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Analytical Methods

Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components

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ABSTRACT

The volatile compounds of four peach cultivars (*Prunus persica* L.) were studied: Sudanell, San Lorenzo, Miraflores and Calanda (two clones, Calante and Jesca). 17–23 Samples of each cultivar with the same maturity level were analyzed, measuring color, firmness, and soluble solids content. The pulp was crushed and mixed with water prior to HS-SPME analysis, and GC–MS was used to determine the volatile compounds. Sixty-five compounds were identified using spectral library matching, Kovat's indices and, when available, pure standards. The main components were lactones and C6 compounds. From the distribution of these compounds, Principal Component Analysis led to the clustering of the samples according to their different cultivars. Finally, Canonical Component Analysis was used to create a classification function that identifies the origin of an unknown sample from its volatile composition. The results obtained will help to avoid fraud and protect the European Designation of Origin 'Melocotón de Calanda'.

1. Introduction

Calanda peach (*Prunus persica* L. cv. Calanda) is a yellow-fleshed indigenous cultivar from Spain, originating from the region around Calanda, Aragón. It is a late season peach, harvested from mid-September to November. It has a uniform pale-yellowish color, large size and intense flavor. At least nine weeks before harvest time, bagging is performed to protect the fruit from insects and diseases. Bagging also improves the fruit skin color and increases volatile aroma content (Jia, Araki, & Okamoto, 2005). The carefully controlled production results in the outstanding gourmet quality of the fruit. The Calanda peach has been registered by the European Union as the Protected Designation of Origin (PDO) "Melocotón de Calanda". Three different clones are included within the PDO: Jesca, Calante and Evaisa. The PDO has led to an expansion of export destinations for which active packaging has recently been developed to extend the shelf-life of the harvested fruit (Montero-Prado, Rodriguez-Lafuente, & Nerin, 2011).

Due to its high added value, "Melocotón de Calanda" is susceptible to fraudulent imitation and the deceptive marketing of similar fruit that lacks the required quality. This results in the image and the business of the genuine fruit being seriously damaged. Identifying the origin of the fruit requires the measurement of an unequivocal characteristic that allows Calanda peach to be clearly differentiated from other peach cultivars. Visual traits or physical properties are not sufficient to certify the origin of the peach, so a more sophisticated approach is needed.

The volatile composition of peach has been thoroughly studied, leading to the identification of more than one hundred volatile compounds. The most abundant components are C6 compounds, linalool, benzaldehyde, esters, terpenoids, C13 norisoprenoids, ketones and lactones (Horvat & Chapman, 1990; Jennings & Sevenant, 1964; Sevenant & Jennings, 1966). The flavoring properties derive from lactones, and particularly γ- and δ-decalactones, with smaller contributions from C6 aldehydes, alcohols and terpenoids (Do, Salunkhe, & Olson, 1969; Horvat & Chapman, 1990; Maga, 1976; Spencer, Pangborn, & Jennings, 1978). The chemical composition of the volatile compounds varies in the different parts of the fruit. In the pulp, volatile compounds such as C6 compounds, C13 norisoprenoids and benzaldehyde are more concentrated than in the inner mesocarp (Aubert & Milhet, 2007). Besides, the composition evolves during the ripening process: C6 compound levels decrease drastically, whilst the content of lactones, benzaldehyde, linalool, C13 norisoprenoids and phenylalanine derivates increase (Aubert, Ambid, Baumes, & Gunata, 2003; Chapman, Horvat, & Forbus, 1991; Do et al., 1969; Eduardo, Chietera, Bassi, Rossini, & Vecchietti, 2010; Engel, Ramming, Flath, & Teranishi, 1988; Visai & Vanoli, 1997). The volatile composition is also affected by the storage conditions of the fruit (Yang, Balandran-Quintana, Ruiz, Toledo, & Kays, 2009; Zhang et al., 2011). The chemical composition ultimately depends on the genetic background of the cultivar

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(Horvat & Chapman, 1990; Montevecchi, Simone, Masino, Bignami, & Antonelli, 2012; Spencer et al., 1978). In a recent work, Wang et al. characterized the composition of volatile compounds in 50 different cultivars of peaches and nectarines, classifying them into four different groups according to the relative abundance of lactones, terpenoids and esters, linalool, and others (Wang et al., 2009).

Due to its practical properties (automation, minimal sample treatment, solvent-free extraction, robustness, etc.), Headspace Solid-Phase Microextraction (HS-SPME) has been widely applied to determine the volatile composition of several fruits and vegetables (Kataoka, Lord, & Pawliszyn, 2000). In this study, HS-SPME combined with gas chromatography–mass spectrometry (GC–MS) was applied to identify and determine the volatile constituents in four peach cultivars produced in Spain: San Lorenzo, Sudanell, Miraflores and Calanda. Two different clones of the Calanda cultivar were studied, Calante and Jesca. To the best of our knowledge, the volatile constituents of these cultivars have not to date been extensively studied. The aim of this study was to detect differences in the volatile composition which could lead to the identification of the cultivar of a peach fruit. Principal Component Analysis (PCA) was carried out to cluster the samples according to their volatile profile and Canonical Discriminant Analysis was used to find a mathematical equation to unequivocally identify the cultivar of one sample. This algorithm could contribute to protect the designation of origin "Melocotón de Calanda".

2. Materials and methods

2.1. Plant material

Four different cultivars of yellow peach were studied: San Lorenzo, Sudanell, Miraflores and Calanda. Two different clones were evaluated within the PDO "Melocotón de Calanda": Calante and Jesca. San Lorenzo, also known as "Yellow of August", is a mid-season cultivar which produces freestone yellow flesh peaches. Sudanell is a mid-season cultivar with hard yellow flesh peaches. Miraflores is a yellow late-season cultivar, with freestone hard yellow flesh peaches. Calanda also comes within the late-season category, with clingstone hard yellow flesh peaches. Calanda was the only one cultivar protected by bagging.

All cultivars were collected at the optimum harvested stage (San Lorenzo August 15th, Sudanell September 12th, Miraflores October 17th, Jesca October 28th and Calante November 8th) and transported to the laboratory within the same day. San Lorenzo, Sudanell and Miraflores were from the same orchard, whereas Calanda was supplied by a local producer. From 17 to 23 pieces of fruit of each cultivar/clone were analyzed (approx. 10 kg each). The samples were carefully selected for their similar physical appearance.

2.2. Physico-chemical analysis

Ripening was controlled by measuring color, firmness and soluble solids content (°Brix). A CR-400 illuminant C colorimeter manufactured by Konica Minolta (Tokyo, Japan) was used to register the color. Two measurements were taken on the skin in the equatorial area of the piece of fruit. The uniform color space CIELAB was used to describe the color. Lightness (L^*), chroma (C^*) and hue angle (h°) values were used to characterize the color of the samples. Chroma represents color saturation and hue angle is defined as a color wheel (McGuire, 1992). Firmness was measured at two opposite points on the equator of the piece of fruit with a firmness tester fitted with a brace of 8 mm FT-327 supplied by TR (Forli, Italy). Soluble solids content was determined from 40 g of pulp, previously crushed and homogenized with a mixer for 15–20 s. After this, 2 mL aliquots were taken for direct measurement with a digital MTD 045nD refractometer supplied by Three-in-one Enterprises (Taipei, Taiwan).

2.3. Chemicals

All the chemicals used were analytical grade reagents. Ethyl nonanoate (internal standard, CAS 123–29-5), 2,4-di-*tert*-butylphenol (CAS 96–76-4), (*E*,*E*)-2,4-decadienal (CAS 25152–84-5), benzaldehyde (CAS 100–52-7), decanal (CAS 112–31-2), dodecanal (CAS 112–54-9), eicosane (CAS 112–95-8), hexanal (CAS 66–25-1), linalool (CAS 78–70-6), nonanal (CAS 124–19-6), and sodium chloride (CAS 7647–14-5) were supplied by Sigma–Aldrich (Madrid, Spain). A mixture of *n*-alkanes (C7-C40, 1000 μg/mL each component in hexane), was also purchased from Sigma–Aldrich (Madrid, Spain). Methanol (HPLC grade, CAS 67–56-1) was provided by Scharlab (Barcelona, Spain). C50 helium was supplied by Carburos Metálicos (Zaragoza, Spain). Water was Milli-Q quality provided by a Milli-Q Plus 185 system from Millipore Iberia (Madrid, Spain).

2.4. Sample treatment for the determination of volatiles

From a piece of fruit, 25 g of homogenized crushed pulp were mixed with 25 mL of 20% (w/v) sodium chloride aqueous solution, containing ethyl nonanoate as internal standard (10 μg/mL). The mixture was stirred with a commercial mixer for approx. 5 s. 3 mL aliquots were placed in 20 mL screw-capped glass vials, ready for analysis by SPME. Once prepared, the samples were immediately frozen and stored at −20 °C. Two hours before the determination, the samples were left to thaw at room temperature in the autosampler. In this way all the parallel samples (17–23 for each cultivar) underwent the same temperature treatment, with no differences between the first and the last processed sample.

2.5. SPME sampling procedure

Sampling was performed by means of a Combi-PAL autosampler from CTC Analytics (Zwingen, Switzerland). The SPME fiber was polydimethylsiloxane/divinylbenzene (PDMS/DVB (StableFlex/SS), 65 μm) from Supelco (Bellefonte, Pennsylvania). The fiber was activated according to the manufacturer's instructions. Samples were incubated at 80 °C for 30 min (optimized parameters). Extraction was carried out at the same temperature for 30 min. In the meantime, vials were shaken at 600 rpm. After extraction, the fiber was thermally desorbed at the GC injection port at 250 °C for 3 min (splitless mode). Memory effects were avoided by baking the fiber at 250 °C for 5 min after desorption.

The different cultivars of peach samples were available during their corresponding harvesting times extending the experimental period over four months. In order to make sure that the method and the instruments kept the same performance during this time, quality control tests were carried out. A standard solution containing linalool, ethyl nonanoate, 2,4-di-*tert*-butylphenol and eicosane was interspersed in every 6 samples. A Shewhart chart was used to find out the 'warning' and 'action' lines (Lopez, Huerga, Batlle, & Nerin, 2006; Miller & Miller, 2005). As a result, five different fibers were used in the course of this work.

2.6. GC–MS

The determination was carried out using a Hewlett–Packard 6890 N GC gas chromatograph coupled to a 5975B Inert XL mass spectrometer, both supplied by Agilent Technologies (Palo Alto, California). A DB5-MS capillary column of 30 m length, 0.25 mm diameter, and 25 μm thickness was used. Helium at 1 mL/min

was used as carrier gas. The temperature program was as follows: 40 °C for 3 min, then the temperature was raised by 5 °C/min to 175 °C and held for 1 min, raised again by 10 °C/min to 300 °C and held for 3 min. The total run time was 46.5 min.

Electronic ionization was used at 70 eV. Detection was performed in scan mode, from 50 to 400 Da. The source and quadrupole temperatures were 230 °C and 150 °C, respectively. When standards were not available, the identification was performed using the WILEY275 and NIST05A libraries (the minimum matching requirement was 80%) and confirmed by Kovat's index. Kovat's indices were found out from the determination of a mixture of *n*-alkanes (C7–C40) under the same conditions.

2.7. Data treatment

Peak areas were normalized to the internal standard area before the statistical analysis. The relative abundance was then found by dividing the peak area between the total area of all the components. PCA ($p < 0.05$) was performed using the Unscrambler v 9.1 program supplied by CAMO Software AS (Trondheim, Norway). Two-dimensional PCA score plots were created on the data. The principal components were orthogonal and linear combinations of the original variables. The principal components were classified depending on the level of information they produced. The PC1 was the axis, which contained the largest possible amount of information, and PC2 was perpendicular to PC1. The aims of the PCA were to reduce the number of variables and to remove the redundant information. All models were validated using the "leave-one-out" method. Canonical Discriminant Component Analysis ($p < 0.05$) was performed using the SPSS v 13.0 program from SPSS Ibérica, an IBM company (Madrid, Spain). Further details are explained in the Section 3.4.

3. Results and discussion

3.1. Physico-chemical analysis

This study aimed to identify the differences in the volatile compound composition of different peach cultivars. Since this composition depends on the maturity level, it was mandatory to carefully select samples at the same ripening state. Thus, physico-chemical analysis was performed to assess the maturity level, discarding the samples far from the average values. Apart from the slight differences in color (lightness and chrome values), no significant differences in firmness or soluble solids content between the cultivars were observed (Table 1).

3.2. Volatile compounds

Sample treatment and SPME sampling were optimized to extract the highest amount of volatile constituents from the pulp. Dilution, salt addition and headspace/sample ratio were studied as sample treatment factors. Besides, three different SPME fibers were evaluated: polydimethylsiloxane (PDMS, 100 μm); divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS (StableFlex), 50/30 μm) and polydimethylsiloxane/divinylbenzene (PDMS/DVB (StableFlex/SS), 65 μm). PDMS/DVB fiber showed the best performance as more compounds were extracted and the signal intensity was higher for most of the compounds (Fig. A1). Previous works dealing with fruit volatiles also used PDMS/DVB fibers (Guillot et al., 2006; Pontes, Marques, & Camara, 2009; Wang et al., 2009). Incubation and extraction times and temperatures were also studied and optimized (Figs. A2 and A3).

108 volatile compounds were detected in all the peach cultivars, 65 of them were identified using mass spectra, Kovat's index and, when available, pure standards. The Kovat's indices were compared to bibliographic data, the highest error observed being 1.4%. Table 2 comprises the compounds identified as well as the concentration. Semi-quantification was performed by direct comparison with the internal standard peak (Eduardo et al., 2010; Wang et al., 2009). Compounds were classified by their chemical families: C6 compounds, alkanes, aldehydes, esters, terpenes, terpenoids, C13 norisoprenoids, lactones and 'other compounds'. To the best of our knowledge, all but five compounds have been previously described in the literature as components of peach aroma. Compounds not reported before in peach are: butyl hexadecanoate, butyl octadecanoate, β-damascone, 6,7,7a-tetrahydro-4,4–7a-trimethyl-2-(4H)-benzofuranone and (*E*,*E*)-farnesyl acetone. Fig. 1 shows the distribution of the main chemical families.

3.2.1. C6 compounds

C6 compounds are major compounds in peach aroma (Engel, Flath et al., 1988). In this study, three C6 compounds were detected: hexanal, (*E*)-2-hexenal and hexen-1-ol. Hexanal or (*E*)-2-hexenal were major compounds (>1%) in Sudanell (20.7%) and Calanda (18.0 and 13.6%) cultivars, but not in San Lorenzo (0.3%) or Miraflores (1.4%). Calanda was the only one cultivar with major quantities of hexen-1-ol (2.4% and 6.2%). Previous studies have reported higher relative abundances of C6 compounds (above 60% of total volatile compounds) (Wang et al., 2009). This is likely due to the fruit in this work was riper than the fruit collected by Wang et al., since the concentration of C6 compounds decreases as the fruit ripens (Do et al., 1969; Engel, Ramming et al., 1988).

3.2.2. Alkanes

A total of eleven different alkanes were identified, from dodecane (C12) to pentacosane (C25). The relative abundance was generally low, from 1.1% (Miraflores) to 7.3% (San Lorenzo). The distribution was different from one cultivar to another. C19–C25 alkanes were mainly found in San Lorenzo (3.0%), but were scarcely present in the Sudanell (0.2%), Miraflores (n.d.) and Calanda (0.0–0.1%) samples. Although up to eight different alkanes were detected in San Lorenzo, Sudanell and Calanda, only three were detected in Miraflores.

3.2.3. Aldehydes

The presence of aldehydes (others than hexanal and (*E*)-2-hexenal) in the cultivars was typically low, from 0.3% to 4.0%, except in the case of Miraflores with a relative concentration of

Table 1
Maturity descriptors of the cultivars studied: color (lightness, chrome and hue angle), firmness and soluble solids content. Values expressed as average ± standard deviation.

Parameter		Sudanell ($n = 23$)	San Lorenzo ($n = 23$)	Miraflores ($n = 23$)	Calanda (Calante) ($n = 18$)	Calanda (Jesca) ($n = 17$)
Color	Lightness (L^*)	63.4 ± 2.9 ac	62.4 ± 2.5 a	71.8 ± 1.9 b	67.5 ± 1.6 cd	69.6 ± 2.0 bd
	Chrome (C)	30.1 ± 2.5 a	29.7 ± 2.6 a	45.3 ± 3.2 b	32.5 ± 1.9 a	41.5 ± 3.2 b
	Hue angle (h°)	78.0 ± 8.6 a	80.1 ± 8.6 a	83.8 ± 4.3 a	84.6 ± 3.3 a	88.7 ± 3.0 a
Firmness (kg)		5.7 ± 0.8 a	6.4 ± 0.9 a	6.3 ± 1.0 a	6.4 ± 0.7 a	5.8 ± 0.5 a
Soluble solids (°Brix)		14.8 ± 2.1 a	15.6 ± 1.5 a	13.1 ± 1.4 a	13.1 ± 1.6 a	12.5 ± 1.7 a

Lowercase letters mean there are not significant differences between populations within the same row.

Table 2
Volatile compounds identified in peach cultivars by SPME and GC–MS. Compounds were identified using Kovat's index, mass spectrum and, when available, pure standards. Concentration (μg g^{-1} equivalent of ethyl nonanoate) is expressed as average ± confidence interval ($\alpha = 0.05$).

Kovat's index (error, %)	Compound	CAS #	Reference	Concentration (μg g^{-1}, equivalent of ethyl nonanoate)				
				Sudanell ($n = 23$)	San Lorenzo ($n = 23$)	Miraflores ($n = 23$)	Calanda (Calante) ($n = 18$)	Calanda (Jesca) ($n = 17$)
C6 Compounds								
810 (0.4)	Hexanal[a]	66–25-1	(Takeoka, Flath, Guntert, & Jennings, 1988)	127 ± 66	n.d.	n.d.	29.5 ± 11.0	6.8 ± 2.7
860 (0.0)	(*E*)-2-Hexenal	6728–26-3	(Takeoka et al., 1988)	1.9 ± 1.0	1.9 ± 0.7	1.7 ± 1.1	248.2 ± 54.9	89.2 ± 26.6
872 (1.4)	Hexen-1-ol[b]	111–27-3	(Takeoka et al., 1988)	1.6 ± 0.7	0.7 ± 0.2	0.2 ± 0.2	33.4 ± 13.7	40.9 ± 11.2
Alkanes								
1200 (0.0)	Dodecane[a]	112–40-3	(Sumitani, Suekane, Nakatani, & Tatsuka, 1994)	n.d.	n.d.	0.2 ± 0.0	0.7 ± 0.1	0.4 ± 0.0
1300 (0.0)	Tridecane[a,b]	629–50-5	(Takeoka et al., 1988)	6.4 ± 0.2	5.2 ± 0.5	n.d.	40.1 ± 5.1	19.9 ± 2.9
1400 (0.0)	Tetradecane[a]	629–59-4	(Takeoka et al., 1988)	6.3 ± 1.8	11.4 ± 2.4	0.7 ± 0.0	1.6 ± 0.2	1.2 ± 0.2
1500 (0.0)	Pentadecane[a,b]	629–62-9	(Takeoka et al., 1988)	n.d.	n.d.	n.d.	1.9 ± 0.4	1.6 ± 0.4
1600 (0.0)	Hexadecane[a]	544–76-3	(Takeoka et al., 1988)	n.d.	n.d.	0.1 ± 0.0	0.5 ± 0.1	0.6 ± 0.2
1700 (0.0)	Heptadecane[a]	629–78-7	(Takeoka et al., 1988)	0.1 ± 0.0	0.8 ± 0.1	n.d.	1.0 ± 0.2	0.9 ± 0.3
1800 (0.0)	Octadecane[a]	593–45-3	(Yamamoto & Ichimura, 1992)	0.2 ± 0.0	0.7 ± 0.1	n.d.	0.1 ± 0.0	0.3 ± 0.1
1900 (0.0)	Nonadecane[a]	36653–82-4	(Wang et al., 2009)	0.7 ± 0.1	3.9 ± 1.2	n.d.	n.d.	n.d.
2100 (0.0)	Heneicosane[a]	629–94-7	(Horvat & Chapman, 1990)	0.3 ± 0.1	3.9 ± 0.9	n.d.	n.d.	n.d.
2300 (0.0)	Tricosane[a,b]	638–67-5	(Horvat & Chapman, 1990)	0.8 ± 0.1	4.5 ± 0.8	n.d.	1.2 ± 0.3	1.1 ± 0.2
2500 (0.0)	Pentacosane[a]	629–99-2	(Horvat & Chapman, 1990)	1.0 ± 0.2	6.9 ± 2.3	n.d.	n.d.	n.d.
Aldehydes								
969 (−1.2)	Benzaldehyde[a]	100–52-7	(Takeoka et al., 1988)	9.5 ± 4.1	0.8 ± 0.4	1.8 ± 1.0	43.1 ± 13.6	17.7 ± 6.1
1005 (−1.0)	(*E*,*E*)-2,4-Heptadienal	4313–03-5	(Wang et al., 2010)	n.d.	n.d.	n.d.	n.d.	2.9 ± 0.9
1052 (0.7)	Benzeneacetaldehyde	122–78-1	(Eduardo et al., 2010)	n.d.	n.d.	n.d.	1.2 ± 0.3	0.7 ± 0.2
1109 (0.5)	Nonanal[a]	124–19-6	(Horvat & Chapman, 1990)	0.7 ± 0.0	0.3 ± 0.0	2.3 ± 0.8	2.6 ± 0.5	1.5 ± 0.2
1210 (0.4)	Decanal[a,b]	112–31-2	(Wang et al., 2009)	0.1 ± 0.0	n.d.	10.2 ± 2.1	0.9 ± 0.1	0.5 ± 0.1
1314 (0.3)	Undecanal[b]	112–44-7	(Wang et al., 2009)	0.2 ± 0.0	0.2 ± 0.0	n.d.	n.d.	n.d.
1333 (0.2)	(*E*,*E*)-2,4-Decadienal[a]	25152–84-5	(Horvat & Chapman, 1990)	n.d.	0.7 ± 0.2	n.d.	n.d.	n.d.
1411 (0.9)	Dodecanal[a]	112–54-9	(Yang, Zhou, & Wei, 2008)	n.d.	n.d.	0.4 ± 0.1	0.3 ± 0.1	0.8 ± 0.2
2243 (0.6)	Eicosanal	2400–66-0	(Eduardo et al., 2010)	n.d.	0.1 ± 0.1	n.d.	n.d.	n.d.
Esters								
1006 (0.1)	3-Hexenyl acetate	3681–71-8	(Takeoka et al., 1988)	n.d.	n.d.	n.d.	4.9 ± 2.1	35.2 ± 6.9
1010 (−0.3)	2-Hexenyl acetate	2497–18-9	(Takeoka et al., 1988)	4.1 ± 1.0	0.9 ± 0.1	10.0 ± 1.8	123.5 ± 22.6	16.2 ± 6.8
1018 (1.0)	Hexyl acetate	142–92-7	(Takeoka et al., 1988)	0.6 ± 0.2	0.2 ± 0.0	0.4 ± 0.1	5.9 ± 2.4	n.d.
1191 (0.4)	Butyl hexanoate	626–82-4	(Riu-Aumatell, Castellari, Lopez-Tamames, Galassi, & Buxaderas, 2004)	1.5 ± 0.3	0.7 ± 0.0	2.7 ± 1.1	2.8 ± 0.5	0.7 ± 0.1
1214 (0.3)	Octyl acetate	112–14-1	(Sumitani et al., 1994)	3.0 ± 1.2	5.4 ± 1.3	n.d.	33.1 ± 9.6	45.4 ± 10.8
1312 (0.0)	Nonyl acetate	143–13-5	(Wang et al., 2009)	n.d.	n.d.	n.d.	2.3 ± 0.4	n.d.
1589 (−0.4)	Ethyl dodecanoate	106–33-2	(Takeoka et al., 1992)	n.d.	n.d.	1.4 ± 0.7	4.2 ± 1.4	0.7 ± 0.2
1736 (0.6)	Methyl tetradecanoate[b]	124–10-7	(Riu-Aumatell et al., 2004)	2.7 ± 1.0	n.d.	n.d.	n.d.	n.d.
2196 (0.9)	Butyl hexadecanoate	111–06-8	Not reported before in peach	0.0 ± 0.0	0.1 ± 0.0	n.d.	n.d.	n.d.
2397 (0.9)	Butyl octadecanoate	123–95-5	Not reported before in peach	n.d.	0.1 ± 0.0	n.d.	n.d.	n.d.
Terpenes								
991 (0.1)	β-Myrcene	123–35-3	(Takeoka et al., 1988)	n.d.	n.d.	1.2 ± 0.3	n.d.	n.d.
1027 (−1.3)	*p*-Cymene	99–87-6	(Takeoka et al., 1988)	0.3 ± 0.0	n.d.	0.1 ± 0.0	n.d.	n.d.
1034 (0.1)	Limonene[b]	138–86-3	(Takeoka et al., 1988)	0.7 ± 0.2	n.d.	0.9 ± 0.2	n.d.	n.d.
1039 (−0.6)	(*Z*)-β-Ocimene[b]	3779–61-1	(Takeoka et al., 1988)	0.4 ± 0.1	n.d.	0.5 ± 0.1	n.d.	n.d.
1049 (0.9)	(*E*)-β-Ocimene	3338–55-4	(Takeoka et al., 1988)	0.7 ± 0.3	n.d.	0.6 ± 0.2	n.d.	n.d.
Terpenoids								
1104 (0.2)	3,7-dimethyl-1,5,7-octatrien-3-ol	29957–43-5	(Takeoka et al., 1988)	5.2 ± 1.6	0.1 ± 0.0	8.7 ± 2.6	3.6 ± 0.5	3.4 ± 0.7
1157 (0.3)	Nerol oxide[b]	1786–08-9	(Riu-Aumatell, Lopez-Tamames, & Buxaderas, 2005)	2.8 ± 1.1	0.0 ± 0.0	0.8 ± 0.1	n.d.	18.2 ± 13.0
1197 (0.3)	α-terpineol	98–55-5	(Takeoka et al., 1988)	5.0 ± 1.0	0.9 ± 0.0	1.4 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
1225 (0.5)	β-Cyclocitral[b]	432–25-7	(Wang et al., 2009)	5.1 ± 0.1	n.d.	n.d.	5.1 ± 2.4	3.9 ± 1.0

(*continued on next page*)

Table 2 (*continued*)

Kovat's index (error, %)	Compound	CAS #	Reference	Concentration (μg g^{-1}, equivalent of ethyl nonanoate)				
				Sudanell (n = 23)	San Lorenzo (n = 23)	Miraflores (n = 23)	Calanda (Calante) (n = 18)	Calanda (Jesca) (n = 17)
1235 (0.3)	*p*-Menth-1-en-9-al	29548–14-9	(Spencer et al., 1978)	21.4 ± 3.3	n.d.	n.d.	n.d.	n.d.
1254 (0.2)	Nerol	106–25-2	(Krammer, Winterhalter, Schwab, & Schreier, 1991)	5.4 ± 0.8	5.8 ± 0.6	7.8 ± 1.5	12.8 ± 2.8	9.6 ± 1.4
1452 (−0.5)	Geranyl acetone[b]	689–67-8	(Wang et al., 2009)	5.2 ± 1.6	0.1 ± 0.0	8.7 ± 2.6	3.6 ± 0.5	3.4 ± 0.7
C13 Norisoprenoids								
1385 (−0.3)	β-Damascenone[b]	23726–93-4	(Eduardo et al., 2010)	4.1 ± 0.7	6.6 ± 0.7	2.6 ± 0.4	6.5 ± 1.3	7.6 ± 1.3
1440 (−1.1)	β-Damascone[b]	85949–43-5	Not reported before in peach	n.d.	n.d.	0.3 ± 0.1	n.d.	n.d.
1485 (0.0)	β-Ionone	79–77-6	(Horvat & Chapman, 1990)	7.8 ± 0.9	12.0 ± 1.2	3.7 ± 0.5	14.8 ± 3.1	10.5 ± 1.9
1647 (−0.5)	(*Z*)-6-Dodecen-4-olide[b]	18679–18-0	(Takeoka et al., 1992)	30.3 ± 5.7	4.9 ± 1.0	n.d.	n.d.	n.d.
Lactones								
1065 (0.1)	γ-Hexalactone[b]	695–06-7	(Takeoka et al., 1988)	0.2 ± 0.0	n.d.	n.d.	n.d.	n.d.
1296 (1.0)	δ-Octalactone	698–76-0	(Takeoka et al., 1988)	n.d.	n.d.	6.7 ± 1.4	33.8 ± 9.3	29.7 ± 9.0
1376 (0.8)	γ-Nonalactone	104–61-0	(Takeoka et al., 1988)	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	10.9 ± 4.7	9.5 ± 4.2
1476 (0.0)	γ-Decalactone	706–14-9	(Takeoka et al., 1988)	258 ± 62	245 ± 70	24.5 ± 7.7	500 ± 116	160.7 ± 55.0
1500 (0.5)	δ-Decalactone	705–86-2	(Takeoka et al., 1988)	25.1 ± 5.8	84.9 ± 10.2	5.2 ± 1.2	59.3 ± 11.5	13.4 ± 3.9
1687 (0.2)	γ-Dodecalactone	2305–05-7	(Do et al., 1969)	4.0 ± 3.1	56.6 ± 14.4	n.d.	22.6 ± 10.5	19.5 ± 9.2
1714 (−0.3)	δ-Dodecalactone	713–95-1	(Do et al., 1969)	1.1 ± 0.1	2.3 ± 0.5	n.d.	n.d.	n.d.
Other compounds								
713 (1.0)	2-Ethyl furan	3208–16-0	(Narain, Hsieh, & Johnson, 1990)	n.d.	n.d.	n.d.	1.8 ± 0.5	n.d.
1180 (0.9)	1-Nonanol	143–08-8	(Takeoka et al., 1988)	n.d.	n.d.	n.d.	0.1 ± 0.0	0.9 ± 0.5
1332 (−1.1)	1,2,3,4-Tetrahydro-1,1,6-trimethyl naphthalene[b]	475–03-6	(Kemp, Stoltz, & Packett, 1971)	n.d.	n.d.	0.6 ± 0.1	1.1 ± 0.4	n.d.
1415 (0.2)	Vanillin	121–33-5	(Aubert & Milhet, 2007)	9.0 ± 2.0	14.3 ± 2.7	0.2 ± 0.0		1.7 ± 0.4
1515 (−0.2)	2,4-Di-*tert*-butyl phenol[a]	96–76-4	(Wang et al., 2009)	4.2 ± 0.7	2.4 ± 0.2	2.7 ± 0.5	8.4 ± 1.7	3.3 ± 0.5
1539 (0.1)	5,6,7,7a-Tetrahydro-4,4–7a-trimethyl-2-(4H)-benzofuranone	15356–74-8	Not reported before in peach	n.d.	n.d.	n.d.	1.2 ± 0.3	1.2 ± 0.2
1900 (0.0)	1-Hexadecanol[b]	629–92-5	(Wang et al., 2010)	n.d.	0.5 ± 0.2	n.d.	n.d.	n.d.
1938 (0.5)	(*E*,*E*)-Farnesyl acetone	1117–52-8	Not reported before in peach	2.0 ± 0.2	7.9 ± 1.4	n.d.	n.d.	n.d.
2155 (−0.2)	Oleic acid	112–80-1	(Sanchez-Vicente, Cabanas, Renuncio, & Pando, 2009)	0.6 ± 0.3	n.d.	n.d.	n.d.	n.d.

'n' stands for the number of parallel measurements, i.e. the pieces of fruit analyzed in each cultivar.
'n.d.' stands for 'not detected'.
[a] Identification confirmed with standard.
[b] Compound used in the function of classification (see Section 3.4).

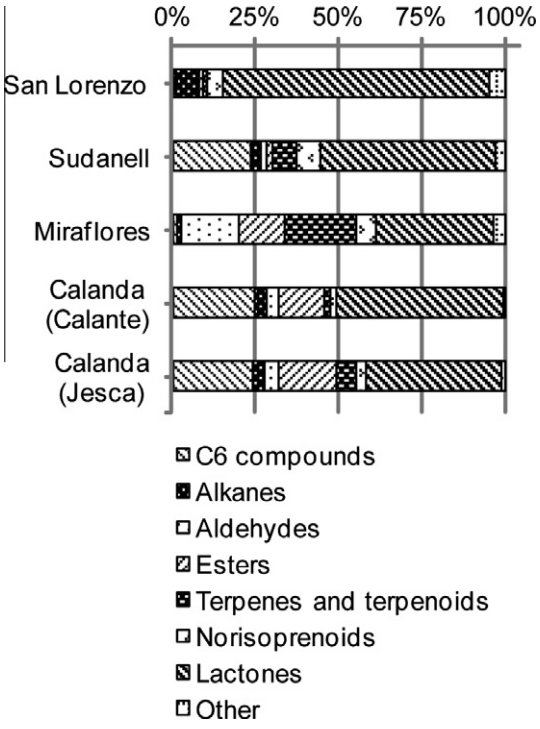

Fig. 1. Distribution of volatile compounds according to their chemical families in four peach cultivars: San Lorenzo, Sudanell, Miraflores and Calanda. Two clones of Calanda were studied: Calante and Jesca.

17%. Benzaldehyde was present in all the cultivars with special significance in Calanda peach. Decanal was only important in the Miraflores cultivar (8.4%), where no significant contribution of C6 aldehydes was observed. This fact could indicate that the same enzymatic decomposition that produces C6 aldehydes in the other cultivars produces decanal and nonanal in the Miraflores cultivar. The reason could be either different starting fatty acid components or different enzymes in the Miraflores cultivar. Further experiments should be performed to address this issue.

3.2.4. Esters

Esters are important flavoring components that contribute with fruity and floral notes. Up to ten ester compounds were found in the samples. Their relative concentration was low in the San Lorenzo and Sudanell cultivars (1.3–1.8%), but significant in the Miraflores and Calanda cultivars (13.8–17.1%). The most abundant compounds were 2-hexenyl acetate, octyl acetate and 3-hexenyl acetate. 2-Hexenyl acetate is mainly present in the Calante clone, whereas octyl acetate and 3-hexenyl acetate are major compounds in the Jesca clone. Thus, these three compounds could help differentiate between the two clones of the Calanda cultivar.

3.2.5. Terpenes and terpenoids

Terpenes and terpenoids contribute to the floral flavor of peach (Engel, Flath et al., 1988). Terpenes were only found in the San Lorenzo and Miraflores cultivars. Terpenoids were found in all cultivars, but were relatively relevant in Miraflores (18.7%). In this cultivar, the most important terpenoids were 3,7-dimethyl-1,5,7-octatrien-3-ol (7.1%) and geranyl acetone (6.4%). *p*-Menth-1-en-9-al was only detected in the Sudanell cultivar (3.4%). Linalool has been reported as an important odorous compound in previous studies (Wang et al., 2009), but it was not detected in any sample.

3.2.6. C13 Norisoprenoids

C13 Norisoprenoids have been described as major peach volatile compounds (Aubert, Gunata, Ambid, & Baumes, 2003). The relative concentration found in this study ranged from 1.6% to 7.5%. The most relatively abundant C13 norisoprenoids were (*Z*)-6-dodecen-4-olide in Sudanell (4.9%). β-Ionone and, at lower concentrations, β-damascenone were present in all cultivars.

3.2.7. Lactones

Lactones, particularly γ-decalactone and δ-decalactone, provide the characteristic flavor of peach (Maga, 1976). They were the predominant family of volatile compounds in all cultivars, from 35.2% in Miraflores to 79.9% in San Lorenzo. Up to seven different lactones were detected. γ-Decalactone was the most abundant volatile compound in all cases (20.1–42.0%), followed by δ-decalactone (2.0–13.9%) and γ-dodecalactone (0.6–9.2%). This is consistent with previous studies (Engel, Flath et al., 1988). γ-Decalactone gives the fruit its characteristic peach odor, and hexanal adds fruity and sweet components to the flavor (Maga, 1976). δ-Octalactone was only found as a major compound in the Miraflores (5.5%) and Calanda cultivars (2.4–4.5%).

3.2.8. Others

The presence of other unclassified compounds was relatively minor in importance, from 0.7% to 4.9%. Major compounds were vanillin in San Lorenzo (2.3%) and Sudanell (1.4%) and (*E*,*E*)-farnesyl acetone in San Lorenzo (1.2%).

3.3. Principal components analysis

Principal components analysis was carried out to help distinguish the peach cultivars under study. Three principal components resulted from the analysis. The principal component 1 was mainly correlated to the relative concentration of benzaldehyde and δ-dodecalactone (positively) and dodecane and pentadecane (negatively). The principal component 2 was mainly correlated to nerol oxide and (*Z*)-β-ocimene (positively) and δ-decalactone and β-ionone (negatively). The principal component 3 was mainly correlated to butyl hexanoate and (*E*)-β-ocimene (positively) and δ-octalactone and 3-hexenyl acetate (negatively). Fig. 2 a) depicts the principal component 2 (PC2) against principal component 1 (PC1). As can be seen, PC1 differentiates the cultivars Calanda and Miraflores from the cultivars Sudanell and San Lorenzo, whereas PC2 differentiates the cultivars Calanda and San Lorenzo from Miraflores and Sudanell. No difference between the Calante and Jesca clones was shown by PC1 and PC2, confirming their common origins as Calanda cultivars. A third principal component (PC3) allows the Jesca and Calante clones to be distinguished to some extent (Fig. 2 b).

3.4. Analysis of canonical variables

Analysis of canonical variables was applied to classify the samples measured according to their cultivar. Three canonical components were needed to explain 100% of the total variance. Table B1 shows the statistical data related to the components found. High values of canonical correlation were obtained, indicating the importance of the variables considered. The measurements of the Wilks' Lambda coefficients and the significance levels (95%) of the three canonical components are also included.

The analysis of canonical variables led to a function of classification that takes into account 18 volatile compounds from the 65 compounds identified in the four cultivars studied (Table B2). One C6 compound was included: hexen-1-ol; three alkanes: tridecane, pentadecane and tricosane; two aldehydes: decanal and unde-

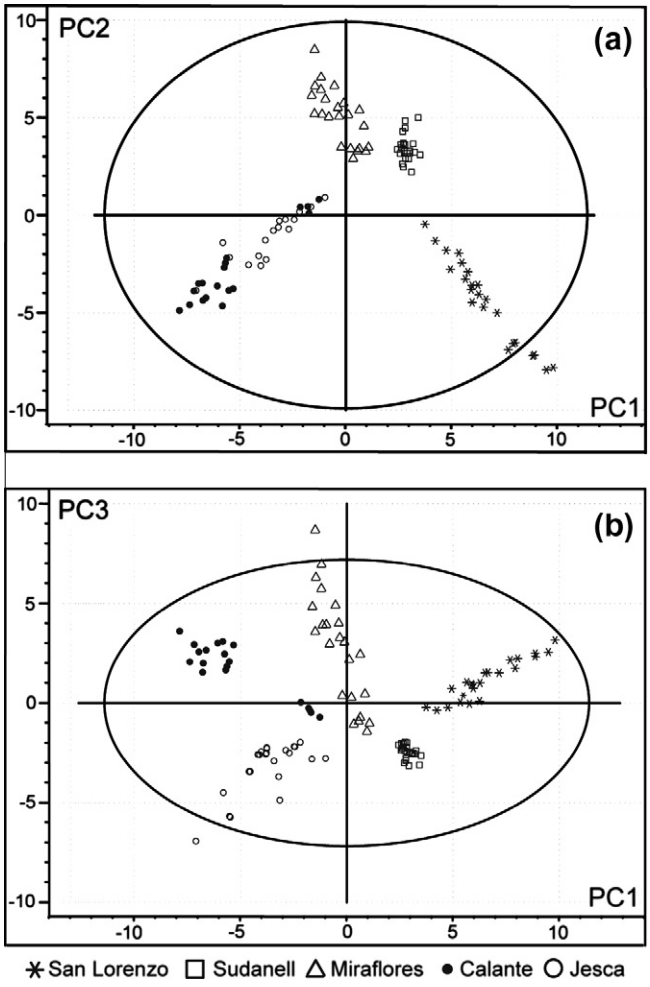

Fig. 2. Principal components analysis of the relative abundance of the volatile compounds in the peach cultivars studied: Sudanell (square), San Lorenzo (asterisk), Miraflores (triangle), Jesca (empty circle) and Calante (full circle): (a) principal component 2 against principal component 1, (b) principal component 3 against principal component 1.

canal; one ester: methyl tetradecanoate; two terpenes: limonene and (*E*)-*β*-ocimene; three terpenoids: nerol oxide, *β*-cyclocitral, and geranyl acetone; two C13 norisoprenoids: *β*-damascone and *β*-damascenone; two lactones: γ-hexalactone and (*Z*)-6-dodecen 4-olide and, finally, 1-hexadecanol and 1,2,3,4 – tetrahydro-1,1,6-trimethyl naphthalene. By applying the method previously explained, the relative abundance of these compounds should be determined in the sample. Then, a decision value (DV) is calculated for each cultivar, according to the following equation:

$$DV_j = K_j + \sum C_{ij} X_i$$

$j = 1 - 4$ cultivars

$i = 1 - 18$ compounds

$K_j =$ constant

$C_{ij} =$ coefficients

$X_i =$ relative abundance of the compound

Table B2 includes the constant values (K_j) and the coefficients (C_{ij}) for each compound in each cultivar. Once the DV_j are calculated, the cultivar with the lowest *DV* corresponds to the cultivar of the sample. Using this function, an unknown peach sample (within these four cultivars) can be identified from the relative response of the volatile components detected. Finally, Fig. 3 shows

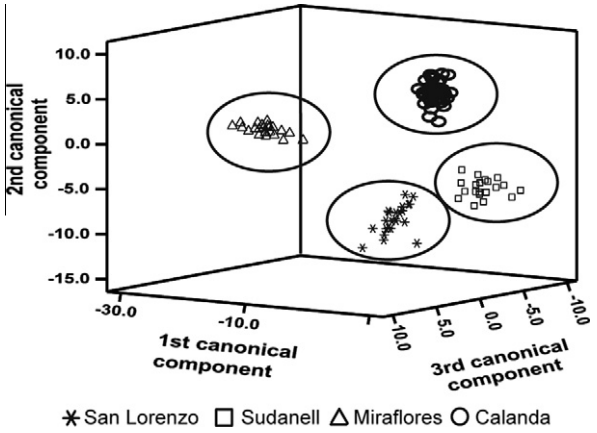

Fig. 3. Canonical components analysis of the samples from the four cultivars: Sudanell (square), San Lorenzo (asterisk), Miraflores (triangle) and Calanda (circle).

the space represented by the canonical components obtained. As can be seen, the different cultivars are clearly separated from each other, indicating the good selection of the set of compounds and the success of the statistical tool for the recognition of the cultivar under study.

4. Conclusions

This study is focused on using the composition of volatile compounds to distinguish the Calanda peach cultivar from other cultivars, in order to protect the Designation of Origin and associated economic implications. Due to the high added value of this specie, forgery and deception is currently increasing in occurrence. This research provides a mechanism to characterize and identify the origin of four common peach cultivars commercialized in Spain: San Lorenzo, Sudanell, Miraflores and Calanda. Besides, two different clones of Calanda cultivar were studied.

HS-SPME has been optimized and used for the extraction of volatile compounds from peach pulp. 65 different volatile compounds have been identified by GC–MS. As previous studies, lactones and C6 compounds were the main volatile compounds detected.

Principal component analysis was able to distinguish between the different cultivars by the application of three principal components. Furthermore, a linear function that allows the classification of a peach sample (with supposed unknown origin) was developed by the use of canonical discriminant components. Using the proposed experimental protocol together with the classification function, the analyst will be able to identify the cultivar of peach. Thus, this procedure could help to detect frauds, in which other cultivars are marketed as Calanda cultivar, protecting the Designation of Origin "Melocotón de Calanda".

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.10.145.

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