Internal Quality Characterization of Fresh Tomato Fruits

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Abstract. The characterization of *Lycopersicon* germplasm for internal quality properties is essential to choose suitable donor parents for breeding programs. When donor parents belong to species of subgenus *Eulycopersicon*, which are phylogenetically closer to *L. esculentum* Mill., the recovery of agronomic traits is faster. When using these materials, a careful selection of donor parents which could improve several internal quality properties allows the acceleration of these breeding programs. In this work, we combine general determinations, such as soluble solid content, titratable acidity, pH, total sugars, pectic substances and total protein contents with precise high-performance liquid chromatography (HPLC), quantitations of individual compounds (vitamin C; citric, malic, fumaric and oxalic acids; glucose, fructose, and sucrose), in order to obtain a more complete characterization of flavor intensity and nutritional properties in *Lycopersicon* germplasm. The multidimensional analysis of all these variables allows classification of several accessions of *L. esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill., according to their usefulness for internal quality breeding programs of fresh tomato. The classification obtained and the comparison of accessions quality characteristics with selected controls show that five of the *L. pimpinellifolium* (Jusl.) Mill. accessions tested can be of great usefulness for being used in breeding for internal quality characteristics. A flavor intensity $\approx 625\%$ higher than commercial hybrids was obtained in the best accession tested. Some of these *L. pimpinellifolium* (Jusl.) Mill. accessions showed better flavor intensity properties than a high SSC *L. cheesmanii* Riley control, traditionally used in internal quality breeding. In addition, three of the *L. esculentum* Mill. accessions tested with medium-to-high flavor intensity value could be useful in advanced stages of breeding programs.

The tomato flavor is directly related to its chemical composition. Two main groups of compounds determining flavor can be distinguished: soluble solids and aromas (Stevens et al., 1977). More than 400 volatile compounds have been identified in tomato, but they represent only 0.1% of dry matter in ripe fruits. Among those, ≈ 30 are mainly responsible for aroma in fresh tomato. The typical tomato aroma is the result of an unknown combination of multiple volatile compounds. Besides, there may yet be some unidentified compounds (Buttery and Ling, 1993).

Several works have established the role of soluble solid content (SSC), acids and sugars in the taste and flavor intensity of tomato fruits. It is well known that sweetness has a high correlation with SSC, pH, and reducing sugars, and sourness has a high correlation with pH and, to a lesser degree, with titratable acidity (TA) (Baldwin et al., 1998; Stevens et al., 1977, 1979). This happens because the major constituents of SSC content in *Lycopersicon* fruits are all soluble sugars (Bisogni et al., 1976; Stevens, 1972). The pH and TA are good measures of free acid and H-ion concentrations, responsible for the sourness of a solution (Stevens et al., 1977). Moreover, SSC, sugars and TA highly contribute to overall flavor intensity (Baldwin et al., 1998; Stevens et al., 1977). Nevertheless, SSC, pH and TA are ambiguous variables since the profile and content of the substances that contribute to them can vary greatly between accessions. These variations can be large if accessions of different species related to cultivated tomato are characterized. The complex nature of SSC, pH, and TA has made their use for selection

in internal quality breeding programs rather inefficient (Hewitt and Garvey, 1987; Triano and St. Clair, 1995).

However, the main reducing sugars [glucose (G), fructose (F), plus some sucrose (S)] and organic acids [citric (CA) and malic (MA) acids in particular] of tomato have proven to contribute to the sweetness and sourness and are also important factors in overall flavor intensity (Hobson and Grierson, 1993; Stevens et al., 1977). Moreover, the accumulation of some of the main sugars or acids present in *Lycopersicon* accessions can be under simple genetic control, as it has been reported for MA and CA (Stevens, 1972; Stevens and Long, 1971) and S (Yelle et al., 1991). In that case, the breeding programs could easily manage those genes. For all these reasons, the determination of individual compounds responsible for flavor could be the best way for selecting germplasm with high flavor intensity. Nevertheless, sweetness was better correlated with total sugars (TS) than with G or F; sourness was better correlated with pH or TA than with CA and MA, and total flavor intensity correlated better with TS and TA than with individual sugar and acids (G, F, CA, and MA). These responses have been attributed to interactions within individual sugars or acids and between sugars and acids that do not reflect individual determinations of sugars and acids, but are reflected somehow in SSC, pH, and TA (Stevens et al., 1977). However, it has been reported that some *Lycopersicon* accessions accumulate important quantities of unidentified acids because the TA value shows higher values than individual acid additions (Sánchez-Mata et al., 2000). For these reasons, until the characterization of *Lycopersicon* sugars and acids profile is complete, the best way to characterize flavor intensity properties in *Lycopersicon* germplasm is to combine general determinations with quantitations of individual compounds.

The objective of this work is to characterize and classify several accessions of *L. esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill., according to their usefulness for internal quality breeding programs of fresh tomato. The variables considered in this study are related to the nutritional and qualitative aspects, emphasizing the sugar and acid composition of the samples. A larger number of variables will be included in characterizations when we have fast, precise methods to quantify the volatile compounds involved in tomato flavor.

Materials and Methods

Plants. Twelve accessions of *L. esculentum* Mill. and eight of *L. pimpinellifolium* (Jusl.) Mill. collected in Ecuador and Peru by the Vegetable Genetics Breeding Group (COMAV, Universidad Politécnica de Valencia, Spain) were tested (Table 1). Four tomato experimental breeding lines (NE-1, Nema-R, FLA-7060 and LA-1563), two Spanish varieties of processing tomato ('Gévora' and 'Guadajira') and one commercial hybrid ('Cambria') were used as controls. The controls LA-1563 (a high SSC *L. esculentum* Mill. breeding line derived from *L. chmielewskii* Rick, Kesicki, Fobes &

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Table 1. Origin of the controls and accessions tested.

Code	Control or accession	Species	Origin
1	LA-530	<i>L. cheesmanii</i> Riley	C.M. Rick TGRC, Davis, Calif.,
2	Guadajira	<i>L. esculentum</i> Mill.	Spanish variety of processing tomato
3	Gévora	<i>L. esculentum</i> Mill.	Spanish variety of processing tomato
4	LA-1563	<i>L. esculentum</i> Mill.	C.M. Rick TGRC, Davis, Calif.
5	Cambria	<i>L. esculentum</i> Mill.	Fresh tomato hybrid SEMINIS vegetable seeds, Almería, Spain
6	NE-1	<i>L. esculentum</i> Mill.	COMAV Fresh tomato breeding line, Spain
7	Nema-R	<i>L. esculentum</i> Mill.	Fresh tomato breeding line SEMINIS vegetable seeds, Almería, Spain
8	FLA-7060	<i>L. esculentum</i> Mill.	Fresh tomato breeding line SEMINIS vegetable seeds, Almería, Spain
9	UPV-14223	<i>L. esculentum</i> Mill.	Loja, Ecuador
10	UPV-16898	<i>L. esculentum</i> Mill.	Zapotebamba, Ecuador
11	UPV-14360	<i>L. esculentum</i> Mill.	Azuay, Ecuador
12	UPV-16907	<i>L. esculentum</i> Mill.	Lambayeque, Peru
13	UPV-16902	<i>L. esculentum</i> Mill.	Huancabamba, Ecuador
14	UPV-16896	<i>L. esculentum</i> Mill.	Zapotebamba, Ecuador
15	UPV-16895	<i>L. esculentum</i> Mill.	Zapotebamba, Ecuador
16	UPV-14324	<i>L. esculentum</i> Mill.	El Oro, Ecuador
17	UPV-16897	<i>L. esculentum</i> Mill.	Zapotebamba, Ecuador
18	UPV-14384	<i>L. esculentum</i> Mill.	Morona-Santiago, Ecuador
19	UPV-14382	<i>L. esculentum</i> Mill.	Morona-Santiago, Ecuador
20	UPV-14326	<i>L. esculentum</i> Mill.	El Oro, Ecuador
21	UPV-14341	<i>L. pimpinellifolium</i> (Jusl.) Mill	Azuay, Ecuador
22	UPV-14345	<i>L. pimpinellifolium</i> (Jusl.) Mill	Cañar, Ecuador
23	UPV-14344	<i>L. pimpinellifolium</i> (Jusl.) Mill	Cañar, Ecuador
24	UPV-14974	<i>L. pimpinellifolium</i> (Jusl.) Mill	Lambayeque, Peru
25	UPV-17049	<i>L. pimpinellifolium</i> (Jusl.) Mill	Piura, Peru
26	UPV-16960	<i>L. pimpinellifolium</i> (Jusl.) Mill	Piura, Peru
27	UPV-16983	<i>L. pimpinellifolium</i> (Jusl.) Mill	Lambayeque, Peru
28	UPV-16903	<i>L. pimpinellifolium</i> (Jusl.) Mill	Lambayeque, Peru

Holle) and LA-530 (*L. cheesmanii* Riley accession used in SSC breeding works), were included in the trials for comparison with previous results (Hewitt and Garvey, 1987; Sanchez-Mata et al., 2000).

Growing conditions. Plants were cultivated under greenhouse conditions with watering, fertilization and appropriate temperature (20 to 30 °C day/15 to 20 °C night) for suitable production in the spring-summer cycle in Va-

lencia, Spain. Environmental variability was reduced by means of a completely randomized block design (4 blocks and 4 replicates per block).

For each sample, several fruits were harvested and pooled together: for the large-fruited accessions a minimum of five fruits (≈100 g each); for the small-fruited accessions up to 120 fruits (≈0.5 g each). The fruits were collected from the low floral clusters (from first to third cluster) at the completely ripe stage.

Analytical methods. Fresh fruits were homogenized in a laboratory blender. Aliquots were taken to analyze dry matter (DM), SSC, pH, TA, organic acids, and vitamin C (VITC). For further analysis, TS, soluble sugars, pectic substances (PS), and total proteins (TP), another portion of homogenized fruit was freeze-dried. DM was determined by desiccation to constant dry weight (0.5 h at 225 °C). SSC was measured with a digital refractometer (ATAGO-PR1, Tokyo) and the results are reported as °Brix at 20 °C. The pH was determined by a pH meter. TA was measured by titration with 0.1 N NaOH and phenolphthalein, and the results were expressed as meq·kg⁻¹ alkali (AOAC, 1990). Oxalic (OA), MA, CA, and fumaric (FA) acids, and VITC were quantified by high performance liquid chromatography (HPLC) (Vazquez-Oderiz et al., 1994). TS were quantified spectrophotometrically (as G) of soluble sugars and available carbohydrates, after hydrolysis of complex carbohydrates with perchloric acid, using antrona reagent (Osborne and Voogt, 1986).

For free sugar analysis, a portion of fresh fruit pulp was homogenized in 85% ethanol using Omnimixer homogenizer model 17106

Table 2. Composition (mean ± SE) of controls and accessions studied. FW = fresh weight, ND = not detectable.

Code	DM (g·kg ⁻¹)	SSC (°Brix)	TS ^a (g·kg ⁻¹ FW)	F (g·kg ⁻¹ FW)	G (g·kg ⁻¹ FW)	S (g·kg ⁻¹ FW)	pH
1	173.1 ± 0.3	13.07 ± 0.09	82.69 ± 0.89	39.98 ± 2.24	42.46 ± 4.28	ND	4.63 ± 0.03
2	71.8 ± 3.1	5.13 ± 0.03	32.06 ± 0.05	17.51 ± 0.51	15.75 ± 0.95	6.11 ± 0.29	4.17 ± 0.03
3	58.9 ± 2.3	5.23 ± 0.07	40.29 ± 0.61	16.25 ± 0.39	14.01 ± 0.46	ND	4.01 ± 0.02
4	70.5 ± 0.7	6.37 ± 0.07	37.90 ± 0.37	11.42 ± 1.19	10.57 ± 1.23	ND	4.92 ± 0.05
5	78.1 ± 0.2	5.87 ± 0.03	59.49 ± 0.95	13.49 ± 1.22	13.30 ± 1.41	ND	4.16 ± 0.02
6	68.0 ± 2.7	5.30 ± 0.00	48.54 ± 0.99	12.13 ± 1.83	11.77 ± 2.25	ND	3.86 ± 0.02
7	64.2 ± 0.2	5.50 ± 0.06	44.57 ± 1.14	9.01 ± 0.31	8.17 ± 0.23	0.45 ± 0.05	3.98 ± 0.02
8	75.7 ± 0.5	6.68 ± 0.04	46.51 ± 0.55	13.77 ± 0.21	13.95 ± 0.51	ND ±	4.75 ± 0.01
9	58.6 ± 0.8	4.03 ± 0.03	44.00 ± 0.95	6.10 ± 0.14	5.33 ± 0.12	ND	4.19 ± 0.01
10	93.7 ± 2.8	7.27 ± 0.12	52.10 ± 0.50	24.03 ± 1.45	24.82 ± 2.22	1.91 ± 0.15	4.20 ± 0.01
11	53.6 ± 0.2	4.03 ± 0.03	33.00 ± 1.07	5.92 ± 0.58	5.42 ± 0.55	ND	4.17 ± 0.03
12	51.0 ± 0.6	3.97 ± 0.03	29.10 ± 0.15	9.32 ± 0.67	7.87 ± 0.55	ND	4.45 ± 0.02
13	62.3 ± 1.3	5.10 ± 0.06	49.97 ± 0.60	10.35 ± 0.52	10.47 ± 1.04	ND	4.79 ± 0.01
14	85.5 ± 2.0	6.13 ± 0.03	44.36 ± 0.47	11.49 ± 0.67	12.62 ± 0.71	1.49 ± 0.30	4.12 ± 0.01
15	66.5 ± 1.3	5.57 ± 0.07	41.95 ± 1.28	8.50 ± 0.38	7.79 ± 0.55	ND	4.26 ± 0.02
16	86.3 ± 0.2	6.70 ± 0.10	64.66 ± 0.89	14.53 ± 0.11	14.71 ± 0.14	ND	4.28 ± 0.02
17	67.8 ± 0.4	5.27 ± 0.03	48.54 ± 1.07	14.35 ± 0.45	13.12 ± 0.22	ND	4.16 ± 0.01
18	84.4 ± 0.2	5.83 ± 0.03	70.59 ± 0.78	8.27 ± 0.58	8.45 ± 0.38	1.28 ± 0.22	4.13 ± 0.02
19	102.0 ± 1.2	7.23 ± 0.12	82.69 ± 0.89	25.38 ± 0.86	31.87 ± 1.73	ND	4.30 ± 0.13
20	62.3 ± 0.7	4.80 ± 0.06	47.53 ± 0.71	3.51 ± 0.42	3.19 ± 0.45	ND	4.23 ± 0.02
21	116.2 ± 0.3	8.10 ± 0.00	52.58 ± 0.89	13.20 ± 1.02	12.42 ± 0.88	2.03 ± 0.46	4.27 ± 0.01
22	126.5 ± 0.8	8.23 ± 0.03	89.61 ± 1.21	40.42 ± 1.83	39.31 ± 2.81	ND	4.31 ± 0.09
23	137.0 ± 0.1	8.90 ± 0.10	93.85 ± 1.22	28.17 ± 1.94	28.43 ± 1.95	ND	4.26 ± 0.00
24	142.9 ± 0.7	11.32 ± 0.12	55.14 ± 0.65	12.04 ± 0.37	12.14 ± 0.91	1.26 ± 0.30	4.09 ± 0.09
25	197.9 ± 1.7	11.35 ± 0.12	88.21 ± 0.46	19.98 ± 0.26	17.74 ± 0.30	0.73 ± 0.09	4.12 ± 0.06
26	133.6 ± 0.4	9.73 ± 0.09	65.32 ± 1.70	17.68 ± 0.20	13.44 ± 0.13	0.63 ± 0.08	4.13 ± 0.01
27	150.1 ± 0.4	9.90 ± 0.08	64.17 ± 1.30	12.13 ± 1.74	11.12 ± 1.49	0.47 ± 0.05	4.24 ± 0.01
28	115.5 ± 1.9	8.55 ± 0.20	74.03 ± 0.87	30.52 ± 1.21	43.56 ± 2.01	ND	2.99 ± 0.05

^aExpressed as glucose.

^bExpressed as L-ascorbic acid.

^cExpressed as galacturonic acid.

(Sorval, Dupont Instruments, Newton, Conn.). To separate the pectin fraction, the ethanol slurry was boiled for 30 min and then centrifuged at -1°C for 15 min at $1900\times g$ in a Gallenkamp laboratory centrifuge. Two phases were obtained: supernatant with free sugar dissolved and an alcohol-insoluble residue containing PS. Soluble sugars, F, G, and S, were analyzed on supernatants by HPLC following the method previously described by Mollá et al. (1994). PS were quantified spectrophotometrically (as galacturonic acid), after hydrolysis of the alcohol-insoluble residue with sulfuric acid, using *m*-phenylphenol reagent (Ahmed and Labavitch, 1977; Blumenkrantz and Asboe-Hansen, 1973). TP were quantified by the Kjeldahl method (AOAC, 1990).

Instrumentation. Equipment used included a Waters Associates (Mildford, Mass.) liquid chromatograph equipped with a 6000A pump and a U6K injector.

For reducing sugar analysis, an R401 differential refractometer was used. The column used was a Waters μ Bondapak/carbohydrate analysis column. The mobile phase was 80 acetonitrile:20 water. Operating conditions were a flow rate of 0.9 mL/min and ambient temperature.

For organic acid and VITC analysis, a Spherisorb ODS2 $250\times 4.6\text{ mm C}_{18}$ column (particle size, $5\text{ }\mu\text{m}$) with a Brownlee Labs Newguard RP-18 $15\times 3.2\text{ mm}$ precolumn (particle size, $7\text{ }\mu\text{m}$), and an Atom 80 rotary Shaker were used. The mobile phase was LC-grade water taken to pH 2.2 with sulfuric acid. The flow rate was 0.5 mL/min. Detection was at 245 nm for VITC and OA and at 215 nm for the other organic acids. All chromatograms

were recorded on a Waters Data Module 745 integrator, (Waters Instruments, Millford, Mass.).

Statistical methods. In order to look for some underlying dimension explaining the variability of the data, a classical Principal Component Analysis (PCA) on the standardized data has been applied (Everitt, 1980).

After the characterization of the population of tomato varieties by means of the PCA, a Cluster Analysis (CIA) was performed to examine the natural linkage of the varieties on the basis of the information provided by the analyzed variables. The procedure applied was an agglomerative hierarchic classification. Euclidean distances and the unweighted pair-group method based on arithmetic averages (UPGMA) was used in the clustering. The maximization of the resemblance coefficient range was used as grouping criterion (Romesburg, 1984). The goodness of fit of the cluster analysis to the data was tested by the cophentic correlation coefficient (Sokal and Rohlf, 1995).

Statistical analysis were performed with the program NTSYS PC 2.02 (Applied Biostatistics, Setauket, N.Y.) for Windows.

Results

The results of the analytical determinations (average values of three determinations from three subsamples of pooled fruits) show a great variation among accessions, mainly in DM, SSC, reducing sugars (G and F) and TA (Table 2). A great DM variability was observed ($51.0\text{--}197.9\text{ g}\cdot\text{kg}^{-1}$) and the results obtained clearly separate *L. esculentum* Mill. acces-

sions from *L. pimpinellifolium* (Jusl.) Mill. and *L. cheesmanii* Riley accessions, which have many small fruits. From our results, the variability in SSC (between $3.97\text{--}13.07^{\circ}\text{Brix}$), F ($3.51\text{--}40.42\text{ g}\cdot\text{kg}^{-1}\text{ FW}$), and G ($3.19\text{--}43.56\text{ g}\cdot\text{kg}^{-1}\text{ FW}$) were closely correlated (lower part of the diagonal in the Table 3); but slight relationships were found between TA ($482.2\text{--}1227.1\text{ mL}$, 0.1 N alkali/kg FW), and pH ($2.99\text{--}4.92$). F and G were found in about equal amounts, while S was always in very small quantities. The main organic acids detected were CA ($1.20\text{--}10.06\text{ g}\cdot\text{kg}^{-1}\text{ FW}$) and MA ($0\text{--}4.85\text{ g}\cdot\text{kg}^{-1}\text{ FW}$) and both showed great variability. The accumulation of the studied acids does not match a shared pattern in all the accessions. The correlation coefficients as well as their *P*-values (in brackets), between the variables on the data were obtained. Numerous significant correlations (>0.7) between the variables considered were found.

The large number of variables studied and the great variability observed in all of them makes a multivariate analysis advisable in order to characterize and classify the accessions according to flavor characteristics. The multidimensional structure of the data was reduced by performing a PCA, which provided a three-dimensional map for explaining the observed variance. Furthermore, a hierarchic classification of samples was carried out in order to group the accessions according to their similar internal quality properties.

The three components of the PCA performed explain 65% of the total variance (39% first, 14% second, and 12% third principal component). As the percentages of the variance explained by the second and third principal

TA ($\text{meq}\cdot\text{kg}^{-1}\text{ FW}$)	CA ($\text{g}\cdot\text{kg}^{-1}\text{ FW}$)	MA ($\text{g}\cdot\text{kg}^{-1}\text{ FW}$)	OA ($\text{g}\cdot\text{kg}^{-1}\text{ FW}$)	FA ($\text{mg}\cdot\text{kg}^{-1}\text{ FW}$)	VITC ^y ($\text{mg}\cdot\text{kg}^{-1}\text{ FW}$)	PS ^x ($\text{g}\cdot\text{kg}^{-1}\text{ FW}$)	TP ($\text{g}\cdot\text{kg}^{-1}\text{ FW}$)
102.20 $\pm$ 5.62	3.58 $\pm$ 0.10	0.61 $\pm$ 0.04	0.94 $\pm$ 0.10	ND	127.3 $\pm$ 1.6	3.11 $\pm$ 0.03	21.84 $\pm$ 0.22
50.85 $\pm$ 3.89	4.26 $\pm$ 0.09	0.48 $\pm$ 0.03	0.75 $\pm$ 0.03	57.57 $\pm$ 0.75	156.1 $\pm$ 3.7	2.14 $\pm$ 0.14	14.55 $\pm$ 0.18
95.18 $\pm$ 6.90	7.69 $\pm$ 0.31	2.49 $\pm$ 0.18	1.06 $\pm$ 0.07	1.81 $\pm$ 0.01	126.1 $\pm$ 2.7	1.03 $\pm$ 0.12	14.48 $\pm$ 0.01
52.26 $\pm$ 5.32	4.72 $\pm$ 0.14	3.02 $\pm$ 0.13	0.87 $\pm$ 0.12	6.95 $\pm$ 0.26	187.1 $\pm$ 1.3	0.76 $\pm$ 0.05	15.14 $\pm$ 0.29
88.74 $\pm$ 1.10	8.12 $\pm$ 0.07	2.95 $\pm$ 0.13	0.79 $\pm$ 0.11	33.51 $\pm$ 1.66	151.3 $\pm$ 3.7	2.62 $\pm$ 0.20	6.44 $\pm$ 0.11
122.70 $\pm$ 2.38	8.04 $\pm$ 0.05	2.90 $\pm$ 0.07	0.61 $\pm$ 0.01	34.94 $\pm$ 2.69	122.9 $\pm$ 0.7	1.23 $\pm$ 0.02	10.12 $\pm$ 0.14
104.16 $\pm$ 2.67	6.79 $\pm$ 0.33	2.46 $\pm$ 0.28	0.85 $\pm$ 0.08	ND	133.2 $\pm$ 1.3	1.30 $\pm$ 0.17	12.52 $\pm$ 0.23
48.22 $\pm$ 1.14	5.78 $\pm$ 0.52	3.71 $\pm$ 0.37	0.53 $\pm$ 0.01	8.06 $\pm$ 1.18	126.6 $\pm$ 1.2	0.93 $\pm$ 0.18	14.43 $\pm$ 0.12
76.27 $\pm$ 3.99	5.19 $\pm$ 0.13	1.46 $\pm$ 0.12	0.55 $\pm$ 0.02	4.68 $\pm$ 0.53	104.6 $\pm$ 1.4	0.85 $\pm$ 0.06	8.71 $\pm$ 0.18
90.25 $\pm$ 0.22	4.81 $\pm$ 0.12	1.73 $\pm$ 0.04	0.94 $\pm$ 0.01	68.60 $\pm$ 0.48	310.6 $\pm$ 4.6	1.39 $\pm$ 0.07	11.46 $\pm$ 0.14
53.68 $\pm$ 2.89	4.42 $\pm$ 0.05	2.45 $\pm$ 0.20	0.77 $\pm$ 0.08	5.76 $\pm$ 0.37	157.9 $\pm$ 0.9	2.09 $\pm$ 0.12	8.91 $\pm$ 0.17
56.00 $\pm$ 1.33	3.49 $\pm$ 0.20	1.49 $\pm$ 0.01	0.77 $\pm$ 0.02	27.97 $\pm$ 2.33	96.2 $\pm$ 1.0	1.58 $\pm$ 0.14	9.72 $\pm$ 0.29
49.57 $\pm$ 1.36	2.48 $\pm$ 0.08	0.75 $\pm$ 0.09	0.27 $\pm$ 0.04	5.09 $\pm$ 1.55	104.6 $\pm$ 0.8	1.00 $\pm$ 0.17	11.81 $\pm$ 0.31
92.24 $\pm$ 1.83	7.82 $\pm$ 0.07	3.16 $\pm$ 0.09	0.71 $\pm$ 0.02	80.37 $\pm$ 1.05	267.2 $\pm$ 0.1	3.50 $\pm$ 0.29	11.06 $\pm$ 0.11
90.77 $\pm$ 3.77	8.67 $\pm$ 0.49	2.12 $\pm$ 0.19	1.91 $\pm$ 0.00	7.65 $\pm$ 0.39	161.9 $\pm$ 0.3	2.11 $\pm$ 0.19	11.11 $\pm$ 0.21
93.30 $\pm$ 2.80	4.00 $\pm$ 0.22	3.25 $\pm$ 0.23	0.60 $\pm$ 0.01	ND	235.7 $\pm$ 1.0	1.59 $\pm$ 0.20	18.07 $\pm$ 2.17
80.05 $\pm$ 2.20	3.50 $\pm$ 0.18	2.01 $\pm$ 0.09	0.50 $\pm$ 0.07	0.82 $\pm$ 0.37	148.3 $\pm$ 0.3	2.96 $\pm$ 0.01	11.82 $\pm$ 0.03
113.64 $\pm$ 3.22	10.06 $\pm$ 0.56	4.64 $\pm$ 0.18	1.59 $\pm$ 0.01	ND	206.0 $\pm$ 1.4	2.73 $\pm$ 0.02	13.40 $\pm$ 0.19
111.50 $\pm$ 1.74	3.76 $\pm$ 0.27	1.68 $\pm$ 0.11	0.74 $\pm$ 0.08	4.76 $\pm$ 0.39	233.7 $\pm$ 2.9	2.35 $\pm$ 0.04	14.91 $\pm$ 0.52
65.69 $\pm$ 2.78	9.97 $\pm$ 0.35	3.22 $\pm$ 0.31	1.25 $\pm$ 0.05	13.06 $\pm$ 0.62	140.6 $\pm$ 0.8	1.25 $\pm$ 0.09	8.00 $\pm$ 0.20
90.51 $\pm$ 3.74	6.83 $\pm$ 0.19	2.38 $\pm$ 0.23	0.88 $\pm$ 0.03	9.16 $\pm$ 0.63	163.3 $\pm$ 3.0	3.58 $\pm$ 0.29	29.34 $\pm$ 0.19
120.29 $\pm$ 3.97	9.35 $\pm$ 0.80	2.37 $\pm$ 0.44	1.23 $\pm$ 0.17	8.61 $\pm$ 1.47	163.7 $\pm$ 1.2	1.56 $\pm$ 0.23	23.68 $\pm$ 0.17
122.78 $\pm$ 2.81	8.15 $\pm$ 0.51	2.34 $\pm$ 0.38	0.96 $\pm$ 0.17	7.38 $\pm$ 2.08	197.5 $\pm$ 2.3	1.50 $\pm$ 0.02	29.04 $\pm$ 1.62
179.00 $\pm$ 3.16	8.40 $\pm$ 0.02	0.00 $\pm$ 0.00	1.30 $\pm$ 0.06	0.67 $\pm$ 0.39	293.6 $\pm$ 2.8	3.60 $\pm$ 0.25	33.88 $\pm$ 0.49
194.65 $\pm$ 18.1	7.58 $\pm$ 0.08	3.33 $\pm$ 0.04	1.67 $\pm$ 0.02	7.51 $\pm$ 0.49	290.1 $\pm$ 1.6	3.13 $\pm$ 0.05	39.56 $\pm$ 2.48
125.99 $\pm$ 6.56	1.20 $\pm$ 0.05	4.85 $\pm$ 0.02	1.00 $\pm$ 0.08	4.19 $\pm$ 0.45	238.2 $\pm$ 0.9	2.23 $\pm$ 0.02	34.65 $\pm$ 0.71
92.55 $\pm$ 3.59	6.55 $\pm$ 0.11	1.77 $\pm$ 0.13	1.42 $\pm$ 0.08	ND	165.6 $\pm$ 3.8	8.58 $\pm$ 0.76	34.05 $\pm$ 1.04
113.80 $\pm$ 0.93	7.45 $\pm$ 0.05	2.91 $\pm$ 0.29	2.58 $\pm$ 0.14	2.65 $\pm$ 0.61	255.4 $\pm$ 2.9	4.53 $\pm$ 0.46	22.22 $\pm$ 0.46

Table 3. Correlation coefficients (*P*-values) between the variables considered on the data of the 28 accessions of the three species (lower part of the diagonal) and on the data of the 19 accessions of only *L. esculentum* Mill. (upper part of the diagonal).

	DM	SSC	TS	F	G	S	pH	TA	CA	MA	OA	FA	VITC	PS	TP
DM		0.894 (<0.001)	0.771 (<0.001)	0.703 (<0.001)	0.786 (<0.001)	0.213 (0.382)	-0.025 (0.918)	0.446 (0.056)	0.027 (0.913)	0.238 (0.327)	0.039 (0.873)	0.311 (0.196)	0.829 (<0.001)	0.389 (0.099)	0.418 (0.075)
SSC	0.960 (<0.001)		0.623 (0.004)	0.708 (<0.001)	0.750 (<0.001)	0.062 (0.802)	0.184 (0.452)	0.316 (0.188)	0.002 (0.993)	0.300 (0.212)	0.040 (0.873)	0.183 (0.454)	0.736 (<0.001)	0.123 (0.617)	0.561 (0.013)
TS	0.780 (<0.001)	0.708 (<0.001)		0.418 (0.075)	0.552 (0.014)	-0.202 (0.406)	-0.095 (0.699)	0.599 (0.007)	0.121 (0.621)	0.325 (0.174)	0.056 (0.821)	-0.207 (0.395)	0.455 (0.050)	0.277 (0.251)	0.260 (0.283)
F	0.609 (<0.001)	0.623 (<0.001)	0.725 (<0.001)		0.973 (<0.001)	0.306 (0.203)	-0.016 (0.949)	0.235 (0.332)	-0.371 (0.118)	-0.272 (0.261)	-0.214 (0.378)	0.322 (0.179)	0.544 (0.016)	0.126 (0.607)	0.476 (0.039)
G	0.551 (0.002)	0.577 (0.001)	0.711 (<0.001)	0.966 (<0.001)		0.220 (0.366)	0.008 (0.974)	0.288 (0.232)	-0.346 (0.147)	-0.219 (0.369)	-0.203 (0.405)	0.280 (0.246)	0.595 (0.007)	0.177 (0.470)	0.446 (0.055)
S	0.035 (0.858)	0.001 (0.997)	-0.227 (0.245)	-0.014 (0.943)	-0.056 (0.777)		-0.173 (0.479)	-0.121 (0.622)	-0.072 (0.770)	-0.342 (0.152)	0.036 (0.883)	0.594 (0.007)	0.254 (0.294)	0.251 (0.301)	0.208 (0.394)
pH	-0.084 (0.670)	-0.036 (0.856)	-0.142 (0.472)	-0.107 (0.589)	-0.228 (0.243)	-0.077 (0.698)		-0.704 (<0.001)	-0.460 (0.048)	-0.084 (0.733)	-0.235 (0.333)	-0.213 (0.382)	-0.098 (0.689)	-0.376 (0.112)	0.272 (0.259)
TA	0.731 (<0.001)	0.676 (<0.001)	0.647 (<0.001)	0.343 (0.074)	0.311 (0.107)	-0.048 (0.810)	-0.420 (0.026)		0.481 (0.037)	0.336 (0.160)	0.302 (0.209)	-0.005 (0.985)	0.311 (0.196)	0.327 (0.172)	0.086 (0.726)
CA	0.081 (0.681)	0.021 (0.915)	0.195 (0.321)	-0.052 (0.794)	-0.023 (0.906)	-0.054 (0.787)	-0.350 (0.068)	0.370 (0.053)		0.225 (0.249)	0.520 (0.005)	0.063 (0.798)	0.023 (0.926)	0.172 (0.481)	-0.309 (0.199)
MA	-0.034 (0.866)	-0.090 (0.650)	0.151 (0.444)	-0.160 (0.416)	ν 0.162 (0.410)	-0.252 (0.197)	-0.171 (0.384)	0.096 (0.628)	0.652 (0.003)		0.197 (0.316)	-0.183 (0.453)	0.216 (0.375)	0.147 (0.550)	0.088 (0.721)
OA	0.408 (0.031)	0.381 (0.046)	0.355 (0.064)	0.233 (0.233)	0.320 (0.097)	-0.035 (0.861)	-0.588 (0.001)	0.465 (0.013)	0.691 (0.001)	0.345 (0.148)		-0.091 (0.711)	0.175 (0.474)	0.178 (0.467)	-0.066 (0.788)
FA	-0.205 (0.295)	-0.239 (0.221)	-0.315 (0.102)	-0.030 (0.880)	-0.039 (0.844)	-0.561 (0.003)	-0.057 (0.774)	-0.198 (0.313)	0.029 (0.883)	-0.078 (0.695)	-0.219 (0.263)		0.465 (0.045)	0.337 (0.158)	-0.221 (0.364)
VITC	0.514 (0.005)	0.502 (0.007)	0.402 (0.034)	0.253 (0.194)	0.294 (0.129)	0.215 (0.272)	-0.331 (0.086)	0.590 (<0.001)	0.106 (0.591)	0.180 (0.360)	0.406 (0.032)	0.231 (0.237)		0.279 (0.150)	0.472 (0.011)
PS	0.535 (0.003)	0.499 (0.007)	0.269 (0.167)	0.103 (0.601)	0.154 (0.435)	0.120 (0.542)	ν 0.315 (0.103)	0.288 (0.137)	0.120 (0.542)	-0.096 (0.625)	0.450 (0.016)	-0.082 (0.680)	0.420 (0.074)		-0.128 (0.603)
TP	0.882 (<0.001)	0.845 (<0.001)	0.594 (<0.001)	0.390 (0.040)	0.316 (0.102)	0.093 (0.637)	-0.107 (0.588)	0.690 (<0.001)	0.027 (0.892)	0.032 (0.873)	0.387 (0.042)	-0.338 (0.078)	0.323 (0.177)	0.520 (0.005)	

components are very close, correct interpretation of the data variability requires considering the three first principal components.

The first principal component is highly correlated to variables considered responsible for tomato flavor intensity (Stevens et al., 1977; Baldwin et al., 1998), such as SSC, TS, and TA and in a minor degree to the variables F and G (positive correlation) (Table 4). It is also positively correlated to DM and TP. Technically, this is due to the high correlation coefficients between these variables (Table 3). The second principal component separates the accessions according to their acidity. The CA is the most important acid that influences the formation of the second principal component. The pH also contributes to the formation of this second principal component, showing

an important and negative correlation (Table 4). Low correlation coefficients of TA to CA and to pH were observed (Table 3). Finally, the third principal component separates the accessions according to their content of S and FA (Table 4).

All *Lycopersicon* accessions are plotted on the reduced space of the three principal components. Two projections, one for the first and second principal components and the other for the first and third principal components (Fig. 1 A and B), were made. The hierarchic

classification performed with all the accessions and controls (cophenetic value = 0.83) shows a good fit between the Euclidean distances of the data and the dendrogram distances. The dendrogram is used as accession classification tool. For this reason, it was cut at the widest range of the resemblance coefficient (Fig. 2) in order to resolve the tradeoff between the desire for detail (many groups) and the desire for simplicity (few groups). So, the most stable accession association was obtained. This cut of the dendrogram gives rise to seven groups,

Table 4. Correlation coefficients between the variables and the three first principal components on the analysis performed on the data of the 28 accessions of the three species.

Variables	PC1	PC2	PC3
DM	0.93	-0.18	0.04
SSC	0.90	-0.25	0.03
TS	0.84	-0.17	-0.25
F	0.69	-0.47	0.08
G	0.68	-0.38	0.07
S	-0.02	0.05	0.86
pH	-0.34	-0.65	-0.15
TA	0.80	0.25	-0.05
CA	0.22	0.68	-0.12
MA	0.04	0.49	-0.38
OA	0.60	0.57	-0.07
FA	-0.24	0.10	0.80
VITC	0.61	0.26	0.38
PS	0.53	0.20	0.19
TP	0.82	-0.07	0.00
Variance explained (%)	39	14	12

Fig. 1. Plot of the tomato cultivars projected on the reduced spaces of the first and second principal components and of the first and third principal components. Groups are derived from a separate cluster analysis.

Fig. 2. Dendrogram of the *Lycopersicon* accessions using the UPGMA criterion method.

four of them with high flavor intensity and three with moderate to low flavor intensity. These new groups are outlined in Fig. 1 in order to clearly observe the relationship within the accessions and between accessions as well as the principal components.

The four high flavor intensity groups are located on the right part of the plot (Fig. 1A) and are made up of *L. pimpinellifolium* accessions and the LA-530 (1) *L. cheesmanii* Riley control. These accessions exhibit high contents on DM, SSC, TS, TP, and TA. UPV-17049 (25) accession shows the highest intensity of flavor characteristics. UPV-14974 (24) accession, grouped with the former, has slightly lower flavor intensity and no MA content. The accession UPV-16903 (28) has the second highest flavor intensity value. It shows lower DM, SSC and TA than UPV-17049 (25) and UPV-14974 (24) accessions, but higher content in F and G. However, this accession is isolated from the other clusters because it has the lowest pH of all the accessions. UPV-14345 (22) and UPV-14344 (23) accessions and *L. cheesmanii* Riley control LA-530 (1) have similar sugar content (TS, F, and G) and TA to UPV-16903 (28), however they are not clustered with it because of its high pH. The *L. cheesmanii* Riley control LA-530 (1) is located on the lower extreme of the plot, because of its high pH and low CA content.

The three moderate-to-low flavor intensity groups are located on the left part of the plot represented by the first and second principal components (Fig. 1A) and are made up of all *L. esculentum* Mill. accessions and controls and two *L. pimpinellifolium* accessions, UPV-14341 (21) and UPV-16960 (26). The dendrogram splits these three groups up according to special features related to the third principal component (S and FA content) and VITC content. 'Guadajira' (2) control do not have special flavor intensity or acidity characteristics, but are cluster isolated since it has the highest S content. UPV-16898 (10) and UPV-16896 (14) accessions are cluster isolated because of their relatively high contents in S and FA as well as their higher VITC content. The last group, six controls and 10 accessions from *L. esculentum*

Mill. and two *L. pimpinellifolium* (Jusl.) Mill. accessions, show quite a wide variability for flavor intensity and acidity; however, in the longitudinal section of this dendrogram no more groups arise. Nevertheless, the next dendrogram stable section (dotted line in Fig. 2), splits the group into three subgroups (dotted line in Fig. 1 A and B). Two of these subgroups have intermediate flavor intensity, but higher than controls. The third subgroup includes controls and accessions with very similar characteristics. One of these subgroups with higher flavor intensity than controls is made up of UPV-14384 (18) *L. esculentum* Mill. accession, which is cluster isolated because of its very high CA, MA, and OA content. The other one is formed by UPV-14324 (16) and UPV-14382 (19) *L. esculentum* Mill. accessions and UPV-14341 (21) and UPV-16960 (26) *L. pimpinellifolium* (Jusl.) Mill. accessions, which have moderate to high VITC content and high TP content (specially *L. pimpinellifolium* (Jusl.) Mill. accessions).

To make a more detailed analysis on the accessions of the species *L. esculentum* Mill. (Table 2), the data corresponding to the species *L. pimpinellifolium* (Jusl.) Mill. and *L. cheesmanii* Riley were excluded and new PCA and CIA were performed. Correlation coefficients between the variables on the 12 accessions and seven controls of *L. esculentum* Mill. have been calculated (upper part of the diagonal of Table 3).

As in previous correlation analysis, DM/SSC and G/F are very closely correlated. The correlation coefficients between DM/VITC and SSC/VITC on the varieties of *L. esculentum* Mill. increase compared to those obtained by considering the three species together. TS are only correlated to DM, and both DM and SSC are correlated to F and G. In contrast to the previous analysis performed with the three tomato species, now TA appears closely associated to pH (correlation coefficient of -0.70) in *L. esculentum* Mill. accessions. The increase of this correlation coefficient is accompanied by a reduction of the variability on the MA/CA ratio, suggesting a relationship between a wide variation

on the MA/CA ratio and the poor correlation between TA and pH.

In the PCA performed on *L. esculentum*, the three first principal components explain 71% of the total variance (Table 5). The first principal component (35% of the variance) is associated to variables responsible for sweet taste (sugar content) together with a high participation of the micronutrient VITC. In addition to CA, variables such as MA, OA, and TA are now correlated to the second principal component (accounting for 21% of the total variance). This axis is an indicator of sourness. The third principal component (15% of the total variance), as in the previous PCA, is strongly correlated to FA and S.

The hierarchic classification performed with 12 accessions and seven controls of *L. esculentum* Mill. (cophenetic value = 0.93) shows a very good fit between the Euclidean distances of the data and the dendrogram distances. The more stable cut of this dendrogram (Fig. 3) allowed a classification of tomato fruits into five groups (outlined in Fig. 4) according firstly to sweetness and VITC content, secondly to acid composition and thirdly to minor compounds (FA and S). Two of these groups (the one formed by control 'Guadajira' (2) and the one formed by UPV-16898 (10) and UPV-16896 (14) accessions) became evident during the former general classification. Two other groups agree with the former subgroups made up of UPV-14384 (18) and of UPV-14324 (16) and UPV-14382 (19) *L. esculentum* Mill. accessions. The last group is formed by six controls and seven accessions showing similar or poorer characteristics than controls.

Discussion

In this work, we present similar variable correlations between general determinations and quantifications of individual compounds to those observed in previous studies (Stevens et al. 1977), but complementary conclusions can be added. Our results suggest that some accessions accumulate important quantities of acids other than CA, MA, OA, and FA or sugars other than G, F, and S because the TA value or TS content show higher values than

Table 5. Correlation coefficients between the variables and the three first principal components on the analysis performed on only the accessions of *L. esculentum* Mill.

Variables	PC1	PC2	PC3
DM	0.97	0.06	0.06
SSC	0.89	-0.05	0.28
TS	0.71	0.27	0.41
F	0.82	-0.41	-0.08
G	0.87	-0.35	-0.01
S	0.26	-0.23	-0.71
pH	-0.12	-0.59	0.49
TA	0.50	0.64	0.02
CA	-0.04	0.91	-0.07
MA	0.14	0.69	0.42
OA	0.03	0.67	-0.03
FA	0.33	-0.09	-0.80
VITC	0.83	0.09	-0.15
PS	0.39	0.36	-0.45
TP	0.50	-0.32	0.40
Variance explained (%)	35	21	15

Fig. 3. Dendrogram of the *L. esculentum* Mill. cultivars using the UPGMA criterion method.

individual acid or sugar additions. Further work is needed in order to identify and quantify the other acids and sugars present in the tomato in order to complete the characterization of *Lycopersicon* sugar and acid profiles.

The results shown suggest that all the variables taken into account on this work, except the PS, are good keys to characterize internal quality properties in red fruited accessions of subgenus *Eulycopersicon*. The multivariate statistical techniques used are suitable tools for examining affinities and differences on the accessions studied and for grouping them based on variables responsible for either sweetness, sourness, flavor intensity, or nutritional value (VITC).

Nevertheless, the environment greatly influences the content of some compounds involved in flavor intensity (Davies and Hobson, 1981, Paterson et al., 1991). Consequently, for a finer interpretation of the characterization results, comparison between accession and control behavior are needed. The results of these comparisons show that five of the *L. pimpinellifolium* (Jusl.) Mill. accessions tested can be very interesting for use in breeding for internal quality characteristics. UPV-17049 (25) is the most interesting accession because it shows the highest flavor intensity characteristics [$\approx 625\%$ higher flavor intensity than 'Cambria' (5), the best *L. esculentum* Mill. control, and $\approx 175\%$ higher flavor intensity than LA-530 (1), the *L. cheesmanii* Riley control]. UPV-14974 (24) accession has similar characteristics, but it has slightly lower flavor intensity [425% higher than 'Cambria' (5) and 27% lower than LA-530(1)] and no MA content. These two accessions are good sources of high flavor intensity and well-balanced taste. Moreover, they are interesting because of their relatively high VITC content [53% higher than LA-1563 (4), the best control]. The accession UPV-16903 (28) also has a high flavor intensity value [535% higher than 'Cambria' (5) and 84% higher than LA-530 (1)] and medium-to-high VITC content (38% higher than the best control). This accession is a good source of high flavor intensity and acid taste. UPV-14345 (22) and

UPV-14344 (23) accessions have similar flavor intensity characteristics to UPV-14974 (24), but its VITC content is comparable to the controls, however they show sweet taste.

It seems interesting to underline the usefulness of the methodology of the study that has shown the very good behavior of some of the *L. pimpinellifolium* (Jusl.) Mill. accessions selected in comparison to the *L. cheesmanii* Riley control. *L. cheesmanii* Riley accessions, specially those from the minor form, which have traditionally been considered very good variability sources for internal quality breeding programs because of their higher SSC (Hewitt and Garvey, 1987; Garvey and Hewitt, 1991, 1992). For this reason, we chose a high SSC *L. cheesmanii* Riley accession [LA-530 (1)] as control. In our study, all the *L. pimpinellifolium* (Jusl.) Mill. accessions tested have lower SSC than *L. cheesmanii* Riley control, but the wide characterization carried out had shown that two of them have really higher flavor intensity characteristics. Moreover, these *L. pimpinellifolium* (Jusl.) Mill. accessions could be a better source to begin a breeding program because this is the species phyletically nearest to *L. esculentum* Mill. and it permits quicker recovering of agronomic traits.

Some of the *L. esculentum* Mill. accessions tested with higher flavor intensity than controls [higher than 120% than 'Cambria' (5)] could be useful in advanced stages of breeding programs. UPV-14382 (19) accession show a medium-to-high flavor intensity value [238% higher than 'Cambria' (5)] and a slightly sweet taste. UPV-16898 (10) accessions have interesting VITC content [63% higher than LA-1563 (4), the best *L. esculentum* Mill. control] and relatively high S and FA contents. UPV-14384 (18) accessions have very high CA [24% higher than 'Cambria' (5)] and MA content [54% higher than LA-1563 (4)].

Nevertheless, it is important to take into account that UPV-16903 (28), UPV-17049 (25), UPV-14384 (18), UPV-14974 (24) and UPV-14345 (22) accessions have an OA content higher than controls, specially UPV-16903 (28). From the nutritional point of view, the main problem which arises for high OA content is the bioavailability of calcium in the alimentary ration (Güil et al., 1996). Although OA content in tomato fruit should be considered together with calcium content in order to determine their bioavailability and, consequently, selecting the best accessions for their use in internal quality breeding programs.

In conclusion, the deep analytical characterization employed proved to be useful for selecting interesting donor parents according to their usefulness for internal quality breeding programs. Nevertheless, identification of other sugars and acids present in *Lycopersicon* germplasm could be interesting for additional research in order to obtain wider characterization of tomato flavor characteristics.

Fig. 4. Plot of the *L. esculentum* Mill. accessions projected on the reduced spaces of the first and second principal components and of the first and third components. Groups are derived from a separate cluster analysis.

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