Differential aroma volatiles in non-climacteric near-isogenic lines of melon as biomarkers of differences of flesh firmness at harvest

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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 multivariate statistical techniques in order to determine the most discriminant aroma volatiles, as analyzed by gas-chromatography mass-spectrometry, which could be associated with differences in ripening and flesh firmness in non-climacteric melons. The NILs and the parental showed non-climacteric behaviour during ripening. SC10-2 was harvested at least 7 days later than the control. At harvest, only the NIL SC10-2 showed 65% higher flesh firmness than PS. Whole fruit hardness of SC7-1 was 34% lower than PS. The results obtained by random forest analysis showed that the aroma discriminated SC10-2 better than SC7-1 from the control, with scarce differences between SC7-1 and PS. The aldehydes and the ketones were the most discriminating volatile groups among the NILs studied and PS. Higher levels of several aldehydes (2-methylpropanal, 2,4-dimethylbenzaldehyde / 3,4-dimethylbenzaldehyde, 2-methylbutanal), not present in PS line, discriminated the NIL SC10-2 from the control PS. Also, SC10-2 displayed lack of some ketones (1-phenylethanone) and lower presence of alcohols (for example 3,5-dimethylcyclohexan-1-ol) compared with PS. The NIL SC7-1 stood out for it higher relative content in alcohols (Z)-non-3-en-1-ol, 3-ethylheptan-2-ol) and one acetate ester (benzyl acetate) than PS and the presence of ketone (pentane-2,3-dione), which was

absent in PS. The results are discussed in terms of the aroma biosynthesis pathways that could be affected by the introgressions.

INTRODUCTION

Fruit texture is an essential quality trait for the melon market and particularly in fresh-cut form (Toivonen and Brummell, 2008). Texture and flavor are the most important quality parameters in melon fruit (Lester, 2006) and both are bound together by the metabolic processes that direct fruit ripening. This tightly interlinked and often inescapable tripartite interaction between ripening, texture change and flavor development means that it is often difficult to specifically attribute consumer preferences either texture or flavor (Harker and Johnston, 2008). Over-mature fruit or with very soft flesh (senescent) are rejected by consumers (Abrahão et al., 2009). Usually, 'Piel de Sapo'-type melons are appreciated for their firm, juicy and crunchy texture (Escribano et al., 2010; Ferrer et al., 2007). However, enhanced flesh firmness in cultivars such as 'Galia' or 'Cantaloupe' is associated by consumers with reduced fruit flavor (Navarro, 1997).

Softening-associated with cell wall degradation and aroma biosynthesis could be a parallel, but independent process. However, some evidence suggests a certain link between softening and aroma biosynthesis. For example, reduced pectolytic enzyme activity, such as polygalacturonase and pectinmethylesterase (PME), affects tomato aroma (Baldwin et al., 2000). The explanation for such effects could be a matrix effect due to the aroma trapped in the network formed by polysaccharides and differences in viscosity (Bezman et al., 2003; Harker and Johnston, 2008; Savary et al., 2006). Another explanation could be a differential substrate supply due to differences in membrane peroxidation, which is catalyzed by lipoxygenases (LOXs) during fruit ripening and senescence (Harker and Johnston, 2008; Whitaker and Lester, 2006).

At least two near-isogenic lines (NILs) of melon contain QTLs with a positive effect on flesh firmness (Moreno et al., 2008). The higher flesh firmness of one of the firmest NILs (SC7-2) was associated with higher neutral sugar (especially galactose) and uronic acid contents, together with a larger cellulose and α-cellulose residue than the control PS (Dos-Santos et al., 2011). When the near-isogenic line SC10-2 containing an introgression in linkage group (LG) X of melon and quantitative trait loci (QTLs) was processed in cubes, firmness and juiciness were improved during the shelf-life at low temperature (Gomes et al., 2010).

The goal of the present paper is to report the potential effects of this enhanced firmness in flesh aroma volatiles, and to identify potential aroma pathways involved in these differences.

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 Korean accession PI 161375 (SC; *Cucumis melo L. var.* 'Shongwan Charmi' *sp. Agrestis,* Conomon group) (Eduardo et al., 2005; Moreno et al., 2008). Two non-climacteric NILs (SC7-1 and SC10-2) with small introgressions of SC in the PS genetic background were used. The first number refers to the linkage group containing the introgression (i.e. in the chromosomes VII and X, respectively), (Dos-Santos et al., 2011; Tijskens et al., 2009). Fruit quality of both NILs was evaluated in comparison to the PS parental.

2.2. Experimental design.

Melon cultivation was carried out 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 (Tijskens et al., 2009).

2.3. Fruit texture.

Whole fruit hardness was determined at the equator as previously reported measuring the compression force to achieve 2 mm deformation (Tijskens et al., 2009). Flesh firmness was measured with a puncture test using cylinders (20 x 15 mm) obtained with stainless steel apple corers (Alexalo 8005, Aleissi SCCL, Barcelona, Spain) snd 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 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 volatiles were measured from vials stored at -80°C with a solution containing melon juice and calcium chloride. The juice, previously

tempered, 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) was added. The volatiles were analyzed by solid phase micro-extraction (SPME). A gas chromatograph HP 6890N GC series II (Hewlett-Packard, Agilent Technologies Inc, Palo Alto, CA, USA) coupled to a mass detector HP 5973 (Hewlett-Packard) was used. The liner was a 78.5 mm × 6.5 mm × 0.75 mm internal diameter (SPME/direct, Supelco, Bellefonte, PA, USA). The total flow 54.4 $mL \cdot min^{-1}$.The chromatograms were manually integrated using the ChemStation software (G1701DA D.02.00.275, Agilent Technologies Inc, USA) and experimental spectra were compared with those of the National Institute for Standards and Technology (NIST05a.L, search version 2.0) data bank. Volatiles were classified in ten groups of compounds (alcohols, aldehydes, ketones, terpenes, acetate esters, non-acetate esters, sulphur-derived compounds, acids, alkanes and others) in a format specially designed in Excel software by Fernández-Trujillo (unpublished) that adds all the areas of the chemical compounds to the corresponding group.

For individual aroma volatile results, the area of each compound was normalized acceding to the internal standard and the results are reported as percent of normalized area of each compound divided by total area counts of the compounds identified. In a separate analysis normalized area were also used. When data were not detected, empty cells were automatically considered as zero for the analysis. The retention times from a series of straight-chain alkanes (C6-C20) supplied by Sigma were used in identical conditions to calculate the linear retention indices for all the identified volatile compounds and compared with data reported in the literature or compiled in the NIST database (http://webbook.nist.gov/chemistry/cas-ser.html).

2.5. Statistical analysis.

Variables representing compounds not exhibiting homogeneous behavior and with a presence below 50% of the replicates analyzed were discarded. Data were examined using univariate and multivariate statistical techniques. First, a graphical exploratory analysis of the volatile aroma data was made by representing the box and whisker plots of the individual aromas volatiles for the control PS and the NILs SC10-2 and SC7-2 to visualize the differences between each group of melons as well as to detect the outliers. Next, we performed a Dunnett's test to identify what means were significantly different between PS (control) and each NIL at significance level of

P=0.05. Sometimes the original data were transformed into their respective logarithm for normalize the variable.

In order to apply the multivariate statistical analysis, the data were means centered and scaled to unit variance and submitted to a Principal Components Analysis (PCA), Random Forest (RF) and Partial Least Square-Discriminant Analysis (PLS-DA). All statistical analysis was made using the R free software environment version 2.14.1 (2011-12-22) and the FactoMineR (Huson et al., 2012), caret (Kuhn, 2013) and randomForest (Liaw and Wiener, 2002) packages for PCA, PLS-DA and RF, respectively. Data of this paper are based on RF results. RF provided two index (*mean decreases in Gini index*, *MDG*; *mean decreases in accuracy*, *MDA*) useful to quantify the relative contribution of each variable to the classification (Liaw and Wiener, 2002).

RESULTS

The NIL SC10-2 had higher flesh firmness (11.2 N) than PS (6.8 N) but similar whole fruit hardness (58±2 $N \cdot mm^{-1}$). The NIL SC7-1, which had lower fruit hardness than PS (36.9 vs. 56.9 $N \cdot mm^{-1}$, respectively), had similar flesh firmness (7.1 N). These textural differences served as a basis for subsequent association between texture and aroma volatiles.

Overall, 444 aroma volatile compounds were tentatively identified in the headspace of the non-climacteric melon NILs and the parental (data not shown), although only 178 compounds were preliminarily considered in the statistical analysis. Here, we only present the results relative to the RF classification method. Multidimensional scaling (MDS) plots visualized the RF dissimilarity graphically (Fig. 1). The first twenty five compounds selected as the most discriminant volatiles among the NILs and the control PS by RF analysis (Table 1) were eight alcohols, six ketones, four aldehydes, three alkanes, two sulphur-derived compounds, one non-acetate esters, one acetate ester.

Other five compounds (reported below by name, CAS, percentage of samples and percentage of total area) were considered qualitatively discriminant (not included in Table 1) because of their presence in traces or consistently in less than 40% of the samples of the lines analyzed. Three compounds were exclusively present in SC7-1: 3,5,5-trimethylcyclopent-2-en-1-one (024156-95-4; < 30% samples, 0.09% total area) , 2-methylpropyl propanoate (000540-42-1; < 20% samples, 0.09% total area), 2-

methylbenzaldehyde / 4-methylbenzaldehyde (000529-20-4 / 000104-87-0 ; < 30% samples, 0.12% total area). Anther compound present in NIL SC10-2 but not in PS was Propan-2-yl hexanoate (002311-46-8; 40% samples, 0.27% total area). Finally, 2-methylbenzofuran (004265-25-2) was present at a higher percentage in NIL SC7-1 (0.29%; < 40% samples) than in PS (0.12%; < 11% samples) or SC7-1 (0.13%; 20% samples). Other compounds such as silane and siloxane derivatives, or volatile organic compounds associated with contamination, plastic composition, and the SPME fiber were discarded (data not shown).

The main results in aroma volatiles using total area or percentages were similar, and, consequently, only percentages are reported here. The firmer NIL SC10-2 was mainly characterized by a higher content in aldehydes (2-methylpropanal, 2,4-dimethylbenzaldehyde / 3,4-dimethylbenzaldehyde, 2-methylbutanal). These volatiles and one ketone ((1-(2,4,5-trimethylphenyl)ethanone)) were not detected in PS (Table 1). The aldehydes 2-methylbutanal and 2-methylpropanal are potent flavor compounds and are regarded as products key-flavor compounds in many foods (Smit et al., 2009). The alcohol 3,5-dimethylcyclohexan-1-ol had a lower content in SC10-2 than in PS, while two ketones (1-phenylethanone; 1-(3-methylphenyl)ethanone / 1-(2-methylphenyl)ethanone) were not present in SC10-2 (Table 1).

The NIL SC7-1 showed greater similarities with the PS aroma than SC10-2. Several compounds showed higher values in NIL SC7-1 than in PS (Table 1): two sulphur-derived esters, methyldisulfanylmethane and 1-methylsulfanylethanone, two alcohols (3-ethylheptan-2-ol; (Z)-non-3-en-1-ol), and benzyl acetate. The ketone pentane-2,3-dione was absent in PS and present in SC7-1 (Table 1).

In the MDS plots (Fig. 1), the first component discriminated the NIL SC7-1 (left MD1 axis) from PS (right MD1 axis) and the second component discriminated the NIL SC10-2 (top MD2 axis) from PS (bottom MD2 axis), and the greater similarity between SC7-1 and PS was evident (Table 1). The results point to an effect of the introgression on the aroma content in the PS genetic background irrespective of the chromosome considered.

The main putative aroma precursors identified for the discriminant compounds were fatty acids (i.e. linolenic or linoleic acid) and the amino acids (methionine, leucine, isoleucine, valine, threonine and phenylalanine) (Table 2). As regards the potential aromatic notes, the main ones were fruity, green, sweet, floral, and burn (Table 2). Finally, all the compounds reported above showing significant differences between each

NIL and PS represent potential QTLs for these compounds mapping in LGs VII or X. In total, three QTLs were tentatively mapped, two mapping in LG VII and related with the methionine pathway and therefore with higher levels of sulphur-derived compounds than PS, and ketones, such as pentane-2,3-dione, obtained from methanethiol following enzymatic degradation of amino acid methionine. Other QTLs were associated with enhanced alcohol content (and perhaps with fatty acid metabolism) in LG VII. Another QTL in LG X was related with higher levels of aldehydes derived from amino acid metabolism than in PS

DISCUSSION

In agreement with previous results (Obando-Ulloa et al., 2010), the non-climacteric parental PS showed many aroma compounds, but particularly the aroma profile was composed of aldehydes and to a lesser extent alcohols and ketones (data not shown). Due the importance of aldehydes as discriminant compounds in this experiment (Table 1), fatty acids and four free amino acids (leucine, isoleucine, valine and phenylalanine) can be considered the putative precursors of aroma (Feussner and Wasternack, 2002; Gigot et al., 2010; Gonda et al., 2010, Smit et al., 2009) and could be the key to finding candidate genes located in LG VII, but especially in LG X. Obando-Ulloa et al. (2010) were not able to find significant differences in aroma profile between SC10-2 and PS, although SC7-1 was not covered by this study**.**

Hexanal showed the highest relative content with respect to other aldehydes, with no differences between the lines (data not shown). The predominance of hexanal is in agreement with previous results with PS and other non-climacteric NILs with introgression in LG III (Obando-Ulloa et al., 2008). No QTLs associated with hexanal content were mapped in linkage groups VII or X. On the other hand, the amino acid methionine has been reported as precursor of several compounds identified in NIL SC7-1, and therefore a potential QTL associated with conversion of methionine into aroma volatiles in LG VII is proposed.

The biosynthetic pathways of valine, isoleucine and other amino acids and free polyunsaturated fatty acids, such as linoleic and linolenic acids, converge to form aldehydes. The beta-oxidation pathway of these fatty acids as substrates of LOXs and hydroperoxide lyases (HPLs) forms these aldehydes, which are then reduced to alcohols in a reaction catalyzed by alcohol dehydrogenase (ADH) (Gigot et al., 2010; Gonda et al., 2010; Smit et al., 2009). Increased activities of LOXs and phospholipases (PLDs)

have been linked with senescence of muskmelon fruit tissues (Whitaker and Lester, 2006). The higher aldehyde values could be explained by delayed senescence and higher flesh firmness in NIL SC10-2 and perhaps the reduced conversion of aldehydes into alcohols and the subsequent conversion into esters. As an example, we show compounds that may result from the lack of conversion of two aldehydes derived from amino acids found in SC10-2 (Table 2). The aldehyde 2-methylbutanal is converted into butanol-2-methyl acetate, a typical acetic ester present in climacteric melon fruits (Pang et al., 2012; Qi et al., 2012), while 2-methylpropanal is converted into 2-methyl propanoic acid or 2-methyl propanol (Smit et al., 2009).

The benzyl acetate found in NIL SC7-1 (Table 1) is among the group of esters that are predominant in certain melon types (Song and Forney, 2007). The benzyl acetates are usually produced via the shikimic pathway. The highest values of benzyl acetate in NIL SC7-1 could be related with a greater conversion of benzaldehyde in benzyl acetate from amino acids via transamination, as indicated by the results in melon cubes (Gonda et al., 2010), although ssome degree of cultivar-dependence exists in the production of benzyl acetate (Mahmuda and Ueda, 2008). Methionine is an amino acid used by plants for ethylene synthesis in the process known as the Yang or methionine cycle. Methanethiol is obtained by enzymatic degradation of cysteine from methionine, which is a sulphur-derived compound that acts as an intermediary in forming other sulphur-derived compound (Varlet and Fernández, 2010), such as methyldisulfanylmethane and 1-methylsulfanylethanone, with higher content in SC7-1 (Table 1). Methanetiol has been previous reported in melon NILs (Obando-Ulloa et al., 2010). The ketone, pentane-2,3-dione present in NIL SC7-1 and absent in PS (Table 1) is another compound obtained in certain microorganisms via the catabolism of methionine as product of 4-methylthio-2-oxobutyric acid degradation (Arfi et al., 2006).

Studies in fruits indicate that the relationship between the physicochemical properties of the volatile and the food matrix can modify the concentration of the aroma compounds in the headspace (Bezman et al., 2003). In the cell wall structure, high levels of uronic acids, neutral sugars (especially galactose), cellulose and α-cellulose residue have been related with higher flesh firmness in another non-climacteric NIL (SC7-2). The role of the cellulosic fraction is important and contributes to maintaining and strengthening the network structure observed microscopically. A higher yield of galactose and uronic acids favors the greater interaction with cellulose microfibers and is probably associated with decreased PME, the hydrolase that acts in the

depolymerization of structural polysaccharides (Dos-Santos et al., 2011; Harker and Johnston, 2008). When cell disruption occurs, previously compartmentalized enzymes and substrates mix and new volatiles are formed (Baldwin et al., 2000).

The results of our study support the hypothesis that different strength among NIL SC10-2 and PS could partly explain the different aromatic composition of both non-climacteric lines. The more resistant matrix of polysaccharides would act to protect aldehydes of enzymatic reactions, thus affecting the formation of esters or alcohols.

Texture, flavor and storability are recognised as important traits in any fruit breeding program. Such programs exploit biodiversity amongst wild and cultivated genotypes to produce progeny with a diverse range of textural and flavor characteristics (Harker et al., 2008). The QTLs identified here could help in the selection of seedlings with promising traits and their subsequent incorporation in crosses for further genetic improvement. Also, the association between texture and aroma volatiles can help in designing new treatments to maintain better flesh firmness in melons.

CONCLUSION

The non-climacteric NIL SC7-1, but particularly SC10-2, showed differences in texture and aroma volatiles in relation to the control PS. The higher firmness in NIL SC10-2 could be related to the higher aldehyde content, but the lower whole fruit hardness in SC7-1 seems to be independent of differences in the flesh aroma content. At least two QTLs located in LG VII and one QTL in LG X could be responsible for the differences of many volatiles, particularly sulphur-derived compounds and ketones related with the methionine metabolic pathway, and other QTL related with the production of alcohols in NIL SC7-1, and one QTL related with aldehyde production and amino acid metabolism in SC10-2.

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TABLES AND FIGURES

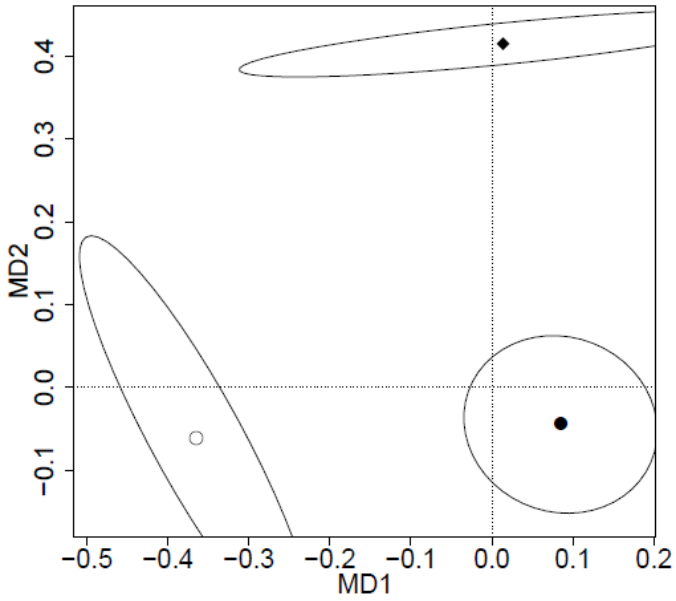

Figure 1. Discrimination among the near-isogenic lines (NILs) SC7-1 (○) and SC10-2 (◆) and the parental control 'Piel de Sapo' (PS,●) using multidimensional scaling (MDS) plots based on random forest (RF) analysis applied to the individual aroma volatiles. Centroids of the datasets for the different lines and 65% confidence ellipses.

Table 1. Main aroma volatiles identified in the near-isogenic line (NILs SC7-1 and SC10-2) and the parental line 'Piel de Sapo' (PSC). The compounds sere arranged according to the random forest (RF) variable importance. Data were the mean relative content in percentage of the probable aromatic compounds identified.

Order	CAS Number	IUPAC Name	Group of compound	PS	SC10-2		SC7-1	
1	000600-14-6	pentane-2,3-dione	KET	0.00	0.00		**0.17**	*
2	000140-11-4	benzyl acetate	ACE	0.31	0.31		**1.76**	*
3	000078-84-2	2-methylpropanal	ALD	0.00	**0.06**	*	0.03	
4	015764-16-6	2,4-dimethylbenzaldehyde	ALD	0.00	**0.73**	*	0.00	
5	002040-07-5	1-(2,4,5-trimethylphenyl)ethanone	KET	0.00	**0.12**	*	0.00	
6	000096-17-3	2-methylbutanal	ALD	0.00	**0.08**	*	0.02	
7	000111-87-5	octan-1-ol	ALC	0.09	0.21		0.58	
8	068920-64-9	methyldisulfanylmethane	SDC	0.27	0.35		**0.52**	*
9	000583-57-3	(1R,2S)-1,2-dimethylcyclohexane	ALK	0.00	**0.14**	*	0.00	
10	019780-39-3	3-ethylheptan-2-ol	ALC	0.06	0.00		**0.49**	*
11	001534-08-3	1-methylsulfanylethanone	SDC	0.09	0.00		**0.42**	*
12	000098-86-2	1-phenylethanone	KET	1.70	**0.00**	*	0.69	
13	000629-59-4	tetradecane	ALK	0.15	**0.42**	*	0.22	
14	005441-52-1	3,5-dimethylcyclohexan-1-ol	ALC	0.33	**0.09**	*	0.29	
15	000112-40-3	dodecane	ALK	0.04	0.09		0.11	
16	018829-55-5	(E)-hept-2-enal	ALD	0.40	0.49		0.53	
17	ND	3-methyloctan-2-ol	ALC	0.36	0.71		0.38	
18	074367-33-2	(1-hydroxy-2,4,4-trimethyl-pentan-3-yl) 2-methylpropanoate	NAE	1.21	0.78		0.59	
19	000624-16-8	decan-4-one	KET	0.15	0.42		0.38	
20	000585-74-0 / 000577-16-2	ethanone, 1-(3-methylphenyl) / ethanone, 1-(2-methylphenyl)-	KET	0.33	**0.00**	*	0.22	
21	003214-41-3	octane-2-5-dione	KET	0.05	0.11		0.12	
22	000617-94-7	2-phenylpropan-2-ol	ALC	2.71	**0.23**	*	2.31	
23	000111-27-3	hexan-1-ol	ALC	0.11	0.07		0.07	
24	010340-23-5	(Z)-non-3-en-1-ol	ALC	0.22	0.24		**0.50**	*
25	004621-04-10	4-propan-2-ylcyclohexan-1-ol	ALC	0.37	**1.73**	*	0.39	

NIL means within rows highlighted with * and bold showed statistical differences from PS data, according to a Dunnett's test at P = 0.05. Abbreviations. Chemical Abstracts Service (CAS), International Union of Pure and Applied Chemistry (IUPAC), Acetate Esters (ACE), Alcohols (ALC), Aldehydes (ALD), Alkanes (ALK), Ketones (KET), Non-Acetate Esters (NAE), Others (OTH), Sulphur-Derived Compounds (SDC), Terpenes (TER).

Table 2. Main aroma volatiles identified in the near-isogenic line (NILs SC7-1 and SC10-2) and the parental line 'Piel de Sapo' (PS). The compounds were arranged according to the random forest (RF) variable importance. Volatile precursors and aromatic notes identified in some of the individual aroma volatiles identified in both near-isogenic lines (NILs SC7-1 and SC10-2) and the control the parental 'Piel de Sapo' (PS).

Order	IUPAC NAME	Group of compound	LRI Cal.	LRI Ref,	Ref.	RT	Precursors		Ref.	Aromatic Notes	Ref.
1	pentane-2,3-dione	KET	698	786	1	2,639	AA	Thr/Met	2,3	Caramel, sweet, fruity, buttery, fresh	27
2	benzyl acetate	ACE	1177	1165	1	21,419	AA	Phe	4	Floral, burnt, boiled zucchini	27
3	2-methylpropanal	ALD	ND	552	1	1,573	AA	Leu/Val	5	Green, pungent, burnt, malty, toasted, fruity	27
4	2,4-dimethylbenzaldehyde	ALD	1220	1175	1	21,892	AA	Phe	6,7	Naphthyl, cherry, almond, spice, vanilla	28
5	1-(2,4,5-trimethylphenyl)ethanone	KET	1189	ND	ND	21,598	ND			ND	
6	2-methylbutanal	ALD	653	682	1	2,239	AA	Iso	4,8, 9	Green, almond, strong burnt, malty, cocoa	27
7	octan-1-ol	ALC	1075	1087	1	18,578	ND		10	Fatty, green, herbal	29
8	methyldisulfanylmethane	SDC	745	743	1	3,430	AA	Met	11	Sulfurous, vegetable, cabbage, onion	28
9	(1R,2S)-1,2-dimethylcyclohexane	ALK	1180	ND	ND	21,454		Cyclohex. Path	12	Fuity, floral, vegetable	30
10	3-ethylheptan-2-ol	ALC	1078	ND	ND	18,870	ND			ND	
11	1-methylsulfanylethanone	SDC	701	ND	1	2,688	AA	Met	13,14,15	Sulfurous, eggy, cheese, dairy ,vegetable, cabbage	28
12	1-phenylethanone	KET	1070	1070	1	18,304		α-Methsty.	16,17,18	Sweet, pungent, hawthorn, mimosa, almond, acacia, chemical	28
13	tetradecane	ALK	1400	1400	1	22,850	ND			Mild herbaceous, sweet, fusel.like	27
14	3,5-dimethylcyclohexan-1-ol	ALC	996	ND	ND	13,535	ND			NDc	
15	dodecane	ALK	1199	1200	1	21,762	ND			Fusel-like	27
16	(E)-hept-2-enal	ALD	956	957	1	11,312	FaOx.	LA	19	Sulfury, grassy, fatty, almond.like, pesticide, onion, mushroom, earthy	27
17	3-methyloctan-2-ol	ALC	1081	ND	ND	18,995	ND			ND	
18	(1-hydroxy-2,4,4-trimethyl-pentan-3-yl) 2-methylpropanoate	NAE	1358	ND	ND	22,675	AA	Cys/Met	20	ND	
19	decan-4-one	KET	1149	ND	ND	20,979	ND			ND	
20	ethanone, 1-(3-methylphenyl) / ethanone, 1-(2-methylphenyl)-	KET	1152	ND	ND	21,020		Acetoph.	21	ND / Sweet, hawthorn, powdery, anisic, coumarinic, phenolic, burnt, nutty, honey	28
21	octane-2-5-dione	KET	988	1118	1	13,069	FaOx.	Eicosap. acd.	22,23	ND	
22	2-phenylpropan-2-ol	ALC	1086	1080	1	19,327	AA	Phe	6, 24	Mild, green, sweet, earthy	28
23	hexan-1-ol	ALC	875	880	1	7,237	Faox.	LA	25,26	Flowery, toasty, dry, fruity, herbal, mild woody, sweet, green grass, leafy	27
24	(Z)-non-3-en-1-ol	ALC	1170	1156	1	21,297	Faox.	LA	26	Sweet, green	28
25	4-propan-2-ylcyclohexan-1-ol	ALC	1150	ND	ND	20,998	ND			Leather, red rose, green, dusty, weedy,	28

metallic

Identificative Compound Number (ID.Nº). International Union of Pure and Applied Chemistry (IUPAC). Linear Retention Time (LRI). Average Retention Time (RT) in minutes. Linear Retention Time References (LRI Ref.). Abbreviations: α-Methsty. (alfa-methylstyrene), Amino acid (AA), Ciclohexene pathway (Ciclohex. Path.), Cysteine (Cys), Eicosapentanoic acid (Eicosp. acd.), Fatty acid oxidation (FaOx), Isoleucine (Iso), Leucine (Leu), Linolenic Acid (LA), Methionine (Met), Mevalonate pathway (Mev. Path.), Not detected (ND), Not detected, but synthetic compound available in the market (NDc), Phenylalanina (Phe), Literature references (Ref.), Terpenes (Terp), Threonine (Thr), Valine (Val).

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