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# Aroma volatiles as biomarkers of textural differences at harvest in non-climacteric near-isogenic lines of melon

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ABSTRACT

The texture and aroma volatiles of two non-climacteric near-isogenic lines (NILs) of melon (*Cucumis melo* L.), SC10-2 and SC7-1, containing introgressions of the Korean cultivar 'Shongwan Charmi' accession PI161375 (SC) in the Spanish cultivar 'Piel de Sapo' (PS) were studied. Data were examined using different supervised and unsupervised univariate and multivariate statistical techniques in order to determine the aroma volatiles most closely associated with differences in melon texture. SC10-2 showed 65% higher flesh firmness, a higher level of pellet juice content and lower flesh juiciness than PS and was harvested at least 7 days later. In SC7-1, only the textural trait, whole fruit hardness, was lower (by 34%) than in PS. About eleven quantitative trait loci for aroma volatiles were mapped in linkage group VII and other nineteen in group X. The aroma compounds discriminated SC10-2 from PS better than SC7-1, as revealed by Partial least squares-discriminant analysis and, to a lesser extent, by Random forest analysis. Around seven aroma volatile compounds, mostly ketones, aldehydes and alcohols, consistently lent support to the discrimination irrespective of the methodology used. Higher levels of several aldehydes (e.g. 2,4-dimethylbenzaldehyde, 2-methylpropanal and 2-methylbutanal), not present in PS, discriminated SC10-2 from PS. Also, SC10-2 lacked some ketones (e.g. 1-phenylethanone), alcohols (e.g. 1-phenylethanol) and one acid (e.g. 2-phenylpropanoic acid) compared with higher content in PS. The former aldehydes and other compounds, such as 3-methylsulfanylpropanal, were the volatiles with the closest association with flesh firmness and harvest, and form part of the phenylalanine, leucine, valine, isoleucine or methionine metabolism and the benzenoid biosynthetic pathway. A negative correlation with the former discriminative compounds of SC7-1 and PS and whole fruit hardness was established. SC7-1 stood out mainly for its higher content of one sulfur-derived compound (S-methyl ethanethioate), one acetate ester (benzyl acetate) and two alcohols (octan-1-ol and (2R, 3S)-3-ethylheptan-2-ol) compared with PS, and one ketone (pentane-2,3-dione), which was absent in PS, but none of these were associated with flesh textural traits.



## 1. Introduction

Melon fruit ripening is a genetically programmed event characterized by a series of physiological and biochemical changes that affect taste traits (sugars, organic acids, amino acids), fruit texture and aroma production, among other quality traits, making the fruit more palatable to consumers (Ezura & Owino, 2008; Saftner & Lester, 2009; Vallone, Sivertsen, Anthon, Barrett, Mitcham, Ebeler, et al., 2013; Villanueva, Tenorio, Esteban, & Mendoza, 2004).

Melon aroma is determined by unique combinations of aroma-active compounds, the proportions of key volatiles as well as the presence or absence of several unique components (Pang, Guo, Qin, Yao, Hu and Wu, 2012; Vallone et al., 2013). Aroma is strongly dependent on the cultivar and physiological behavior of the fruit (Obando-Ulloa, Moreno, Garcia-Mas, Nicolai, Lammertyn, Monforte, et al., 2008). Volatiles derived from amino acids as precursors, particularly esters, are the major contributors to melon aroma (Gonda, Bar, Portnoy, Lev, Burger, Schaffer, et al., 2010).

Texture is one of the most important quality parameters and is partly responsible for consumer preferences of edible fruit (Harker & Johnston, 2008), while softening – partly associated with cell wall polysaccharide changes – is a determining factor in the quality and postharvest life of

*Abbreviations:* ANOVA, Analysis of variance; FDR, False discovery rate test; FF, flesh firmness; FJ, flesh juiciness; GC-MS, gas-chromatography mass-spectrometry; JD, juice density; JJ, juiciness; LG, linkage group; LRI Cal, calculated linear retention index; LRI, linear retention index; NID, unidentified compound; NILs, near-isogenic lines; NIST, National Institute for Standards and Technology; PJ, pellet content of the juice; PLS-DA, Partial least square-discriminant analysis; PS, Spanish cultivar 'Piel de Sapo'; RF, Random forest; SC, Korean cultivar 'Shongwan Charmi' accession PI161375; SPME, Solid phase micro-extraction; QTL, quantitative trait loci; VIM, Variable importance measures; VIP, Variable importance in the projection; WFH, whole fruit hardness.

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fruit (Dos-Santos, Jiménez-Araujo, Rodríguez-Arcos, & Fernández-Trujillo, 2011). Harvesting firm and early mature fruits is a commercial practice commonly adopted to maximize post-harvest life, but this practice can modify the fruit aroma profile, and is counterproductive to the flavor and quality, especially in climacteric fruit (Navarro, 1997; Vallone et al., 2013). Fruits with very soft flesh or that are over-mature are also rejected by consumers (Abrahão, Miguel, Dias, Spoto, & da Silva, 2009), particularly for the fresh market, due to loss of volatiles during storage (Amaro et al., 2013).

Aroma biosynthesis and textural changes during fruit ripening and senescence have a certain correlation due to a matrix effect. The aromas trapped in the network formed by the polysaccharides modify flesh and juice viscosity (Bezman, Mayer, Takeoka, Buttery, Ben Oliel, Rabinowitch, et al., 2003; Harker & Johnston, 2008; Savary, Guichard, Doublier, & Cayot, 2006). Matrix effect also can result in differential substrate supply due to differences in membrane peroxidation catalyzed by lipoxygenases (Harker & Johnston, 2008; Whitaker & Lester, 2006).

One near-isogenic line (NIL) of melon (SC10-2) contains quantitative trait loci (QTL) with a positive effect on flesh firmness (Moreno, Obando, Dos-Santos, Fernández-Trujillo, Monforte and Garcia-Mas, 2008) and also in juiciness retention in refrigerated fresh-cut cubes of SC10-2 (Gomes, Fundo, Obando-Ulloa, Almeida, & Fernández-Trujillo, 2009). Also, the aroma profile and, to a lesser extent textural traits, are efficient tools for discriminating climacteric NILs from non-climacteric ones (Obando-Ulloa, Jowkar, Moreno, Souri, Martínez, Bueso, et al., 2009; Obando-Ulloa et al., 2008).

The goal of this work was to map QTLs and select the most discriminant flesh aroma volatiles as potential biomarkers of textural differences (especially flesh firmness) due to introgressions in melon chromosomes VII and X. The usefulness of combining univariate and multivariate statistical methods and aroma pathways potentially involved in textural differences between PS and NILs are also discussed.

## 2. Materials and methods

### 2.1. Plant material

The non-climacteric melon near-isogenic lines were obtained through repeated backcrossing between non-climacteric parentals, the Spanish melon *Cucumis melo* L., Inodorus group, cultivar T111, of the 'Piel de Sapo'-type (PS) and the exotic Korean accession PI 161375 (SC; *Cucumis melo* L. var. 'Shongwan Charmi' sp. *Agrestis*, Conomon group) (Eduardo, Arús, & Monforte, 2005; Moreno et al., 2008). Two non-climacteric NILs (SC7-1 and SC10-2) with introgressions of SC in a single linkage group (LG) of the PS genetic background as mapped by Eduardo et al. (2005) were tested. The first numbers (7 or 10) refer to the LG containing the introgression (i.e. in the chromosomes VII and X, respectively) (Dos-Santos et al., 2011; Tijskens, Dos-Santos, Jowkar, Obando, Moreno, Schouten, et al., 2009). Fruit quality traits of both NILs were compared with those of the PS parental.

### 2.2. Experimental design

Melons were cultivated in Mediterranean conditions in Torre Pacheco (Murcia, Spain) according to the growing practices commonly used for this crop. The number of replicates was 21 for PS and 3 and 5 replicates, respectively, for NILs SC10-2 and SC7-1 (Fernández-Trujillo, Obando, Martínez, Alarcón, Eduardo, Arús and Monforte, 2005; Obando, Fernández-Trujillo, Martínez, Alarcón, Eduardo, Arús, et al., 2008; Tijskens et al., 2009).

### 2.3. Textural traits

Flesh juiciness (FJ), juice density (JD), juiciness (JJ) and pellet content of the juice (PJ) were measured according to the methodology described in Dos-Santos et al. (2011) and Obando et al. (2008). The results were expressed in grams of juice per kg fresh weight, grams of juice per mL juice, grams of juice per kg juice and grams of pellet per kg juice.

Whole fruit hardness (WFH) was determined at the equator as previously reported measuring the compression force to achieve 2 mm deformation (Tijskens et al., 2009). Flesh firmness (FF) was measured with a puncture test using cylinders (L = 20 mm; Ø = 15 mm) obtained with stainless steel apple corers from the equator of the fruit and a 4.6 mm wide probe (TG83, SAE Ibertest, Madrid, Spain) adapted to a testing machine (ELIB-5K, SAE Ibertest) (Fernández-Trujillo et al., 2005).

### 2.4. Juice sampling and volatile compounds analysis

The methodology to determine the volatile aroma composition of melon juice by constant flow gas-chromatography mass-spectrometry (GC–MS) was adapted from Obando-Ulloa et al. (2008). The volatile compounds were measured from vials stored at −80 °C with a solution containing melon juice and saturated calcium chloride. The previously tempered juice was poured into a 10 mL glass vial (Gerstel, Germany) and then an internal standard (10 μL of phenyl–ethyl alcohol 0.01% v/v; Merck, Spain) dissolved in 1-mL deionized water (Type I) was added. We added this amount to each vial before analysis. The volatiles were analyzed by solid phase micro-extraction (SPME) and identified by gas-chromatography mass-spectrometry, according to Obando-Ulloa et al. (2008). Briefly, the 1-cm long SPME fiber was a 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane coating (57329-U DVB/Carboxen™/PDMS Stable Flex™ Fiber, Supelco, Bellefonte, PA, USA). Fiber was automatically preconditioned before extraction in the injection port at 250 °C for 1 h and remained for 30 min at 35 °C absorbing the volatiles of the vial.

The analyses were conducted with a MPS2 Gerstel Multipurpose sampler coupled to the 6890 GC coupled to a mass spectrometer 5975 with a hyperbolic quadrupole (Agilent Technol.). The injection port was operated at 280 °C in a splitless mode and subjected to a pressure of 80 psi. The liner was a 78.5 mm × 6.5 mm × 0.75 mm internal diameter (SPME/direct, Supelco). Volatiles were separated according to Obando-Ulloa et al. (2008) on a 30 m × 0.25 mm id × 0.25 μm thickness capillary column (HP-5MS UI, Agilent Technol.). The inlet liner used was a 2637505 SPME/direct (Supelco), 78.5 mm × 6.5 mm × 0.75 mm. Chromatographic-grade helium was used as the carrier gas with a flow rate of 1.5 mL/min. The variable temperature program was an initial oven temperature of 35 °C, followed by a ramp of 2 °C/min up to 75 °C, and then at 50 °C/min to reach a final temperature of 250 °C, which was held for 5 min. Mass spectra were obtained by electron ionization (EI) at 70 eV, and a spectrum range of 40–450 m/z was used. The detector worked at 230 °C and in full scan with data acquisition and ion mass captured between 30 and 300 amu. The total flow 54.4 mL/min.

The chromatograms and mass spectra were evaluated using the ChemStation software (G1791CA, Version C.00.00, Agilent Technol.). The compounds were tentatively identified by comparing their mass spectra with those included in the National Institute for Standards and Technology (NIST05a.L, search version 2.0) data bank and by comparing with linear retention indices (LRI) reported in the literature or NIST database (http://webbook.nist.gov/chemistry/cas-ser.html) (Obando-Ulloa et al., 2008).

Volatiles were classified into ten classes of compounds (acetate esters, acids alcohols, aldehydes, alkanes, ketones, non-acetate esters, sulfur-derived compounds, others and terpenes) and all the areas of the compounds were added to the corresponding class and percentages were based on total area of the identified compounds.

For individual aroma volatile compounds, the area of each compound was normalized according to the internal standard and the results are reported as percent of normalized area of each compound divided by total area counts of the identified compounds. In a separate