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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.

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 sate of ripeness which are very similar to

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the 3/3 yellow skin color sate 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-mircene, 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 others 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 °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 °C), with traps containing 100 mg of Porapak Q (150–180 μm, Waters Associates, Milford, U.S.A) previously conditioned in an ultra pure N_2 flow (40 mL/min) at 170 °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 μL 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 μm film thickness) stationary phase column (J&W, Folsom, USA) was used. The column temperature was initially maintained at 40 °C for 10 min and then raised to 200 °C at 3 °C/min, remaining at this temperature for a further 10 min. Injection was in the splitless mode (2 μL) with a hydrogen gas flow rate of 1.3 mL/min. The injector and detector temperatures were 230 °C and 250 °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 μL of pentadecane standard solution (4 μL/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 (Wilimington, 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 μm film thickness) was maintained at 40 °C for 10 min, then programmed to rise to 200 °C at 3 °C/min, and held for 10 min. Helium was used at a flow rate of 1.3 mL/min. The injector temperature was 230 °C and the detector was 240 °C. The DB-5 column (60 m length, 0.25 mm i.d., 0.25 μm film thickness), was maintained at 50 °C, then programmed to rise to 250 °C at 3 °C/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 °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