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Sugar-and-acid profile of Penjar tomatoes and its evolution during storage

Joan Casals Missio[1]*, Raul Marti Renau[2], Francesc Casañas Artigas[1,3], Jaime Cebolla Cornejo[4]

[1]Miquel Agustí Foundation – Campus Baix Llobregat, Av. Canal Olímpic, 15 – 08860 – Castelldefels – Spain.
[2]University Jaume I – Dept. of Agricultural Sciences and Natural Environment – Campus Riu Sec – 12071 – Castellón – Spain.
[3]Polytechnic University of Catalonia – Dept. of Agri-Food Engineering and Biotechnology – Campus Baix Llobregat, Av. Canal Olímpic, 15 – 08860 – Castelldefels – Spain.
[4]Polytechnic University of Valencia – Institute for Conservation & Improvement of Valentian Agrodiversity (COMAV) – Camino de Vera, s/n – 46022 – Valencia – Spain.
*Corresponding author <j.casalsmissio@gmail.com>

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ABSTRACT: The *alcobaça* mutation in the Penjar tomato (*Solanum lycopersicum* L.) variety alters the ripening process and confers a long shelf life (more than four months). Storage of Penjar tomatoes leads to a distinctive sensory profile valued by local consumers, who prefer aged tomatoes to fresh ones. To study chemical changes occurring during storage, we characterized the complete sugar-and-acid profile of 25 accessions at harvest and at 2 and 4 months after harvest. We found considerable variability in the sugar-and-acid profile within the Penjar variety, especially for fructose and glucose. Some accessions presented exceptionally high values for sugars, making them especially interesting for breeding programs. During postharvest, the concentration of glucose, fructose, and citric acid decreased, whereas the concentration of malic and glutamic acids increased. Data from this study offer novel insights into postharvest changes in tomato quality parameters and help elucidate the reasons for the appreciation of this variety by consumers.

Keywords: tomato landrace, alcobaça, ripening mutant, postharvest, quality

Introduction

Ripening and postharvest behavior are strongly disrupted in ripening mutants of tomato (*Solanum lycopersicum* L.) (Klee and Giovannoni, 2011). A number of mutations related to these disruptions have been found in scientific breeding programs (e.g.: *ripening inhibitor* (*rin*) (Robinson and Tomes, 1968)) and others have been found in landraces. In Europe, long-storage landraces (Bota et al., 2014; Casals et al., 2012; Siracusa et al., 2012) are highly valued by local consumers, especially in Italy and Spain, where they are traditionally consumed after a storage period that confers a specific sensory profile. For these special tomatoes, the postharvest period could be referred to as the "aging phase" (Watada et al., 1984), because it involves a series of changes in quality parameters after maturity and before consumption.

In the Penjar variety, long shelf life (mean, 126.8 days) is mainly due to the *alcobaça* (*alc*) mutation in conjunction with small fruit size (Casals et al., 2012). During the aging phase, Penjar tomatoes undergo a rearrangement of the concentrations of volatiles, resulting in fruits with a distinctive "sharp-floral" aroma that is very intense in some genotypes (Casals et al., 2011), and consumers prefer aged tomatoes (between one and four months) over recently harvested ones. In addition to changes in the aroma profile, aging in Penjar tomatoes probably entails changes in the concentrations of molecules related to sweetness and acidity. During the first stages of the postharvest period in standard tomatoes, reducing sugars (glucose and fructose) continue to accumulate due to degradation of stored carbohydrates, lipids, and proteins (Beckles, 2012), but within a few days the concentration of sugars falls due to the respiratory metabolism (Getinet et al., 2008). On the other hand, the concentration of organic acids (malic and citric acids) decreases throughout the postharvest period (Getinet et al., 2008). Differences between standard tomatoes and *alc* mutants during the ripening period have been well described (Kopeliovitch et al., 1980; Mutschler, 1984), but much less is known about differences in the postharvest period. Thus, we sought to describe the evolution of malic, glutamic, and citric acids and of glucose and fructose during the long aging phase. Moreover, because the *alc* allele in the Penjar tomatoes occurs in a variety of genetic backgrounds (Casals et al., 2012), we also sought to ascertain the reasons for consumers' postharvest preferences of this variety so that this information could be transferred to breeding programs.

Materials and Methods

Plant materials

In a previous study, we analyzed genetic variability within the Penjar variety (Casals et al., 2012). Characterization of 118 accessions provided by local farmers enabled us to select 21 accessions representing the major part of the genetic variability found in the variety. In the current study, we used these 21 accessions plus two additional Penjar accessions widely cultivated by local farmers (Table 1). The previous study confirmed the presence of the *alc* allele in all the Penjar accessions using a cleaved amplified polymorphism (CAP) marker (Casals et al., 2012). All these materials are available from the germplasm bank of the Miquel Agustí Foundation (Spain). We included as controls four ripening mu-

Table 1 – Tomato accessions used in this study.

Type	Accession	Origin	Fruit shape	Fruit weight[1]	Shelf life[1]
Landraces				g	days
	CDP01203	Sant Vicenç de Montalt, NE Spain	Cylindrical	34.4	139.3
	CDP05566	Cassà de la Selva, NE Spain	Cylindrical	37.9	129.4
	CDP01254	Llinars del Vallès, NE Spain	Heart-shaped	25.2	133.9
	CDP01447	Pineda de Mar, NE Spain	Cylindrical	42.2	128.8
	CDP00235	Mollet del Vallès, NE Spain	Slightly flattened	95.0	94.3
	CDP01255	Arenys de Munt, NE Spain	Rounded	60.7	132.3
	CDP07789	Argentona, NE Spain	Ellipsoid	34.6	132.0
	CDP01114	Creixell, NE Spain	Rounded	53.6	118.3
	CDP07531	Sant Quintí de Mediona, NE Spain	Highly rounded	50.8	117.9
	LC77	Castellbell i el Vilar, NE Spain	Ellipsoid	34.5	40.6
	CDP03512	Palafolls, NE Spain	Rounded	51.5	129.8
	CDP02569	Sabadell, NE Spain	Heart-shaped	54.0	126.0
	CDP03365	Sabadell, NE Spain	Slightly flattened	74.1	117.9
	CDP02588	*Unknown*	Flattened	78.0	106.9
	CDP06432	Sabadell, NE Spain	Flattened	47.7	138.6
	CDP00023	*Unknown*	Rounded	100.5	118.4
	CDP01245	Estanyol, NE Spain	Slightly flattened	46.5	137.6
	CDP01475	Santa Coloma de Farners, NE Spain	Cylindrical	62.3	115.2
	CDP05468	*Unknown*	Heart-shaped	31.2	133.9
	CDP01127	Mallorca, Balearic Islands	Heart-shaped	120.2	98.7
	CDP03698	*Unknown*	Cylindrical	71.0	99.3
	LC313	Blanes, NE Spain	Heart-shaped	68.4	127.9
	CDP06989	Blanes, NE Spain	Heart-shaped	60.9	131.5
Commercial varieties (*Penjar*)					
	CDP03366	Hortícola Alavesa SL	Slightly flattened	120.9	98.4
	CDP02356	Diamond Seeds SL	Rounded	57.0	123.6
Ripening mutants (*controls*)[3]					
	LA3770 (*nor*)	TGRC[2]	Rounded	51.9	124.8
	LA3012 (*rin*)	TGRC[2]	Rounded	128.2	18.1
	LA3134 (*alc*)	TGRC[2]	Rounded	130.7	20.8
	LA0162 (*Nr*)	TGRC[2]	Rounded	122.0	11.2
Commercial hybrid					
	Bodar	Royal Sluis	Rounded	131.96	19.2

[1]Data extracted from Casals et al. (2012); [2]Tomato Genetics Resource Center (TGRC, University of California, Davis, CA); [3]Allele names: non-ripening (*nor*), ripening inhibitor (*rin*), alcobaça (*alc*), never ripe (*Nr*).

tants from the Tomato Genetics Resource Center (TGRC, University of California, Davis, CA; LA0162 (Never ripe (*Nr*)), LA3770 (non-ripening (*nor*)), LA3012 (ripening inhibitor (*rin*)), and LA3134 (alcobaça (*alc*)), and one normal-ripening commercial hybrid widely grown in NE Spain (Bodar, Royal Sluis).

As described by Casals et al. (2012), plants were grown in an experimental field in Sabadell (NE Spain, 41°32′ N 2°4′ E) in the open air at a density of 25,000 plants ha^{-1} using standard local farming practices (canes for support, pruning every 15 days, with local irrigation and fertigation) and a random three-block design with 12 plants per plot. Agromorphological traits for each variety have been published in a previous work (Casals et al., 2012). From each plot, fruits were collected at the red ripe stage in one single harvest. Fruits were stored in a dark room at 20 ± 5 °C and 68 % to 75 % relative humidity. Shelf life was calculated for each plot as the mean number of days from harvesting to discarding of fruits (Casals et al., 2012).

Previous studies based on aroma profile (Casals et al., 2011) showed that Penjar tomatoes maintain their high commercial value for 4 months after harvesting (Figure 1). Thus, we targeted this period for the present study, and selected three sampling dates: at harvest (0 months), at 2 months postharvest, and at 4 months postharvest. At each sampling date, we froze three replicates per accession and kept them at -20 °C until analysis. Each replicate consisted of three fruits with good external appearance from a single plot.

Sample preparation

Samples were thawed in a refrigerator in the dark. Then the three replicates were blended and homogenized at a low temperature. Samples were centrifuged at 2,500 rpm (510 x *g*) for 5 min. The upper phase was diluted (1:10) in deionized water and the internal standard D-fructose 1.6-diphosphate added. The solution was filtered using centrifuge tube filters with 0.22 µm membranes and analyzed.

Chemical analysis

Sucrose, fructose, glucose, malic acid, citric acid, and glutamic acid were quantified by capillary electrophoresis following the method described by Cebolla-Cornejo et al. (2012) on an Agilent 7100 system using fused silica capillaries with a 50 µm internal diameter, 363 µm external diameter, 67 cm total length, and 60 cm effective length. Capillaries were initially conditioned with consecutive rinses at 95000 Pa and 50 °C, as follows: 1 M NaOH (5 min), 1 M NaOH (5 min), and deionized water (10 min). At the beginning of each working session, the capillary was flushed at 20 °C with the background electrolyte (20 mM 2.6-piridin dicarboxylic acid at pH 12.1 and 0.1 % w/v hexadimethrine bromide) running for 30 min. Between runs, it was flushed with 58 mM SDS solution (2 min) followed by the background electrolyte running for 5 min. Samples were injected hydrodynamically at 3447 Pa for 20 s. Separations were performed at -25 kV and 20 °C. Indirect detection was done at 214 nm. Results are expressed in grams per kilogram of fresh weight (FW).

Statistical analysis

As proposed by Sakiyama and Stevens (1976), to avoid presumed increases in acid and sugar concentration due to water loss during postharvest, concentrations at 2 and 4 months' postharvest were corrected according to the following formula: Reported value = Measured value × Weight after storage/Weight at harvest. We used the SAS statistical package (Statistical Analysis System, Cary, NC, USA, version 8.02) for ANOVA. Differences between storage times and between accessions were determined by the Student-Newman-Keuls test with significance set at 0.05. Genotypic correlations (between mean phenotypic values of each accession) were estimated using the Pearson coefficient. Using this approach, we analyzed the correlations between chemical composition and agromorphological traits to evaluate whether the chemical composition of Penjar tomatoes was related to shelf life.

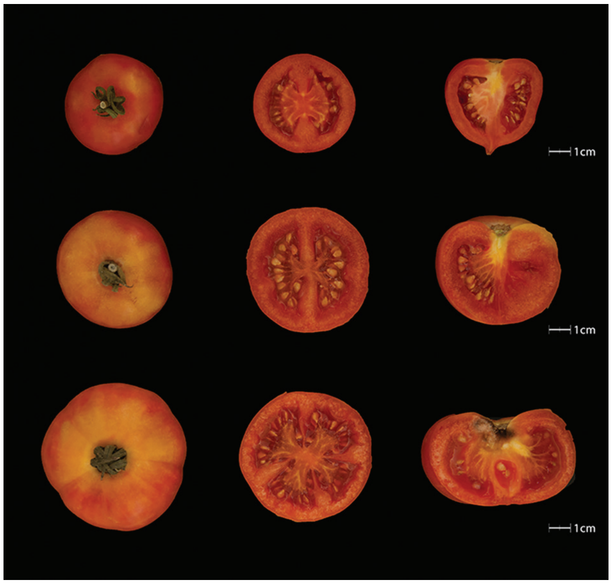

Figure 1 – External appearance, longitudinal and transversal sections of fruits from three Penjar accessions (CDP05468, CDP01255, CDP00235) after 4 months' storage.

Results

The controls Bodar, *rin*, *alc*, and *Nr* lost their commercial value at one month postharvest and were therefore discarded as controls for the long-term postharvest evolution of sugars and acids. However, like the Penjar accessions, accession LA3770 carrying the *nor* allele provided fruits that maintained their commercial value after 4 months storage.

The average weight loss for the accessions of the Penjar variety at 4 months postharvest was 17 %. The *nor* control lost less weight (13 %). For the Penjar accessions, the average weight loss in the first period (11 %, 0-2 months postharvest) was significantly higher (1.6 fold, $p < 0.0001$) than in the second (7 %, 2-4 months postharvest) (Figure 2). All the Penjar accessions had the same pattern of weight loss, and no significant interaction between accession and postharvest period was detected ($p = 0.49$). In the *nor* control, weight loss was also greater in the first period (8 %) than in the second (5 %) ($p < 0.01$).

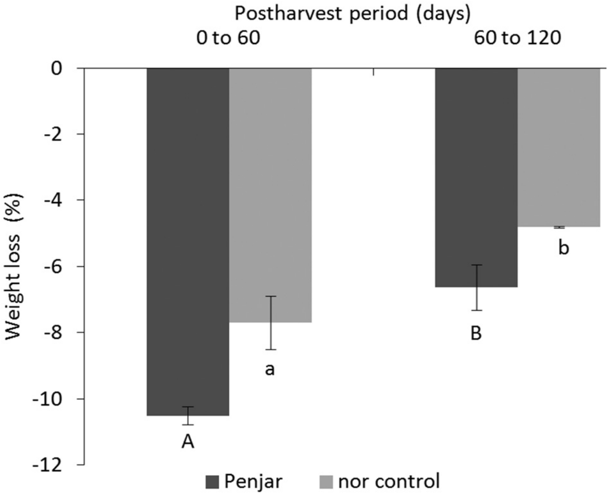

Figure 2 – Fruit weight loss in the 0-2 months and 2-4 months postharvest periods for the Penjar variety and for the LA3770 non-ripening (*nor*) control. Data represent the mean ± standard error of the mean of 25 accessions for the Penjar variety and of three replicates for the *nor* control. Within each variety, different letters indicate differences between periods (Student-Newman-Keuls test, $p < 0.05$).

The concentration of sugars and acids varied widely between accessions at harvest ($p < 0.0001$) (Table 2). Sucrose concentrations were not evaluated because they were below the detection threshold (8.99 µg mL^{-1}) of the analytical method (Cebolla-Cornejo et al., 2012) in both the Penjar accessions and the controls. For the other metabolites, concentrations ranged from 0.59 to 1.57 g kg^{-1} FW for malic acid, from 3.94 to 7.11 g kg^{-1} FW for citric acid, from 0.92 to 3.82 g kg^{-1} FW for glutamic acid, from 14.41 to 34.76 g kg^{-1} FW for fructose, and from 11.62 to 29.64 g kg^{-1} FW for glucose. The range of variation in acid concentrations at harvest within the Penjar variety was similar to that observed in the controls.

Maximum sugar concentrations at harvest were lower in the controls than in the Penjar accessions with the highest concentrations. Specifically, the highest fructose concentration in a Penjar accession (CDP07789 = 34.76 g kg^{-1} FW) was 1.5-fold higher ($p < 0.0001$) than in the control with the highest concentration (*nor* = 20.17 g kg^{-1} FW), and the highest glucose concentration in a Penjar accession (LC77 = 29.64 g kg^{-1} FW) was 1.6-fold higher ($p < 0.0001$) than the highest concentration in a control (Bodar = 18.44 g kg^{-1} FW). Values of the fructose-to-glucose ratio in the Penjar accessions at harvest ranged from 1.11 to 1.37, although no differences were detected between accessions. The ratio was lowest in the control Bodar (1.00), where it was lower than the four highest rated Penjar accessions ($p = 0.0038$).

During the 4 months after harvest, the concentrations of sugars evolved differently from the concentrations of acids (Figure 3). Concentrations of sugars decreased sharply during the first two months (in the Penjar accessions, 43 % for glucose and 39 % for fructose) and then more gradually in the third and fourth months (in the Penjar accessions, 28 % for glucose and 12 % for fructose). The evolution in the *nor* control was similar to that in the Penjar variety. In Penjar tomatoes, the average fructose-to-glucose ratio increased slightly ($p < 0.0001$) during postharvest, from 1:1.22 at harvest to 1:1.32 two months after harvest and 1:1.62 four months after harvest.

Table 2 – Sugar and acid concentrations for table-ripe tomatoes at harvest. Data represent means from three replicates ± standard error of the mean.

Accession	Malic acid	Citric acid	Glutamic acid	Fructose	Glucose	Fructose to glucose ratio
	g kg^{-1} fresh weight (FW)					
Bodar	0.89 ± 0.05	4.23 ± 0.43	1.50 ± 0.13	18.39 ± 1.23	18.43 ± 1.04	1.00 ± 0.03
LA0162 (*Nr*)[1]	1.20 ± 0.10	6.04 ± 0.28	1.71 ± 0.15	12.82 ± 0.72	10.28 ± 0.90	1.25 ± 0.04
LA3012 (*rin*)[1]	1.32 ± 0.13	3.16 ± 0.01	2.86 ± 0.83	16.44 ± 1.82	13.66 ± 1.28	1.20 ± 0.03
LA3134 (*alc*)[1]	0.89 ± 0.12	6.44 ± 0.20	2.22 ± 0.07	16.77 ± 3.61	13.56 ± 4.18	1.24 ± 0.12
LA3770 (*nor*)[1]	1.63 ± 0.28	5.11 ± 0.37	0.85 ± 0.29	20.17 ± 4.32	18.18 ± 3.83	1.11 ± 0.03
CDP03512	1.01 ± 0.10	5.26 ± 0.68	3.21 ± 0.87	28.16 ± 6.43	21.19 ± 3.35	1.33 ± 0.11
CDP01203	1.05 ± 0.10	5.30 ± 0.30	3.58 ± 0.67	28.18 ± 2.62	22.34 ± 1.41	1.26 ± 0.06
CDP02569	0.84 ± 0.13	6.41 ± 1.07	2.38 ± 0.43	23.10 ± 3.09	20.29 ± 2.49	1.14 ± 0.04
CDP03365	1.57 ± 0.30	4.95 ± 0.43	3.49 ± 0.16	22.71 ± 4.13	19.26 ± 3.94	1.18 ± 0.05
CDP05566	1.05 ± 0.07	4.92 ± 0.65	3.13 ± 0.14	33.67 ± 5.19	29.23 ± 5.47	1.15 ± 0.09
CDP02588	0.81 ± 0.13	7.11 ± 0.56	2.78 ± 0.39	17.13 ± 1.23	13.83 ± 0.62	1.24 ± 0.03
CDP06432	0.96 ± 0.13	4.42 ± 0.03	3.21 ± 0.39	22.39 ± 1.56	17.28 ± 0.32	1.30 ± 0.11
CDP00023	0.86 ± 0.22	4.90 ± 0.71	2.30 ± 0.33	18.78 ± 3.28	14.70 ± 3.25	1.28 ± 0.19
CDP01245	1.04 ± 0.11	4.58 ± 0.44	2.40 ± 0.72	25.02 ± 5.50	19.70 ± 4.36	1.27 ± 0.09
CDP01254	0.85 ± 0.16	4.83 ± 0.37	3.17 ± 0.32	24.80 ± 4.42	19.44 ± 3.99	1.28 ± 0.08
CDP01475	1.17 ± 0.21	4.14 ± 0.42	3.42 ± 0.29	27.82 ± 2.28	22.95 ± 2.59	1.21 ± 0.07
CDP05468	1.09 ± 0.14	4.24 ± 0.13	2.60 ± 0.32	21.15 ± 3.72	15.42 ± 2.05	1.37 ± 0.12
CDP01127	0.84 ± 0.20	4.11 ± 0.78	1.21 ± 0.44	14.41 ± 0.92	11.62 ± 0.30	1.24 ± 0.11
CDP03698	1.39 ± 0.13	5.06 ± 0.07	3.79 ± 0.45	25.24 ± 1.13	22.65 ± 2.48	1.11 ± 0.09
CDP01447	0.85 ± 0.12	4.48 ± 1.18	2.75 ± 0.87	29.71 ± 9.83	23.60 ± 7.96	1.26 ± 0.07
LC313	1.21 ± 0.13	4.89 ± 0.36	3.13 ± 0.25	32.15 ± 4.26	28.05 ± 3.65	1.15 ± 0.01
CDP06989	0.96 ± 0.14	4.23 ± 1.04	3.82 ± 0.08	33.29 ± 1.56	29.25 ± 0.56	1.14 ± 0.03
CDP00235	0.71 ± 0.01	4.00 ± 0.79	2.40 ± 0.04	19.90 ± 3.57	16.29 ± 3.16	1.22 ± 0.03
CDP01255	1.29 ± 0.34	4.56 ± 0.73	3.67 ± 0.62	29.22 ± 2.83	25.20 ± 2.59	1.16 ± 0.02
CDP07789	0.59 ± 0.09	5.48 ± 0.33	3.74 ± 0.51	34.76 ± 2.83	28.26 ± 4.08	1.23 ± 0.07
CDP01114	1.29 ± 0.15	4.41 ± 0.48	3.17 ± 0.45	26.50 ± 2.98	20.07 ± 3.32	1.32 ± 0.05
CDP07531	1.44 ± 0.06	4.15 ± 0.37	0.92 ± 0.25	17.28 ± 2.70	12.96 ± 1.78	1.33 ± 0.04
LC77	1.10 ± 0.09	6.15 ± 1.20	3.20 ± 0.89	33.78 ± 6.48	29.64 ± 5.43	1.14 ± 0.07
CDP03366	0.93 ± 0.10	3.94 ± 0.52	2.62 ± 0.21	16.03 ± 0.05	12.61 ± 1.84	1.27 ± 0.19
CDP02356	1.20 ± 0.25	4.42 ± 0.87	3.77 ± 0.61	27.11 ± 5.08	22.86 ± 3.92	1.19 ± 0.02
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0038

[1] Allele names: non-ripening (*nor*), ripening inhibitor (*rin*), alcobaça (*alc*), never ripe (*Nr*).

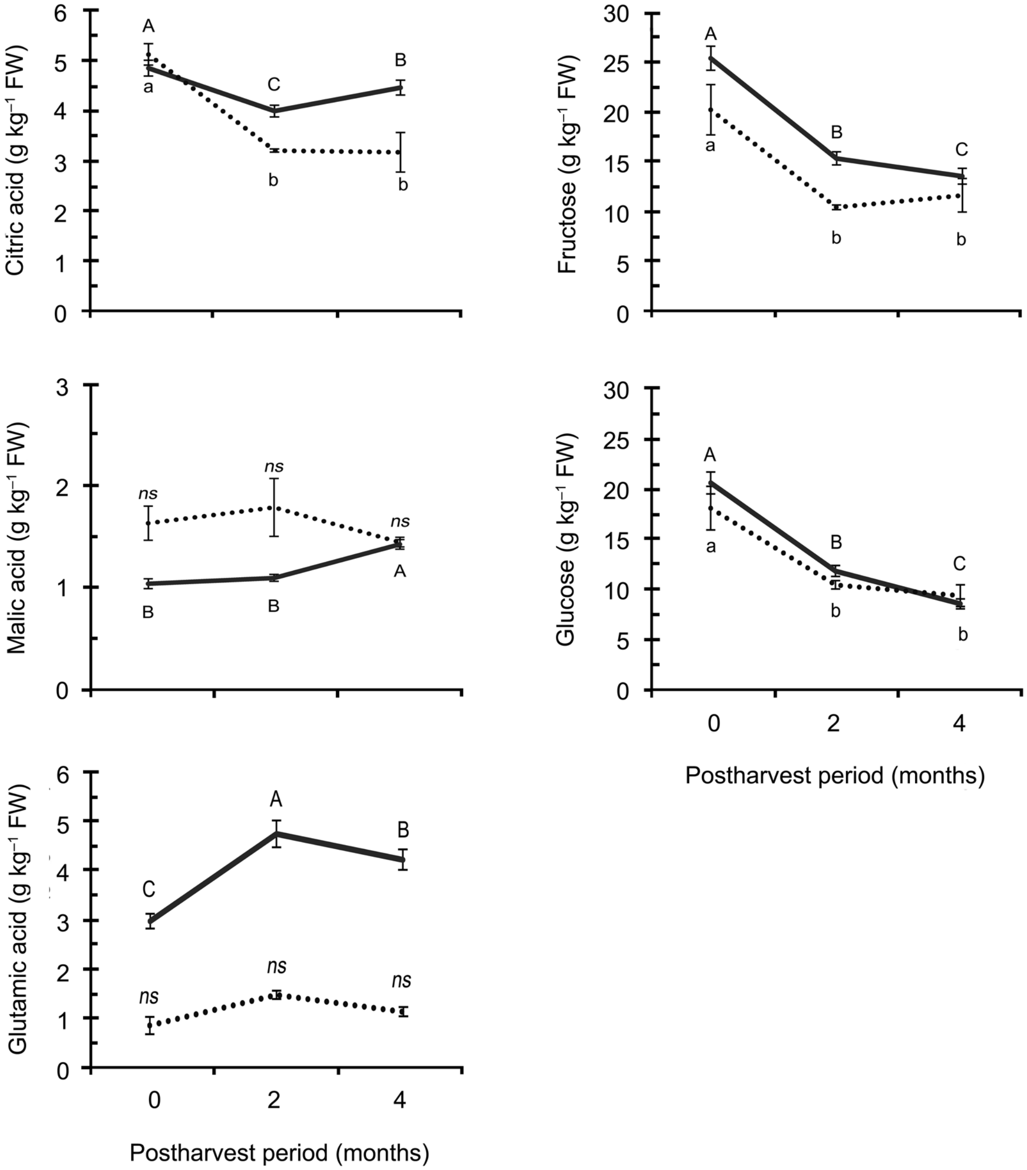

Figure 3 – Evolution of sugars and acids during long-term storage for the Penjar variety and the LA3770 non-ripening (*nor*) control. Data represent the mean ± standard error of the mean of 25 accessions for the Penjar variety (solid line) and of three replicates for the n*or* control (dotted line). Within each variety, different letters indicate differences between periods (Student-Newman-Keuls test, $p < 0.05$); *ns* = not significant, FW = fresh weight.

Of the three acids analyzed, only the citric decreased in concentration during the postharvest period in Penjar accessions (8 % over the 4-month period) (Figure 3). Similar results were observed in the *nor* control (Figure 3). The other two acids increased in concentration during postharvest in the Penjar accessions. The concentration of malic acid remained unchanged in the first 2 months postharvest and increased 29 % in the remaining 2 months (Figure 3). The concentration of glutamic acid increased 60 % in the first 2 months after harvest and then decreased 11 % in the remaining 2 months. By contrast, in the *nor* control no differences in the concentrations of malic and glutamic acids were observed during postharvest (Figure 3).

Shelf life had significant correlations with malic acid concentration at 2 months postharvest (r = 0.53, $p = 0.0076$) and with malic acid (r = 0.47, $p = 0.027$), fructose (r = 0.59, $p = 0.003$), and glucose (r = 0.51, $p = 0.01$) concentrations at 4 months postharvest. Glucose and fructose were negatively correlated with fruit weight at each postharvest time (e.g.: at harvest, r = -0.54 and -0.63, respectively, $p < 0.001$). Regarding correlations between the concentrations of compounds at harvest.highly significant correlations were found between fructose and glucose (r = 0.98, $p < 0.0001$) and between glutamic acid and both fructose (r = 0.69, p = 0.0002) and glucose (r = 0.67, p = 0.0004).

Discussion

Genetic background, agromorphological characteristics (Casals et al., 2012) and aroma profile (Casals et al., 2011) vary widely between accessions of Penjar tomatoes. In the present study, the concentrations of sugar and acid at harvest varied widely between accessions. The mean concentrations of malic, citric, and glutamic acids in the Penjar accessions were similar to those reported for tomatoes in general (Causse et al., 2007), but the concentrations of sugars in the Penjar accessions were on average higher than in conventional tomatoes. Glucose concentrations in Penjar ranged from 11.62 to 29.64 g kg^{-1} FW, compared to 9 to 17 g kg^{-1} FW in conventional tomatoes (Causse et al., 2007). Similarly, fructose concentration in Penjar accessions ranged from 14.41 to 34.76 g kg^{-1} FW, compared to 11 to 20 g kg^{-1} FW in conventional tomatoes (Causse et al., 2007). Likewise, the sugar content in Penjar is clearly higher than in other tomato landraces from the Mediterranean basin (Ercolano et al., 2008; Loiudice et al., 1995).

The high sugar concentrations of some accessions could be related to their low fruit weight (mean 64.1 g). We found a negative correlation between these traits, and this relation has been reported in other tomato varieties (Prudent et al., 2009). In fact, the degree of sugar accumulation in Penjar fruits seems comparable to that found in cherry tomatoes (Rosales et al., 2007). Although low fruit weight is an undesirable trait for most tomato breeding programs and the high glucose and fructose traits could be partly lost when transferred to breeding lines with higher fruit weight (Levin and Schaffer, 2013), a number of the Penjar accessions evaluated could provide germplasm for developing new cultivars with improved quality and shelf life. Particularly interesting were the accessions CDP06989 (fructose = 33.29 g kg^{-1} FW; glucose = 29.25 g kg^{-1} FW) and LC313 (fructose = 32.15 g kg^{-1} FW; glucose = 28.05 g kg^{-1} FW), which also had good aroma and taste characteristics according to the trained sensory panel's descriptions (data not shown).

Average fruit weight loss during storage of Penjar tomatoes (17 % in 120 days) is far lower than that reported in standard varieties. For instance, Getinet et al. (2008) reported that the cultivars Roma and Marglobe lost 14 % to 19 % fruit weight after 32 days of storage, and Javanmardi and Kubota (2006) reported that the cultivar Clermon lost 1 % to 5 % fruit weight after 7 days of storage at different temperatures. Although the effects of temperature and other environmental factors on fruit weight loss make it difficult to compare results across studies, the Penjar variety's pattern of transpiration clearly differs from that of standard tomatoes. Reduced polygalacturonase activity (Mutschler et al., 1988) and changes in cuticle composition (Saladie et al., 2007) described in *alc* mutants could be the basis of the low postharvest fruit weight loss of the Penjar variety. Nevertheless, the presence of *alc* is insufficient to explain the long shelf life, as the shelf life of the *alc* control included in the study was only about one month. As reported by Casals et al. (2012), shorter shelf life in the *alc* control is probably related to its larger fruit size.

Long-term storage of Penjar tomato results in a large decrease in sugars, a slight decrease in citric acid, and an increase in malic and glutamic acids. Fructose and glucose, found in approximately equimolar concentrations in cultivated tomatoes (Davies and Hobson, 1981; Levin et al., 2000), are involved in the initial oxidative step of the Embden-Meyerhof-Parnas pathway; thus, their decrease during postharvest must be related to respiration (Wills et al., 1982). The increase in the fructose-to-glucose ratio during storage is probably due to the different activity of the two molecules in metabolic pathways during postharvest, which results in higher glucose consumption (Patching et al., 1975).

Similarly, the decrease in citric acid during postharvest can be explained mainly by its participation in the first steps of the Krebs cycle. Contrary to citric acid, malic acid and glutamic acid increased during postharvest. Malic acid is the second most abundant acid in tomato fruit, and in normal tomatoes its content decreases markedly during ripening (Davies, 1966) and more slowly during postharvest (Thorne and Efiuvwevwere, 1988). However, malic acid metabolism is under ethylene regulation; Gao et al. (2007) reported increased malic acid during ripening in transgenic tomatoes with suppressed ethylene biosynthesis. The *alc* allele induces changes in the ethylene content (Mutschler, 1984) similar to those reported by Gao et al. (2007), so this may explain the postharvest increase in malic acid in Penjar tomatoes.

Glutamic acid is one of the major amino acids present in tomato fruits (Kader et al., 1978; Oruna-Concha et al., 2007), and its content increases 10-to-40-fold during ripening (Boggio et al., 2000; Oms-Oliu et al., 2011). However, less information is available about changes in glutamic acid content during postharvest, making it difficult to compare our results. Oms-Oliu et al. (2011) reported an increase in glutamic acid concentration during postharvest; however, the period analyzed was much shorter (10 days) than the 4 month period considered in our study. We found that glutamic acid continued to accumulate during extended storage in all the Penjar accessions. It seems that the metabolic pathways related to glutamic acid accumulation are reinforced during storage (Freeman and Woodbridge, 1960; Sorrequieta et al., 2010).

The positive correlation between shelf life and fructose and glucose levels at 4 months postharvest most likely reflects an indirect relationship between shelf life and fruit weight rather than a role for reducing sugars in the fruits' storage ability. Moreover, we found a significant correlation ($p < 0.05$) between glutamic acid and fructose and glucose. Some authors have hypothesized that glutamic acid uptake in fruit is coupled to the mass flow of sucrose while the fruit is attached to the plant (Valle et al., 1998; Winter et al., 1992), and this hypothesis might explain the correlation observed between the concentration of sugars and glutamic acid.

During the aging phase, the sensory profile of Penjar tomatoes varies with changes in sugar and acid levels, as well as with changes in volatile concentrations (Casals et al., 2011). On the one hand, sweetness decreases with the reduction of glucose and fructose, although the unusually high concentrations of these molecules at harvest ensure adequate levels of sweetness during the first months of storage. On the other hand, although citric acid content decreased, the combined level of citric and malic acids remained unchanged during storage. This more acidic profile of aged Penjar tomatoes could explain the enhancement of some aroma descriptors in Penjar tomatoes reported by Casals et al. (2011), as acidity probably affects the perception of some volatiles (Baldwin et al., 2008). Finally, the increased glutamic acid concentration may also contribute to the appreciation of the consumer for aged Penjar tomatoes.

Glutamate is a powerful taste enhancer (Bellisle, 1999), but its role in tomato flavor has been scarcely studied since Bucheli et al. (1999) who reported its negative effect on fruitiness attribute. The combined effect of high concentrations of sugars and acid, together with the appearance of new flavors due to the rearrangement of the relative concentrations among volatiles and the increase in glutamic acid content, makes the period between 1 and 4 months after harvest the best time to consume Penjar tomatoes. These findings on compositional changes during the aging phase and their relation to the commercial success of Penjar tomatoes could help breeders develop new cultivars with better flavor and longer shelf life.

Conclusions

The sugar-and-acid profile varies widely between accessions of the Penjar variety, but on average, sugar concentrations at harvest are higher than in conventional tomatoes; higher concentrations of sugar are probably related to Penjar's medium-small fruit size. Some of the studied accessions have good quality characteristics at harvest, making them particularly interesting as non-aged materials for quality markets or for use in breeding programs. During storage, concentrations of the compounds involved in the first steps of respiration (glucose, fructose, and citric acid) decrease, and concentrations of malic acid and glutamic acid increase significantly. Nevertheless, due to the high concentrations of sugars at harvest, acceptable levels of sweetness are maintained for several months after harvest. These postharvest changes contribute to the characteristic sensory profile of Penjar tomatoes, which is highly appreciated by local consumers. Penjar tomatoes reach their best sensory quality between 1 and 4 months postharvest, although the shelf life of many accessions may exceed this period.

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