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Article Sub-Title		
Article CopyRight	Springer-Verlag (This will be the copyright line in the final PDF)	
Journal Name	European Food Research and Technology	
Corresponding Author	Family Name	**Nuez**
	Particle	
	Given Name	**Fernando**
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	Division	Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV)
	Organization	Universitat Politècnica de València
	Address	Cno. de Vera, s.n., València, 46022, Spain
	Email	fnuez@btc.upv.es
Author	Family Name	**Casals**
	Particle	
	Given Name	**Joan**
	Suffix	
	Division	Deptartament d'. Enginyeria Agroalimentària i Biotecnologia
	Organization	Universitat Politècnica de Catalunya
	Address	c/Esteve Terrades 8, Campus del Baix Llobregat, Castelldefels, 08860, Spain
	Email	
Author	Family Name	**Cebolla-Cornejo**
	Particle	
	Given Name	**Jaime**
	Suffix	
	Division	Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV)
	Organization	Universitat Politècnica de València
	Address	Cno. de Vera, s.n., València, 46022, Spain
	Email	
Author	Family Name	**Roselló**
	Particle	
	Given Name	**Salvador**
	Suffix	
	Division	Departamento de Ciencias Agrarias y del Medio Natural
	Organization	Universitat Jaume I
	Address	Campus de Riu Sec, Castellón, 12071, Spain
	Email	
Author	Family Name	**Beltán**
	Particle	
	Given Name	**Joaquim**

	Suffix	
	Division	Instituto Universitario de Plaguicidas y Aguas (IUPA)
	Organization	Universitat Jaume I
	Address	Campus de Riu Sec, Castellón, 12071, Spain
	Email	
Author	Family Name	**Casañas**
	Particle	
	Given Name	**Francesc**
	Suffix	
	Division	Deptartament d'. Enginyeria Agroalimentària i Biotecnologia
	Organization	Universitat Politècnica de Catalunya
	Address	c/Esteve Terrades 8, Campus del Baix Llobregat, Castelldefels, 08860, Spain
	Email	

	Received	30 March 2011
Schedule	Revised	25 May 2011
	Accepted	28 May 2011

Abstract

The postharvest evolution of Penjar tomatoes has been studied in four accessions representative of the variability of the varietal type. The long-term shelf life of these materials, which carry the *alc* allele, was confirmed with 31.2–59.1% of commercial fruits after 6 months of effective conservation at room temperature and a limited loss of weight (21.1–27.9%). Aroma in Penjar tomatoes is differentiated from other tomato varieties by a characteristic 'sharp-floral' aroma descriptor. The evolution of the 'sharp-floral' aroma during postharvest showed a peak of intensity at 2 months of postharvest, though in one accession a delay of 2 months in this response was detected. Out of 25 volatiles analysed, including main and background notes, a reverse iPLS variable selection revealed that the main candidates behind this aromatic behaviour are α-terpineol, *trans*-2-hexenal, 6-metyl-5-hepten-2-one, trans-2-octenal, α-pinene, β-ionone, 2 + 3-methylbutanol and phenylacetaldehyde. Between harvest and 2 months postharvest, most compounds reduced considerably their concentration, while the intensity of the 'sharp-floral' descriptor increased, which means that probably there is a rearrangement of the relative concentrations among volatiles that may lead to masking/unmasking processes.

Keywords (separated by '-') Alcobaça - Aroma - Postharvest - Ripening mutants - Sensory analysis - Tomato landrace

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Eur Food Res Technol
DOI 10.1007/s00217-011-1517-6

ORIGINAL PAPER

Long-term postharvest aroma evolution of tomatoes with the alcobaça (*alc*) mutation

**Joan Casals · Jaime Cebolla-Cornejo ·
Salvador Roselló · Joaquim Beltán ·
Francesc Casañas · Fernando Nuez**

Received: 30 March 2011 / Revised: 25 May 2011 / Accepted: 28 May 2011

Abstract The postharvest evolution of Penjar tomatoes has been studied in four accessions representative of the variability of the varietal type. The long-term shelf life of these materials, which carry the *alc* allele, was confirmed with 31.2–59.1% of commercial fruits after 6 months of effective conservation at room temperature and a limited loss of weight (21.1–27.9%). Aroma in Penjar tomatoes is differentiated from other tomato varieties by a characteristic 'sharp-floral' aroma descriptor. The evolution of the 'sharp-floral' aroma during postharvest showed a peak of intensity at 2 months of postharvest, though in one accession a delay of 2 months in this response was detected. Out of 25 volatiles analysed, including main and background notes, a reverse iPLS variable selection revealed that the main candidates behind this aromatic behaviour are α-terpineol, *trans*-2-hexenal, 6-metyl-5-hepten-2-one, trans-2-octenal, α-pinene, β-ionone, 2 + 3-methylbutanol and phenylacetaldehyde. Between harvest and 2 months postharvest, most compounds reduced considerably their concentration, while the intensity of the 'sharp-floral' descriptor increased, which means that probably there is a rearrangement of the relative concentrations among volatiles that may lead to masking/unmasking processes.

Keywords Alcobaça · Aroma · Postharvest · Ripening mutants · Sensory analysis · Tomato landrace

Introduction

More than 400 volatiles have been reported in tomato (*Solanum lycopersicum* L.) [1], and at least 10 of these compounds are required to reproduce its aroma: *cis*-3-hexenal, *cis*-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole and β-ionone [2].

The deficient aroma profile of fruits being commercialized at the moment [3] is mainly due to three factors: first, the aroma is a complex polygenic trait with a difficult selection and is usually neglected in breeding programmes. Nevertheless, it should be noted that the elucidation of volatile precursors [3] and of genes related to the accumulation of volatiles [4, 5] opens promising opportunities to tomato breeders. Second, handling procedures might play an important role in the aroma profile. In this sense, harvesting in mature-green stage [6] and low-temperature storage procedures [7] lead to a decrease in fruit volatile concentrations. Third, breeding for shelf life has had collateral effects, and at the moment it is one of the main causes of the lower aroma levels in modern varieties.

In fact, the use of ripening mutants *rin* (ripening inhibitor) [8] and *nor* (non-ripening) [9], which operate upstream of ethylene biosynthesis, increases shelf life with a delay in the ripening process but in return they cause

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J. Casals · F. Casañas
Deptartament d'. Enginyeria Agroalimentària i Biotecnologia,
Universitat Politècnica de Catalunya, c/Esteve Terrades 8,
Campus del Baix Llobregat, 08860 Castelldefels, Spain

J. Cebolla-Cornejo · F. Nuez (✉)
Instituto Universitario de Conservación y Mejora de la
Agrodiversidad Valenciana (COMAV), Universitat Politècnica
de València, Cno. de Vera, s.n., 46022 València, Spain
e-mail: fnuez@btc.upv.es

S. Roselló
Departamento de Ciencias Agrarias y del Medio Natural,
Universitat Jaume I, Campus de Riu Sec, 12071 Castellón, Spain

J. Beltán
Instituto Universitario de Plaguicidas y Aguas (IUPA),
Universitat Jaume I, Campus de Riu Sec, 12071 Castellón, Spain

Journal : Large 217 | Dispatch : 6-6-2011 | Pages : 12
Article No. : 1517 | ☐ LE | ☐ TYPESET
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negative effects on aroma profiles, lowering the levels of many important volatiles in the red ripe (RR) stage [10–12]. This effect may be a consequence of the impairment of ethylene and lycopene biosynthesis, compounds implied in the metabolic pathways of a great number of volatile compounds [13, 14]. Alcobaça (*alc*) is another mutation with a similar effect on ripening [15], and it is allelic to *nor* [16]. But this mutation seems to have a lower negative impact on fruit quality [15] and the use of *alc* has been described as a more appropriate strategy than the use of *rin* and *nor* in the development of long shelf life quality cultivars of tomato [17]. Despite this potential benefit, this mutation has been disregarded in breeding programmes, which have been focused on the use of the *rin* mutant mainly in the development of large-sized fresh-market cultivars and of the *nor* mutant in the case of cherry cultivars [18].

In the north east of Spain, the *alc* allele is widely distributed in different genetic backgrounds making up a varietal type called Penjar. These tomatoes are characterized by a long shelf life (mean storage ability of 126.8 days) and a reduced fruit size (mean fruit weight of 64.1 g). In a recent analysis of the genetic diversity in the varietal type using amplified fragment length polymorphism (AFLP), 18.07% of polymorphism was found, revealing the broad genetic base of Penjar landrace [16]. Considering the importance of the genetic background in the aroma profile of tomato fruits, it would be logical to expect that the great diversity found in the Penjar type might lead to considerable differences in the aroma profiles of different accessions, even though all of them carry the *alc* allele.

This type of tomatoes is mainly used to prepare ‘pan con tomate’, a traditional dish prepared rubbing the tomato on a slice of toasted bread, and to cook fried tomato sauces. It is usually grown in the open field, harvested during August–October, and it is commercialized during the traditional low-temperature and non-producing period ranging from December to March. This time span represents a conservation period between 2 and 6 months, with storage at room temperature. Local consumers usually consider that Penjar tomatoes have better aroma properties when compared with other tomato varieties, a consideration quite unusual in the appreciation of the aroma of the ripening mutants, and this fact justifies higher selling prices in the local market.

There are no detailed works on the effect of the ripening mutant *alc* on tomato aroma, and studies regarding aroma evolution during storage in other varieties are carried only on a short-term basis. The Penjar tomato is a good model to analyse both effects, as it includes a variety of genetic backgrounds and more than 6 months of effective conservation [16]. In this context, the main purpose of this work is to obtain a sensory and analytical description of the aroma of Penjar tomatoes and to track its evolution during its storage (0–6 months).

Materials and methods

Plant material

In previous works, an extensive prospection and collection of accessions belonging to the traditional varietal-type Penjar was carried out in its area of cultivation on the east coast of Spain. The collected accessions were characterized examining their morphologic, agronomic and genetic diversity [16]. Using this information, four accessions, conserved at the COMAV Seedbank, with an outstanding long shelf life and representing different shapes, colours and agronomic characteristics were selected (Table 1). All these accessions had previously been genetically analysed, and the presence of the *alc* allele was confirmed [16].

Field trials

The accessions were cultivated in open field conditions in Castellar del Vallès (UTM: Latitude 41°36′ 57″; Longitude 2°4′15″; Zone 31). In order to check the homogeneity of growing conditions, a randomized complete block design was selected with 4 repetitions and 20 plants per plot. Cultivation was carried out using the traditional practices

Table 1 Agronomic and morphologic characteristics of the Penjar accessions assayed (mean ± standard deviation)

Accession	Yield (kg plant^{-1})[a]	Fruit weight (g)[b]	Soluble solids (°Brix)[b]	Fruit colour	Fruit shape	Fruit blossom end shape	Other traits
CDP-1245	2.31 ± 0.33	61.7 ± 8.2	4.8 ± 0.8	Yellow	Flattened	Flat	Potato-leaf
CDP-1240	2.07 ± 0.66	115.8 ± 31.8	4.9 ± 1.0	Orange–red	Heart-shaped	Pointed	High sensibility to fruit cracking
CDP-8268	3.06 ± 0.86	59.2 ± 17.4	4.7 ± 0.4	Orange–red	Heart-shaped	Pointed	Multiparous inflorescence
CDP-5468	1.71 ± 0.11	31.4 ± 4.1	6.6 ± 0.7	Pink	Heart-shaped	Pointed	Multiparous inflorescence

[a] Mean from 16 plants

[b] Fruit traits were evaluated on a random sample of 20 fruits from different plants

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applied for tomato cultivation in the area, including drip irrigation, staking, fortnight pruning, integrated pest management and initial manure fertilization. The characteristics of the accessions were checked, and mean yield, mean fruit weight, soluble solids (°Brix), fruit colour (visual estimation), fruit shape, fruit blossom end shape and other interesting traits were recorded. Yield was recorded in 20 randomly selected plants per accession, while fruit traits were evaluated in 20 randomly selected fruits from different plants per accession. All the fruits from the second to the fourth truss were harvested and stored in darkness at room temperature (20 ± 5 °C) and humidity (68–75% relative humidity). During postharvest, a screening of the fruits was performed every 2 weeks. Fruits were discarded if they showed external signs of desiccation, loss of turgor or fungal infection, and the rest of the fruits were considered commercial. Shelf life was calculated as the percentage of commercial fruits at 6 months of postharvest storage. The percentage loss of weight was determined at 2, 4 and 6 months of postharvest storage using 16 fruits per accession, on a per fruit basis.

Sample preparation and aroma analysis

Sample preparation

Samples were obtained at harvest (0 months postharvest) and at 2, 4 and 6 months of postharvest storage. Each sample was kept frozen in order to analyse the aromatic profile of the whole collection at the same time and in the same conditions. Each sample was made up by 10 fruits with good conservation (without external signs of deterioration) and with weights near to the estimated mean weight calculated for the accession (Table 1). The lack of internal bruising was established as an additional criterion in order to select the fruits for the sample [19]. The lignified area surrounding the pedicel scar was discarded, and the fruits were ground and homogenized, adding a saturated solution of $CaCl_2$ to inactivate volatile degrading enzymes [20]. Samples were instantly kept frozen at −80 °C until analysis.

Sensory analysis

Sensory analysis was conducted to discriminate the odour between accessions and between postharvest storages (0, 2, 4 and 6 months). Sensory analysis was performed with 10 trained panellists with previous experience in tomato and bean evaluation [21]. The panellists were specifically trained to evaluate tomato odour descriptors using Penjar populations. Firstly, in order to reach a consensus in the odour descriptors more appropriate for Penjar tomatoes, the panellists were presented during 4 sessions with Penjar tomato samples with 2 and 4 months of postharvest storage, as well as with samples belonging to commercial fresh tomatoes obtained from the local market (4 sessions). These sessions enabled an initial consensus on a limited set of odour descriptors. During other 8 sessions, the panellists were presented with numerous samples including different genotypes and storage periods in order to get familiar with the range of variation in the intensity of the selected descriptors. Finally, during 2 additional sessions, the optimal serving temperature was evaluated. Four collections with 0, 2, 4 and 6 months of postharvest storage were evaluated at four different serving temperatures: 15, 17.5, 20 and 25 °C.

Once the best serving temperature was selected, the following thawing procedure was adopted: samples were taken out of the ultra-low freezer (−80 °C) the day before the evaluation session and hermetically sealed and placed in a refrigerator (8 °C) for 12 h. The samples were introduced in a chamber at 20 °C 3 h before the evaluation session.

Tasting sessions were carried out twice a week in a room designed for sensory analyses (ISO 8589) that was illuminated with green light to mask the colour of the samples. Accessions were evaluated in quadruplicate and were randomly distributed in 16 sessions (4 accessions per session). The samples were presented in sealed cylindrical vials (diameter: 50 mm; height: 43 mm). Vials were unsealed 2 min before starting the sensory analysis. All scoring took place on a semi-structured scale ranging from 0 to 10 with the endpoints anchored and marked with the descriptors.

Volatile analysis

Twenty-five tomato volatiles were chromatographically determined in the samples: 2-phenylethanol, *trans*-2-hexenal, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, 2 + 3-methyl-1-butanol, hexanal, 1-hexanol, *cis*-3-hexenol, *cis*-3-hexenal, *trans*-2-heptenal, R-limonene, nonanal, eugenol, geranyl acetone, methyl salicylate, linalool, guaiacol, β-ionone, *trans*-2-octenal, α-pinene, phenylacetaldehyde, benzaldehyde, α-terpineol, camphor and β-cyclocitral. Reference aroma compounds were obtained from Sigma–Aldrich Química S.A. (Madrid, Spain) as pure compounds. Stock solutions of the aroma standards at 500 mg L^{-1} were prepared in acetone and stored at −18 °C. Working solutions were prepared by volume dilution in diethyl ether-hexane (1:1). The internal standard methyl salicylate-D_4 of 99.5% purity was purchased from Sigma–Aldrich Química S.A. (Madrid, Spain). Calcium chloride 97% (Riedel–de–Haen) was purchased from Supelco (Sigma–Aldrich Química S.A., Madrid, Spain). Organic solvents (hexane, ethyl acetate and diethyl ether) of trace residue analysis quality were purchased from Scharlab (Barcelona, Spain).

SPE cartridges (Supelco, Sigma–Aldrich Química S.A., Madrid, Spain) were prepared by the manufacturer packing 500 mg of Tenax TA (80–100 mesh,) in 6-mL polyethylene cartridges retained using two polietilene fruits.

The extraction system developed in a previous work [22] consisted in a 50-mL Erlenmeyer flask attached to a glass cap with two connexion tubes: the inlet connected to a dry N_2 gas supply and the outlet fitted to the Tenax trap. Dry nitrogen (99.7%) was used to carry out the purge process and was led to flow into the flask at a flow of 1 L min^{-1}. Thirty grams of tomato sample together with 5% (w:w) $CaCl_2$ and with addition of 50 μL of 15 μg mL^{-1} methyl salicylate-D_4 (surrogate/internal standard) was magnetically stirred (350 rpm) and heated at 35 °C for 120 min in order to allow the volatile analytes to be retained in the Tenax trap (maintained at ambient temperature). The trap was removed and eluted with 3.5 mL of hexane-ether (1:1) mixture. The final volume extract was adjusted to 1 mL by means of a gentle stream of nitrogen.

Chromatographic determination was carried out using a Varian CP-3800 gas chromatograph (Varian Inc. Palo Alto, USA) coupled to an ion trap mass spectrometry detector (Saturn 4000, Varian Inc. Palo Alto, USA). Separation of the analytes was carried out on a 30 m × 0.25 mm DB-5MS (0.25 μm film thickness) Varian capillary column, using helium at a constant flow of 1 mL min^{-1} as carrier gas. The temperature programme was as follows: 45 °C for 5 min, then raised to 96 °C at a rate of 3 °C min^{-1}, then raised to 150 °C at a rate of 6 °C min^{-1} and finally raised up to 240 °C at a rate of 30 °C min^{-1}, with a final isothermal stage of 1.5 min (total chromatographic analysis time of 36 min). Injection in the splitless mode of a volume of 1 μL (injection port temperature 200 °C, splitless time 1 min) was carried out using an autosampler Varian 8400 (Varian Inc. Palo Alto, USA) equipped with a 10 μL syringe. The gas chromatograph was directly interfaced with the Varian 4000 mass-spectrometer, ion trap, (Varian Inc. Palo Alto, USA) in the external ionization mode with electron ionization energy of 70 eV in the positive ion mode. Transfer line temperature was established at 250 °C, and ion source and trap temperatures were adjusted to 200 °C.

Quantification of analytes in the sample extracts was performed using an external calibration curve obtained after direct injection of solvent standards containing internal standard and plotting relative areas to internal standard methyl salicylate-D4 against concentration (ng mL^{-1}) as described by Beltran et al. [22]. Quantification ion used for the internal standard methyl salicylate-D_4 was 155. This ion corresponded to the molecular mass of the compound after having changed the deuterium in the alcohol group by hydrogen, which occurs due to the contact with the aqueous sample.

Statistical analysis

For sensory data analysis, ANOVA procedure was conducted using SAS statistical package v.8.02 (SAS Institute Inc, Cary, NC, USA). A lineal model considering all the factors and their interactions was selected: $x_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + s_l + \alpha\beta_{ij} + \alpha_i\gamma_k + \beta_j\gamma_k + \alpha_i\beta_j\gamma_k + \varepsilon_{ijk}$, where $\alpha_i =$ panellist, $\beta_j =$ accession, $\gamma_k =$ postharvest storage, $s_l =$ session (random factor) and $\alpha\beta_{ij}$, $\alpha_i\gamma_k$, $\beta_j\gamma_k$ and $\alpha_i\beta_j\gamma_k$ are the interactions between fixed factors. A Student–Newman–Keuls mean comparison test was performed after checking effect significance with the ANOVA.

To perform the statistical analysis of the concentrations of the volatile compounds being determined, log odour units were calculated using commonly accepted odour thresholds for all volatiles. This transformation was selected to scale the relative importance of each compound in aroma perception. In order to study the relation between sensory data and volatile composition, a Partial Least Square (PLS) regression was used [23]. Prior to the PLS regression, the data were autoscaled with mean-centring and division by the standard deviation of the variable [24] to avoid the distortion caused by different variable scaling. The PLS regression model was calculated using full cross-validation resampling method. The goodness of the model fit was tested using the root mean square error of calibration (RMSEC) and the root mean square error of cross-validation (RMSECV).

In order to select the number of latent variables of the PLS model, two criteria were used: an additional latent variable was only chosen when the RMSECV was improved by at least 2% and the number of new variables was minimized as possible. In order to improve model precision, an aromatic variable selection was performed using an interval PLS (iPLS) variable selection which performs a hierarchical, sequential and exhaustive search for the best combinations of variables. iPLS was performed in reverse mode, with intervals successively removed from the analysis [24].

The calculations of PLS regressions were made using PLS_Toolbox v 6.0 (Eigenvector Research Inc, Wenatchee, WA, USA) for Matlab v 7.6.0 (Mathworks Inc, Natick, MA, USA).

Results

Shelf life evolution

Field trials confirmed that there were no statistical agro-morphological differences between blocks; thus, samples from the same accession were pooled. Postharvest storage behaviour (Table 2) showed significant differences between accessions. The highest shelf life was recorded in accession CDP-1245, which showed 59.1% of commercial fruits after 6 months of conservation, a value that was significantly different to that of accession CDP-5468, which showed the lowest shelf life (31.2%). Accessions CDP-1240 (42.4%) and CDP-8268 (42.8%) showed no significant differences between them and between the rest of accessions. The higher weight loss was detected in the accession CDP-1245, with 12.1, 19.2 and 27.9% of weight loss at 2, 4 and 6 months postharvest, respectively, values significantly higher than the weight loss recorded for CDP-1240 and CDP-5468 and CDP-8268 at 6 moths postharvest.

Panel training and consensus of odour attributes

With the lexicon proposed by Hongsoongnern and Chambers [25] as a starting point, different descriptors were suggested by the panel to describe the odour perceived in the accessions assayed. Panellists identified a characteristic odour in most of the Penjar tomatoes samples, and it was described as ‘sharp’ with ‘floral notes’. Other descriptors cited by the panellists in the Penjar samples were ‘green’, ‘fermented’, ‘pharmaceutical’ and ‘earthy’. Out of all these descriptors, only the odours ‘sharp-floral’ and ‘earthy’ were not found in the samples of commercial standard fresh tomatoes. These descriptors also appeared in different intensities in the different accessions and storage periods. The odour descriptor ‘sharp-floral’ was the most cited by the panellists during the training sessions. Other suggested descriptors were discarded: ‘earthy’ was considered as important but not frequent, the odour descriptors ‘fermented’ and ‘pharmaceutical’ were judged as negative and the odour descriptor ‘green’ was judged as occasional. Therefore, the rest of the training and the evaluation sessions were performed using only the descriptor ‘sharp-floral’. During the training, all the panellists indicated that the aromas were better perceived at 20 °C among the four temperatures tested, and this serving temperature was selected for the sensory analysis.

Sensory analysis

The odour descriptor ‘sharp-floral’ increased its intensity during postharvest storage of the Penjar tomatoes ($p < 0.0001$), with a maximum observed at 2 months of postharvest storage (Fig. 1). After this peak (4 months postharvest), the intensity of this descriptor decreased to similar values to those recorded at the harvest (0 months postharvest). Finally, at 6 months postharvest, the intensity of the ‘sharp-floral’ descriptor was very low in all the accessions. Out of the four accessions assayed, accessions CDP-1240 and CDP-5468 recorded the highest intensities of the ‘sharp-floral’ descriptor with higher values than CDP-1245 at 0, 2 and 4 months postharvest and to CDP-8268 at 2 months postharvest ($p < 0.0001$). Only accession CDP-8268 showed a different pattern in the evolution of aroma perception, with a maximum intensity of the ‘sharp-floral’ descriptor at 4 months postharvest. This unusual delay caused the significance of the accession x postharvest storage interaction ($p = 0.0229$).

Volatile compounds

Twenty-four volatiles were detected in the samples analysed. *Cis*-3-hexenal remained under detection limits in all the accessions and storage periods. This absence was unusual as it has been considered as one of the main aroma volatiles in other tomato varieties [2].

At the harvest (0 months postharvest storage), the compound with the highest concentration was 2-phenylethanol (Table 3). Other abundant compounds were *trans*-2-hexenal, *cis*-3-hexenol, hexanal and 2-isobutylthiazole. Accessions CDP-5468 and CDP-1240 registered the higher concentrations of volatiles at harvest, and 4 of the most important volatiles, including, *cis*-3-hexenol, *trans*-2-hexenal, hexanal and 2-isobutylthiazole, reached a concentration more than 5 times higher than those found in the accessions CDP-1245 and CDP-8268.

The data obtained for postharvest storages of 2, 4 and 6 months showed that there is a generalized decrease in the

Table 2 Mean values for postharvest traits. In the same column, different letters indicate significant differences (Student–Newman–Keuls, at $p \leq 0.05$)

Accession	Shelf life (%)[a]	Loss of weight 2 months (%)[b]	Loss of weight 4 months (%)[b]	Loss of weight 6 months (%)[b]
CDP-1245	59.1 a	12.1 a	19.2 a	27.9 a
CDP-8268	42.8 ab	10.4 ab	16.6 ab	23.9 b
CDP-1240	42.4 ab	9.0 b	14.8 b	21.1 b
CDP-5468	31.2 b	9.8 b	15.9 b	24.0 b

[a] % commercial fruits at 6 months postharvest

[b] % of weight loss with respect to initial weight at harvest

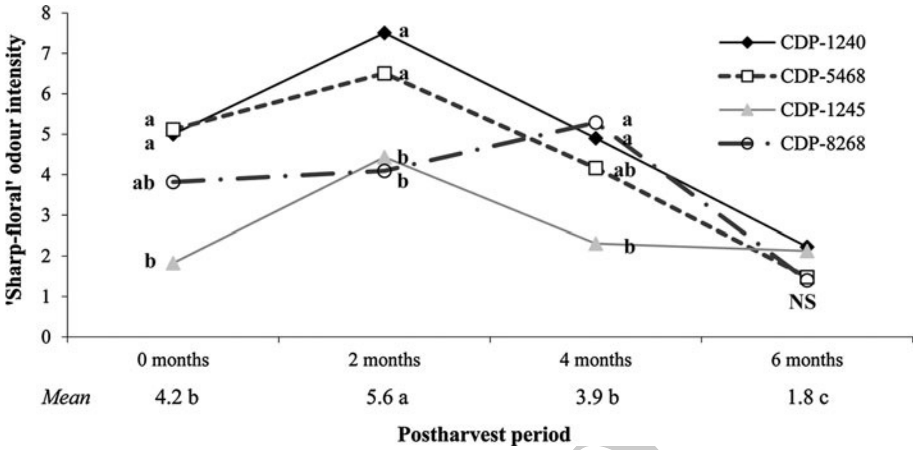

Fig. 1 Evolution of the intensity of the 'sharp-floral' odour descriptor during postharvest of four Penjar accessions. Inferior abscise legend indicates mean intensity for each postharvest period (*different letters* significant differences, Student–Newman–Keuls at $p < 0.05$). Inside the figure, *different letters* significant differences between accessions within each postharvest time (same statistical procedure)

concentration of all the volatiles determined, excluding some cases such as nonanal and α-pinene, with very low concentration at harvest. The most important reduction in the concentration occurred during the period between harvest and 2 months postharvest, when a mean reduction of 50% was registered (Table 3), except for accession CDP-1245 where, in average, no considerable reduction was recorded in this period, a result probably related to the smaller concentrations detected at harvest in this accession. After this initial reduction, between 2 and 4 months postharvest the decrease in concentration was small. Finally, in most cases concentration remained stable between 4 and 6 months.

In order to obtain a better interpretation of the relation between volatile composition and the sensory perception by the panellists, a PLS analysis using all the detected volatile components was carried out. The two first latent variables were selected to minimize calibration (RMSEC) and cross-validation (RMSECV) errors. With the first two latent variables, the model captured a 64.53% of the variation of sensory panel response using 62.89% of the variation in the volatiles composition matrix. The determination coefficient obtained in the calibration model was moderate ($R^2 = 0.63$) with a REMSEC of 1.08 and a REMSECV of 1.69 sensory units. The first latent variable was positively correlated with all the volatiles with similar loadings, but negatively correlated with α-pinene. The second latent variable was positively correlated mainly with volatiles 1-hexanol, hexanal and phenylacetaldehyde mainly and negatively correlated with volatiles camphor, α-terpineol, 2-phenylethanol, linalool and β-ionone.

Despite the good prediction response, the model still could not clearly establish which of the original variables were really important to explain the variability of the sensory panel response. Therefore, a selection of a subset of aromatic compounds was performed using reverse interval PLS (iPLS) [26] in order to obtain a superior prediction model. The results of the iPLS variable selection indicated that the main volatiles related with the variation in the sensory matrix were α-terpineol, *trans*-2-hexenal, 6-metyl-5-hepten-2-one, *trans*-2-octenal, α-pinene, β-ionone, 2 + 3-methylbutanol and phenylacetaldehyde. Using these set of volatiles, the model minimized RMSEC and RMSECV with the two first latent variables, which captured 65.19% of the variation in the sensory matrix using 73% of the variation in the volatiles matrix. A higher determination coefficient was obtained ($R^2 = 0.73$) with lower errors (RMSEC = 0.93 sensory units and RMSECV = 1.33 sensory units). Thus, the reduction in the number of initial volatiles enabled the development of a better model, confirming the good selection of the main volatiles involved in the sensory matrix variation. This time, the first component was positively correlated with similar loadings with volatiles *trans*-2-hexenal, 6-metyl-5-hepten-2-one, *trans*-2-octenal, 2 + 3-methylbutanol, phenylacetaldehyde and β-ionone and with a lower loading with α-terpineol and again negatively correlated with volatile α-pinene (Table 4). The second latent variable was positively correlated with volatiles α-pinene, 2 + 3-methylbutanol and phenylacetaldehyde and negatively with volatiles 6-metyl-5-hepten-2-one, *trans*-2-octenal and β-ionone; a value close to 0 was obtained for volatile *trans*-2-hexenal (Table 4).

In the PLS model obtained (Fig. 2), it was easier to identify clusters of points associated with postharvest storage duration than to accessions. The points corresponding to the peaks of intensity of the odour descriptor 'sharp-floral' were clustered in the upper right quarter of the graph, even the point corresponding to the intensity peak of the accession CDP-8268 that showed an unusual delay in the response was in the same area. Other samples with high values of 'sharp-floral' intensity (Fig. 1) were also clustered in the same quarter (Fig. 2). This was the case of the accession CDP-1240 at 4 months postharvest and of the accession CDP-5468 at harvest. Accession CDP-1240 at harvest with high intensity in the descriptor (Fig. 1) was placed in the lower

Table 3 Mean concentration (mg kg^{-1}) of main volatiles related to tomato aroma at different postharvest storage periods

Months	CD-P1245				CDP-1240				CDP-8268				CDP-5468			
	0	2	4	6	0	2	4	6	0	2	4	6	0	2	4	6
2-Phenylethanol	0.7950	0.4337	0.1975	0.2573	0.9388	0.3878	0.1882	0.3787	0.7580	0.3760	0.3859	0.2282	0.3505	0.3563	0.4130	0.3712
trans-2-hexenal	0.0120	0.1136	0.0260	0.0743	0.6158	0.0823	0.0209	0.0033	0.0442	0.0099	0.0324	0.0283	0.9818	0.3072	0.0103	0.0103
2-Isobutylthiazole	0.0154	0.0208	0.0038	0.0059	0.4603	0.1160	0.0279	0.0012	0.0380	0.0004	0.0007	0.0008	0.2904	0.1153	0.0012	0.0012
6-Methyl-5-hepten-2-one	0.0471	0.0686	0.0328	0.0534	0.2911	0.0724	0.0502	0.0356	0.1800	0.0432	0.0472	0.0474	0.2265	0.1045	0.0471	0.0471
2 + 3-Methylbutanol	n.d.	0.0145	n.d.	n.d.	0.2537	0.0785	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0270	n.d.	n.d.
Hexanal	0.0424	0.2790	0.0718	0.1640	0.2383	0.2007	0.1150	0.0553	0.0409	0.0509	0.1130	0.0660	0.5090	0.2867	0.0569	0.0569
1-Hexanol	0.0141	0.0350	0.0159	0.0841	0.1658	0.0482	0.0678	0.0145	0.0135	0.0531	0.0281	0.0838	0.2091	0.1611	0.0563	0.0563
cis-3-Hexenol	0.0051	0.0351	0.0057	0.0312	0.1580	0.0469	0.0121	0.0048	0.0087	0.0043	0.0159	0.0105	0.5440	0.0670	n.d.	n.d.
trans-2-heptenal	0.0594	0.0701	0.0122	0.0389	0.1382	0.0700	0.0695	0.0125	0.0473	0.0360	0.0130	0.0120	0.0575	0.0517	0.0671	0.0671
R-Limonene	0.0216	0.0371	0.0081	0.0148	0.1079	0.0330	0.0087	0.0142	0.0158	0.0343	0.0086	0.0128	0.0119	0.0115	0.0352	0.0352
Nonanal	0.0283	0.0255	0.0245	0.0307	0.0641	0.0283	0.0253	0.0250	0.0246	0.0250	0.0244	0.0223	0.0252	0.0316	0.0279	0.0279
Eugenol	0.0276	0.0135	0.0030	0.0151	0.0604	0.0132	0.0146	0.0118	0.0497	0.0094	0.0423	0.0111	0.0338	0.0225	0.0088	0.0088
Geranyl acetone	0.0212	0.0141	0.0010	0.0179	0.0490	0.0171	0.0012	0.0042	0.0403	n.d.	0.0133	0.0109	0.0331	0.0406	0.0036	0.0036
Methyl salicylate	0.0013	0.0247	0.0091	0.0186	0.0486	0.0178	0.0356	0.0110	0.0647	0.0016	0.0330	0.0098	0.0312	0.0273	0.0131	0.0131
Linalool	0.0176	0.0081	0.0047	0.0126	0.0337	0.0084	0.0037	0.0020	0.0395	0.0033	0.0073	0.0034	0.0134	0.0066	0.0041	0.0041
Guaiacol	0.0274	0.0108	0.0026	0.0115	0.0317	0.0099	0.0108	0.0063	0.0642	0.0050	0.0173	0.0083	0.0888	0.0198	0.0049	0.0049
Benzaldehyde	0.0151	0.0129	0.0123	0.0197	0.0293	0.0196	0.0132	0.0122	0.0251	0.0112	0.0125	0.0098	0.0189	0.0224	0.0126	0.0126
α-Terpineol	0.0126	0.0064	0.0037	0.0105	0.0267	0.0056	0.0027	0.0013	0.0313	0.0026	0.0051	0.0028	0.0011	0.0053	0.0031	0.0031
β-Cyclocitral	0.0069	0.0029	0.0020	0.0031	0.0120	0.0041	0.0027	0.0015	0.0087	0.0012	0.0022	0.0011	0.0043	0.0027	0.0015	0.0015
β-Ionone	0.0086	0.0025	0.0016	0.0025	0.0101	0.0031	0.0020	0.0011	0.0060	0.0009	0.0017	0.0011	0.0042	0.0026	0.0012	0.0012
trans-2-octenal	0.0037	0.0038	0.0025	0.0039	0.0073	0.0041	0.0044	0.0025	0.0062	0.0029	0.0033	0.0022	0.0035	0.0050	0.0035	0.0035
α-Pinene	0.0077	0.0087	0.0077	0.0061	0.0065	0.0091	0.0090	0.0085	0.0085	0.0065	0.0077	0.0060	0.0062	0.0063	0.0080	0.0080
Camphor	0.0019	0.0011	0.0012	0.0018	0.0035	0.0018	0.0013	0.0010	0.0036	0.0008	0.0011	0.0008	0.0019	0.0018	0.0011	0.0011
Phenylacetaldehyde	n.d.	n.d.	n.d.	0.0011	0.0013	0.0029	0.0005	n.d.	n.d.	n.d.	0.0009	n.d.	0.0005	0.0005	n.d.	n.d.
Total	1.192	1.2425	0.4497	0.879	3.7521	1.2808	0.6873	0.6085	1.5188	0.6785	0.8169	0.5794	3.4468	1.6833	0.7805	0.7387

n.d. not detected

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Table 4 Loadings of the volatiles included in the PLS model optimized with reverse iPLS variable selection considering the first two latent variables

Volatile	Loading on latent variable 1	Loading on latent variable 2
α-Terpineol	0.255	−0.582
trans-2-hexenal	0.426	−0.046
6-Metyl-5-hepten-2-one	0.413	−0.276
trans-2-octenal	0.413	−0.243
α-Pinene	−0.061	0.359
β-Ionone	0.366	−0.473
2 + 3-Methylbutanol	0.379	0.239
Phenylacetaldehyde	0.361	0.338

right quarter, but close to the other samples with high intensity. In the upper right quarter of the model, only accessions with high 'sharp-floral' intensity could be found (Fig. 2).

Discussion

As expected, a considerable variation in shelf life was detected among the accessions assayed. Although all of them offered good conservation in long-term storage, it was possible to identify outstanding accessions such as CDP-1245 with almost 59.1% commercial fruits after 6 months of storage at room temperature. The differences detected confirmed the good selection of the materials as the objective was to evaluate a representative sample of the variation in the varietal type. It should be noted the good response of the Penjar tomatoes, especially if the loss of weight is compared with results provided by other authors. In this sense, Javanmardi and Kubota [27] reported a loss of weight ratio at room temperature of 0.68% per day, and that would mean a 40.8% in 2 months, while in our study Penjar tomatoes showed only a 9.0–12.1% reduction in this period.

Despite different aroma notes such as 'green', 'sharp', 'floral', 'earthy', 'fermented' and 'pharmaceutical' being identified in the collection of Penjar tomatoes with the *alc* mutation, it was the 'sharp with floral notes' descriptor the one that clearly and continuously was associated with this particular varietal type. This descriptor would represent an 'identification mark' for the varietal type as it was not found in reference commercial fresh tomato varieties. The intensity of this descriptor, as expected, varied during postharvest storage, reaching a maximum not at harvest, but generally at 2 months postharvest. This is an unusual but interesting result, as it is usually suggested that a reduction of postharvest storage minimizes the typical loss of the characteristic tomato aroma [28, 29].

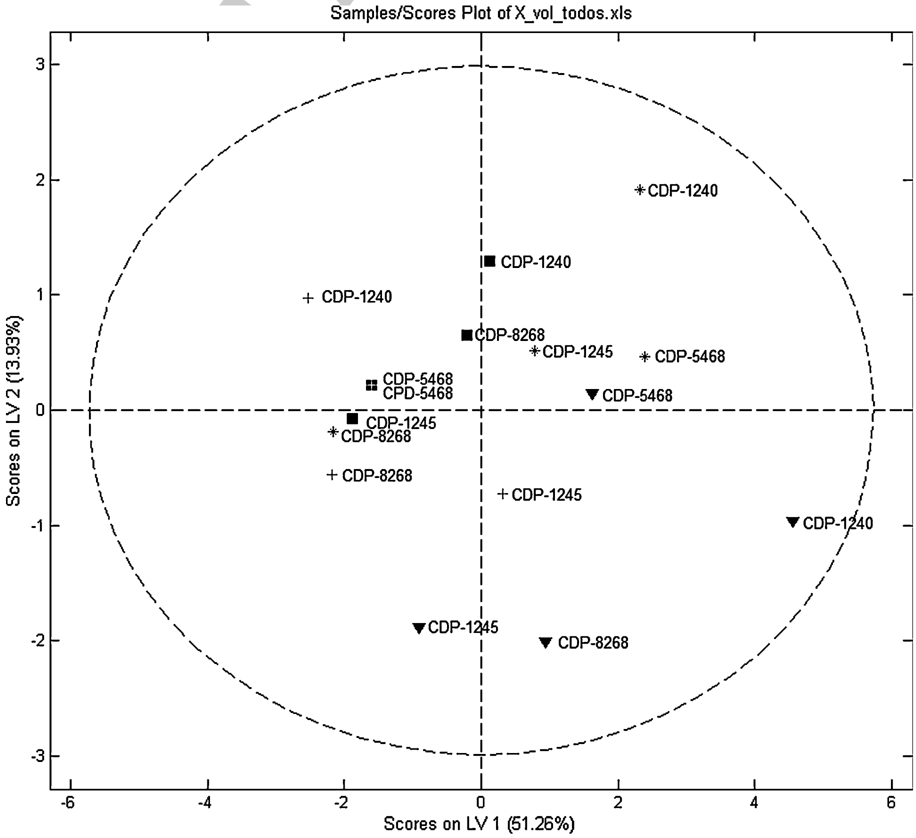

Fig. 2 PLS model optimized with reverse iPLS variable selection relating volatile concentration and sensory evaluation. First latent positively correlated with similar loadings with volatiles *trans*-2-hexenal, 6-metyl-5-hepten-2-one, *trans*-2-octenal, 2 + 3-methylbutanol, phenylacetaldehyde and β-ionone, and with a lower loading with α-terpineol and negatively correlated with α-pinene. Second latent variable positively correlated with volatiles α-pinene, 2 + 3-methylbutanol and phenylacetaldehyde, and negatively with volatiles 6-metyl-5-hepten-2-one, *trans*-2-octenal and β-ionone. Postharvest storage *filled inverted triangle* 0 months, *asterisk* 2 months, *filled square* 4 months, +6 months

The existence of a characteristic odour descriptor possibly contributes to the preservation of a local market associated with this varietal type, as well as to the association with the variety with traditional dishes. On the other hand, the identification of intensity peaks for the descriptor enables the determination of the best moment to release the stored materials with the maximum quality. In general, the best aromatic properties would be obtained at 2 months postharvest.

The fact that Penjar varietal type is formed by a wide variety of genetic backgrounds, in which the *alc* allele has been inserted, enabled the identification of accessions with high odour scores, such as CDP-1240 and CDP-5468. It also enabled the identification of unusual patterns of aroma evolution. In this sense, the accession CDP-8268 showed a delay in the 'sharp-floral' descriptor intensity at 4 months instead of the 2 months peak identified in the rest of the accessions.

The existence of genotypic variability among the Penjar tomatoes, as odour intensity is concerned, also leads to a further conclusion related to the structure of traditional or landrace populations. It is known that these materials are usually configured as population varieties with a high level of diversity, maintained through mass selection processes. It is also known that the materials that have survived the genetic erosion processes are usually related to quality markets because the consumer identifies in them a higher level of organoleptic quality. In the case of the Penjar tomato, the main morpho-agronomic characteristic of the varietal type is due to its long shelf life as a consequence of the introgression of the *alc* allele in different varietal types [16]. Therefore, this is the characteristic that has been traditionally associated with a higher organoleptic quality. But the considerable variation in odour intensity detected in this work results in the existence of low-quality populations, which are probably maintained in the market through the generalization of a higher quality traditionally assigned to the varietal type. The association of the ideas 'traditional' and 'high quality' is not always true, especially in species such as the tomato where the existence of a certain degree of cross-pollination may contribute to varietal degeneration. Therefore, in order to consolidate quality markets and to promote on-farm conservation of these genetic resources, it is necessary to purge the existing populations, fostering those with better organoleptic profiles.

Regarding volatile concentration, it is unusual to find tomato fruits with low levels of *cis*-3-hexenal as in this case. This compound has been described as the most important in tomato in several studies [20, 30, 31], with a major contribution to the aroma descriptors 'fresh green', 'sweet' [30] and 'tomato-like' [31]. It has been reported the instability of *cis*-3-hexenal and its isomerization to *trans*-2-hexenal during isolation and analysis [20], though it does not seem that this is the case of this study. In fact, we have found *cis*-3-hexenal using exactly the same methodology in other tomato varieties [32]. The absence of this compound may be important in the characteristic aroma of the Penjar tomatoes, as it may be related to the emergence or unveil of other compounds which typically show lower log odour units.

Apart from the deficiency in *cis*-3-hexenal, it does not seem that the introgression of the *alc* allele affects the concentration of other volatiles, as it has been reported in the ripening mutant *nor* [10–12], which is allelic to *alc* [16]. The comparison of the results obtained in this study and the analyses performed with the same methodology or the previously published results by other groups in other varietal types [2, 33, 34], apart from the lack of *cis*-3-hexenal, only evidenced reduced levels of hexanal and phenylacetaldehyde.

The lightness of the external colour typical of this varietal type made logical to expect reduced levels of volatiles derived from the carotenoid degradation pathway such as 6-methyl-5-hepten-2-one and geranyl acetone [14], especially considering that the *alc* mutation has been related to low levels of this carotenoid [15]. But on the contrary, the values obtained in the Penjar tomatoes at harvest (Table 1) were similar to those reported by other authors in conventional varieties: 0.13 mg kg^{-1} [2], 0.1–0.3 mg kg^{-1} [20] or 0.05–0.2 mg kg^{-1} [33] in the case of 6-methyl-5-hepten-2-one, and 0.057 mg kg^{-1} [2] in the case of geranyl acetone. It should also be highlighted that the concentration obtained of 2-isobutylthiazole at harvest in the accessions CDP-1240 and CDP-5468 (Table 1) is more than 10 times higher than the previously reported in other varieties: 0.04 mg kg^{-1} [2], 0.01 mg kg^{-1} [6] or 0.03 mg kg^{-1} [33].

In some fruits, a single compound dominates aroma perception, but in tomato no single compound dominates and more than 10 volatiles have been described as having positive log odour units. Even compounds with negative log odour units should not be neglected, as they may still contribute to the overall flavour as background notes [11]. It has even been determined that some of the last, such as eugenol, may have an impact on tomato aroma upon release from their glycosidic conjugates [6].

In this complex context, with so many compounds, and relations between them, conditioning odour perception, it is extremely difficult to elucidate a direct relation between aroma perception by the panellists and volatile composition of the fruit, and its evolution during storage period. The best alternative found was to carry out Partial Least Square regression (PLS) analysis. PLS attempts to find factors which both capture the greatest amount of variance in the

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aromatic composition and achieve the best correlation between the panel ‘striking’ odour intensity evaluation (predicted variable) and the volatile composition matrix (predictor variables) including storage evolution. In other words, PLS maximize covariance between predictor and predicted variables. This statistical procedure is frequently used in several complex chemometric applications and has also been applied to identify the most important descriptors in aroma perception [35]. Following this methodology, optimized with iPLS variable selection, the volatiles α-terpineol, *trans*-2-hexenal, 6-metyl-5-hepten-2-one, *trans*-2-octenal, α-pinene, β-ionone, 2 + 3-methylbutanol and phenylacetaldehyde were identified as important compounds to consider in order to explain the postharvest odour evolution of the Penjar tomatoes.

The contribution of each compound to the descriptor is really difficult to ascertain. Several compounds may change the induced aroma perception at different concentrations and some of them may interact with others masking or unmasking aroma notes [1]. Additionally, not only each compound may be responsible for different attributes at different concentrations, but their perception may vary with changes in alcohol content such as the increase in ethanol during ripening and this may add complexity to tomato aroma evaluation [31].

Regarding the perception of the selected volatiles, α-terpineol has been described as ‘floral/fruity’ [36], *trans*-2-hexenal might induce a ‘green’ or ‘stale’ perception [31], 6-metyl-5-hepten-2-one as ‘sweet-floral’ [31], *trans*-2-octenal as ‘sweet/phenolic’ [37], α-pinene as ‘stem-like’ [38], β-ionone as ‘sweet fruity’ [31], 2 + 3-methylbutanol as ‘tomato-like’ [39] and phenylacetaldehyde as ‘sweet’ [30]. In short, most of them may contribute to the ‘sharp-floral’ descriptor found in the Penjar tomatoes.

In the PLS model, the first latent variable had positive and similar loadings with almost all these selected volatiles and it may be related with overall volatile content, while in the second latent variable 5 volatiles had negative loadings and 3 had positive loadings, and it would be related to aroma nuance. As the samples corresponding to the higher ‘sharp-floral’ intensity had positive values of the first two latent variables of the optimized PLS model (Fig. 2), a higher impact would be ascribed to volatiles with high loadings in both latent variables. This was the case of 2 + 3-methylbutanol and phenylacetaldehyde (Table 4). Nevertheless, it may also be possible that some of the compounds with negative loadings in the second latent variable might be masking other compounds, and thus should not be disregarded. It should also be pointed that between harvest and 2 months postharvest most compounds reduced considerably their concentration, while the intensity of the ‘sharp-floral’ descriptor increased, which means that probably there is a rearrangement of the relative concentrations among volatiles that may lead to masking/unmasking processes.

Berna et al. [38] studying the evolution of aroma profiles from harvest to 19 days postharvest storage reported an initial shift with terpenoids, produced in the stem, holding an important participation in the overall aroma at the beginning of conservation, to a more important role of compounds such as 1-nitropentane and 6-methyl-5-hepten-2-one related to fresh tomato and fruity aroma, respectively, as storage progressed. They also found an increase in 2-methylbutanol at ending stages of maturity.

It is difficult to extrapolate similarities between these findings related to the first weeks of conservation and our work, as the Penjar tomatoes are adapted to longer storage periods and therefore time span evaluated is much larger. Nevertheless, it is interesting to see that compounds selected as important in the evolution of the aroma profiles with the reverse iPLS such as 6-methyl-5-hepten-2-one and 2 + 3-methylbutanol are highlighted in both studies.

Krumbein et al. [40] monitoring the postharvest aroma evolution during 21 days on different cultivars, some of them with reported long shelf life, found that the increase in hexanal and 2-isobutylthiazole during postharvest was connected with an increase in the mouldy descriptor, whereas the attribute tomato-like increased simultaneously, maybe linked with the concentration of geranyl acetone, a compound related to this attribute. In the present study, the content of hexanal evolved differently in each accession, but 2-isobutylthiazole decreased rapidly. Nevertheless, it is important to highlight that β-ionone and 6-metyl-5-hepten-2-one, compounds derived from carotenoid metabolism as geranyl acetone, were also selected as important in the explanation of the aroma evolution of Penjar tomatoes.

The evaluation of aroma profiles in tomato is extremely complex. Despite the attempts to generalize the volatile and aroma profiles correlation as a common model for all the tomato varieties, it seems clear that at least in the varieties with long-term conservation such as the Penjar tomatoes, the standard conclusions are not justified. Specific aroma notes may be variety dependent and masking/unmasking relations may reveal the effect of volatiles usually disregarded in the evaluation of tomato aroma.

Conclusions

The aroma of Penjar tomatoes is mainly characterized by the ‘sharp-floral’ descriptor, although other notes as ‘earthy’ contribute to its typical aroma. The ‘sharp-floral’ aroma note evolves during postharvest (0–6 months), increasing during the period 0–2 months, when it reaches its maximum. The broad genetic basis of this varietal type results in considerable differences between accessions: two of the 4 accessions studied (CDP-1240 and CDP-5468) showed a significantly higher 'sharp-floral' intensity, and one accession (CDP-8268) showed a delay in the development of the intensity peak of the 'sharp-floral' note. These results are very interesting in order to emphasize the added value of this landrace and to determine the better time for its commercialization (2 months).

Despite the volatile concentration decrease during the first 2 months of conservation, there is an increase in 'sharp-floral' aroma perception, a result with difficult explanation. The use of iPLS variable selection revealed that 8 of the 24 volatiles detected play a prevalent role, and it seems that the rearrangement of the relative concentrations during the postharvest period and the consequent masking/unmasking processes is the most plausible explanation for the changes in odour intensity during the postharvest of the Penjar tomato.

Acknowledgments This work was supported by grants from the Conselleria de Agricultura, Pesca y Alimentació de la Comunidad Valenciana, the Fundación de la Comunidad Valenciana para la Investigación Agroalimentaria (AGROALIMED) and from the Departament d'Agricultura, Alimentació i Acció Rural (DAR) de la Generalitat de Catalunya.

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