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Profiling of passion fruit volatiles: An effective tool to discriminate between species and varieties

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ABSTRACT

The aim of this work was to gain insights on the volatile composition of nine passion fruits grown at Madeira Island (Portugal) – Yellow, Purple, Lemon, Orange, Pineapple, Peach, Melon, Banana and Tomato – and discriminate between them. The volatile composition of these fruits has been investigated using the same analytical technique, HS–SPME/GC–MS and multivariate analysis (MVA). The selected SPME methodology (DVB/CAR/PDMS fiber at 40 ± 1 °C for 30 min and 10% (*w*/*w*) of NaCl under stirring mode (47 ×*g*)) was applied in the profiling of nine different passion fruit samples by GC–MS, allowing the identification of up to 169 volatile compounds belonging to different chemical groups, namely linear and branched esters, terpenes, alcohols and others. Esters were found to be the dominant metabolites regardless of passion fruit sample, with hexyl hexanoate (ranging from 6 to 31%), methyl hexanoate (14–75%) ethyl hexanoate (12–53%) and hexyl butanoate (11–26%) being the principal volatile compounds found, followed by *cis*-β-ocimene (from 8 to 55%), (E)-2-hexenal (4 to 10% for Banana and Tomato passion fruit samples) and eucalyptol (18% for Tomato passion fruit). The results revealed that the differences in the volatile profile among the studied passion fruits were essentially qualitative, with only 7 common volatiles found in all samples, in different abundance. Advanced statistical techniques (PCA and PLS-DA) were used to explore data. Characteristic markers were successively identified using the NIST library, thus showing that the volatile profile was able to differentiate all nine species and varieties. Profiling of passion fruit volatile metabolites can provide an effective tool to characterize the product and to extract useful information concerning its quality or geographic origin.

1. Introduction

Passiflora L., originated from the tropical and warm climates of South America, is the largest genus in the family Passifloraceae, with around 500 different species. This genus is most common in warm, temperate and tropical regions, of which Madeira Island is an example. Passion fruit is usually consumed as fresh fruit or juice and is very popular due to its organoleptic properties — it possesses an exotic, flowery and fruity aroma (Dhawan, Dhawan, & Sharma, 2004). The interest of researchers and producers in this fruit has been stimulated due to its good nutritional and bioactive characteristics. It constitutes a powerful source of vitamins A, C and D (Amata et al., 2009), alkaloids, carotenoids and flavonoids (Cerqueira-Silva, Jesus, Santos, Corrêa, & Souza, 2014). Moreover, it is a good source of nicotinic acid, riboflavin and a fair source of mineral matter. There has also been evidence of antioxidant, anti-inflammatory and antipyretic properties due to the high content in polyphenols (Montanher, Zucolotto, Schenkel, & Fröde, 2007; Saravanan, Arunachalam, & Parimelazhagan, 2014; Zeraik & Yariwake, 2010). In addition, some studies show that passion fruit has antihypertensive, sedative and analgesic properties in a dose-dependent manner (Cerqueira-Silva et al., 2014; Deng, Zhou, Bai, Li, & Li, 2010; Konta et al., 2014).

Actually, several different passion fruit cultivars are known. Hybridization is a key factor for the existence of many species of *Passiflora* L. It has been practiced in breeding programs between two populations of the same species (*Passiflora edulis*) or between populations of different species in the same genus. These experiments originated many hybrids with intermediate fruit or corona colors (Cerqueira - Silva, Cardoso-Silva, Nonato, Corrêa, & Oliveira, 2009; Li et al., 2011). One of the reasons for this practice is due to improvement of economical properties, yield production and organoleptic characteristics, as well as higher resistance to diseases (Li et al., 2011; Ramaiya, Bujang, & Zakaria, 2014). Other factors that influence the existence of so many species and varieties include cross-pollination, environmental factors (Ramaiya et al., 2014), genetic differences and cultivation conditions (Cerqueira-Silva et al., 2014).

A major factor contributing to the popularity of passion fruit is its flavor, arising from a complex combination of several secondary metabolites which collectively define the aroma of the fruit and juice

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(Janzantti, Macoris, Garruti, & Monteiro, 2012; Riu-Aumatell, Castellari, Lopez-Tamames, Galassi, & Buxaderas, 2004). Different esters (linear, branched and substituted) have been reported, including ethyl butanoate and ethyl hexanoate, as being characteristic components of passion fruit. Higher alcohols, such as hexanol and heptan-2-ol, which constitute an important source for the flowery and green aromas, represent the second largest chemical group identified in passion fruits. Terpenes, like β-ocimene, are significantly involved in the characteristic aroma of passion fruit, conferring *floral* notes, while aldehydes contribute for the *green* and *citrus* odors. Other minor chemical groups include ketones, ethers, hydrocarbons and phenols (Janzantti et al., 2012; Leão, Sampaio, Pagani, & Da Silva, 2014; Murray, Shipton, & Whitfield, 1972). The volatile fraction can present considerable modifications according to species and variety, geographical region where the fruit was cultivated, processing procedure and degree of maturation.

In the early 1990s, headspace–solid-phase microextraction (HS–SPME) emerged as a promising alternative to the conventional techniques for the isolation of volatile compounds from headspace (Ma, Hamid, Bekhit, Robertson, & Law, 2013). It presents some advantages over the conventional extraction techniques such as high sensitivity, low cost, solventless and simplicity, as it combines extraction and pre-concentration into a single step, reflecting a truer volatile profile (Ma et al., 2013; Pontes, Marques, & Câmara, 2009; Wani et al., 2011). As HS–SPME is a technique based on a physico-chemical processes of equilibrium between the sample-headspace-fiber coating, its performance depends on different experimental factors and on the nature of the target analytes (Ma et al., 2013; Pawliszyn, 1999; Pontes et al., 2009; Wani et al., 2011). According to Pontes et al. (2009), the HS–SPME technique coupled with GC–MS is an appropriate and selective way of characterizing the volatile profile of different passion fruits.

Currently, consumers are more interested in the provenance and authenticity of their food products. Since the aroma is one of the most typical features of a food product, the characterization of the aromatic profile can represent a useful tool to evaluate the organoleptic quality and it could be used to guarantee its authenticity. Therefore, the fast identification of potential markers, suitable to qualitatively differentiate food products from various countries and regions, is a crucial requirement in regulations and for consumer confidence.

The main purpose of this study was to establish the volatile profile of passion fruit samples by HS–SPME/GC–MS and multivariate analysis (MVA), as an effective tool to characterize the product, to extract useful information concerning its quality and to differentiate between the investigated fruits. The passion fruits investigated belong to three different species, *P. edulis* (Purple passion fruit), *Passiflora ligularis* (Orange passion fruit) and *Passiflora mollissima* (Banana passion fruit), three varieties, *P. edulis* var. *flavicarpa* (Yellow passion fruit), *P. edulis* var. *violette* (Pineapple passion fruit), *P. edulis* var. *panama gold* (Lemon passion fruit) and two hybrids from the cross *P. edulis* var. *flavicarpa* × *P. ligularis* (Peach and Melon passion fruits). In addition, a sample of *Solanum betaceum*, commonly known in Madeira Island as Tomato passion fruit, was also analyzed, in order to determine a possible relationship with the other passion fruits (*Passiflora* spp.) analyzed in this study. SPME was selected for capturing the volatiles from the samples and to provide efficient and high throughput sample preparation. The effect of SPME variables, namely SPME fiber coatings, extraction time (10–40 min), sample amount (2–5 g) and salt amount (0–0.75 g *w*/*w*), on the equilibrium headspace concentration of passion fruit volatile metabolites, was also investigated.

This is the first time that passion fruits from different species and varieties are analyzed using the same analytical technique, which allowed us to describe the volatile profile of each sample studied in this work. It is also the first time that the volatiles from Pineapple, Lemon and Melon passion fruits are described.

2. Experimental

2.1. Chemical reagents

All chemicals used were analytical quality and all solvents were HPLC grade. Sodium chloride (99.5%), used to adjust the ionic strength, was supplied by Merck (Darmstadt, Germany). All the standard substances used for confirmation have a purity level higher than 98.5% and were obtained from Sigma-Aldrich. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, PA, USA). The n-alkanes mixture containing C_8–C_{20} straight-chain alkanes in hexane was also purchased from Sigma-Aldrich. Helium, ultra-pure grade (Air Liquide, Portugal) was used as carrier gas in the GC system.

Clear glass screw cap vials for SPME with PTFE/silica septa were purchased from Supelco. The SPME fiber optimization step was carried out by testing commercially available silica SPME fibers purchased from Supelco (Bellefonte, PA, USA) and coated with the following polymers: polyethylene glycol (PEG, 60 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm), polyacrylate (PA, 85 μm), divinylbenzene/carboxen on polydimethylsiloxane (DBV/CAR/PDMS; StableFlex, 50/30 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm) and polydimethylsiloxane (PDMS, 100 μm).

Prior to their first use, all fibers were conditioned according to the manufacturer's instructions. Before each initial sampling, blank runs were completed to ensure there was no carry-over of analytes from the previous extractions.

2.2. Sample preparation

Nine mature passion fruits, at ripe stage, of different species and varieties – *P. edulis* (Purple passion fruit), *P. ligularis* (Orange passion fruit), *P. mollissima* (Banana passion fruit), *P. edulis* var. *flavicarpa* (Yellow passion fruit), *P. edulis* var. *violette* (Pineapple passion fruit), *P. edulis* var. *panama gold* (Lemon passion fruit), two hybrids from the cross *P. edulis* var. *flavicarpa* × *P. ligularis* (Peach and Melon passion fruits) and *S. betaceum* (Tomato passion fruit) – grown at Madeira Island (Portugal) were obtained from a local market in Funchal. All fruit samples were grown in the same region and subjected to homogenous cultivar conditions, reducing the environmental effects on the volatile profile. The passion fruit samples were opened and the pulp was separated from the seeds to make juice. The juice was homogenized, aliquoted in glass vials and stored at −80 °C in glass bottles until analysis. All analysis were carried out in triplicate.

2.3. HS–SPME extraction conditions

Extractions were carried out using 5.0 g of each sample of passion fruit, previously homogenized and put into 10 mL headspace glass vials covered with PTFE/silicone septum and containing a micro stirring bar. All the experiments were performed under constant stirring (47 × *g*) in order to improve the extraction, since the static layer resistant to mass transfer is destroyed (facilitating mass transport between the bulk of the aqueous sample and the fiber). In order to improve extraction efficiency, the samples ionic strength was adjusted with NaCl. This step decreases the solubility of hydrophilic metabolites in the aqueous phase. The vials were immersed in a thermostat bath fixed at 40 ± 1 °C, temperature selected for the extraction according to Figueira, Câmara, Pereira, and Câmara (2014). After the exposition period of each sampling, the SPME fibers were withdrawn into the needle, removed from the glass vial and immediately inserted into the injection port of the gas chromatograph at 250 °C, where the extracted volatiles were thermally desorbed for 7 min and transferred directly to the GC system equipped with a quadrupole mass analyzer. All the samples were analyzed in triplicate, and the results are presented in mean values.