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Volatile profile of green and ripe curriola fruit [Pouteria ramiflora (Mart.) Radlk

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ABSTRACT
Curriola fruit (Pouteria ramiflora) is a fruit of the Brazilian Cerrado (a type of savannah) that is recognized for its pronounced and remarkable aroma. In this work, the effect of ripening on the volatile profile of curriola fruit was evaluated using solid phase micro extraction (SPME) combined with gas chromatography and massspectrometry (CG-MS). Ester was the major class of compounds identified in the volatile profile of curriola fruit. Followed alcohols and sesquirterpenes. The major volatile compounds identified in curriola fruit were ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethanol, ethyl-(Z)-3-hexenoate, β-curcumene, δ-curcumene,α-bergamotene (cis), α-curcumene, and (E)-2-hexenal. The largest changes observed in volatile constituents during ripening of curriola fruit were decreases in alcohols, aldehydes, and sesquiterpenes as well as an increase in esters.

Keywords – Brazilian fruit, CG-MS, ripening, SPME, volatile compounds

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I. INTRODUCTION

The Cerrado (a type of savannah) is the second largest biome in South America and one of the six major Brazilian biomes, and the Brazilian Cerrado, includes several fruit species normally used by local people. The nutritional, functional and sensory potential of fruits grown in the Cerrado is immense, although information about these fruits is scarce in the scientific literature. Nevertheless, the Brazilian Cerrado biodiversity is threatened by its irrational and irresponsible use as pasture and monoculture, thus jeopardising the possibility of exploiting its huge potential. Pouteria ramiflora (Mart.) Radlk. is among the main species of the Cerrado of the Brazilian Midwest [1], and this species also exists in southern Brazil, extending north and west to the Amazon and Bolivia [2]. Belonging to the family Sapotaceae, the tree of P. ramiflora measures approximately 10 m height and produces approximately 100 - 400 fruits [3]. In the Brazilian Cerrado, there are at least seven general and twenty species of the family Sapotaceae, and researchers point to this family as a potential source of polyphenol antioxidants [1] [5]. The fruits of Pouteria ramiflora, which are commonly called curriola, are bacoid-type inde his cent berries that can be up to 4.9 cm long when mature with a green is external colour and white fleshy gelatinous pulp. The berry has a single ellipsoid seed with an approximate length of 3 cm and a diameter of 1.8 cm [1] [6].

The curriola fruit (Pouteria ramiflora) has a sweet and pleasant taste, but its main attraction is its odour, which is striking and attractive to humans and the wild life that frequently visit the plant sat the time of fruiting. Although edible and appreciated, the curriola fruit is a commercially neglected fruit, but it has great potential. The study of this fruit is crucial for the dissemination of its attributes and recognition of its value both in Brazil and in other countries.

Aroma is an important quality attribute that sensitises the olfactory sense of the consumer. The emanation of a complex mixture of volatile compounds usually during fruit ripening markedly influences the degree of acceptance of the fruit by the consumer. Although a range of volatile compounds can be determined, one or a few of these compounds are generally associated with the peculiar aroma of each individual fruit, and such compounds are known as character impact compounds. The volatile profile of curriola fruit (Pouteria ramiflora) has not been studied, and its elucidation may be useful in commercialising the fruit not only in Brazil but in many countries, thus

contributing to its wide use and preservation of the species.

Several researchers have concentrated efforts in recent years to determine the volatile profile of fruit using solid phase micro-extraction (SPME) in head space and gas chromatography-mass spectrometry. Currently, the technique of micro-solid phase extraction is widely used for analysis of volatile compounds in fruits and their products. With this technique, it is common to use composite fibres, such as divinylbenzene/polydimethylsiloxane (DVB/ PDMS) [7] [8], carboxen/polydimethylsiloxine (CAR/PDMS) [9] and divinylbenzene/carboxen/ polydimethylsiloxine (DVB/CAR/PDMS) [10] [11] [12] [13]. This technique typically uses a low temperature of 50°C for preserving the original aroma, which prevents the fruit from baking, and it also uses an equilibration time of 10 to 80 minutes [7] [8] [9] [10] [11] [12] [13] [14] [15] [16]. SEME/GC/MS is a technique that extracts a wide range of substances both in the mature and immature stages with little loss of volatile compounds, such as monoterpenes, which are lost by hydrodistillation [14]. The major advantage of SPME/GC/MS is that it is fast and responsive as well assuitable for the detection of volatile substances. The aim of this study was to evaluate the volatile profile of green and ripe curriola fruit [Pouteria ramiflora (Mart.) Radlk.] using SPME/GC/MS.

II. MATERIAL AND METHODS

2.1 Fruit samples

The experiment was conducted between August and December 2015. The fruits were picked in a preserved area of Stricto sensu native Cerrado located in Santo Antônio de Leverger, Mato Grosso, Brazil (Midwest Brazil; Latitude 15° 43' 45.83" S; Longitude 56° 4' 14.82" W) with various specimens of Pouteria ramiflora. Green and ripe fruits were picked, washed and sanitised. Unblemished fruits were placed in sterile plastic bags and frozen for later analysis.

2.2 HS-SPME

Samples of green ripe fruits (1 g) were taken in six replicates and placed in10 mL septum-capped vials and magnetised with PTFE (Supelco).

The divinylbenzene/ polydimethylsiloxane (DVB/PDMS) fibre (65 µm) was introduced into the vial and exposed for 40 minutes at a temperature of 40°C with agitation (250 rpm). After extraction, the fibre was retracted and removed from the vial and inserted in to the injector for desorption for 2 minutes. The fibre was conditioned for 20 minutes at 250°C for further extraction.

2.3 Gas chromatography

The head space analysis by solid phase micro-extraction was performed using gas chromatography equipment coupled to a mass spectrometer (Shimadzu QP2010 Plus and CGMS solution software version 2.5). An Rtx-5MS column was used (30 m x 0.25 mm ID, 0.25 µm; RESTEC). The injection was performed in splitless mode, and helium was used as the carrier gas at a flow rate of 16 mL/min. The programming of the oven began at 40°C with a heating rate of 4°C/minute up to 200°C, resulting in a run of 40 minutes. All mass spectra were obtained using electron impact, and the mass was acquired in scan mode. The compounds were identified by comparing the retention times of C8-C20 alkane standards purchased from Sigma Aldrich® under the same conditions using retention indices (Kovats indices) from the literature. The mass fragments were compared using Willey8NIST10 and NIST11 libraries as well as specific literature [17].

III. RESULTS AND DISCUSSION

The analysis of the volatile profile from curriola fruit [Pouteria ramiflora (Mart.) Radlk] at the green and ripe stages using solid phase micro-extraction identified 47 compounds, as shown in Table 1. The identified substances belong to the following chemical classes: alcohols (10), aldehydes (3), esters (22), carboxylic acids (2), sesquiterpenes (8), hydrocarbon (1), and furans (1).

The volatile profiles of the fruits varied qualitatively and in relative abundance of areas, depending on the stage of development. Chromatograms characteristic of each stage of development are presented in Fig. 1.

Fruits at the green stage had a lower number of substances (26) when compared with those at the ripe stage (33). The most abundant chemical classes at the green stage were alcohols (7; 31%), sesquiterpenes (7; 27%), esters (5; 19%) and aldehydes (4; 15%), and the least abundant chemical classes at this stage were carboxylic acids (1; 4%) and furan (1; 4%). At the green stage, the volatile compounds that showed higher relative areas were ethylbutanoate (57%), α-bergamotene (9.7%), δ-curcumene (3.38%), ethylhexanoate (2.1%), n-hexane (1.9%), ethanol (1.79%), α-curcumene (1.1%), 2-ethylhexan-1-ol (1.1%) and (E)-2-hexenal (1%). The sum of the areas of these substances represented 79% of the total area of the volatile compounds of green curriola fruit (Pouteria ramiflora). The remaining components did not have detectable areas or areas less than 1%.

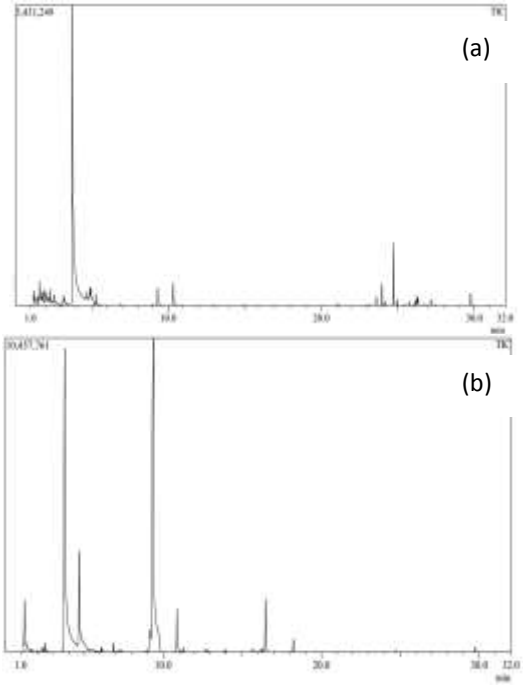

Figure 1 Chromatogram of volatile compounds from (a) green and (b) ripe fruits of Pouteria ramiflora using a HS-SPME DVB/PDMS fibre.

When compared with fruit at the green stage, the fruit at maturity showed a greater number of compounds (33). The chemical class of greatest abundance at maturity was esters (21; 62%), followed by alcohols (7; 18%) and, to a lesser extent, carboxylic acids (2; 6%), hydrocarbon (1; 3%) and aldehydes (1; 3%) (Fig. 2). The volatile compounds that showed higher relative areas were ethylhexanoate (44.9 %), ethylbutanoate (34.2 %), 2-methyl, pent-4-enoic acid (4.3 %), ethyl octanoate (4.3 %), ethanol (2.9 %), ethyl (Z)-3-hexenoate (2.1 %) and β-curcumene (1.4 %). These substances represented 96% of the relative area of the volatile profile of ripe curriola fruit (Pouteria ramiflora), and the remaining substances presented no detectable area or areas between 0.03 and 0.5%.

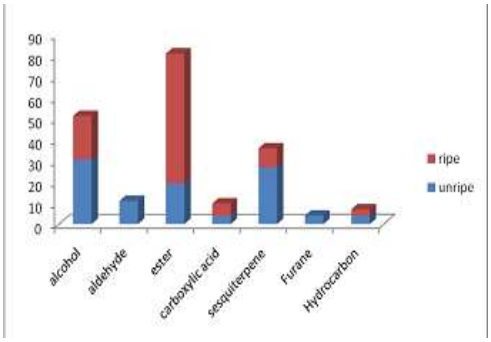

Figure 2 Change in relative abundance of chemical classes identified in the maturation of Pouteria ramiflora.

The greatest changes observed in the volatile constituents during ripening of curriola fruit (Pouteria ramiflora) were decreases in alcohols, aldehydes and sesquiterpenes as well as increases in esters as the fruit ripened. According to [18], the volatile profile of apple demonstrates a change of aldehydes ("greenbacks") to esters ("fruity notes") during ripening.

A turnover of alcohols during ripening was observed, as follows: 2-methyl-1-butanol, 3-methyl-1-pentanol, cyclopentanol, (E)-4-methyl cyclohexanol, (Z)-3-hexen-1-ol, n-hexan-1-ol and 2-ethylhexan-1-ol decreased or disappeared in the ripe fruit; 2-propanol increased in the ripe fruit; and 3,3-dimethyl-2-butanol and 3-methyl-3-buten-1-ol appeared in the ripe fruit. During ripening, 3-methyl-1-pentanol was reduced by 7-fold, and (Z)-3-hexen-1-ol and n-hexan-1-ol were not detected in the ripe fruit. In fact, these three compounds are normally associated with green odour.

Aldehydes were present mainly in the green stage with relative areas ranging from 0.60 to 1.90, and they were not present at the ripe stage.

The (Z)-3-hexen-1-ol, n-hexan-1-ol and (E)-2-hexenal compounds present only in the green stage are known as "green leaf volatiles" (GVLs) [19], which are reported as green notes with odour characteristics associated with fruits and vegetables, and they are present in most studies, indicating that they are substances common to the metabolism of a great variety of fruits such as blackberries [20] [21], cupuaçu (Theobroma grandiflorum Schumann fruit) [22], passion fruit (Passiflora edulis Sims f. flavicarpa Deg.) [23] [20], soursop (Annona muricata) [24], jackfruit (Artocarpus heterophyllus L.) [11], apricot (Prunus armeniaca L. cv. Bergeron) [9] [25] and umbu (Spondias tuberosa) [26]. Alcohols and aldehydes (C6-type compounds) such as (Z)-3-hexen-1-ol and (E)-2-hexenal are formed through a lipoxygenase pathway and can be esterified with acyl-CoA [27]. In fact, aldehydes can be synthesised via lipoxygenase from unsaturated fatty acids [7] [26] [27] or from amino acids via the alpha-keto acid dehydrogenase/decarboxylase. The decrease in the concentration of aldehydes during ripening may be associated with their transformation into alcohols via alcohol dehydrogenase. Moreover, the reduction of alcohols may be related to their esterification with fatty acids. The esterification reaction during fruit ripening may be based on the reduction of (Z)-3-hexen-1-ol and the increase of ethyl (Z)-3-hexenoate when comparing green and ripe fruits.

The ester chemical class was highlighted, especially at the ripe stage. Of the twenty-two substances identified, twenty-one were present at the ripe stage, and five were present at the green stage. Among the esters identified, 1,1-dimethylethyl

benzoate was the only one not detected in the ripe fruits.

A predominance of C5 to C10 saturated esters and branched esters with ethyl groups existed. The presence of ethyl esters may be indicative of the formation of esters by the amino acid metabolism of alanine. There was a 40% increase in total esters during ripening. This behaviour has also been observed in other species such as soursop (Annona muricata) and apricot (Prunus armeniaca L. cv. Bergeron) [24] [25]. Esters were prominent both in relative abundance and in percentage of area. The substance with the largest relative area was ethylbutanoate (57.6%) in the green stage with a decrease in the mature stage (34.2%), and the same behaviour was exhibited by esters, namely methylbutanoate and 1,1-dimethylethylbenzoate. The opposite behaviour occurred with ethylhexanoate, which showed a dramatic increase from the green to ripe stage (2.1 to 44. 9%). Ethylhexanoate has been associated with the fruity aroma of passion fruit (Passiflora edulis Sims f. flavicarpa Deg.) [23] and the aroma of apple, banana and pineapple [28]. Methylhexanoate also showed an increase but to a lesser extent. The ethylhexanoate and ethylbutanoate ester compounds have also been reported as the majority of the volatile compounds of cupuaçu (Theobroma grandiflorum Schumann fruit) [22] and have been determined as the main contributors to the sweet fruity aroma of passion fruit (Passiflora edulis Sims f. flavicarpa Deg.) [23]. Ethyl butanoate is common in fruits and has been found in the volatile profile of araçá-boi (Eugenia stipitata) [22], soursop (Annona muricata) [24], passion fruit (Passiflora edulis Sims f. flavicarpa Deg.) [23], five jackfruit varieties (Artocarpus heterophyllus L.) [11], fourteen cultivars of apricot (Prunus armeniaca L. cv. Bergeron) [9], cupuaçu (Theobroma grandiflorum Shumann) [22], umbu (Spondias tuberosa) [26] and three varieties of blackberries [20] [21]. Ethyl hexanoate, in turn, is less common, being found in the volatile profiles of soursop (Annona muricata) [24], passion fruit (Passiflora edulis Sims f. flavicarpa Deg.) [23], cupuaçu (Theobroma grandiflorum Shumann) [22], araçá-boi (Eugenia stipitata) [22] and two varieties of blackberries [21]. During ripening of soursop (Annona muricata), [24] observed an increase of esters from C6, C4 and C8 saturated and unsaturated acids with (Z)-3-hexanol and (Z)-3-hexanal being the major compounds at the green stage and methylhexanoate and methyl-(E)-2-hexanoate esters being the major compounds at the ripe fruit stage. The post-ripe stage was characterised by the increase in ethylacetate, ethyl butanoate and methylbutanoate. Of these substances, the C6 compounds and the last two compounds mentioned were also present in the current volatile profile studied. [18] detected eight major compounds during ripening of papaya fruit, including ethanol, ethyl butanoate, 2-ethyl-1-hexanol, ethyl hexanoate, limonene, and ethyl octanoate. These authors also observed that ethyl esters are predominant in the last stages of maturation, and they also reported that ethyl butanoate (56.87 % area) and ethyl hexanoate (34.07 % area) were substances with greater relative area in the last stage of maturation.

Only two carboxylic acids were observed in curriola fruit (Pouteria ramiflora) as follows: 2-methylpent-4-enoic acid, found at both stages but in greater abundance in ripe fruits; octanoic acid, observed only in ripe fruits. [26] analysed volatile compounds of umbu (Spondias tuberosa) and their odour descriptors during ripening and observed that umbu showed a small amount of carboxylic acids, similar to the profile found in the present study with curriola fruit (Pouteria ramiflora).

Eight sesquiterpenes were identified in curriola fruits (Pouteria ramiflora), with 7 found at the green stage and 3 found at the ripe stage. β-curcumene was observed only in ripe fruits, and α-curcumene, γ-curcumene and α-bergamotene decreased with ripening. The other sesquiterpenes were observed only in green fruits. The sesquiterpenes found at the green stage, namely (Z)-β-farnesene, (E)-β-farnesene, β-acoradiene, γ-curcumene, and α-curcumene, are formed by the metabolism of carbohydrates through them evalonic acid (MVA) pathway. These same compounds were not observed or they had reduced concentrations at the ripe fruit stage, which suggested that they participate in the synthesis for the formation of esters [24] [27]. [29] analysed volatile compounds during ripening of 2 cultivars of avocado and observed a decrease in the concentration of sesquiterpenes, which was similar behaviour compared to curriola (Pouteria ramiflora). However, [26] observed an increase in the concentration of terpenes during the ripening of umbu (Spondias tuberosa), which was different behaviour to that observed in the present study for curriola (Pouteria ramiflora).

Unlike curriola fruit (Pouteria ramiflora), ripening of umbu (Spondias tuberosa) is marked by a considerable increase in terpenes and decreases in alcohols and esters, among others [26]. [30] identified 33, 34 and 32 volatile compounds in the green, intermediate and mature stages, respectively, in mangaba (Harconia speciosa). Fruits at the green and intermediate stages showed 33.8% esters, 16.4% alcohols, 25.3% aldehydes, 3.0% ketones and 13.4% monoterpenes. These authors noted an increase in esters (40.9%) and a reduction in monoterpenes (1.9%) during ripening, similar to the behaviour of curriola fruit (Pouteria ramiflora). During the ripening of Arbutus unedo, [7] observed reductions

in alcohols, aldehydes and esters as well as a moderate increase in monoterpenes. As in curriola fruit (Pouteria ramiflora), ethyl hexanoate was formed only at the ripe stage, and (Z)-β-farnesene was consumed at the intermediate stage. The identification of volatile compounds in curriola fruit (Pouteria ramiflora) showed a marked presence of alcohols and esters. The alcohols may contribute to the "green notes" at the green stage, and the esters and alcohols may contribute to the sweet and fruity aroma reported in the aroma descriptors.

Table 1 Volatile compounds from green and ripe fruits of Pouteria ramiflora.

Compound	RT (min)	QI (m/z)	**Green** (area %)	SD	**Ripe** (area %)	SD	**Odour descriptions**
alcohols							
Ethanol	1.217	45/57	1.79	1.00	2.90	1.20	
2-Methyl-1-butanol	1.516	57/86	0.29	0.13	0.07	0.00	oily, whiskey(a), butter, sweet, valeric(b) wine onion (h)
3-Methyl-1-pentanol	1.749	56/84	0.7	0.30	0.10	0.01	chocolate, wine-like, green(a), fruity, rancid, pungent(b)
3,3-dimethyl-2-butanol	1.917	45/86	n.d.		0.04	0.00	
3-methyl-3-buten-1-ol	1.984	56/86	n.d.		0.05	0.02	
Cyclopentanol	2.140	57/69	0.40	0.20	n.d.		
(E)-4-methyl Cyclohexanol	2.309	57/96	0.60	0.50	n.d		
(Z)-3-Hexen-1-ol	4.949	67/101	0.80	0.20	n.d		green(a), sulphur-like, passion fruit, grass(e) (h)
n-Hexan-1-ol	5.282	56/84	0.90	0.30	n.d		green, herbaceous, woody, sweet(a) citrus eucalipt(e) resin, flower (h)
2-ethylhexan-1-ol	10.333	57/98	1.10	1.00	0.03	0.03	oil, rose, sweet(a) weak rose odour: fatty floral taste (g)
Aldehydes							
3-methylbutanal	1.925	56/86	0.90	0.40	n.d.		fruity, peach, sour(a)
2-methylbutanal	1.990	57/86	0.60	0.20	n.d.		chocolate, coffee (a)
(E)-2-Hexenal	4.875	69/83	1.00	0.50	n.d.		almond, apple, green, plum, sweet, vegetable (a)
Hydrocarbon							
n-Hexane	1.583	57/86	1.90	0.80	0.10	0.20	
Esters							
2-methylpropyl 2-hydroxy propanoate	2.417	45/102	n.d.		0.14	0.04	
Methyl butanoate	2.542	74/102	0.80	0.10	0.37	0.08	apple, ethereal, pineapple (b) sweet, fruity, strawberry(e)
1,1,-dimethylethyl benzoate	3.232	57/91	0.30	0.10	n.d.		
Ethylbutanoate	3.760	71/116	57.60	5.70	34.20	3.90	banana, ethereal pineapple, fruity (b)(a) sweet, strawberry(e) ripe fruity note (g)
Ethyl 2-methyl-2-propenoate	4.867	69/116	n.d.		0.10	0.00	
Ethyl (E)-2-butenoate	5.275	69/99	n.d.		0.10	0.00	ethereal, wine-like (a)
Propyl butanoate	6.049	71/102	n.d.		0.30	0.10	apple, creamy, honey, wine-like, waxy (a)

Ethyl pentanoate	6.133	88/103	n.d.		0.14	0.03	Apple(a)
methyl hexanoate	6.833	74/99	0.14	0.03	0.50	0.10	ethereal, pineapple (a)
Ethyl 3-hydroxybutanoate	7.145	45/117	n.d.		0.12	0.04	grape, sweet (a)
Ethyl (E)-2-methyl-2-butenoate	7.318	55/128	n.d.		0.08	0.03	caramel, raspberry, fruity(a)
2-methylpropyl butanoate	9.174	71/116	n.d.		0.90	0.10	fruity, ethereal (a)
Ethyl hexanoate	9.391	88/281	2.10	0.50	44.9	1.90	apple, banana, pineapple, wine-like (a) sweet, fruit (e) ethereal (g) peel (h)
Isopropyl hexanoate	10.617	99/117	n.d.		0.03	0.02	cheese, wine-like (a)
Ethyl (Z)-3-hexenoate	10.867	97/142	n.d.		2.10	0.87	pineapple (a)
2-methylbutyl isobutyrate	11.284	70/115	n.d.		0.14	0.03	apricot, honey (a)
Ethyl-2,4-Hexadienoate	12.692	67/140	n.d.		0.24	0.04	fruity, ethereal (a)
Ethyl heptanoate	12.841	88/140	n.d.		0.19	0.04	berry, melon, peach, pineapple, plum (a)
Methyl Octanoate	13.801	74/127	n.d.		0.04	0.01	fruity, green, citrus (a)
ethyl 3-hydroxyhexanoate	13.909	71/117	n.d.		0.11	0.02	grape, citrus, smoky (a)
Ethyl octanoate	16.467	88/172	n.d.		4.30	1.00	apricot, floral, pear, pineapple (a), grass earthy(e) sweet odour, winey, fruity (g)
Ethyl (E)-2-octenoate	18.233	55/125	n.d.		0.50	0.30	fatty, fruity, green (a)
Carboxylic acids							
2-methyl, pent-4-enoic acid	4.658	69/207	0.80	0.10	4.32	1.42	cheese
octanoic acid	15.682	60/115	n.d.		0.18	0.05	oily (a)
Sesquiterpenes							
□-Farnesene (Z)	24.954	69/161	0.80	0.20	n.d.		wood, green(a)(f), lavender apple, herbaceous (a)
β-Farnesene (E)	26.322	69/161	0.88	0.25	n.d.		oily, fruity, citrus-like, woody (d) sweet (f)
β-Curcumene	23.592	119/204	n.d.		1.40	0.30	
δ-Curcumene	23.944	119/204	3.38	0.62	0.10	0.00	
α-Bergamotene (cis)	24.699	119/204	9.70	2.30	0.20	0.10	warm, tealeaflike (d)(f)
β-Acoradiene	25.756	119/204	0.51	0.13	n.d.		
γ-Curcumene	26.141	119/204	0.72	0.16	n.d.		
α-Curcumene	26.238	132/202	1.10	0.20	n.d.		
Furans							
2-pentyl furan	8.99	81/131	0.13	0.05	n.d.		

(a) Description of pure substances by [28]
(b) Reported in five cultivars of jackfruit (Artocarpus heterophyllus L.) by [11]
(c) Reported in passion fruit juice by [20]
(d) Description of odours by [26]
(e) Reported in passion fruit by [23]
(f) Reported in avocado in West-Indian and Guatemala-West-Indian areas by [29]
(g) Reported in papaya ‘Pluk Mai Lie’ by [31]
(h) Reported in Viburnum opulus fruit by [32]

IV. CONCLUSION

Changes in the volatile profile of curriola fruits [Pouteriaramiflora (Mart.) Radlk] during ripening are marked by the turnover of alcohols and the reduction of aldehydes and sesquiterpenes as well as the accumulation of esters and carboxylic acids.

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