA of metabolites in yellow pitanga in three groups of ripening: GP, IP MP; (c) PCA of volatile compounds in red pitanga in three groups of ripening: GP, IP MP; (d) PCA of metabolites compounds in red pitanga in three groups of ripening: GP, IP MP; (e) PCA of volatile compounds in purple pitanga in three groups of ripening: GP, IP MP; (f) PCA of metabolites compounds in purple pitanga in three groups of ripening: GP, IP, MP.

pitanga (yellow, red and purple) were separated in three groups of ripening: green pitanga (GP), intermediate pitanga (IP) and mature pitanga (MP).

Left side of the Figure 1 shows the two principal components (PC1 and PC2) of PCA for the volatile compound profile. Figure 1a explains 98.70% of the total variability for yellow pitanga at three ripening stages (GP, IP and MP); Figure 1c explains 99.50% for red pitanga at three ripening stages (GP, IP and MP); and Figure 1e explains 98.80% for purple pitanga at three ripening stages.

Right side of the Figure 1 shows the two principal components (PC1 and PC2) of PCA for the metabolites. Figure 1b explains 93.30% of the total variability for yellow pitanga at three ripening stages (GP, IP and MP); Figure 1d explains 91.50% for red pitanga at three ripening stages (GP, IP and MP); and Figure 1f explains 96.10% for purple pitanga at three ripening stages (GP, IP and MP).

Brazilian yellow pitanga: volatile and non-volatile compounds (polar and non-polar metabolites)

The metabolite analysis of yellow pitanga fruits identified 84 compounds, comprising 35 polar metabolites (41.67%) and 49 non-polar metabolites (58.33%). Among the 35 polar metabolites, the following were found: 13 sugars (37.14%), 12 organic acids (34.29%), 7 amino acids (20%) and 3 other substances (quinone, short-chain fatty acids and aldehyde) (8.57%). Among the 49 non-polar metabolites, the following were identified: 16 saturated fatty acids (32.65%), 13 terpenes (26.53%), 7 unsaturated fatty acids (14.29%), 5 phytosterols (10.20%) and 4 tocopherols (8.16%).

Tables 2, 3 and 4 show all volatile compounds of yellow, red and purple pitanga presented at different ripening stages, respectively. Each volatile compound, from each type of pitanga and ripening stage, was expressed as a percentage (%) of its peak area in relation to the sum of all volatile peaks in the chromatogram. Table 2 shows volatile compounds of yellow pitanga fruit at different ripening stages.

Figures 2a and 2d show changes in the abundance of volatile compounds and metabolites at the intermediate stage compared to green stage in yellow pitanga. ChemRich analysis revealed significant differences (FDR < 0.05) in a total of 3 clusters of volatile compounds (Figure 2a) and 6 clusters of metabolites (Figure 2d) at the intermediate stage compared to green stage in yellow pitanga. Esters (red cluster, Figure 2a), terpenes (red cluster, Figure 2d) and unsaturated fatty acids (red cluster, Figure 2d) significantly increased at the intermediate stage compared to the green stage in yellow pitanga. However, amino acids (blue cluster, Figure 2d) significantly decreased at the intermediate stage compared to the green stage.

Table 2. Volatile compounds of yellow pitanga fruit (*Eugenia uniflora* L.) at different ripening stages

Volatile compound	Maturity stage / (% of the area)		
	Green	Intermediate	Ripe
Terpene			
β-Elemene	51.26	31.86	11.59
β-Pinene	21.97	31.19	23.90
δ-Elemene	13.11	14.31	11.50
γ-Elemene	–	2.47	15.54
α-Cadinene	2.06	2.06	–
Cubenol	1.57	–	–
α-Gurjunene	1.42	–	–
Limonene	0.92	–	1.30
Aldehyde			
3-Hexenal	1.85	1.86	–
Hexanal	0.99	1.10	0.51
3-Methylhexanal	0.05	–	–
Pentanal	0.05	–	–
Acetaldehyde	–	–	0.88
Ester			
Ethyl acetate	3.07	11.07	23.21
Methyl acetate	–	0.33	0.76
Ethyl propanoate	–	0.09	0.18
3-Methylbutyl acetate	–	0.08	0.75
Isobutyl acetate	–	0.06	0.24
Butyl acetate	–	0.02	–
Ethyl-(*E*)-but-2-enoate	–	–	4.62
Ethyl butanoate	–	–	0.56
Phenethyl isobutyrate	–	–	0.53
Ethyl-hex-3-enoate	–	–	0.13
Ethyl-2-methylbutanoate	–	–	0.09
Ethyl-3-methyl-butanoate	–	–	0.02
Others			
Acetic acid	1.08	2.67	2.27
Toluene	0.49	0.35	–
Methyl-heptenone	0.11	0.38	0.72
Propan-2-one	–	0.07	0.05
2-Phenyletanol	–	–	0.67
Total	100	100	100

Analyzing Figure 2a, the main metabolic pathways involved in volatile compound formation are related to fatty acids and/or amino acids. Figure 2d highlights that amino acids were utilized as precursors for aroma formation, as their abundance significantly decreased. Terpenes showed a significant increase (red cluster, Figure 2d) at the intermediate stage compared to green, indicating isoprenoids as the primary precursors.

Figures 2b and 2e show changes in the abundance of volatile compounds and metabolites at the ripe stage compared to intermediate stage in yellow pitanga. ChemRich analysis revealed that a total of 3 clusters of volatile compounds (Figure 2b) and 7 clusters of metabolites (Figure 2e) were significantly different

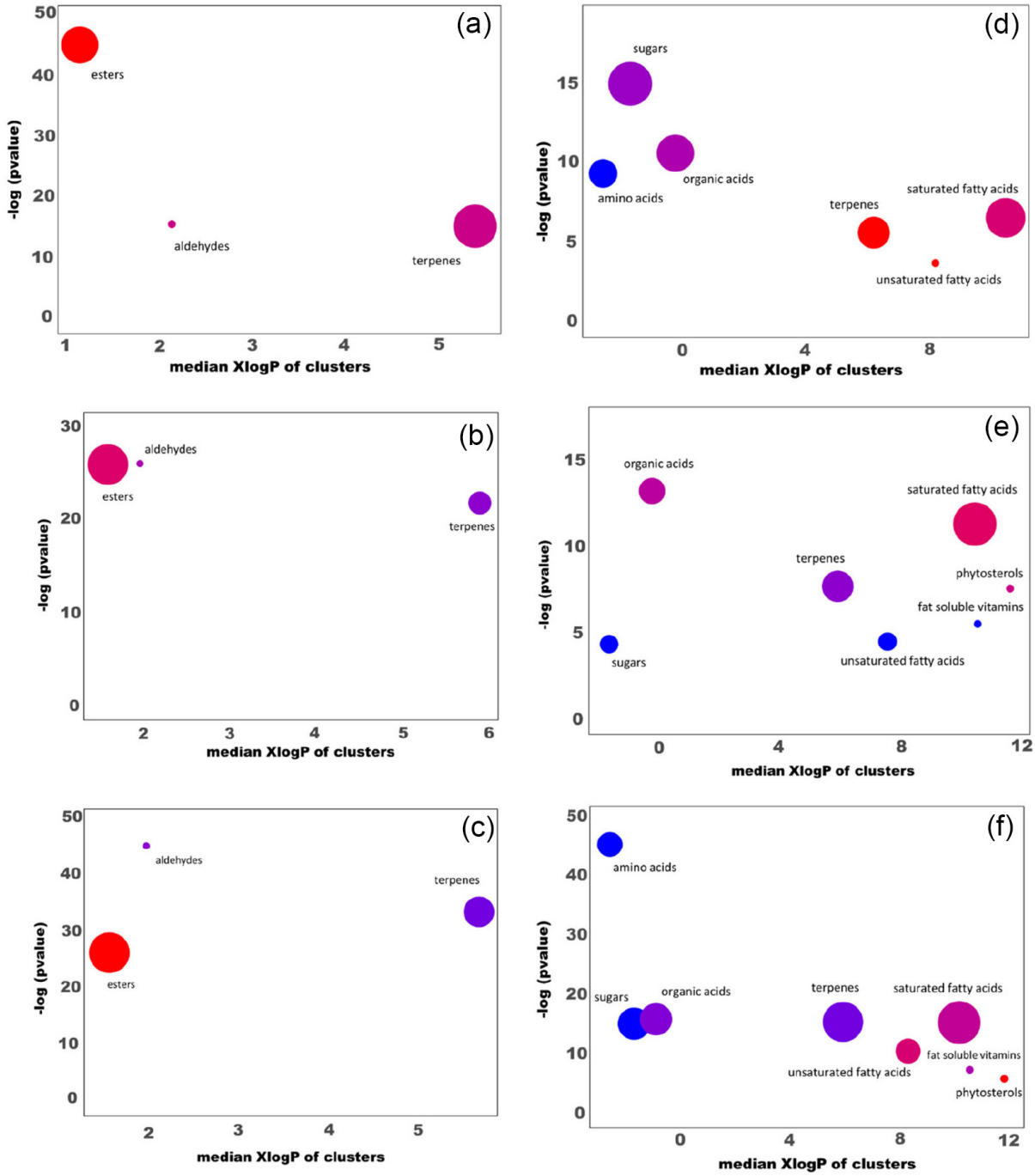

Figure 2. ChemRICH analysis of volatile compounds (a, b and c) and metabolites (d, e and f) in yellow pitanga at three ripening stages. Figures from letter (a) to (f) show the ChemRICH analysis that compare data from pairs of ripening stage, being the first stage as a reference, as follow: intermediate (reference) *versus* green; mature (reference) *versus* intermediate; and mature (reference) *versus* green. (a) ChemRich analysis of volatiles compounds in intermediate stage compared to green stage in yellow pitanga; (b) ChemRICH analysis of volatiles compounds in ripe stage compared to intermediate stage in yellow pitanga; (c) ChemRICH analysis of volatiles compounds in ripe stage compared to green stage in yellow pitanga; (d) ChemRICH analysis of metabolites compounds in intermediate stage compared to green stage in yellow pitanga; (e) ChemRICH analysis of metabolites compounds in ripe stage compared to intermediate stage in yellow pitanga; and (f) ChemRICH analysis of metabolites compounds in ripe stage compared to green stage in yellow pitanga. Red cluster = significantly increased abundance of volatile compounds and metabolites. Blue cluster = significantly decreased abundance of volatile compounds and metabolites. Circle size = number of detected compounds in the cluster. Y-axis: significance of the enriched groups of volatile compounds and metabolites (FDR < 0.05). X-axis: lipophilicity of the groups of volatile compounds and metabolites.

(FDR < 0.05) at the ripe stage compared to intermediate stage in yellow pitanga. Esters (red cluster, Figure 2b) and saturated fatty acids (red cluster, Figure 2e) significantly increased at the ripe stage compared to the intermediate stage. However, sugars (blue cluster, Figure 2e), unsaturated fatty acids (blue cluster, Figure 2e) and fat-soluble vitamins (blue cluster, Figure 2e) decreased significantly at the ripe stage compared to the intermediate stage.

An increase in esters at the ripe stage compared to the intermediate stage, indicating that isoprenoids were the primary precursors utilized during the metabolic pathway of terpenoids, is observed in Figure 2b. Figure 2e highlights that sugars and unsaturated fatty acids may have been the main substrates for ester formation at the ripe stage compared to the intermediate stage.

Figures 2c and 2f show changes in abundance of volatile compounds and metabolites at the ripe stage compared to green stage in yellow pitanga. ChemRich analysis revealed that a total of 3 clusters of volatile compounds (Figure 2c) and 8 clusters of metabolites (Figure 2f) were significantly different (FDR < 0.05) at the ripe stage compared to green stage in yellow pitanga. Esters (red cluster, Figure 2c) and phytosterols (red cluster, Figure 2f) significantly increased at the ripe stage compared to the green stage, while amino acids (blue cluster, Figure 2f) and sugars (blue cluster, Figure 2f) significantly decreased at the ripe stage compared to the green stage. Moreover, a significant increase in ester production during ripening, comparing two extreme ripening stages (ripe *vs.* green) in yellow pitanga fruits, was observed in Figure 2c. Therefore, the primary metabolic pathway directed towards the ripe stage involved terpenoid biosynthesis, utilizing isoprenoids as substrates. Figure 2f demonstrates that amino acids and sugars were the main substrates significantly altered for aroma formation in yellow pitanga fruits.

Brazilian red pitanga: volatile and non-volatile compounds (polar and non-polar metabolites)

The metabolite analysis of red pitanga fruits identified 81 compounds, with 38 polar (46.91%) and 43 non-polar metabolites (53.09%). Among the 38 polar metabolites, the following were found: 10 organic acids (26.32%), 16 sugars (42.10%), 8 amino acids (21.05%) and 4 other compounds (10.53%) (quinone, short-chain fatty acid, aldehyde and alcohol). Among the 43 non-polar compounds, the following were identified: 11 terpenes (25.58%), 18 saturated fatty acids (41.86%), 6 unsaturated fatty acids (13.95%), 5 other compounds (11.63%) (tocopherol, quinones, oleic acid and fatty alcohols) and 3 phytosterols (6.98%). Volatile compounds of red pitanga at different ripening stages are presented in Table 3.

Table 3. Volatile compounds of red pitanga (*Eugenia uniflora* L.) at different ripening stages

Volatile compound	Maturity stage / (% of the area)		
	Green	Intermediate	Ripe
Terpene			
(*E*)-β-Ocimene	3.24	4.34	–
δ-Elemene	–	9.72	–
β-Elemene	70.25	36.56	7.66
α-Cubebene	1.26	–	–
Linalool	10.99	3.45	–
Limonene	0.99	0.93	–
Gama elemene	–	20.58	–
Aldehyde			
3-Hexenal	0.96	1.54	–
Hexanal	0.86	1.56	1.28
Nonanal	0.17	0.26	–
Pentanal	0.05	0.06	–
Ester			
Ethyl(*E*)-but-2-enoate	–	0.32	5.05
Ethyl-acetate	7.13	14.70	68.56
Methyl-acetate	0.13	0.36	2.31
3-Methylbutyl acetate	0.54	0.54	4.72
Isobutyl acetate	0.23	0.39	1.15
Ethyl-2-methylbutanoate	–	–	0.40
Ethyl butanoate	–	–	2.79
Ethyl propanoate	0.01	0.06	0.57
Others			
Acetic acid	1.25	2.46	4.95
Toluene	0.57	0.77	–
Methyl-heptenone	0.13	1.20	–
2-Phenylethanol	–	–	0.55
2-Ethyl 1-hexanol	0.19	–	–
Espatulenol	1.04	–	–
Estirene	–	0.19	–
Total	100	100	100

Figures 3a and 3d show changes in the abundance of volatile compounds and metabolites at the intermediate stage compared to green stage in red pitanga. ChemRich analysis revealed that a total of 2 clusters of volatile compounds (Figure 3a) and 7 clusters of metabolites (Figure 3d) were significantly different (FDR < 0.05) at the intermediate stage compared to green stage in red pitanga. Esters (red cluster, Figure 3a), organic acids (red cluster, Figure 3d) and unsaturated fatty acids (red cluster, Figure 3d) significantly increased in the intermediate stage

compared to green. Figure 3a shows a significant increase in esters, indicating that the intermediate stage (compared to green) utilized terpenoid metabolic pathways.

Figures 3b and 3e show changes in the abundance of volatile compounds and metabolites at the intermediate stage compared to ripe stage in red pitanga. ChemRich analysis revealed that a total of 3 clusters of volatile compounds (Figure 3b) and 6 clusters of metabolites (Figures 3e) were significantly different (FDR < 0.05) at the ripe stage compared to intermediate stage in red pitanga. Terpenes (red cluster, Figure 3b) and aldehydes (red cluster, Figure 3b) significantly increased in the ripe stage compared to the intermediate stage. However, saturated fatty acids (blue cluster, Figure 3e) and phytosterols (blue

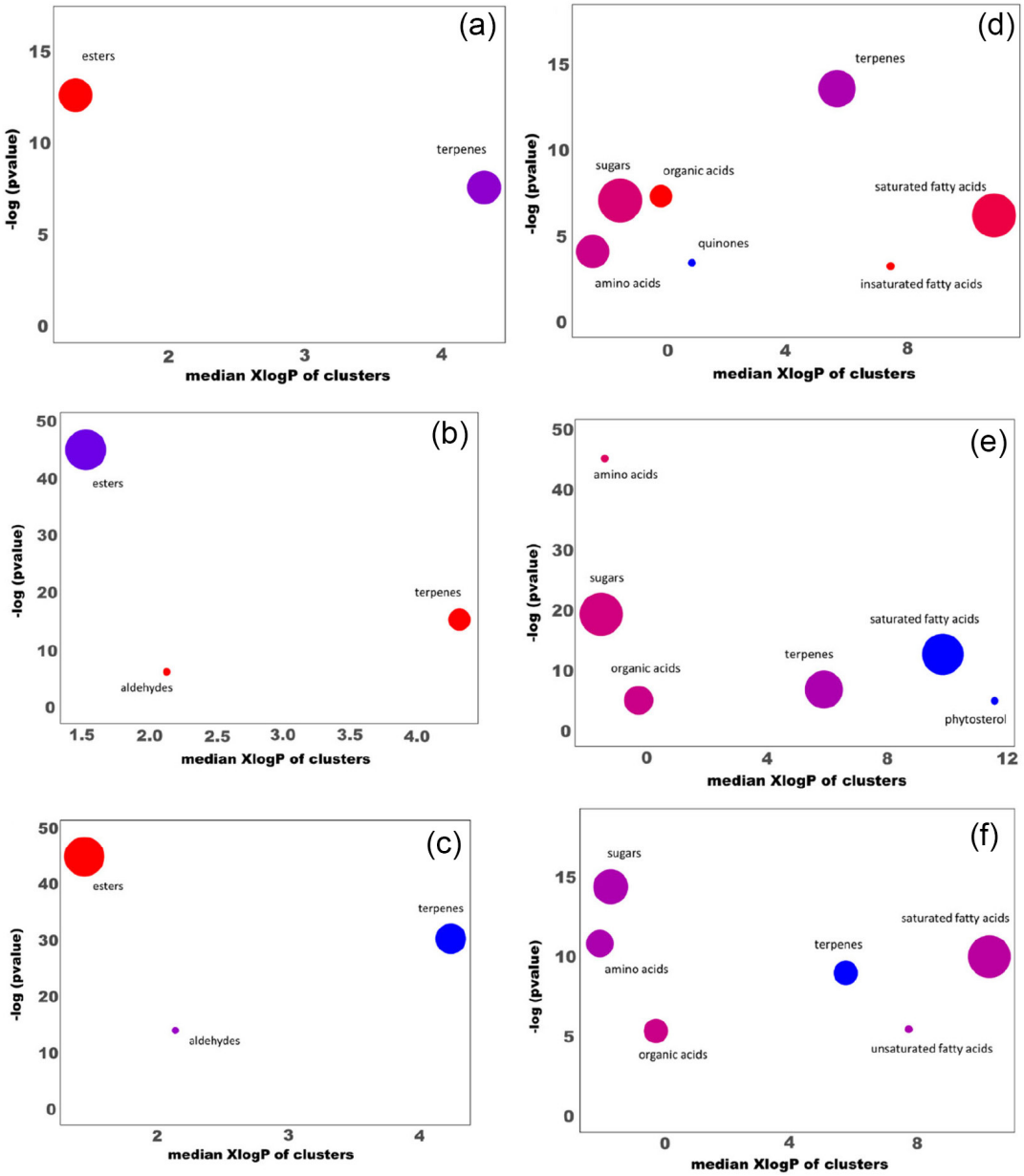

Figure 3. ChemRICH analysis of volatile compounds (a, b and c) and metabolites (d, e and f) in red pitanga at three ripening stages. Figures from letter (a) to (f) show the ChemRICH analysis that compare data from pairs of ripening stage, being the first stage as a reference, as follow: intermediate (reference) *versus* green; mature (reference) *versus* intermediate; and mature (reference) *versus* green. (a) ChemRich analysis of volatiles compounds in intermediate stage compared to green stage in red pitanga; (b) ChemRICH analysis of volatiles compounds in ripe stage compared to intermediate stage in red pitanga; (c) ChemRICH analysis of volatiles compounds in ripe stage compared to green stage in red pitanga; (d) ChemRICH analysis of metabolites compounds in intermediate stage compared to green stage in red pitanga; (e) ChemRICH analysis of metabolites compounds in ripe stage compared to intermediate stage in red pitanga; and (f) ChemRICH analysis of metabolites compounds in ripe stage compared to green stage in red pitanga. Red cluster = significantly increased abundance of volatile compounds and metabolites. Blue cluster = significantly decreased abundance of volatile compounds and metabolites. Circle size = number of detected compounds in the cluster. Y-axis: significance of the enriched groups of volatile compounds and metabolites (FDR < 0.05). X-axis: lipophilicity of the groups of volatile compounds and metabolites.

cluster, Figure 3e) decreased significantly in the ripe stage compared to the intermediate stage.

A significant increase in terpenes, indicating stimulation of the terpenoid pathway and use of isoprenoids as precursors was shown in Figure 3b. Figure 3e demonstrates a decrease in saturated fatty acids, likely to facilitate the synthesis of esters.

Figures 3c and 3f show changes in the abundance of volatile compounds and metabolites at the ripe stage compared to green stage in red pitanga. ChemRich analysis indicated that a total of 3 clusters of volatile compounds (Figure 3c) and 6 clusters of metabolites (Figure 3f) were significantly different (FDR < 0.05) at the ripe stage compared to green stage in red pitanga. Esters (red cluster, Figure 3c) increased significantly in the ripe stage compared to green, whereas terpenes (blue cluster, Figures 3c and 3f) decreased significantly in the ripe stage compared to green. Figure 3c illustrates that the ripe stage of red pitanga fruits exhibited a significant increase in esters, which may be synthesized through fatty acid and/or amino acid metabolism. Figure 3f shows a decrease in terpene accumulation, potentially accumulating more carotenoids for the red color formation of pitanga fruits.

Brazilian purple pitanga: volatile and non-volatile compounds (polar and non-polar metabolites)

The metabolite analysis of purple pitanga identified 97 compounds, comprising 43 polar metabolites (44.33%) and 54 non-polar metabolites (55.67%). Among the 43 polar metabolites, the following were found: 20 sugars (46.51%), 11 organic acids (25.58%), 8 amino acids (18.60%) and 4 other substances (quinones, short-chain fatty acids and aldehyde) (9.31%). Among the 54 non-polar metabolites, the following were identified: 16 terpenes (29.63%), 18 saturated fatty acids (33.33%), 9 other compounds (16.67%) (fatty alcohols, alkaloid, ketones, lactones, quinones and tocopherol), 6 unsaturated fatty acids (11.11%) and 5 phytosterols (9.26%). Volatile compounds of purple pitanga at different ripening stages are presented in Table 4.

Figures 4a and 4d show changes in the abundance of volatile compounds and metabolites at the intermediate stage compared to green stage in purple pitanga. ChemRich analysis revealed that a total of 3 clusters of volatile compounds (Figure 4a) and 7 clusters of metabolites (Figure 4d) were significantly different (FDR < 0.05) at the intermediate stage compared to green stage in purple pitanga. Aldehydes (blue cluster, Figure 4a) significantly decreased at the intermediate stage compared to the green stage. However, sugars (red

Table 4. Volatile compounds of purple pitanga (*Eugenia unifl*ora L.) at different ripening stages

Volatile compound	Maturity stage / (% of the area)		
	Green	Intermediate	Ripe
Terpene			
(*E*)-Germacrene D	73.41	–	–
(*E*)-β-Ocimene			46.90
Valencene	7.21	–	–
δ-Elemene	5.88	10.70	4.20
β-Elemene		–	–
Aromadendrene	–	7.26	3.18
α-Guaiene	–	8.99	–
α-Cubebene	1.20	2.98	9.11
α-Cadinene	1.81	3.94	1.26
β-Cubebene	–	3.18	–
Linalool	1.47	–	–
α-Humulene	0.64	7.32	–
Limonene	0.52	1.21	0.50
Carotol	0.45	–	–
Aldehyde			
3-Hexenal	3.03	5.93	0.11
Hexanal	1.93	3.92	0.56
Nonanal	0.15	1.10	–
Octanal	0.06	0.68	–
3-Methyl hexanal	0.04	0.23	–
Pentanal	0.04	0.17	–
Propanal	0.003	–	–
Ester			
Ethyl-(*E*)-but-2-enoate	–	7.64	7.70
Ethyl-acetate	1.57	23.88	15.78
Methyl-acetate	0.05	0.82	0.20
3-Methyl-butylacetate	0.10	0.75	1.36
Isobutyl acetate	0.06	0.94	0.18
Ethyl 2-methyl-butanoate	0.01	0.13	0.15
Ethyl 2-hexenoate	–	0.01	0.01
Ethyl butanoate	–	0.87	0.76
Ethyl isobutyrate	–	0.16	–
Ethyl propanoate	–	0.27	0.15
Ethyl 3-methylbutanoate	–	–	0.03
Ethyl phenyl acetate	–	–	0.81
cis-3-Hexenyl acetate	–	–	0.15
Methyl(*E*)-2-butenoate	–	–	0.08
Others			
Acetic acid	–	3.50	1.40
Toluene	0.28	1.51	0.36
Methyl-heptenone	0.10	1.91	–
2-Phenyl ethanol	–	–	0.79
Total	100	100	100

cluster, Figure 4d) and saturated fatty acids (red cluster, Figure 4d) significantly increased at the intermediate stage compared to the green stage. Figure 4a indicates that the main metabolic pathways involved are related to amino acids and/or fatty acids. Figure 4d shows that sugars and saturated fatty acids were the main metabolites found at the green stage for ester synthesis.

Figures 4b and 4e show changes in the abundance of volatile compounds and metabolites at the ripe stage compared to intermediate stage in purple pitanga. ChemRich analysis showed that a total of 3 clusters of volatile compounds (Figure 4b) and 5 clusters of metabolites (Figure 4e) were significantly different (FDR < 0.05) at the ripe stage compared to intermediate

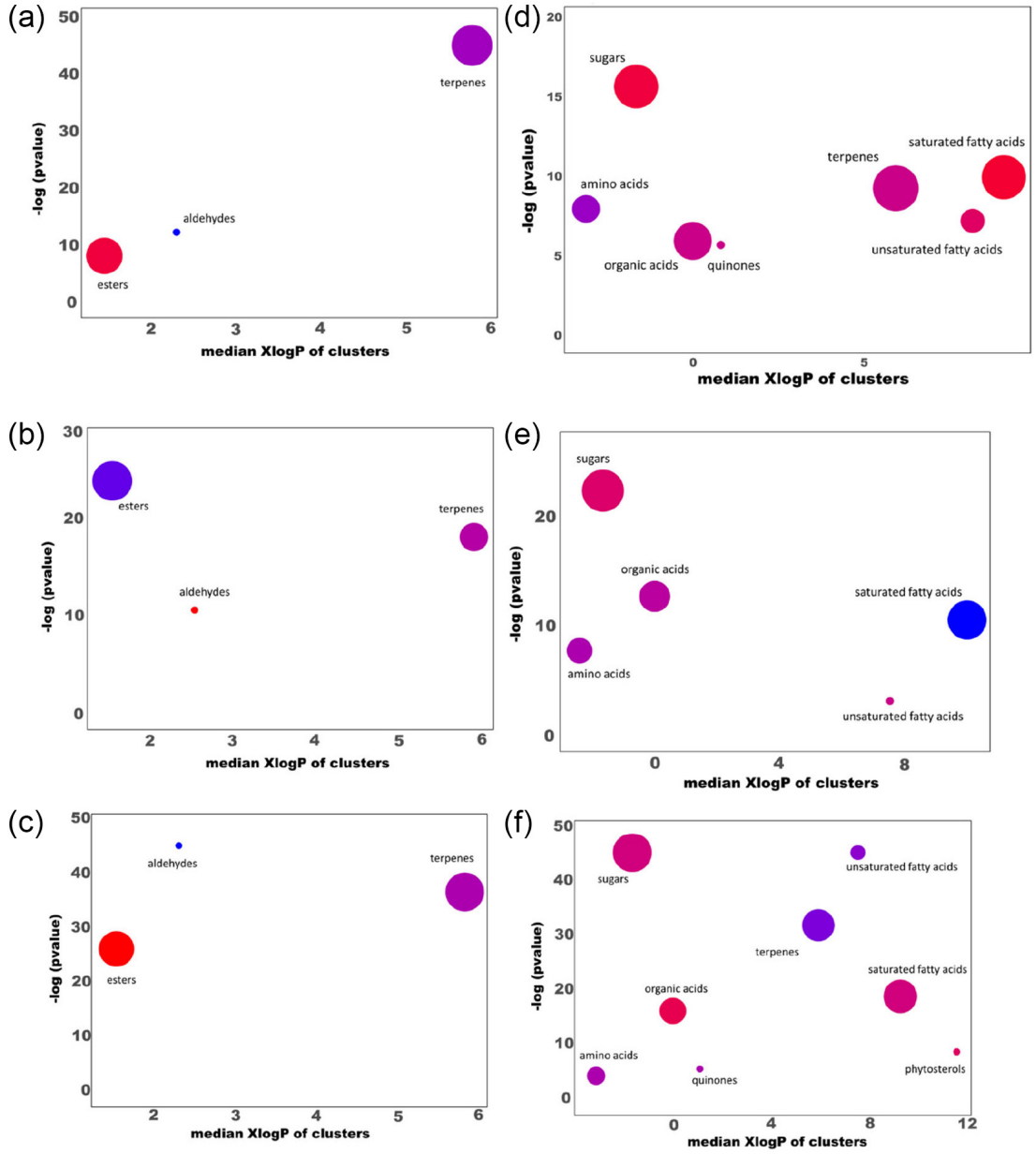

Figure 4. ChemRICH analysis of volatile compounds (a, b and c) and metabolites (d, e and f) in purple pitanga at three ripening stages. Figures from letter (a) to (f) show the ChemRICH analysis that compare data from pairs of ripening stage, being the first stage as a reference, as follow: intermediate (reference) *versus* green; mature (reference) *versus* intermediate; and mature (reference) *versus* green. (a) ChemRich analysis of volatiles compounds in intermediate stage compared to green stage in purple pitanga; (b) ChemRICH analysis of volatiles compounds in ripe stage compared to intermediate stage in purple pitanga; (c) ChemRICH analysis of volatiles compounds in ripe stage compared to green stage in purple pitanga; (d) ChemRICH analysis of metabolites compounds in intermediate stage compared to green stage in purple pitanga; (e) ChemRICH analysis of metabolites compounds in ripe stage compared to intermediate stage in purple pitanga; (f) ChemRICH analysis of metabolites compounds in ripe stage compared to green stage in purple pitanga. Red cluster = significantly increased abundance of volatile compounds and metabolites. Blue cluster = significantly decreased abundance of volatile compounds and metabolites. Circle size = number of detected compounds in the cluster. Y-axis: significance of the enriched groups of volatile compounds and metabolites (FDR < 0.05). X-axis: lipophilicity of the groups of volatile compounds and metabolites.

stage in purple pitanga. Aldehydes (red cluster, Figure 4b) significantly increased at the ripe stage compared to the intermediate stage. However, saturated fatty acids (blue cluster, Figure 4e) significantly decreased at the ripe stage compared to the intermediate stage.

Figure 4b shows that aldehydes increased significantly at the ripe stage, indicating that the main metabolic pathways for aroma formation are related to fatty acids. Figure 4e indicates a significant decrease in saturated fatty acids at the ripe stage for the production of esters.

Figures 4c and 4f show changes in the abundance of volatile compounds and metabolites at the ripe stage compared to green stage in purple pitanga. ChemRich analysis showed that a total of 3 clusters of volatile compounds (Figure 4c) and 8 clusters of metabolites (Figure 4f) were significantly different (FDR < 0.05) at the ripe stage compared to green stage in purple pitanga. Esters (red cluster, Figure 4c) increased significantly at the ripe stage compared to the green stage, whereas aldehydes (blue cluster, Figure 4c) decreased significantly at the ripe stage compared to the green stage.

Figure 4c indicates that the main metabolic pathways are related to fatty acids, as there was an increase in esters and a decrease in aldehydes during ripening (ripe stage compared to green one). Figure 4f shows that there was no significant difference among the 8 clusters of metabolites compared to the green stage.

The results of the present research demonstrated that most of the volatile compounds of pitanga (yellow, red and purple) at three ripening stages (green, intermediate and mature) originated from esters, terpenes and aldehydes, corresponding to almost 90% of the substances. The results from each type of pitanga at three ripening stages were shown in detail. However, we can make certain generalizations, as various behaviors of the volatile compounds were repeated throughout the ripening process.

Generally, the results showed that both abundance and type of volatile ester compounds during ripening of pitanga were increased, meaning esters were more abundant at the mature stage. However, volatile compounds containing aldehyde or terpene groups decreased in abundance, particularly at the mature stage. Volatile compounds containing aldehyde groups presented a smaller number and variety of substances compared to terpenes and esters.

Various studies[6,23-26] have shown that esters are present in high concentration and variety in fruits, corroborating with the present work. For instance, in pears (*Pyrus pyrifolia* Nak.) about 300 volatile compounds have been recognized, mainly esters (hexyl acetate, 2-methyl-propyl acetate, butyl butanoate, pentyl acetate, ethyl hexanoate, among others).[6] In melons, 240 volatile compounds have been identified in various cultivars, mainly esters, terpenes, alcohols, aldehydes and sulfur compounds.[23] In bananas, there is also an appreciable number of esters, with more than 250 volatile compounds identified and esters correspond to the main compounds responsible for the aroma, particularly isoamyl acetate and isobutyl acetate. Corroborating the present work, esters in bananas increase in abundance during ripening.[24]

The volatile compounds of peaches (*Prunus persica*) include more than 100 substances, highlighting C6-aldehydes, alcohols, esters and lactones. Esters (hexyl acetate and *Z*-3-hexenyl acetate) influence the flavor of peaches, especially at the mature stage.[6] In apricots (*Prunus armeniaca* L.), more than 200 volatile compounds have been identified, with aldehydes (hexanal and *E*-2-hexenal) being the main compounds in green apricots and their concentrations significantly decreasing during ripening. Terpenes and alcohols, although in lower concentration, also decreased at the mature stage.[25]

In avocados (*Persea americana* Mill), there is a higher abundance of alcohols, aldehydes and terpenes in green fruit, declining with ripening. Thus, mature avocados also present a high concentration of esters.[26,27] In summary, the behavior of the volatile compounds in the present study corroborates the literature, which portrays that aldehydes and terpenes are generally found in green fruits and their concentrations decrease throughout the ripening process. Ester production, on the other hand, occurs in later ripening stages due to the greater availability of primary substrates, especially unsaturated fatty acids.[6,28]

As demonstrated in the present study, the ripening process generated a biochemical variation, evidenced by the abundance and type of volatile compounds formed during the process. Studies on pitangas have particularly focused on the leaves and essential oils of the fruits, so there is little research on the pitanga fruit itself.[8,29,30] So far, no studies have been found on pitanga fruits that focused mainly on the changes in the abundance and type of substances (volatile compounds and metabolites) at different ripening stages and in some varieties of pitanga (yellow, red and purple).

According to the obtained results, it is clear that the volatile compounds of pitanga, in any variety, were influenced during ripening, represented by the green, intermediate and mature stages. Many compounds were detected exclusively at a specific stage (Tables 2, 3 and 4). Compounds such as cubenol, α-gurjunene, 3-methyl hexanal and pentanal were found only in the green stage of yellow pitangas, meaning they were not found in the other stages of this variety. The ester group presented several compounds found exclusively in mature yellow pitangas, such as ethyl (*E*)-but-2-enoate, ethyl butanoate,

phenethyl isobutyrate, ethyl hex-3-enoate, ethyl-2-methyl butanoate and ethyl-3-methyl butanoate. The green stage of purple pitangas also presented several exclusive compounds, such as (*E*)-germacrene D, linalool, carotol and propanal; while mature purple pitangas exclusively presented (*E*)-β-ocimene and β-elemene. In red pitanga, α-cubebene was identified in the green stage; γ-elemene in the intermediate stage; and ethyl-2-methyl butanoate, ethyl butanoate and 2-phenyl ethanol were identified in the mature stage.

The study managed by Santos-Silva *et al*.[5] identified several volatile compounds in red pitangas at four ripening stages which were not observed in the present study: carene, curzerene, cymene, epizonarene, maaliol, myrcene, ocimene, selinene, silvestrene, terpinene, terpinolene, tujopsene, triciclene and valerianol. However, several compounds identified in the present study were also not found by Santos-Silva *et al*.:[5] alpha-cubebene, beta-pinene, cubenol, alpha-gurjunene, valencene, beta-cubebene, alpha-humulene and carotol. It is important to emphasize and highlight that Santos-Silva *et al*.[5] analyzed only the profile of the volatile emitted during the ripening process of red pitanga, not analyzing any metabolites during the experiment.

Marin *et al*.[31] identified dozens of volatile compounds in essential oils of pitanga, many of which were not identified in the present study, particularly substances belonging to the class of terpenoids: α-phellandrene, α-terpineol, β-caryophyllene, santalene, β-chamigrene, γ-himachalene, bicyclogermacrene, bisabolene, calamenene, bulnesol, nerolidol, elemol and ledol. The variation in volatile compounds identified in the present study, compared to the literature, may be due to the extraction process, analysis method, genetic differences, fruit maturity, edaphoclimatic conditions[5] and mainly due to this present research focused in to analyze only fruits from pitangas, that are used as food or as product by industry. The volatile compounds found in essential oils or leaves in previous studies[26-29] did not have the same composition because they used distinct parts from *Eugenia uniflora* L. In addition, these studies did not analyze the metabolites from essential oils or leaves from *Eugenia uniflora* L.

The present study demonstrated that three main groups of volatile compounds (esters, terpenes and aldehydes) underwent noticeable changes during ripening. Tables 2, 3 and 4 show that the aroma is predominantly composed of terpenoids (except for the mature stage of red pitanga). Terpenoids undergo abrupt changes during ripening, substantially reducing their abundance. A similar result was found by Santos-Silva *et al*.,[5] who observed a substantial reduction in abundance of terpenes during ripening.

When the pitangas were compared by ripening stage, it was found that the green stage had a predominance of terpenes and aldehydes. This result was expected, as terpenes and aldehydes are considered compounds synthesized in the early ripening stages. The yellow pitanga can be considered a biotype or a fruit in the initial stages of ripening. Some fruits acquire only a yellow or orange color, while others continue the color change process, eventually reaching a purple color. From this perspective, considering the purple and red pitangas as biotypes that have undergone various color changes, the yellow pitanga is at the beginning of the process and, for this reason, has accumulated a lot of terpenes and aldehydes.

The mature stage of pitangas showed a predominance of esters compared to the green and intermediate stages. Terpenes and aldehydes were reduced during ripening. From this point, the results of primary metabolites identified in pitangas (yellow, red and purple) during ripening will be integrated with the biosynthesis of volatile compounds, which are formed specially from sugars, amino acids and fatty acids during complex metabolic pathways.[5,32]

Analyzing the overall biochemical behavior of volatile compound formation in the three types of pitanga (yellow, red and purple), terpenes and aldehydes were indicative substances of green pitangas, while esters were indicative of the mature stage. Terpenes in the aroma of ripe tomatoes have a negligible impact and they can be considered as indicator compounds of fruits in the earlier ripening stages.[33] The decrease in synthesis of terpenes during ripening of pitangas (considering the three biotypes) was also a pattern observed in the present study.

Regarding metabolites, the green stage of pitanga predominantly presented sugars, terpenes and amino acids; the intermediate stage did not show a uniform characteristic and, finally, the mature stage presented notable presence of esters and sugars. In other words, regardless of biotype of pitanga, the present study showed that the mature stage compared to the green one (two antagonistic ripening stages) clearly exhibited a completely different physiological, hormonal and biochemical behavior. Then, our data showed that the mature stage decreased amino acids abundance to form organic acids and aldehydes and, consequently, esters during the ripening. Thus, amino acids may also be responsible for the formation of esters.

The results of the present research showed that all type of pitanga increased their sugar abundance during ripening, agreeing with other studies on fruits whose sugar content also increased during ripening.[34] Pitanga ripening can change some quality characteristics, mainly the flavor and texture, reflecting on consumer acceptance. Pitanga fruit is quite popular and it is originally from Brazil and it has not

been domesticated yet on a national scale and EMBRAPA has studied this plant since the 1980s. Studying deeply the compounds, particularly volatile and metabolites, from various biotype of pitanga, is extremely crucial and urgent, as we need to know about our biodiversity and spread and publicize about the composition of pitanga and its potential use as a food (*in natura*) or food product (liquor, ice cream, jam, etc.).

The present study is promising as monitoring the changes in volatile compounds and metabolites in a high-potential native fruit, like pitanga, is essential to understand pre- and post-harvest physiology, create regional food products (jam, ice cream, fruit pulp, etc.) and, mainly, explore its potential nutritional value as a healthy food.

The findings of the present study value the Brazilian biodiversity and bring up some inedited compounds of pitanga. Research about Brazilian native little-known fruits is crucial to promote and contribute to innovative approaches related to technological and health explorations of them. In addition, exploring Brazilian biodiversity can rescue flora and fauna and preserve our food culture in order to ensure sovereignty and food security to the local population.

Conclusions

Brazil is renowned for its extensive biodiversity of native edible fruits, which are recognized for their health-promoting bioactive compounds and diverse flavors. Among these, Brazilian pitanga fruits are particularly notable. The fruit displays a range of colors from yellow to purple and is appreciated for its strong aroma and versatile taste profile, which can vary from sweet to sour depending on its ripeness. However, the specific bioactive compounds responsible for its nutritional and sensory characteristics remain largely unexplored. This study employed a metabolomics approach to analyze the volatile compounds and metabolites present in yellow, red and purple pitanga fruits at various ripening stages. Yellow, red and purple pitanga fruits exhibited an enormous number of volatile compounds and metabolites at three ripening stages, highlighting terpenes, esters, aldehydes, fatty acids and sugars. Future research should provide detailed nutritional information, suggest consumption methods and explore the industrial applications of pitanga fruits. Additionally, studies should focus on sensory analysis, health benefits, potential use as a food product and on breeding programs to incentivize and give due importance to the Brazilian biodiversity, which is still so underexplored, enhancing the bioactive compounds and flavor profiles of pitanga fruits and ensuring sovereignty and food security to the local population.

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Author Contributions

Grazieli B. Pascoal was responsible for conceptualization, formal analysis, investigation and writing original draft; Silvia L. R. Meza for investigation, validation, writing original draft, review and editing; Eric C. Tobaruela for data curation, methodology, validation, writing review and editing; Rodrigo C. Franzon for resources and visualization; Isabel L. Massaretto for formal analysis, investigation, software and validation; Eduardo Purgatto for project administration, resources, supervision, visualization and writing review and editing. All authors discussed the results and contributed to the final manuscript.

References

1. Bailão, E. F. L. C.; Devilla, I. A.; Da Conceição, E. C.; Borges, L. L.; *Int. J. Mol. Sci*. **2015**, *16*, 23760. [Crossref]
2. de Araújo, F. F.; Neri-Numa, I. A.; Farias, D. P.; Cunha, G. R. M. C.; Pastore, G. M.; *Food Res. Int*. **2019**, *121*, 57. [Crossref]
3. Celli, G. B.; Pereira-Netto, A. B.; Beta, T.; *Food Res. Int*. **2011**, *44*, 2442. [Crossref]
4. Sviech, F.; Ubbink, J.; Prata, A. S.; *LWT* **2022**, *156*, 113013. [Crossref]
5. Santos-Silva, J.; Damiani, C.; Cunha, M. C.; Carvalho, E. E. N.; Boas, E. V. B. V.; *Sci. Hortic*. **2019**, *250*, 366. [Crossref]
6. El Hadi, M. A. M.; Zhang, F. J.; Wu, F. F.; Zhou, C. H.; Tao, J.; *Molecules* **2013**, *18*, 8200. [Crossref]
7. Biazotto, K. R.; Mesquita, L. M. S.; Neves, B. V.; Braga, A. R. C.; Tangerina, M. M. P.; Vilegas, W.; Mercadante, A. Z.; De-Rosso, V. V.; *J. Agric. Food Chem*. **2019**, *67*, 1860. [Crossref]
8. Ramalho, R. R. F.; Barbosa, J. M. G.; Ferri, P. H.; Santos, S. C.; *Food Res. Int*. **2019**, *119*, 850. [Crossref]
9. *Mass Hunter Workstation Software Qualitative Analysis*, version B.06.00; Agilent Technologies Inc, USA, 2012.
10. The Pherobase: of Pheromones and Semiochemicals, http://www.pherobase.com, accessed in September 2024.
11. The Good Scents Company Information System, http://www.thegoodscentscompany.com/, accessed in September 2024.
12. Meza, S. L. R.; Massaretto, I. L.; Sinnecker, P.; Schmiele, M.; Chang, Y. K.; Noldin, J. A.; Marquez, U. M. L.; *Int. J. Food Sci. Technol*. **2021**, *56*, 3218. [Crossref]

13. Metaboanalyst, https://www.metaboanalyst.ca/home.xhtml, accessed in September 2024.
14. Pang, Z.; Lu, Y.; Zhou, G.; Hui, F.; Xu, L.; Viau, C.; Spigelman, A. F.; MacDonald, P. E.; Wishart, D. S.; Li, S.; Xia, J.; *Nucleic Acids Res*. **2024**, *52*, W398. [Crossref]
15. Barupal, D. K., Fiehn, O.; *Sci. Rep*. **2017**, *7*, 14567. [Crossref]; ChemRICH, https://chemrich.fiehnlab.ucdavis.edu/, accessed in September 2024.
16. Bajaj, J. S.; Fan, S.; Thacker, L. R.; Fagan, A.; Gavis, E.; White, M. B.; Heuman, D. M.; Fuchs, M.; Fiehn, O.; *PLoS One* **2019**, *14*, e0223061. [Crossref]
17. Bizzo, H. R.; Brilhante, N. S.; Nolvachai, Y.; Marriott, P. J.; *J. Chromatogr. A* **2023**, *1708*, 464376. [Crossref]
18. Zellner, B. A.; Bicchi, C.; Dugo, P.; Rubiolo, P.; Dugo, G.; Mondello, L.; *Flavour Fragr. J*. **2008**, *23*, 297. [Crossref]
19. Zenkevich, I. G.; Babushok, V. I.; Linstrom, P. J.; White, V. E.; Stein, S. E.; *J. Chromatogr. A* **2009**, *1216*, 6651. [Crossref]
20. National Institute of Standards and Technology (NIST) Chemistry WebBook, https://webbook.nist.gov/chemistry/, accessed in August 2024.
21. ChemSpider: Search and Share Chemistry, https://www.chemspider.com/, accessed in August 2024.
22. PubChem: National Library of Medicine, https://pubchem.ncbi.nlm.nih.gov/, accessed in August 2024.
23. Aubert, C.; Bourger, N.; *J. Agric. Food Chem*. **2004**, *52*, 4522. [Crossref]
24. Jayanty, S.; Song, J.; Rubinstein, N. M.; Chong, A.; Beaudry, R. M.; *J. Am. Soc. Hort. Sci*. **2002**, *127*, 998. [Crossref]
25. González-Agüero, M.; Troncoso, S.; Gudenschwager, O.; Campos-Vargas, R.; Moya-León, M. A.; Defilippi, B. G.; *Plant Physiol. Biochem*. **2009**, *47*, 435. [Crossref]
26. Beaulieu, J. C.; *Hort. Sci*. **2006**, *41*, 65. [Crossref]
27. El-Mageed, M. A. A.; *Arab Univ. J. Agric. Sci*. **2007**, *15*, 89. [Crossref]
28. Ortiz, A.; Graell, J.; Lara, I.; *Sci. Hortic*. **2011**, *131*, 6. [Crossref]
29. Costa, D. P.; Alves Filho, E. G.; Silva, L. M. A.; Santos, S. C.; Passos, X. S.; Silva, M. R. R.; Seraphin, J. C.; Ferri, P. H.; *J. Braz. Chem. Soc*. **2010**, *21*, 851. [Crossref]
30. Mesquita, P. R. R.; Nunes, E. C.; Santos, F. N.; Bastos, L. P.; Costa, M. A. P. C.; Rodrigues, F. M.; de Andrade, J. B.; *Microchem. J*. **2017**, *130*, 79. [Crossref]
31. Marin, R.; Apel, M. A.; Limberger, R. P.; Raseira, M. C. B.; Pereira, J. F. M.; Zuanazzi, J. A. S.; Henriques, A. T.; *Lat. Am. J. Pharm*. **2008**, *27*, 172. [Crossref]
32. Hui, Y. H.; *Handbook of Fruit and Vegetable Flavors*, vol. 1, 1st ed.; Wiley & Sons: Hoboken, USA, 2010.
33. Rambla, J. L.; Tikunov, Y. M.; Monforte, A. J.; Bovy, A. G.; Granell, A.; *J. Exp. Bot.* **2014**, *65*, 4613. [Crossref]
34. Martínez-Rivas, F. J.; Fernie, A. R.; *J. Exp. Bot.* **2024**, *75*, 1726. [Crossref]

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