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# Changes in the aroma of organic passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) during ripeness



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## ABSTRACT

The odoriferous importance of volatile compounds during maturation of organic passion fruit was studied. The volatile compounds were analyzed using GC-FID-OSME and GC-MS. The volatile profile changed throughout maturation. Relative peak area improved from the 1/3 to 2/3 yellow skin color state of ripeness and was still improved from the 2/3 to 3/3, but the profile remained. Ethyl butanoate showed the highest relative peak area and importance for the aroma of the organic passion fruit in the 1/3 yellow skin color. Ethyl butanoate and hexanoate, propyl acetate and alpha-terpineol showed odoriferous importance from the 2/3 yellow skin color, and together with diethyl carbonate and *cis*-3-hexen-1-ol were the most important compounds in the 3/3 yellow skin color state of ripeness. PCA allowed clearly differentiate all ripening states, indicating that hexanal and caramel, earthy and synthetic aroma were most closely associated with the unripe passion fruit. 2-methylpropyl acetate characterized the passion fruit at the 2/3 yellow skin color together with *cis*-beta-ocimene, benzaldehyde and aroma sweet and passion fruit, skin, which also were associated with the 3/3 yellow skin color. Esters, terpenes, alcohols, octanal, dodecanol and aroma of passion fruit, fruity, citric, candy and plastic were closely associated with the whole maturation.

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## 1. Introduction

The world market of organic foods had an annual turnover of around US\$ 60.9 billion in 2012. In the same year the organic food market was about US\$ 250 million in Brazil. The main organic Brazilian products are sugar cane, cocoa, coffee, soybean and tropical fruits like passion fruit, pineapple, mango, papaya, guava, banana, grape, strawberry and citrus. Most Brazilian organic food is exported, around 70%, to Europe, the United States, Canada and Japan. There are 90 thousand organic producers in Brazil, the major part small producers and most of the certified organic growers are concentrated in the southeast and southern regions, especially in the state of São Paulo (Folha de São Paulo, 2012; IFOAM, 2013; Santos & Monteiro, 2004).

Brazil is the largest producer and consumer of passion fruit in the world, with 920 thousand ton in 2010 (IBGE, 2011). The economically most important form of passion fruit (*P. edulis* Sims f. *flavicarpa* Deg.) is responsible for 95% of the cultivation area, grown

by organic or conventional systems. Passion fruit is consumed as in nature fruit and used to produce industrialized juice and other fruit products, being much appreciated mainly because of the exotic, flowery and fruity aroma. The fruits are generally harvested when they fall to the ground, leading to dehydration and microbial contamination, which reduces their shelf life and commercial value, resulting in economic prejudice (Meletti, 2011). Harvesting should be carried out when the fruits are ripe but still connected to the plant, allowing one to obtain fruits with better phytosanitary conditions, more uniform and with better quality, thus reducing losses and microbiological contamination (Amaro & Monteiro, 2001; De Marchi, Monteiro, Benato, & Silva, 2000). As a result, the sensorial and nutritional characteristics of the fruit are less compromised, so less drastic conditions can be used for heat treatment. It is important to recognize the ideal point to harvest the fruit. The skin color has been widely used as an indicator of the ideal harvesting point, since this is considered to be an easy and practical procedure to identify the adequate ripeness for harvesting. The ripe passion fruit shows whole yellow skin color. But, it was observed that fruits not completely ripe show physicochemical and sensorial characteristics close to the ripe fruits, mainly those from the 2/3 yellow skin color state of ripeness which are very similar to

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the 3/3 yellow skin color state of ripeness (Amaro & Monteiro, 2001; De Marchi et al., 2000; Janzantti, Santos, & Monteiro, 2014). There is no information regarding the aroma and volatile compounds.

The main volatile compounds responsible for the aroma of fruits are from the classes of esters, terpenes and aldehydes. The esters ethyl butanoate and ethyl hexanoate contribute to the sweet, fruity and floral aroma, characteristic of passion fruit (Jordán, Goodner, & Shaw, 2002). The terpenes alpha-terpineol, beta-myrcene, limonene and gamma-terpinolene have been described as important to the floral and fruity aroma of passion fruit, and the aldehydes hexanal and octanal for the green aroma of the fruit (Jales et al., 2005; Janzantti, Macoris, Garruti, & Monteiro, 2012). Aldehydes and alcohols ( $C_6$ ) have also been described as major volatile compounds in ripe fruits (Defilippi, Manríquez, Luengwilai, & González-Agüero, 2009). The volatile composition of yellow passion fruit depends on the edaphoclimatic conditions, cultivar and cultivation system, among other factors. Many studies in literature report the volatile composition of yellow passion fruit, few of them used GC-O (gas chromatography-olfactometry) techniques to evaluate the odoriferous contribution of each volatile compound to the overall passion fruit aroma (Janzantti et al., 2012; Jordán, Goodner, & Shaw, 2000; Pontes, Marques, & Câmara, 2009).

The aim of this work was to investigate the odor and volatile compounds of the organic passion fruit according to the skin color during ripeness. The study also aimed to characterize the volatile compounds responsible for the flavor of each state of ripeness.

## 2. Material and methods

### 2.1. Material

Yellow passion fruits (*P. edulis* Sims f. *flavicarpa* Deg.) from Feltrin selection were organically cultivated in 2008, in Morungaba (coordinates: 22°52'48" S and 46°47'30" W, altitude of 765 m), SP, Brazil. The organic fruits were certified by the Biodynamic Institute (IBD), SP, Brazil. The fruits were harvested in different states of ripeness corresponding to 1/3, 2/3 and 3/3 yellow skin color (Amaro & Monteiro, 2001; De Marchi et al., 2000). About 15 kg of passion fruit from each state of ripeness were harvested at random, taking care to choose fruits of similar size. The fruits were placed in wooden crates and transported to the laboratory, where they were selected, classified according to the state of ripeness, and washed. The pulp obtained was packed into hermetically closed glass flasks and stored frozen at  $-18\text{ }^{\circ}\text{C}$  until analyzed.

### 2.2. Reagents

All reagents were GC-analytical grade, supplied by Merck (Darmstadt, Germany) and J. T. Baker (Philipsburg, USA). The volatile standards were of analytical grade. Ethyl butanoate, hexyl acetate, hexyl butanoate and acetic acid were purchased from Sigma–Aldrich (St. Louis, USA) and ethyl propanoate, propyl acetate, methyl butanoate, 2-methylpropyl acetate, butyl acetate, methyl hexanoate, butyl butanoate, ethyl hexanoate, butyl hexanoate, ethyl octanoate, hexyl hexanoate, hexanal, octanal, 1-butanol, 1-hexanol, 1-octanol, *cis*-3-hexen-1-ol, beta-myrcene, limonene, beta-linalool and alpha-terpineol were purchased from Fluka (Steinheim, Germany).

### 2.3. Isolation of volatile compounds from passion fruit

The volatile compounds from fruits in each state of ripeness were isolated in triplicate using dynamic headspace by suction by vacuum (79.99 mm Hg) at room temperature ( $25\text{ }^{\circ}\text{C}$ ), with traps containing 100 mg of Porapak Q (150–180  $\mu\text{m}$ , Waters Associates,

Milford, U.S.A) previously conditioned in an ultra pure  $\text{N}_2$  flow (40 mL/min) at  $170\text{ }^{\circ}\text{C}$  for 8 h. Passion fruit pulp (300 g) was placed in the flask of the volatiles capture system and NaCl p.a. (30 g/100 g) was added. An isolation time of 2 h followed by elution with 300  $\mu\text{L}$  dichloromethane was used to provide a representative isolate (Macoris, Janzantti, Garruti, & Monteiro, 2011).

### 2.4. High resolution gas chromatography (GC)

A gas chromatograph (GC), model 2010 Shimadzu (Kyoto, Japan), with a flame ionization detector (FID) equipped with a DB-Wax (30 m length, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness) stationary phase column (J&W, Folsom, USA) was used. The column temperature was initially maintained at  $40\text{ }^{\circ}\text{C}$  for 10 min and then raised to  $200\text{ }^{\circ}\text{C}$  at  $3\text{ }^{\circ}\text{C}/\text{min}$ , remaining at this temperature for a further 10 min. Injection was in the splitless mode (2  $\mu\text{L}$ ) with a hydrogen gas flow rate of 1.3 mL/min. The injector and detector temperatures were  $230\text{ }^{\circ}\text{C}$  and  $250\text{ }^{\circ}\text{C}$ , respectively.

The relative peak area of each volatile compound was obtained in relation to the peak area of the internal standard, pentadecane ( $C_{15}$ ). 10  $\mu\text{L}$  of pentadecane standard solution (4  $\mu\text{L}/\text{mL}$ ) was added into the isolated. The volatile compounds were quantified by GC-FID in triplicate.

### 2.5. Gas chromatography-mass spectrometry (GCMS)

A 5975C gas chromatograph-mass spectrometer from Agilent (Wilmingon, USA), with an electron impact ionization source (70 eV) was used in scan mode. The mass range was from 35 to 350  $m/z$ . The DB-Wax column (30 m length, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness) was maintained at  $40\text{ }^{\circ}\text{C}$  for 10 min, then programmed to rise to  $200\text{ }^{\circ}\text{C}$  at  $3\text{ }^{\circ}\text{C}/\text{min}$ , and held for 10 min. Helium was used at a flow rate of 1.3 mL/min. The injector temperature was  $230\text{ }^{\circ}\text{C}$  and the detector was  $240\text{ }^{\circ}\text{C}$ . The DB-5 column (60 m length, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness), was maintained at  $50\text{ }^{\circ}\text{C}$ , then programmed to rise to  $250\text{ }^{\circ}\text{C}$  at  $3\text{ }^{\circ}\text{C}/\text{min}$ , and held for 10 min. Helium was used at a flow rate of 1.0 mL/min. The injector and detector temperatures were  $250\text{ }^{\circ}\text{C}$ .

The volatile compounds of the organic passion fruit in different states of ripeness were identified by (1) GC-MS with DB-Wax and DB-5 columns, comparing the mass spectra obtained with those of pure standards and those found in the NIST library (vers. 1.7), (2) comparing the experimentally obtained retention indices with those of pure standards chromatographed under the same GC-FID conditions in both columns and those found in the literature (Acree & Arn, 2004), and (3) comparing the description of the odor of the volatile compounds with those of pure standards chromatographed under the same GC-FID conditions in DB-Wax column and those found in the literature (Acree & Arn, 2004; Janzantti et al., 2012; Jordán et al., 2002). The retention indices of the volatile compounds were calculated by injecting a solution containing a series of alkanes ( $C_8$  to  $C_{30}$ ) in dichloromethane, under the same chromatographic conditions, in DB-Wax and DB-5 columns.

### 2.6. Gas chromatography-Olfactometry (GC-O)

The OSME olfactometric technique was used to analyze the odoriferous importance of the volatile compounds of the organic passion fruit in different states of ripeness (McDaniel, Miranda-Lopez, Watson, Micheals, & Libbey, 1990), using the SCDTI (time-intensity data collection system) data collection program (Da Silva, 1999).

Data concerning each aroma perceived during the olfactometric analysis were registered using a 10-point hybrid scale anchored

with the terms “none”, “moderate” and “strong” in the points 0, 5 and 10, respectively. The judges were requested to use the scale available on the computer screen so as to record the intensity of each compound and to describe the perceived odor. The organic passion fruit was analyzed in each state of ripeness, in triplicate. The duration of each olfactometric analysis was 60 min, divided into two 30-min sessions.

The sensory panel consisted of four trained and selected female, 23–37 year old judges. Odor recognition and passion fruit aroma intensity tests were used to select the judges. In the aroma recognition tests, essences of cloves, strawberry, orange, aniseed, passion fruit pulp, passion fruit flower, grass and guarana syrup were offered to the judges, each placed in a 300 mL black china mug coded with a three digit number and covered with aluminum paper with some holes, covered with a watch glass. Each judge was requested to describe the perceived odor after inhaling the content of each mug. Judges capable of identifying at least 70% of the aromas were selected (ASTM, 1981). The judges showed good discrimination ( $p \leq 0.005$ ) and repeatability ( $p > 0.05$ ) in respect to the intensity of the passion fruit aroma (Macoris et al., 2011). After selection, the sensory panel carried out two training sessions in the use of the OSME technique, in order to familiarize them with the characteristic aromas of passion fruit and with the use of the scale to record the odoriferous peaks.

The same chromatographic conditions to separate the volatile compounds in the GC-FID were used in the GC-O. The equipment was modified for the GC-O analysis as follows: the GC column was disconnected from the FID and connected to a flow splitter installed in the chromatograph oven, which directed the emerging volatile compounds to the nose of the judge, via an inactive column (without stationary phase). The splitter also introduced a carrier gas to complete the volume of the column and ensure the compounds were delivered quickly from the system. The SGE ODO-II system (Texas, USA) was used to deliver the outlet gas stream to the nose of the judge and to warm the tube surrounding the inactive column. The system included a device to introduce moistened air into the outlet stream, in order to minimize any discomfort caused by drying of the nasal mucosa during the olfactometric tests.

To evaluate the odoriferous importance of the volatile compounds, data of each compound were collected using the SCDTI software in each GC-O analysis session, for each judge and passion fruit from each state of ripeness. In order to construct an aromagram for each judge and passion fruit from each state of ripeness, the inclusion criterion used was that the odoriferous peaks should be detected in at least two of the three repetitions. In sequence, a consensual aromagram was constructed by the sensory panel as a whole based on the individual aromagrams. The inclusion criterion was that the peaks should be detected by at least two of the four judges.

The qualitative description of each aroma was established from the descriptions of all the judges. To ensure that the chromatographic data and compound identities were correctly related to the olfactometric data, the retention indices of each aroma described in the consensus aromagram were calculated. Furthermore, the judges' descriptions of the aroma of each compound were checked against their published descriptions (Acree & Arn, 2004; Janzantti et al., 2012; Jordán et al., 2002).

## 2.7. Statistical analysis

The olfactometric data were analyzed using Excel (Microsoft Office, 2007). The principal component analysis (PCA) was performed based on the correlation matrix, using the relative peak area of the volatile compound and the odoriferous intensity of

passion fruit in each state of ripeness. The volatile compounds were represented by the name and/or chemical class and the aroma description of the organic passion fruit in each state of ripeness. The PCA was carried out using the Statistica 7.0 software (StatSoft, Tulsa, USA).

## 3. Results and discussion

### 3.1. Profile of volatiles of the organic passion fruit

The isolation conditions of volatiles of the organic passion fruit were established in a previous work (Macoris et al., 2011). Isolation for 2 h and elution with 300  $\mu$ L dichloromethane were the best conditions to provide the most representative isolate, besides DB-Wax column for the best separation.

Sixty-six volatile compounds were detected in the organic passion fruit in all the states of ripeness (Table 1 and Figs. 1a–3a). Amongst the compounds identified, the major chemical class was esters (26), followed by alcohols (7), terpenes (7), aldehydes (3), hydrocarbon (1) and acids (1) (Table 1). The volatile compounds identified in this study were also reported in other studies concerning passion fruit (Janzantti et al., 2012; Jordán et al., 2002; Werkhoff, Guntert, Krammer, Sommer, & Kaulen, 1998).

The major volatile compounds of the organic passion fruit in the 1/3 yellow skin color state of ripeness were hexanal, ethyl butanoate, *cis*-beta-ocimene, 2-methyl-3-buten-2-ol and a non-identified compound (peak 21, RI 1172). Ethyl butanoate, ethyl hexanoate, hexyl acetate, 1-hexanol, *cis*-3-hexenyl acetate, *cis*-beta-ocimene, hexyl butanoate, 2-methyl-3-buten-2-ol, beta-myrcene and the non-identified compounds from peak 8 (RI 1016) and peak 21 were dominant in the 2/3 yellow skin color. Moreover the hexyl hexanoate, the same majority compounds as the 2/3 yellow skin color were in the 3/3 yellow skin color passion fruit, with exception of the non-identified compound from peak 21. Ethyl butanoate, *cis*-beta-ocimene and 2-methyl-3-buten-2-ol were the major compounds in the organic passion fruit in all the states of ripeness (Table 1, Figs. 1a–3a).

There were changes in the volatile profile throughout the maturation process (Table 1). Most volatile compounds increased relative peak area during ripeness of the organic passion fruit. There was an expressive increase in relative peak area from the 1/3 to 2/3 yellow skin color state of ripeness with changes in the volatile profile, especially of ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate, *cis*-3-hexenyl acetate, hexyl butanoate, hexyl hexanoate, *cis*-3-hexenyl butanoate, beta-myrcene, limonene, 1-hexanol, *cis*-3-hexen-1-ol and the non-identified compounds from peak 3 (RI 938), peak 8 (RI 1016) and peak 31 (RI 1294). Then, relative peak area was still improved from the 2/3 to 3/3 yellow skin color state of ripeness, but the volatile profile remained. *Cis*-beta-ocimene showed a slight increase in relative peak area and 2-methylpropyl acetate reduced relative peak area from the 2/3 to 3/3 yellow skin color state of ripeness. Hexanal, methyl butanoate and the non-identified compounds from peak 13 (RI 1071) and peak 21 (RI 1172) reduced relative peak area during ripeness, although methyl butanoate increased up to the 3/3 yellow skin color state of ripeness.

The reduction of relative peak area of hexanal during ripeness could be related to the appearing of hexanol. Aldehydes and ketones are converted from lipoxygenase into the correspondent alcohols. Hexanol shows higher threshold and odoriferous importance than the carboxylic compound progenitor, hexanal (Damodaran, Parkin, & Fennema, 2007).

The same major compounds as ours were found in the organic yellow passion fruit from Afruvec material in the 3/3 yellow skin color state of ripeness (Janzantti et al., 2012; Macoris et al., 2011).

**Table 1**

Volatile and odoriferous compounds of the organic passion fruit in different states of ripeness, corresponding to the 1/3, 2/3 and 3/3 yellow skin color state of ripeness detected by GC-FID and GC-O.

Peak	RI <sup>d</sup>	RI <sup>e</sup>	Compound	Aroma description	GC-FID <sup>f</sup>			GC-O <sup>g</sup>		
					1/3	2/3	3/3	1/3	2/3	3/3
1	922	<800	ethyl propanoate <sup>a,b,c</sup>	not detected by OSME	nd – 0.14	1.45	3.55	–	–	–
2	932	<800	propyl acetate <sup>a,b,c</sup>	skin, passion fruit	nd – 0.12	3.59	5.51	4.07	6.68	7.33
3	938	–	ni	not detected by OSME	3.15	7.74	15.50	–	–	–
4	939	<800	methyl butanoate <sup>a,b,c</sup>	passion fruit/fruity	6.93	2.76	5.75	2.01	3.91	3.53
5	945	–	ni	not detected by OSME	0.66	4.00	10.66	–	–	–
6	974	<800	2-methylpropyl acetate <sup>a,b,c</sup>	passion fruit	7.49	16.46	10.42	–	1.12	0.98
7	997	–	ni	not detected by OSME	13.47	18.77	19.97	–	–	–
8	1016	–	ni	not detected by OSME	11.96	314.24	1058.71	–	–	–
9	1019	803	ethyl butanoate <sup>a,b,c</sup>	sweet/strawberry	31.77	874.77	2691.18	9.81	9.82	9.93
10	1027	<800	2-methyl-3-buten-2-ol <sup>b,c</sup>	not detected by OSME	18.92	27.22	36.81	–	–	–
11	1064	–	ni	not detected by OSME	0.79	4.71	5.91	–	–	–
12	1066	815	butyl acetate <sup>a,b,c</sup>	green, sweet	1.29	15.00	21.40	–	4.23	4.63
13	1071	–	ni	not detected by OSME	13.08	3.66	2.68	–	–	–
14	1073	<800	hexanal <sup>a,b,c</sup>	not detected by OSME	41.26	7.46	0.73	–	–	–
15	1096	<800	diethyl carbonate <sup>b,c</sup>	plastic	tr	0.63	0.97	2.11	4.06	6.97
16	1105	–	amyl isobutanoate <sup>b,c</sup>	not detected by OSME	nd – tr	0.29	0.92	–	–	–
17	1125	886	o-xylene <sup>b,c</sup>	plastic	nd – tr	tr – 0.42	tr – 1.25	–	–	1.18
18	1131	<800	methyl 2-methylbutanoate <sup>b,c</sup>	not detected by OSME	nd – 0.64	3.53	8.25	–	–	–
19	1155	<800	1-butanol <sup>a,b,c</sup>	sweet/fruity	1.05	3.30	5.82	1.77	3.06	3.32
20	1172	993	beta-myrcene <sup>a,b,c</sup>	citric/fruity	6.33	23.88	46.75	–	–	2.46
21	1172	–	ni	not detected by OSME	45.41	21.79	12.76	–	–	–
22	1175	934	methyl hexanoate <sup>a,b,c</sup>	strawberry, earthy	0.80	5.19	12.84	–	1.48	1.50
23	1184	1029	limonene <sup>a,b,c</sup>	not detected by OSME	5.29	11.90	17.50	–	–	–
24	1210	972	butyl butanoate <sup>a,b,c</sup>	sweet	0.38	2.92	7.35	–	–	1.23
25	1228	1001	ethyl hexanoate <sup>a,b,c</sup>	syrup, guarana	8.75	151.49	455.24	5.02	6.43	8.14
26	1244	1052	cis-beta-ocimene <sup>b,c</sup>	not detected by OSME	21.14	34.59	35.81	–	–	–
27	1249	914	3-methyl-2-butenyl acetate <sup>b,c</sup>	not detected by OSME	1.12	3.47	4.26	–	–	–
28	1268	1019	hexyl acetate <sup>a,b,c</sup>	floral	7.83	65.79	100.87	–	1.09	1.78
29	1274	1006	octanal <sup>a,b,c</sup>	sweet, acid	tr – 0.78	2.68	4.64	–	1.71	2.34
30	1275	990	ethyl cis-3-hexenoate <sup>b,c</sup>	fruity/floral	tr	tr – 0.43	0.72	2.40	2.18	2.79
31	1294	–	ni	not detected by OSME	tr – 0.30	4.49	17.88	–	–	–
32	1300	1009	trans-3-hexenyl acetate <sup>b,c</sup>	fruity, citrus	tr – 0.24	1.81	2.80	–	1.23	1.52
33	1309	991	cis-3-hexenyl acetate <sup>b,c</sup>	fruity, green	10.83	41.81	56.77	2.89	2.58	3.06
34	1328	1021	ethyl trans-2-hexenoate <sup>b,c</sup>	floral	1.19	2.27	2.66	–	–	1.19
35	1339	–	ni	plastic, sweet	nd – tr	tr – 0.22	2.21	–	1.85	3.24
36	1355	870	1-hexanol <sup>a,b,c</sup>	passion fruit, skin	8.02	43.09	77.61	–	3.27	3.14
37	1362	1039	trans-3-hexen-1-ol <sup>b,c</sup>	not detected by OSME	0.50	2.27	5.21	–	–	–
38	1382	855	cis-3-hexen-1-ol <sup>a,b,c</sup>	passion fruit, grass	4.22	10.67	16.60	4.36	4.77	5.37
39	1402	1193	butyl hexanoate <sup>a,b,c</sup>	unripe fruit	0.50	2.40	5.72	–	–	1.53
40	1406	1193	hexyl butanoate <sup>a,b,c</sup>	not detected by OSME	2.89	27.88	72.75	–	–	–
41	1444	1187	trans-3-hexenyl butanoate <sup>b,c</sup>	not detected by OSME	nd – 0.43	tr – 0.40	1.33	–	–	–
42	1449	1198	ethyl octanoate <sup>a,b,c</sup>	earthy	nd – tr	0.57	2.35	3.50	3.18	3.20
43	1452	1185	cis-3-hexenyl butanoate <sup>b,c</sup>	not detected by OSME	1.35	6.32	16.30	–	–	–
44	1463	–	ni	not detected by OSME	tr – 0.35	0.51	0.78	–	–	–
45	1466	<800	acetic acid <sup>a,b,c</sup>	not detected by OSME	tr – 0.18	0.63	1.56	–	–	–
46	1495	1385	alpha-copaene <sup>b,c</sup>	green, fruity	Tr	0.46	1.82	–	–	1.44
47	1515	971	benzaldehyde <sup>b,c</sup>	lavander	0.92	1.78	1.83	–	0.81	0.92
A	1537	–	ni	citric	nd	nd	nd	1.37	1.61	1.17
B	1570	–	ni	citric, lavender	nd	nd	nd	–	1.40	2.40
48	1575	1100	beta-linalool <sup>a,b,c</sup>	sweet, citrus	1.56	1.93	2.57	2.25	2.01	2.72
49	1580	1072	1-octanol <sup>a,b,c</sup>	not detected by OSME	0.43	0.98	2.00	–	–	–
50	1591	–	ni	not detected by OSME	0.48	1.67	2.85	–	–	–
51	1598	1387	hexyl hexanoate <sup>a,b,c</sup>	synthetic, rubber	1.31	11.03	37.35	0.80	0.95	0.74
52	1646	1381	cis-3-hexenyl hexanoate <sup>b,c</sup>	green, citric	0.53	2.83	7.83	–	1.10	1.44
53	1689	1487	germacrene D <sup>b,c</sup>	passion fruit	tr – 0.14	nd – 0.37	tr – 0.27	–	1.70	1.68
C	1705	–	ni	passion fruit	nd	nd	Nd	1.21	1.01	0.88
54	1715	1195	alpha-terpineol <sup>a,b,c</sup>	passion fruit/leafy	tr – 1.27	1.62	3.00	1.30	5.91	7.35
55	1727	–	ni	not detected by OSME	tr – 0.45	nd – 1.40	1.33	–	–	–
56	1753	–	ni	green, passion fruit	nd – 0.18	tr – 0.15	tr – 0.51	–	2.12	3.75
57	1771	–	ni	green, leafy	tr – 0.18	tr – 0.11	tr – 0.32	0.82	1.71	1.89
D	1785	–	ni	green, sweet	nd	nd	nd	–	1.22	1.68
E	1813	–	ni	passion fruit	nd	nd	nd	1.45	1.50	1.55
58	1840	–	ni	sweet, molasses	nd – 0.54	tr	tr – 0.17	1.83	3.61	3.91
F	1913	–	ni	rubber, passion fruit	nd	nd	nd	–	1.43	1.34
59	1941	–	ni	sweet	0.92	1.21	1.44	0.90	–	1.41
G	1960	–	ni	fruity, peach	nd	nd	nd	1.63	1.50	1.40
60	1962	–	ni	passion fruit, sweet	tr	tr – 0.33	1.07	1.07	0.95	3.23
61	1963	1487	dodecanol <sup>b,c</sup>	caramel	2.61	1.69	3.94	0.63	–	–
62	2011	–	ni	not detected by OSME	0.48	0.49	tr – 1.62	–	–	–
63	2072	–	ni	candy floss	0.58	0.47	0.91	1.20	3.66	8.44
64	2086	–	ni	candy floss/caramel	0.89	1.15	1.39	–	–	2.04

(continued on next page)

**Table 1** (continued)

Peak	RI <sup>d</sup>	RI <sup>e</sup>	Compound	Aroma description	GC-FID <sup>f</sup>			GC-O <sup>g</sup>		
					1/3	2/3	3/3	1/3	2/3	3/3
65	2188	–	methyl hexadecanoate <sup>b,c</sup>	metallic/solvent	tr – 0.34	nd – 0.18	0.60	1.19	0.97	1.12
66	2217	–	ni	solvent	tr – 0.33	tr	tr	2.47	1.05	3.86

<sup>a</sup> Compound identified by pure standards.

<sup>b</sup> Compound identified by mass spectrometry.

<sup>c</sup> Compound identified by calculating the retention index.

<sup>d</sup> RI = retention index of peak in the DB-Wax column.

<sup>e</sup> RI = retention index of peak in the DB-5 column.

<sup>f</sup> GC-FID: relative area of peak ( $n = 3$ ) in the GC-FID, multiplied by 100.

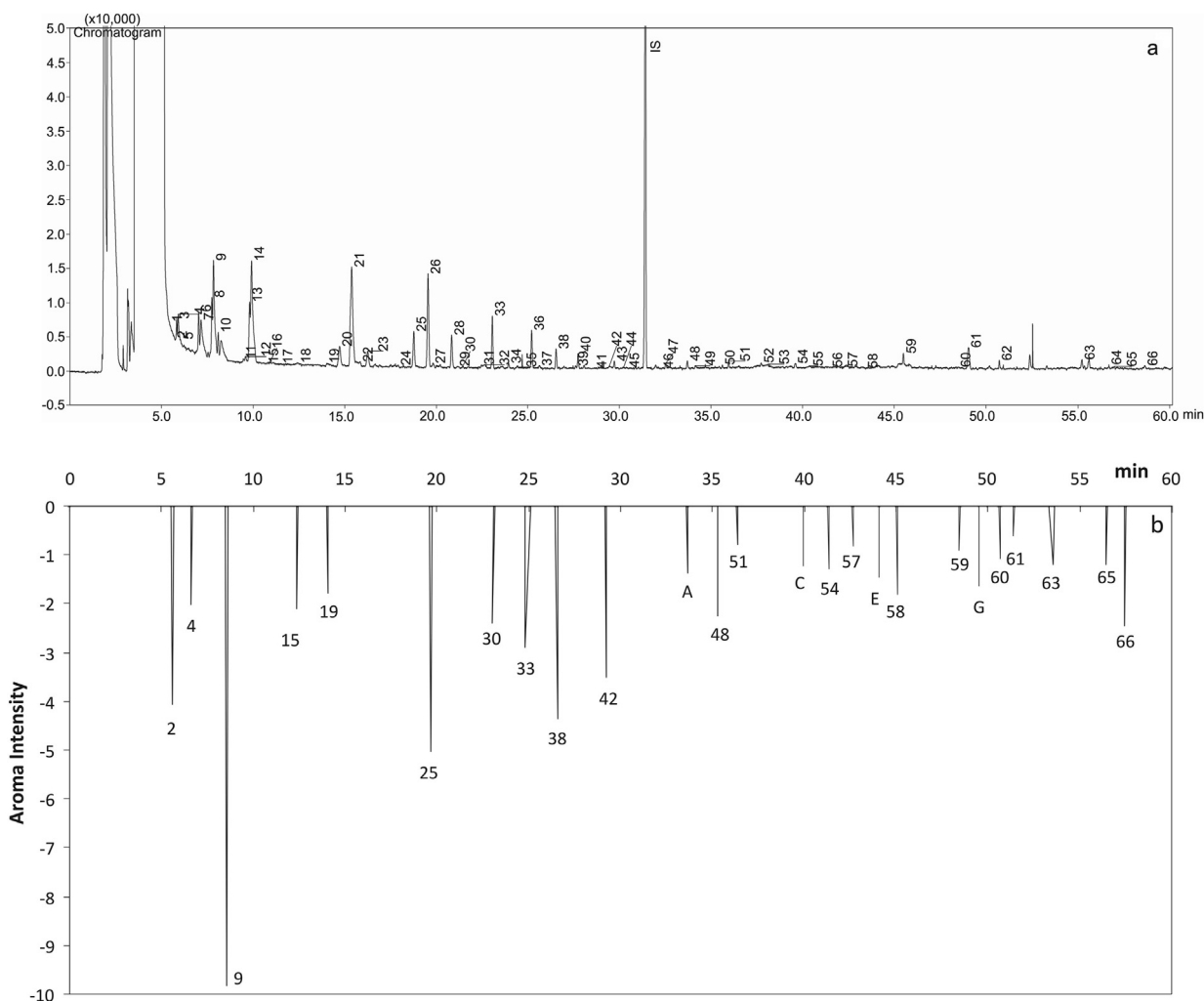
<sup>g</sup> GC-O: maximum odoriferous intensity, 0 = none, 5 = moderate and 10 = strong. nd = compound not detected. ni = compound not identified. tr = trace, relative area of peak <0.1.

Different from the present work, vinyl benzene and diethyl carbonate were included among the major compounds of the organic passion fruit (Janzantti et al., 2012) and *cis*-3-hexenyl acetate was not (Janzantti et al., 2012; Macoris et al., 2011).

### 3.2. GC-O-OSME of the organic passion fruit

The odoriferous compounds of the aroma perceived in the organic passion fruit during ripeness received identical number to those attributed to the peaks on the chromatogram, whilst letters were attributed only to the odoriferous compounds not correlated

to the volatile compounds detected by GC-FID (Table 1, Figs. 1b–3b). Twenty-five compounds were detected in the organic passion fruit from the 1/3 yellow skin color state of ripeness, and 38 and 46 odoriferous compounds in the 2/3 and 3/3, respectively (Table 1). The odoriferous compounds with intensity of 5.0 or more, in the half part of the 10-point hybrid scale, between the terms “moderate” and “strong”, were considered to be of major odoriferous importance for the aroma of the organic passion fruit. The odoriferous compounds with intensity between 3.0 and 4.9 were considered to be of moderate odoriferous importance and those between 0.1 and 2.9 of weak odoriferous importance.



**Fig. 1.** Chromatogram (a) and consensual aromagram (b) of the volatile compounds of the organic passion fruit in the 1/3 yellow skin color state of ripeness.



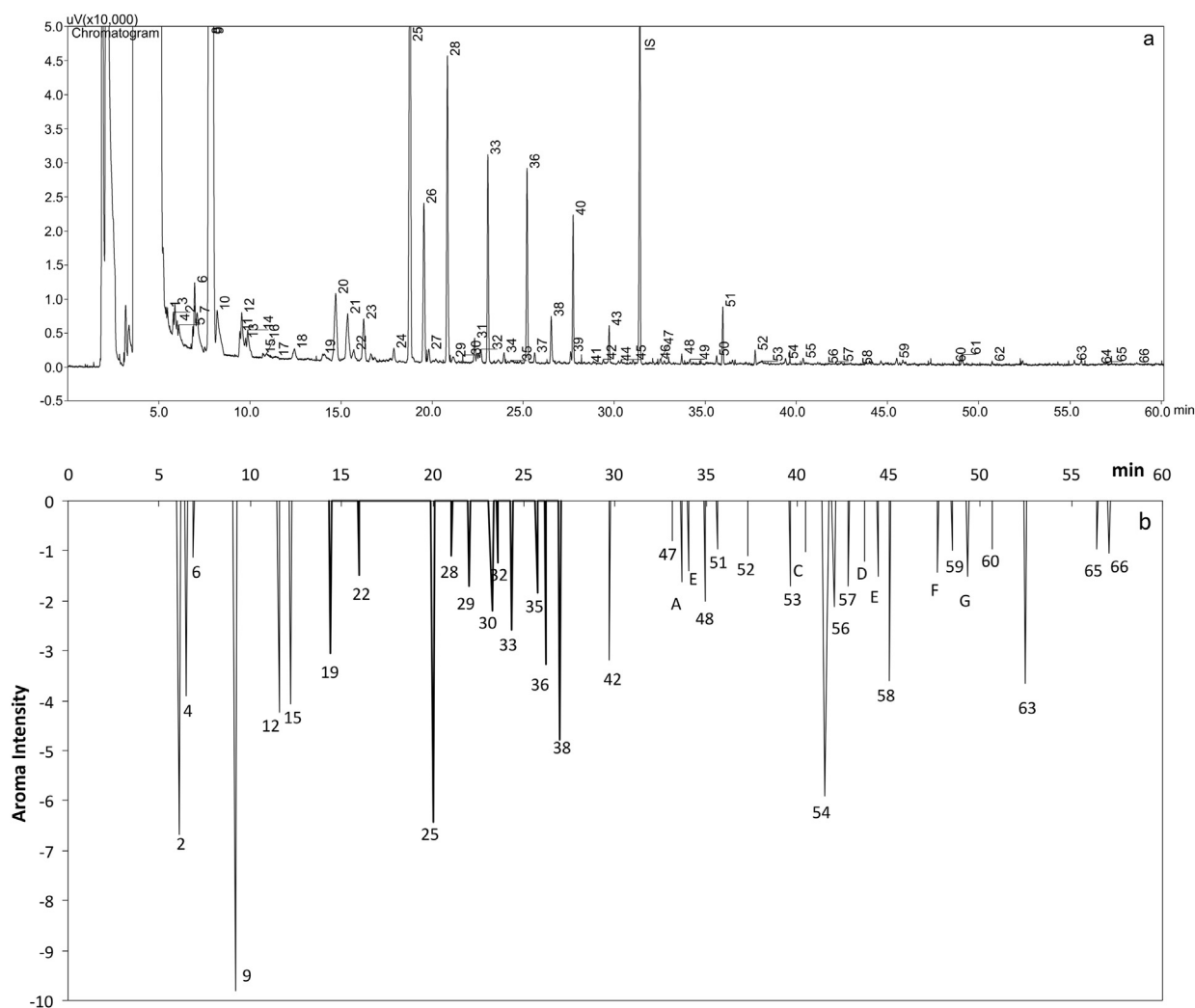


Fig. 2. Chromatogram (a) and consensual aromagram (b) of the volatile compounds of the organic passion fruit in the 2/3 yellow skin color state of ripeness.

The odoriferous intensity of the volatile compounds increased during ripeness. The volatile compounds showing the highest odoriferous importance for the organic passion fruit in the 1/3 yellow skin color state of ripeness were ethyl butanoate and ethyl hexanoate, described as “sweet, strawberry” and “guarana, syrup”, respectively. These compounds also showed major odoriferous importance in the organic passion fruit at the 2/3 yellow skin color state of ripeness, together with propyl acetate, described as “skin, passion fruit”, and alpha-terpineol, with aroma of “passion fruit/leafy”. Also, diethyl carbonate, described as “plastic”, *cis*-3-hexen-1-ol, described as “passion fruit, grass” and a non-identified compound (peak 63, RI 2072), described as “candy floss”, showed odoriferous importance in the organic passion fruit at the 3/3 yellow skin color state of ripeness (Table 1, Figs. 1b–3b).

During ripeness, methyl butanoate, described as “passion fruit/fruity”, 1-butanol, described as “sweet/fruity” and the non-identified compound from peak 58 (RI 1840), described as “sweet, molasses”, showed weak odoriferous importance in the 1/3 yellow skin color, but moderate in the 2/3 and 3/3. *Cis*-3-hexenyl acetate, described as “fruity, green”, the non-identified compounds from peak 60 (RI 1962), described as “passion fruit, sweet”, and peak 66 (RI 2217), described as “solvent”, showed weak odoriferous importance in the 1/3 and 2/3 yellow skin color state of ripeness, and moderate odoriferous importance in the 3/3. Butyl acetate,

described as “green, sweet” and 1-hexanol, described as “passion fruit, skin”, were not detected in the 1/3 yellow skin color state of ripeness, but showed moderate odoriferous intensity in the 2/3 and 3/3. Ethyl octanoate, described as “earthy”, showed moderate odoriferous intensity in all the states of ripeness. Propyl acetate, methyl and ethyl butanoate, ethyl hexanoate, alpha-terpineol, 1-butanol, 1-hexanol, and the non-identified compounds from peak 58, 60 and 63 were the most important for the aroma of “passion fruit”, “sweet” and “fruity” in all states of ripeness.

Dodecanol, described as “caramel” appeared only in the organic passion fruit from the 1/3 yellow skin color state of ripeness, showing weak intensity. O-xylene, described as “plastic”, beta-myrcene, described as “citric/fruity”, butyl butanoate, described as “sweet”, ethyl *trans*-2-hexenoate, described as “floral”, butyl hexanoate, described as “unripe fruit”, alpha-copaene, described as “green/fruity” and the non-identified compound from peak 64 (RI 2086), described as “candy floss/caramel”, were found only in the 3/3 yellow skin color state of ripeness, all with weak intensity.

Ethyl butanoate is a major compound according to both the GC-FID and GC-OSME. Ethyl hexanoate is a major compound in passion fruit from the 2/3 and 3/3 yellow skin color state of ripeness according to the GC-FID. Although in the GC-OSME ethyl hexanoate improves intensity during ripeness, it has showed high odoriferous importance since in the 1/3 yellow skin color even with a low

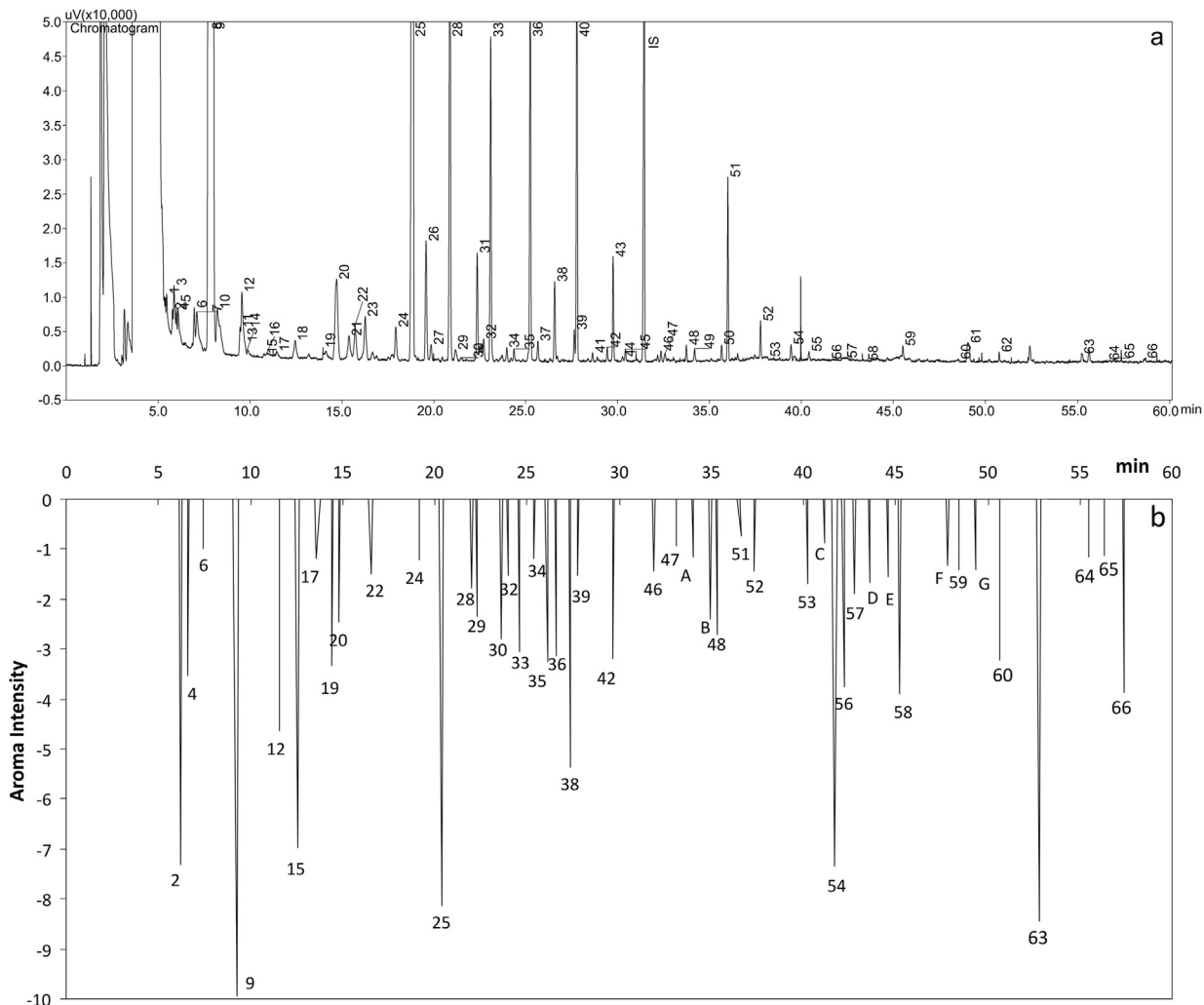


Fig. 3. Chromatogram (a) and consensual aromagram (b) of the volatile compounds of the organic passion fruit in the 3/3 yellow skin color state of ripeness.

relative peak area. Relative peak area of hexanal reduced during ripeness, but it was not detected by GC-OSME.

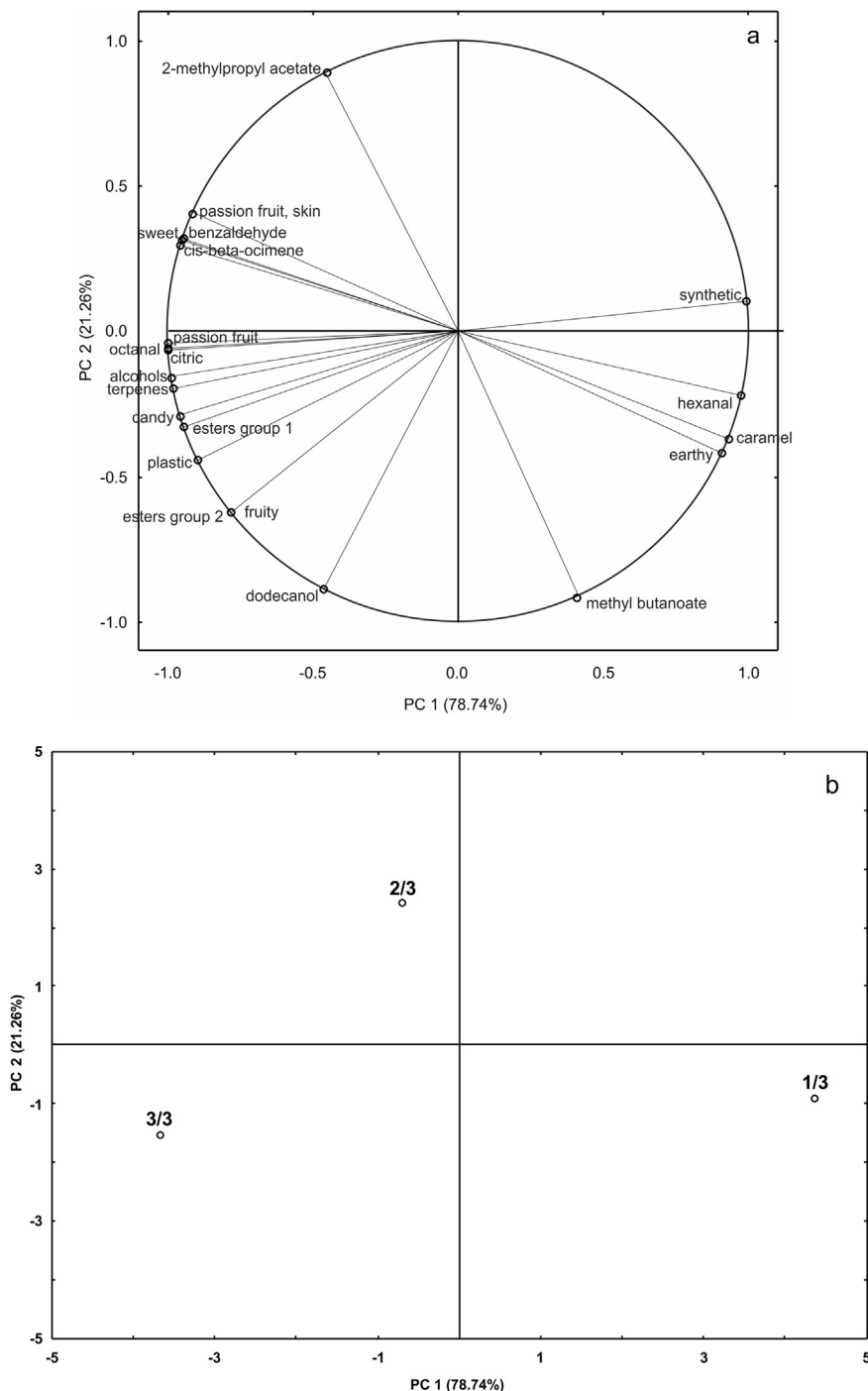
Janzanti et al. (2012) reported that methyl butanoate, ethyl butanoate, ethyl hexanoate, propyl acetate, diethyl carbonate and *cis*-3-hexen-1-ol showed the highest odoriferous importance for the organic yellow passion fruit from Afruvec material. Ethyl butanoate, ethyl hexanoate, propyl acetate, diethyl carbonate and *cis*-3-hexen-1-ol also showed the highest odoriferous importance for the aroma of the organic yellow passion fruit from the present work. Alpha-terpineol had low odoriferous intensity for the aroma of passion fruit from Afruvec material (Janzanti et al., 2012), but it was considered to be one of the most important for the organic passion fruit in this work. These differences could be attributed to the different passion fruit material.

Principal component analysis was performed to visualize the data in two dimensions, in order to differentiate the odoriferous and volatile compounds of organic passion fruit among the states of ripeness (Fig. 4). The volatile compounds were represented by the name and/or chemical class, where the sum of relative peak area of the compounds at each state of ripeness was used. The odoriferous compounds were grouped by identical or similar description; the aroma intensities were summed at each state of ripeness (Fig. 4a). The passion fruit from the 1/3 yellow skin color state of ripeness was loaded positively on PC 1 and negatively on PC 2. The passion

fruit from the 2/3 yellow skin color state of ripeness was loaded negatively on PC 1 and positively on PC 2, and the passion fruit from the 3/3 yellow skin color state of ripeness was loaded negatively on PC 1 and PC 2 (Fig. 4b).

Hexanal and aroma of caramel, earthy and synthetic were loaded near to the 1/3 yellow skin color state of ripeness, indicating they are higher in the unripe passion fruit, thus characterizing the passion fruit from the 1/3 yellow skin color state of ripeness (Fig. 4). Hexanal is associated with the unripe fruit, but with no odor contribution, because the threshold value may play the major role. The 2/3 yellow skin color state of ripeness showed higher level of 2-methylpropyl acetate, characterizing the passion fruit from the 2/3 yellow skin color state of ripeness. *Cis*-beta-ocimene, benzaldehyde and aroma sweet and passion fruit, skin showed similar level in the 2/3 and 3/3 yellow skin color state of ripeness, characterizing both the 2/3 and 3/3 yellow skin color state of ripeness. At the end of maturation, esters, terpenes, alcohols, octanal, dodecanol and aroma of passion fruit, fruity, citric, candy and plastic were loaded near to the 3/3 yellow skin color state of ripeness, in higher levels, thus characterizing the whole ripe fruit. Methyl butanoate showed similar level in the 1/3 and 3/3 yellow skin color states of ripeness (Fig. 4).

Another important aspect of passion fruit is the presence of marker volatile compounds. Concerning to the organic passion fruit



**Fig. 4.** Principal component analysis (PCA) of volatile and odoriferous compounds from organic passion fruit in the 1/3, 2/3 and 3/3 yellow skin color state of ripeness. 1/3 = organic passion fruit from the 1/3 yellow skin color state of ripeness, 2/3 = organic passion fruit from the 2/3 yellow skin color state of ripeness, 3/3 = organic passion fruit from the 3/3 yellow skin color state of ripeness.

from Afruvec material (Janzantti et al., 2012), *trans* and *cis*-3-hexenyl acetate, alpha-copaene, alpha-terpineol, limonene, *trans*-beta-ocimene, and delta-cadinene showed major odoriferous contribution for the aroma of the organic passion fruit when compared to the conventional passion fruit, being considered the marker volatile compounds. In this work, almost the same odoriferous compounds were also responsible for the aroma of the organic passion fruit from the Feltrin selection. *Trans* and *cis*-3-hexenyl acetate, alpha-copaene and alpha-terpineol showed the

major contribution for the aroma. It should be noticed that these compounds showed the same odoriferous intensity in both organic passion fruit (Afruvec material and Feltrin selection), with exception of alpha-terpineol, which had strong odoriferous intensity in the organic passion fruit from Feltrin selection and moderate in the Afruvec material.

The volatile compounds most important for the overall aroma of the organic passion fruit at different states of ripeness were identified by GC-OSME. Additional investigation should be done in



order to determine compounds with high odoriferous contribution, which were not identified by GC-MS and/or detected by GC-FID.

The results of this study provide information to allow extend the harvest period, indicating that passion fruit harvest can begin in the 2/3 yellow skin color because there already are volatile compounds responsible for the passion fruit aroma, although the whole passion fruit aroma could only be obtained at the 3/3 yellow skin color state of ripeness.

#### 4. Conclusions

There were changes in the volatile profile throughout the maturation process of the organic passion fruit. Relative peak area of the volatile compounds improved during ripeness from the 1/3 to 2/3 yellow skin color state of ripeness, especially ethyl butanoate, butyl acetate, beta-myrcene, limonene, ethyl hexanoate, hexyl acetate, *cis*-3-hexenyl acetate, 1-hexanol, *cis*-3-hexen-1-ol, hexyl butanoate, hexyl hexanoate, *cis*-3-hexenyl butanoate. Relative peak area of the volatile compounds was still improved from the 2/3 to 3/3, especially *cis*-beta-ocimene and 2-methylpropyl acetate, but the profile remained. Hexanal reduced during ripeness.

Ethyl butanoate (“sweet/strawberry”), important in the 1/3 yellow skin color state of ripeness, showed the highest odoriferous importance for the characteristic aroma of the organic passion fruit and also the highest relative peak area, improving during ripeness. Ethyl butanoate, ethyl hexanoate (“syrup, guarana”), propyl acetate (“skin, passion fruit”) and alpha-terpineol (“passion fruit/leaf”), important in the 2/3 yellow skin color state of ripeness, together with diethyl carbonate (“plastic”) and *cis*-3-hexen-ol (“passion fruit, grass”) showed high odoriferous importance at the 3/3 yellow skin color state of ripeness.

PCA allowed all ripening states to be clearly differentiated one from another. It showed that the variables selected were able to explain the differences observed between the states of ripeness. PCA analysis indicated that hexanal and caramel, earthy and synthetic aroma were most closely associated with the unripe passion fruit, the 1/3 yellow skin color state of ripeness. 2-methylpropyl acetate characterized the passion fruit in the 2/3 yellow skin color state of ripeness together with *cis*-beta-ocimene, benzaldehyde and aroma sweet and passion fruit, skin, which also was associated with the 3/3 yellow skin color state of ripeness. Esters, terpenes, alcohols, octanal, dodecanol and aroma of passion fruit, fruity, citric, candy and plastic were closely associated with the whole maturation, the 3/3 yellow skin color state of ripeness.

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#### References

- Acree, T. E., & Arn, H. (2004). *Flavornet*. Available from <http://www.flavornet.org/flavornet.html> Accessed 16.06.13.
- Amaro, A. P., & Monteiro, M. (2001). Rendimento de extração da polpa e características físico-químicas do maracujá amarelo (*Passiflora edulis* f. *Flavicarpa* Sims. Deg.) produzido por cultivo orgânico e convencional em relação à cor da casca. *Alimentos e Nutrição*, 12, 171–184.
- ASTM. (1981). *American Society For Testing and Materials. Guidelines for the selection and training of sensory panel members*. Philadelphia: ASTM, 77p. (STP 758).
- Da Silva, M. A. A. P. (1999). Avaliação de atributos sensoriais por técnicas tempo-intensidade. In T. C. A. Almeida, G. Hough, M. H. Damásio, & M. A. A. P. Da Silva (Eds.), *Avanços em Análise sensorial* (pp. 49–61). São Paulo: Livraria Varela.
- De Marchi, R., Monteiro, M., Benato, E. A., & Silva, C. A. R. (2000). Uso da cor da casca como indicador de qualidade do maracujá amarelo (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) destinado à industrialização. *Ciência e Tecnologia de Alimentos*, 20(3), 381–387.
- Defilippi, B. G., Manríquez, D., Luengwilai, K., & González-Agüero, M. (2009). Aroma volatiles: biosynthesis and mechanisms of modulation during fruit ripening. *Advances in Botanical Research*, 50, 1–37.
- Damodaran, S., Parkin, K. L., & Fennema, O. R. (2007). *Fennema's food chemistry* (4th ed.). Boca Raton: CRC Press, 1160p.
- Folha de São Paulo. (2012). *Mercado de alimentos orgânicos no Brasil movimentou R\$ 500 milhões*. Available from <http://classificados.folha.uol.com.br/negocios/1187639-mercado-de-alimentos-organicos-no-brasil-movimentou-r-500-mil-hoes.shtml> Accessed 12.02.14.
- IBGE. (2011). *Banco de dados agregados. Quantidade produzida, valor da produção, área plantada e área colhida da lavoura permanente*. Instituto Brasileiro de Geografia e Estatística. Available from <http://www.sidra.ibge.gov.br/bda/tabela/protabl.asp?z=t&o=11&i=P> Accessed 12.02.14.
- IFOAM. (2013). *International Federation of Organic Agriculture Movements*. Available from <http://www.ifoam.org/en/node> Accessed 12.02.14.
- Jales, K. A., Maia, G. A., Garruti, D. S., Souza Neto, M. A., Janzantti, N. S., & Franco, M. R. B. (2005). Evaluación de los compuestos odoríferos del jugo de maracujá amarillo por GC-MS y GC-O (OSME). *Notícias técnicas del laboratorio*, 3, 12–14.
- Janzantti, N. S., Macoris, M. S., Garruti, D. S., & Monteiro, M. (2012). Influence of the cultivation system in the aroma of the volatile compounds and total antioxidant activity of passion fruit. *LWT – Food Science and Technology*, 46, 511–518.
- Janzantti, N. S., Santos, G. C., & Monteiro, M. (2014). Shelf Life of Fresh and Pasteurized Organic Passion Fruit (*Passiflora Edulis* F. *Flavicarpa* Deg.) Pulp. *Journal of Food Processing and Preservation*, 38, 262–270.
- Jordán, M. J., Goodner, K. L., & Shaw, P. E. (2002). Characterization of the aromatic profile in aqueous essence and fruit juice of yellow passion fruit (*Passiflora edulis* Sims F. *Flavicarpa degner*) by GC-MS and GC/O. *Journal of Agricultural and Food Chemistry*, 50(6), 1523–1528.
- Jordán, M. J., Goodner, K. L., & Shaw, P. E. (2000). Volatile components in tropical fruit essences: yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Degener) and banana (*Musa sapientum* L.). *Proceedings of the Florida State Horticultural Society*, 113, 284–286.
- Macoris, M. S., Janzantti, N. S., Garruti, D. S., & Monteiro, M. (2011). Volatile compounds from organic and conventional passion fruit (*Passiflora edulis* F. *flavicarpa*) pulp. *Ciência e Tecnologia de Alimentos*, 31, 430–435.
- McDaniel, M. R., Miranda-Lopez, R., Watson, B. T., Micheals, N. J., & Libbey, L. M. (1990). Pinot noir aroma: a sensory/gas chromatographic approach. In G. Charalambous (Ed.), *Flavors and off-flavors* (pp. 23–36). Amsterdam: Elsevier.
- Pontes, M., Marques, J. C., & Câmara, J. S. (2009). Headspace solid-phase micro-extraction-gas chromatography-quadrupole mass spectrometric methodology for the establishment of the volatile composition of *Passiflora* fruit species. *Microchemical Journal*, 93, 1–11.
- Meletti, L. M. M. (2011). Avanços na cultura do maracujá no Brasil. *Revista Brasileira de Fruticultura*, 33, 83–91.
- Santos, G. C., & Monteiro, M. (2004). Sistema orgânico de produção de alimentos. *Alimentos e Nutrição*, 15(1), 73–86.
- Werkhoff, P., Güntert, M., Krammer, G., Sommer, H., & Kaulen, J. (1998). Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. *Journal of Agricultural and Food Chemistry*, 46(3), 1076–1093.