Food Research International 100 (2017) 234–243

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Volatile metabolite profiling reveals the changes in the volatile compounds of new spontaneously generated loquat cultivars

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ARTICLE INFO

Keywords:
Loquat
Volatilome
Bud mutation
Chance seedling
Flavour

ABSTRACT

In recent years, the advantageous traits of three new loquat cultivars have drawn the attention of breeders and growers. All three have spontaneously arisen from the 'Algerie' cultivar: the new 'Xirlero' cultivar is a bud mutant of 'Algerie', while 'Amadeo' and 'Raúl' arose as chance seedlings. Following a non-targeted approach based on HS-SPME-GC–MS, the volatile compounds profile of the fruits from the new cultivars were obtained and compared to the original 'Algerie' cultivar. Carboxylic acids clearly dominated the volatile profile of all the loquat cultivars, but esters, aldehydes, ketones and alcohols were also predominant compounds. Interestingly when the bud mutant event did not lead to marked changes in the volatile compounds complement, pronounced changes in the volatile composition of chance seedling-generated cultivars 'Amadeo' and 'Raúl' were observed. 'Amadeo' fruits showed lower levels of 2-methyl butanoic acid and much higher levels of methylhexanoate, methylbutanoate and 2-hydroxy-5-methylacetophenone. The 'Raúl' cultivar also had a distinctive volatile profile characterised by high levels of C6-aldehydes, (E)-2-hexanal, 2-hexenal, (Z)-3-hexenal and hexanal, and several carotenoid-derived volatiles; e.g. 2-pentene-1,4-dione 1-(1,2,2-trimethylcyclopentyl), (*S*)-dihydroactinidiolide, isodurene, cis-geranyl acetone, β-damascenone, β-ionone, α-ionone and 3,4-dehydro-β-ionone. These changes in volatiles were associated with a more intense flavour in cultivars 'Amadeo' and 'Raúl', according to the sensory evaluation of the flavour intensity carried out by a semi-trained panel. A metabolomic correlation network analysis provided insights as to how volatiles were regulated, and revealed that the compounds modified in 'Amadeo' were uncoupled from the rest of the volatilome, while the volatiles modified in 'Raul' changed according to specific groups. To conclude, this work provides a holistic view of how the loquat volatilome was affected, and this information was integrated with the physical-chemical-sensory attributes to understand the changes that occur in the new cultivars.

1. Introduction

The increasing interest shown in loquat crops (*Eriobotrya japonica* Lindl.) worldwide has prompted the development of breeding programmes to obtain premium quality loquat varieties (Badenes et al., 2013). In the particular case of Spain, which is the main loquat-producing country in the Mediterranean region and the main exporter in the world, production concentrates on the 'Algerie' cultivar. This cultivar accounts for > 80% of total production, which comes together during a short period (from mid-April to the end of April). Thus one of the main objectives of Spanish breeding programmes is to increase the diversity of loquat cultivars to obtain early cultivars with high fruit quality (Badenes et al., 2013). According to Kader (2008), providing better-flavoured fruit and vegetables at affordable prices is likely to increase consumption, which would be good for not only producers and handlers, but also for consumers. Fruit and vegetable flavour depends on taste (striking a balance between sweetness and sourness or acidity, and low or no astringency) and aroma (concentrations of odour-active volatile compounds). Although taste and aroma are well integrated into their contribution to overall flavour, aroma is often considered to play a dominant role in flavour (Goff & Klee, 2006). Baldwin (2004) concluded that the bottom line for flavour quality is still genetic, and breeders need more information and analytical tools to select flavour quality.

Volatile compounds are low-molecular-weight compound, of which a large number has been identified in many fruit and vegetables, especially in those of much commercial importance. Over the last decade, the nature of metabolic pathways and their contribution to the aroma profile of fruits, such as tomato or peach, are starting to be

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http://dx.doi.org/10.1016/j.foodres.2017.06.068
Received 27 March 2017; Received in revised form 27 June 2017; Accepted 27 June 2017
Available online 29 June 2017

understood (Mathieu et al., 2009; Rambla et al., 2015; Sánchez, Besada, Badenes, Monforte, & Granell, 2012), and is providing breeders with valuable information. However, our knowledge of the volatile composition of loquat is limited (Besada, Salvador, Sdiri, Gil, & Granell, 2013; Jiang et al., 2014). The evolution of ripening traits and volatile compounds during maturation was studied in two cultivars, 'Algerie' and 'Golden Nugget', and specific volatiles with a strong association with loquat aroma-flavour, including those closely linked to the physiological-quality-related traits, were identified (Besada et al., 2013). The heritability of loquat aromas has been evaluated by Jiang et al. (2014) by examining the composition of the volatile compounds of cultivars 'Xiantgtian' and 'Jiefangzhong', and two hybrid progenies, 'Xiangzhong No.11' and 'Zhongxiang No. 25', to conclude that the volatiles levels in the progeny fruit was between the values known in their parents.

Exploring the heritability of aromas in the progeny after hybridisation can provide interesting information (Jiang et al., 2013, Rambla et al., 2014, Sánchez et al., 2014). Moreover, exploring new spontaneously generated varieties can be of scientific and commercial value (Koornneef, Alonso-Blanco, & Vreugdenhil, 2004) as many of them can be more desirable than the original variety. This is the case of three new spontaneously generated varieties in Spain from the 'Algerie' cultivar. These new varieties have captured the attention of growers and breeders in recent years given their desirable traits and commercial interest: one bud mutant named 'Xirlero' and two chance seedlings named 'Amadeo' and 'Raúl'. The most interesting trait of 'Xirlero' is that its fruits ripen earlier and are bigger than those of 'Algerie'. 'Amadeo' fruits are slightly bigger than those of 'Algerie' and their flavour is greatly appreciated, while 'Raul' fruits' stand out positively for their intense flavour. While 'Raúl' and 'Algerie' share ripening dates, 'Amadeo' fruits ripen 10 days later. These vegetal materials represent an opportunity to explore changes in volatile compounds associated with the generation of new loquat cultivars, and to obtain new information about the biosynthetic pathways of flavour compounds and their regulatory mechanisms in this crop.

It is in this context that the present research aimed to explore variation in the volatile composition of 'Algerie' and the three new varieties: 'Xirlero', 'Raúl' and 'Amadeo'. To this end, we took into account that the fruit maturity stage determines the loquat aroma profile (Besada et al., 2013), thus the study was conducted with fruits at two commercial maturity stages. The information generated from the present study can help to identify markers that track flavour-related chemicals. Besides, knowledge of fruit genetic diversity is important for quality improvement, and would be useful for breeding programmes, planning and germplasm, and for developing conservation strategies (Gisbert et al., 2009).

2. Material and methods

2.1. Fruit material

'Algerie' and the three new varieties generated from it, 'Raúl', 'Amadeo' and 'Xirlero', were evaluated herein. Fruits from each cultivar were harvested from adult trees in an orchard located at the Experimental Station of the Cooperativa Agrícola of Callosa d'Ensarria´ (Alicante, Spain). From 17 April to 5 May 2014, fruits were harvested in two commercial maturity stages. The first maturity stage was harvested when fruits had a minimum 10 °Brix content, which is the quality standard to harvest 'Algerie' fruits required in the Spain's main loquat packing-house to avoid over-acid fruits (Pinillos, Hueso, Marcon Filho, & Cuevas, 2011). Since external colour is the non-destructive maturity index for harvesting, pickers are instructed to harvest fruits when the external colour corresponds to an internal TSS 10 °Brix content.

The second maturity stage was harvested when fruits displayed the colour characteristic of each variety namely the colour corresponding to consumption maturity (generally fruits in the second stage were collected 5–7 days after the first stage). Sets of 24 fruits per cultivar were carefully selected from three trees on each harvest day. In each maturation/ripening stage, homogenous lots of six fruits each were assembled, of which three (three replicates per maturity stage) were used for the weight, colour, firmness, acidity, total soluble solids and sensory evaluations. Pulp samples were flash-frozen in liquid N_2 and stored at − 80 °C until the volatile analyses were done (see Section 2.3). The remaining lot of six fruits was used for ethylene and CO_2 measurements. All the analyses were done within 8 h of harvesting.

2.2. Physico-chemical and sensory analysis

Fruit skin colour was evaluated in a Minolta Colorimeter (Model CR-300, Ramsey, NY, USA). The 'L', 'a', 'b' Hunter parameters were measured and the results were expressed as a Skin Colour Index (CI) = (1000a)/(Lb) (Jiménez-Cuesta, Cuquerella, & Martínez-Jávega, 1981).

Flesh firmness was determined after removing peel by a Texturometer Instron Universal Machine model 4301 (Instron Corp., Canton, MA, USA) using an 8-mm flat plunger. The results are expressed as the load in Newtons (N) required for breaking fruit flesh.

Immediately after texture measurements were taken, each fruit was cut into two longitudinal pieces. To determine titratable acidity and total soluble solids (°Brix), one fruit quarter was ground in a mortar and filtered through cheese cloth. The juice obtained from the six fruits of each replicate was analysed. The other quarter was immediately frozen with liquid N_2, and the samples obtained from the six fruits of each replicate were pooled, ground together and kept at − 80 °C until the volatile compounds analysis was done.

The opposite half-fruit was used for the sensory evaluation, which was performed as soon as possible after measuring texture; to this end, the composite samples of six fruits were evaluated from each replicate (three replicates per maturity stage and cultivar). Loquat samples were presented to a panel on trays labelled with 3-digit codes and were served at room temperature (25 ± 1 °C). A semi-trained panel of 8–10 people, who were familiar with loquat fruit, was asked to evaluate the typical loquat flavour intensity on an independent unstructured anchored line scale, where 0 was 'not perceptible' and 10 was 'strongly perceptible'. Panellists were provided with a knife, and were asked to remove the peel and the fruit surface that came into contact with air, to evaluate the flavour intensity of flesh. Panellists were asked to taste several segments of each sample in order to compensate, as far as possible, any biological variation in the material.

The total soluble solids contents of each sample were measured twice with a digital refractometer (model PR1, Atago, Tokyo, Japan) and the results were expressed as °Brix. Titratable acidity was determined with a 0.1 N sodium hydroxide solution and reported as the equivalent g of malic acid per 100 mL of juice.

The ethylene and respiration rates were recorded in three replicates of two fruits per cultivar/harvest date, and were respectively analysed by GC-FID and GC-TCD, as described by Salvador et al. (2005). Ethylene production was expressed as μL C_2H_4 kg^{-1} h^{-1} and the respiration rate was denoted as mL CO_2 kg^{-1} h^{-1}.

2.3. Volatile compounds analysis

2.3.1. The HS-SPME extraction conditions

A volatile analysis was performed essentially as described by Besada et al. (2013), with minor modifications: 500 mg of frozen tissue powder were weighed in a 10-ml crimp cap phial, which was capped and incubated at 30 °C for 10 min. Immediately after incubation, 500 μL of 100 mM EDTA-NaOH (pH 7.5) solution and 1.1 g of $CaCl_2 2H_2O$ were added to terminate endogenous enzyme activity. Phials were closed, mixed by vortex for 1 min and transferred to a GC autosampler to be analysed. A 65-μm PDMS/DVB (Supelco, Bellefonte, PA, USA) fibre was used in all the analyses. Preincubation and extraction were done for 10 and 40 min, respectively, at 70 °C. Desorption was performed at 250 °C