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Postharvest Quality Evolution in Long Shelf-Life "Vesuviano" Tomato Landrace

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Abstract: "Vesuviano" or "piennolo" tomato is among the most famous Italian small tomato landraces and is cultivated on the slopes of Vesuvio volcano (Southern Italy). The "piennolo" tomato is an interesting case with regard to its potential sustainability, as it is traditionally grown in water-deficit conditions with a low fertilizer input. Fruits with a high firmness and a thick skin can be stored for 3–4 months at room temperature ("long shelf-life" or LSL tomato) without postharvest fungicide applications. The aim of this research was to study the retention, changes in quality, and nutritional traits of "Vesuviano" tomatoes over 120 days of "natural" storage. The dry matter, soluble sugar, organic acids, volatile compounds, and carotenoid contents were evaluated at harvesting and in fruits stored for 40, 80, and 120 days. Slight decreases in dry matter content, soluble sugars, and sweetness index were found, while the organic acids levels remained relatively stable. Moreover, interesting increases in the concentrations of certain flavor volatiles, alcohols, aldehydes, and terpenes were detected. Regarding carotenoids, the total lycopene levels exhibited a 1.5-fold increase from harvest to 120 days. The unchanged lycopene *cis*-isomer levels and the β-carotene/total lycopene ratio is characteristic of relatively stable isomerization activity and indicated an optimal ripening pattern up until the end of the "natural" storage period. These results, which demonstrate good overall quality retention of this LSL tomato, represent a well-grounded reason to enhance the cultivation and marketing of this genetic resource, the fruits of which can be appreciated by consumers during the winter–early spring, when high-quality fresh tomatoes are not available on the markets.

Keywords: piennolo; organic acids; carotenoids; genetic resources; reducing sugars; VOCs

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1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) was introduced into Europe in the early 16th century. As a result, the Mediterranean area became an important secondary center for diversification, which resulted in a wide array of variations in terms of adaptation to different agro-climatic conditions, fruit shape and color, market destination, and nutritional and functional quality [1–6].

Among this germplasm, landraces are often characterized by outstanding organoleptic quality. This is appreciated by consumers who are willing to pay higher prices than for commercial modern varieties, which are more homogeneous in taste. Moreover, several tomato landraces are important sources of nutritional and functional compounds in the human diet [7].

Reducing sugars (fructose and glucose, mainly) and organic acids (firstly citric acid, followed by malic acid) represent the main constituents of the dry matter content of tomato fruit [8]. These two chemical classes affect both the tomato taste attributes and the overall flavor [9], which is determined by a complex mixture of primary and secondary metabolites, including minerals and volatile substances (VOCs).

The latter hugely contributes to the flavor; indeed, hexanal, (*E*)2-hexenal, and 2-isobutylthiazole are considered characteristic tomato compounds [10]. As reported by Tandon et al. [11], terpenes also play an important role in product quality, as do other volatiles, commonly named "apocarotenoids" (6-methyl-5-hepten-2-one, geranyl acetone, β-ionone), which result from the catabolism of carotenoids [12,13].

Tomatoes are one of the most important sources of health-related compounds (vitamin C, carotenoids, and flavonols), which are associated with reduced risks of certain types of cancer and cardiovascular diseases [14–16]. Carotenoids represent the main liposoluble antioxidant found in fresh tomatoes and processed tomato products, and among these tetraterpenoid compounds, the most abundant are lycopene, α- and β-carotene, lutein, zeaxanthin, and β-cryptoxanthin. Lycopene, as the all-*trans* form, constitutes about 80–90% of the total carotenoids content of red-ripe tomatoes, followed by β-carotene [17,18].

Long shelf-life (LSL) or "long-storage" tomatoes are typical cherry-like landraces traditionally cultivated in the Eastern Iberian Peninsula (the Balearic Islands) and in southern Italy (the Campania, Sicilia, and Apulia regions). This is due to the textural properties of the fruits (such as high firmness and a thick skin), which affords an extended shelf-life of up to 4–6 months under ambient conditions [19–24]. This germplasm, having been selected for under reduced nitrogen and water availability conditions that are typical in marginal Mediterranean areas, represents a valuable genetic resource for selection and breeding for resilience [25–28].

Among the Italian LSL landraces, the most important and profitable is the "Vesuviano" tomato, which is labeled as "Pomodorino del piennolo del Vesuvio" (Protected Designation of Origin according to the Regulation EC No 1238/2009). This landrace is traditionally cultivated in volcanic soils on the slopes of Vesuvio volcano (southern Italy) and is characterized by red small-sized fruits, weighing 20–30 g, with a pointed blossom-end shape. The high firmness, the dry pulp matter, and the thick, coriaceous skin extends the shelf-life of the fruits, which are stored after harvest in ambient conditions in typical hanging bunches ("piennolo") (Supplementary Figure S1) for 4–6 months in dry, ventilated rooms [29]. The canned and "naturally-stored" "Vesuviano" tomatoes are commercialized throughout the Italian peninsula and demand from top food brands worldwide is increasing. A wide ranging agronomic [30], morphological [3], and genetic [5,21] assessment, and a characterization of the sensorial [31] and quality [5,32] aspects of fresh fruits has been conducted on PV-ISCI 10, that is a biotype selected by CREA Research Center for Vegetables and Ornamental Crops, which belongs to the "piennolo" landrace. Moreover, an appreciable variability in the fresh fruits from a chemical and physical perspective at the intra-landrace level was reported by Carillo et al. [33] and Fratianni et al. [29] when analyzing different "piennolo" tomatoes.

To the best of our knowledge, there is a lack of research concerning quality indicators and the evolution of the overall quality of "Vesuviano" tomatoes during the extended postharvest traditional storage ("piennoli") period [34,35]. For this reason, the aim of the present study was to evaluate the retention, changes in quality, and nutritional traits (dry matter (DM), soluble sugar (SS), organics acid (OA), volatile compounds (VOCS), and carotenoid contents (CAR)) of "Vesuviano" tomatoes over 120 days of "natural" storage, both in the framework of local genetic resource conservation and in order to promote them to traders and consumers during the winter–early spring, when high-quality fresh tomatoes are not available.

2. Materials and Methods

2.1. Plant Growth Conditions, Fruit Storage Conditions, and Sampling Procedure

The study was performed in 2016 on the "Vesuviano" tomato (PV-ISCI 10 biotype) grown in open-field conditions on the slopes of Vesuvio volcano (Massa di Somma, 40°50′0″ N, 14°22′0″ E, 180 m a.s.l.) in a sandy loam soil (84% sand, 12% silt, and 4% clay) characterized as follows: pH 8.0, electrical conductivity 0.25 dS m^{-1}, organic matter 1.5% (*w*/*w*), total N 0.03%, available P 50 mg kg^{-1}, and exchangeable K 1510 mg kg^{-1}. The transplant

was carried out in the early spring (March 30th) wherein the plants were arranged in single rows with a density of 5.0 plants m^{-2}. Two drip irrigation treatments were applied in support of rainfall through the growing season (115 mm). Fertilization, plant protection, and weed control were carried out according to local practices, which included stakes as support and galvanized wires. At harvest (denoted hereafter as T0), which occurred on 30th July, 12 kg of ripe bunched fruits were harvested, taking care not to detach them from the peduncles. Moreover, the berries were selected based on the uniformity of the external red color and the absence of physical injury or lesions caused by pathogens. The collected fruits were stored under ambient conditions until 120 days after harvesting (T120) in a well-ventilated room by hanging from the ceilings in three clusters (traditionally called "piennoli") of 4 kg, which were held together by hemp twine (Supplementary Figure S1).

Fruit sampling for chemical and physical determinations was carried out at 40 (September 9th), 80 (October 19th), and 120 (November 29th) days postharvest (T40, T80, and T120). At each time point, 200 gr of healthy fruit (ripe fruits without abiotic/biotic damage or rot caused by physiological decay) were collected from each of three "piennoli". The temperature and relative humidity from T0 to T120 were recorded every 60 min by a wireless data logger (EL-USB-2 model, Lascar Electronics Ltd.-United Kingdom) (Figure 1).

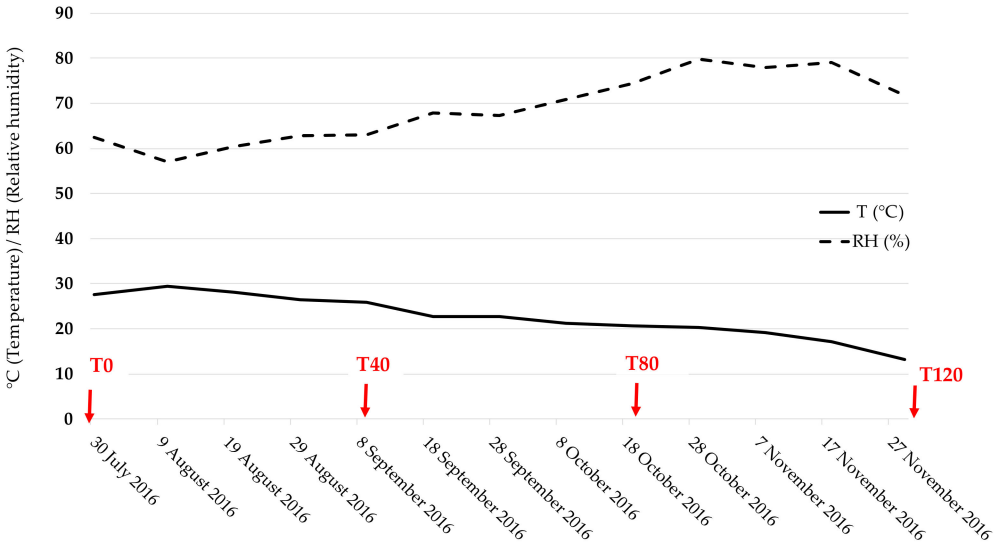

Figure 1. Mean temperature and relative humidity (average decadal values) from harvest (T0 = 30 July 2016) until the end of storage (T120 = 29 November 2016) of "piennolo" tomatoes.

2.2. *Chemical Analyses*

2.2.1. Sampling

For all analytical parameters, the analyses were made in triplicate on a representative sample of uniform and healthy fruits for each sampling time (T0, T40, T80, and T120).

2.2.2. Dry Matter

Tomato berries were analyzed for dry matter (DM) according to the AOAC (1980) methods [36].

2.2.3. Soluble Sugars and Organic Acids

The rationale for the analyses of soluble sugars (SS) and organic acids (OA) was described by Paolo et al. [37]. Briefly, 5 g of frozen sample were homogenized with 20.0 mL deionized water and separated by centrifugation. Thereafter, the residue was blended with an additional 20.0 mL of water and centrifuged. The two extracts were brought to volume in 50.0 mL volumetric flasks. Before injection, the samples were filtered through a millipore 0.45 µm nylon filter and diluted.

The identification and quantification of SS were performed by isocratic high-performance liquid chromatography (Jasco-Italy, Lecco, Italy models PU980 equipped with a RI 930 refractive index detector), as described by Forni et al. [38]. The adopted column was a Biorad Aminex HPX-87C, the mobile phase was ultrapure water (HPLC grade), and the flow was 0.70 mL min^{-1}. The column temperature was maintained at 85 °C.

The HPLC analysis of OA was performed using a Jasco-Italy, model PU980, equipped with a UV detector (UV1570) set at 214 nm, and with a GL Sciences Inertsil ODS-3 C18 (Microcolumn, Monza, Italy) column. The mobile phase was H_3PO_4 0.02 M, the flow rate was 0.60 mL min^{-1}, and the column temperature was constantly maintained at 30 °C, as described by Lo Scalzo et al. [39].

The quantification was made using external standards; the systems were calibrated with pure sugars and organic acids at known concentrations. The measure units used were g 100 g^{-1} of dry weight.

The sweetness index ratio was calculated on the basis of previous work [40,41].

2.2.4. Volatiles (VOCs)

The VOC fraction from the tomato samples was extracted and concentrated using a combined microwave-resin-solvent and the extracts obtained were analyzed using GC/MS (Agilent Mod. 6890N and MS 5973N, Agilent Technologies Italia, Milano, Italy) equipped with a capillary column (DB-1, 60 m, 0.25 mm, 0.25 µm film thickness, J &W Scientific, Folsom, CA, USA), following an already validated protocol [42]. The volatile components were identified using the retention times of the chromatographic peaks, by comparing their mass spectra with those in a commercial library (Wiley 7 n. 1 Library, Mass Spectral Data Base, Hewlett-Packard, Vienna, Austria) and by using commercial standards when available. For the quantitative analysis, standard solutions of the components at known concentrations were used to calculate the response factors. For the components whose standards were not commercially available, the internal standard procedure was followed using methyl palmitate solutions.

The identified VOCS were divided into the main chemical classes and their respective sums and potential differences were evaluated: alcohols (ALC), carbonyl compounds (CaC), phenolic derivates (PHE), terpenes (TER), and 2-isobuthyl thiazole (a sulphur-containing heterocyclic compound) (HeC).

2.2.5. Carotenoids (CAR)

These lipophilic compounds were extracted under controlled conditions (in darkness at 0–1 °C to avoid sample decomposition) from a 10 g frozen homogenized sample, using 100 mL n-hexane/acetone/ethanol (2:1:1 *v/v/v*) solution, with 1 mg mL^{-1} butylated hydroxytoluene (BHT), as described by Shi et al. [17]. The mixture was separated by centrifugation at 5000 rpm for 10 min at 4 °C. As the method followed was for only one extraction, it was validated by laboratory trials with subsequent extractions, checking the color of the pellet, which remained uncolored after the first solvent treatment. Chromatograms of the second and subsequent extracts gave no significant peaks. The clean-colored organic layer was filtered (a 0.45 µm regenerated cellulose filter) and analyzed using HPLC (Jasco-Italy, Lecco, Italy model PU980), with a diode array detector mod MD-2010; the column used was a C30 YMC Carotenoid 0.80 mL min^{-1}, at 35 °C, using the chromatographic methodology as described by Ishida et al. [43]. The concentration of β-carotene, *cis* isomers, and all-trans isomers of lycopene were calculated from the area of the experimental peaks by analytical interpolation, using standard calibration curves; these were expressed as mg 100 g^{-1} dw.

2.3. Statistical Analyses

Data were subjected to the analysis of variance (ANOVA) and the comparison of the average values was carried out using the Tukey test. Statistical differences at $p \leq 0.05$ were considered significant. Statistical analysis was performed using Statistica version 6.0 (Tulsa, OK, USA).

3. Results

As reported in Table 1, DM decreased during the postharvest from 8.43 mg 100 g^{-1} (T0) to 6.85 mg 100 g^{-1} (T120); in particular, starting from T80, significant lower values were detected as compared with T0. Similar behavior was also recorded for glucose (GLU) content, the amounts of which did not differ statistically between 80 and 120 days (17.3 mg 100 g^{-1} dw and 18.1 mg 100 g^{-1} dw, respectively). Decreases in GLU and fructose (FRU) contents were detected during the "natural" storage, with no significant differences occurring from 40 days postharvest. Higher FRU/GLU ratio values were noted at T80 and T120 as compared to harvest (T0) (1.56, 1.53, and 1.26, respectively). With regard to organic acids, the malic acid (MAL) content decreased after harvest (1.66 mg 100 g^{-1} dw) up until T80; however, a significantly higher content was detected at T120 (1.80 mg 100 g^{-1}) as compared with the previous value. The citric acid concentration (CITR) (6.21 mg 100 g^{-1} dw, on average) exhibited an increasing trend from T0 to T80, although it was not significant; this was followed by a weak decreasing at the end of storage, without significant differences among the subsequent time-points. Finally, similar to CITR, the lowest total organic acids content (TAC) was noticed at the end of storage (7.77 mg 100 g^{-1} dw for T120). The main consequence of SS and TAC trends was that their reciprocal ratio did not significantly change over the storage period (Table 1), although a slightly decreasing trend was noted (data not shown). On the other hand, the evidence concerning a decrease in the sweetness index was clear, i.e., it was significantly lower at T80 and T120 (Table 1).

Table 1. Postharvest changes in dry matter, soluble sugars, organic acids content, and sweetness index of "Vesuviano" tomatoes from harvest until 120 days postharvest.

Treatment	DM	GLU	FRU	SS	FRU/GLU	MAL	CITR	TAC	SWI
	g 100 g^{-1}	g 100 g^{-1} dw	g 100 g^{-1} dw	g 100 g^{-1} dw		g 100 g^{-1} dw	g 100 g^{-1} dw	g 100 g^{-1} dw	
T0	8.43 a	24.4 a	30.8 a	55.3 a	1.26 b	1.66 a	6.20 a	7.86 a	80.4 a
T40	7.59 ab	20.4 ab	28.3 b	48.7 b	1.39 ab	1.45 b	6.34 a	7.79 a	64.9 ab
T80	7.00 b	17.3 b	27.1 b	44.4 b	1.56 a	1.41 b	6.40 a	7.81 a	55.8 b
T120	6.85 b	18.1 b	27.7 b	45.9 b	1.53 a	1.80 a	5.90 a	7.70 a	56.1 b
Average	7.47	18.6	27.7	46.3	1.44	1.55	6.21	7.77	64.3
p-value	*	*	*	*	*	*	NS	NS	*

T0 = harvest; T40 = 40 days postharvest; T80 = 80 days postharvest; T120 = 120 days postharvest; DM = dry matter; FRU = fructose; GLU = glucose; SS = GLU + FRU; MAL = malic acid; CITR = citric acid; TAC = total acids; SWI = sweetness index; dw= dry weight; NS, *, not significant or significant at $p \leq 0.05$. Different letters within each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

The volatile fraction extracted from tomato samples included alcohols (ALC), carbonyl compounds (CaC), phenolic derivates (PHE), terpenes (TER), and 2-isobuthyl thiazole (a sulphur-containing heterocyclic compound (HeC)), which are considered an important biomarker of tomato aroma [10,44]. Through the storage period (T0–T120), within the ALC class, a significant increase was detected until T80 for 1-butanol-3-methyl, 1-pentanol, (*Z*)3-hexen-1-ol, 1-hexanol, and benzyl alcohol (231, 31.1, 33.2, 39.4, and 53.5 µg 100 g^{-1} dw, respectively, at T80), with an average of an 11-fold increase, although there was a great variability among the detected alcohols. Interestingly, the 1-pentanol exhibited an interesting trend: from absence at T0 to a maximum of 31.1 µg 100 g^{-1} dw at T80. Contrarily, a significant decrease in the phenethyl alcohol content was detected in the same postharvest period. Forty days later, the 1-butanol-3-methyl and benzyl alcohol contents decreased, and no variations or no detectable amounts were noticed for the remaining alcohols. Regarding CaC, nine (hexanal, heptanal, (*E*)2-heptenal, 6-methyl-5-hepten-2-one, (*E*)2-octenal, nonanal, (*E*)2-nonenal, (*E*,*E*)2,4-decadienal, (*E*,*Z*)2,4-decadienal) of 15 molecules were detected from the harvest that were increased at T120, with the most significant of these being 6-methyl-5-hepten-2-one and hexanal. The highest amounts of the other three volatile substances ((*E*)2-heptenal, 5-heptenal-2,6-dimethyl, 6-methyl-3,5-heptadien-2-one)

were also found at the end of the "natural" storage period, i.e., T120 (34.9, 41.9, 32.9 μg 100 g^{-1} dw, respectively).

A significant increase in two PHE, i.e., 2-methoxyphenol and methylsalicylate, was noted from the harvest (T0) up until T80, while the highest amount of eugenol (35.8 μg 100 g^{-1} dw) was detected at the end of the experiment period (T120), as was the case with the most relevant compound in this class: methylsalicilate (144 μg 100 g^{-1} dw).

Regarding TER, which comprises various apocarotenoids produced by the carotenoid cleavage, six (2,3-epoxygeranial, neral, geranial, nerylacetone, (*E*)β-ionone, (*E*,*E*)pseudoionone) of 10 molecules significantly increased up until T120, while for the other three compounds (α-terpineol, β-damascenone and (*Z*)β-ionone), the significant increasing trend stopped at T80 (67.7, 18.4, and 23.0 μg 100 g^{-1} dw, respectively). The most plentiful TER compound, from those identified and quantified, was geranial, an apocarotenoid compound. 2-Isobutylthiazole, as a characteristic volatile compound of tomato fruit, significantly increased up until T80, i.e., the amounts detected at this time-point (527 μg 100 g^{-1} dw) were three times higher than at harvest (T0) (167 μg 100 g^{-1} dw). With regard to the time-course of the other two volatile compounds characteristic of tomato fruit, an increase in hexanal levels was noted up until T120, while for (*E*)2-hexenal, a significant decrease was detected 40 days after harvest.

In Figure 2, the postharvest time-course profiles of VOCs for ALC, CaC, PHE, and TER, as the sum of the overall molecules belonging to the four volatile classes (Table 2), are reported. Total volatile compounds (TVC) were also obtained as the sum of ALC, CaC, PHE, TER, and 2-isobutyl thiazole, at each time-point. Significant increases in the ALC content were detected from harvest up until T80; this had then halved by the end of storage. Contrarily, CaC, PHE, and TER contents reached the highest values at T120; the increases detected during postharvest (from T0 to T120) were equal to +326%, +158%, and +577% for the three volatile classes, respectively. Finally, considering the volatile substances as a whole (TVC), they clearly increased during postharvest (+216%, from T0 to T120) with the strongest increase being noted between harvest and the T40 time-point.

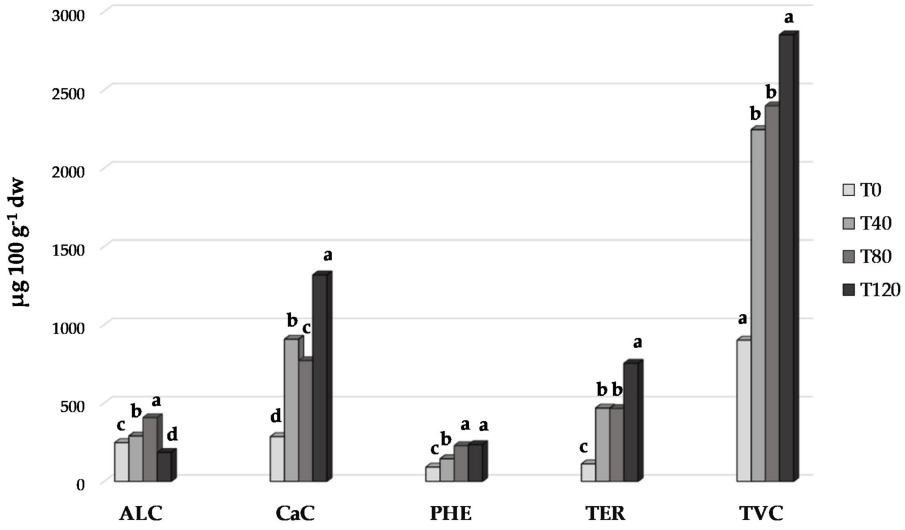

Figure 2. Postharvest time-course of alcohol (ALC), carbonyl compound (CaC), phenolic derivate (PHE), and terpenes (TER) contents at three time-points (T0 = at harvest, T40 = 40, T80 = 80, T120 = 120 days postharvest). Total volatile compounds (TVC) were calculated as the sum of ALC, CaC, PHE, TER, and 2-isobutyl thiazole (data from Table 2). Means followed by the same letter do not significantly differ at $p < 0.05$.

Table 2. Postharvest time-course profile of volatiles organic compounds found in "Vesuviano" tomatoes under "natural" storage conditions (concentrations are reported as μg 100 g^{-1} dw).

Volatile Class	Molecule	Time-Point							
		T0		T40		T80		T120	
ALC	1-penten-3-ol	9.00	b	21.3	a	–		–	
	1-butanol-3-methyl	173	b	181	b	231	a	110	c
	1-pentanol	–		23.9	b	31.1	a	29.2	ab
	(*Z*)3-hexen-1-ol	1.83	b	–		33.2	a	–	
	1-hexanol	7.25	b	8.64	b	39.4	a	–	
	benzyl alcohol	25.9	d	38.7	c	53.5	a	45.5	b
	phenethyl alcohol	29.7	a	16.1	b	19.0	b	–	
CaC	1-penten-3-one	7.63		–		–		–	
	pentanal	9.34		–		–		–	
	hexanal	78.5	c	134	a	104	b	141.5	a
	(*E*)2-hexenal	11.3	c	19.9	a	17.2	b	13.4	c
	heptanal	5.87	d	17.9	c	29.6	b	43.7	a
	benzaldehyde	6.33		–		–		–	
	(*E*)2-heptenal	14.1	c	25.6	b	15.4	c	34.9	a
	6-methyl-5-hepten-2-one	87.7	d	498	b	405	c	769	a
	(*E*)2-octenal	16.9	c	37.9	b	36.4	b	53.6	a
	5-heptenal-2,6-dimethyl	–		39.8	a	27.3	b	41.9	a
	6-methyl-3,5-heptadien-2-one	–		26.0	b	–		32.9	a
	nonanal	10.1	c	18.9	b	30.4	a	33.4	a
	(*E*)2-nonenal	11.5	d	28.2	c	36.2	b	69.7	a
	(*E,E*)2,4-decadienal	11.2	c	33.8	b	36.6	b	44.8	a
	(*E,Z*)2,4-decadienal	14.8	d	27.5	c	32.6	b	39.0	a
PHE	2-methoxyphenol	20.7	c	33.4	b	62.9	a	54.1	a
	methylsalicylate	48.0	c	110	b	138	a	144	a
	eugenol	22.1	b	–		26.7	b	35.8	a
HeC	2-isobutylthiazole	167	d	436	b	527	a	360	c
TER	4-terpineol	9.63		–		–		–	
	α-terpineol	11.7	c	46.9	b	67.7	a	77.5	a
	2,3-epoxygeranial	11.2	d	94.3	b	75.9	c	138	a
	neral	9.3737	c	46.4	b	40.2	b	76.7	a
	geranial	18.7	d	138	c	112.6	b	220	a
	β-damascenone	5.1	c	8.85	b	18.4	a	6.30	bc
	nerylacetone	22.8	d	39.8	c	58.0	b	95.2	a
	(*Z*)β-ionone	5.22	c	17.9	b	23.0	a	26.5	a
	(*E*)β-ionone	12.2	c	34.9	b	37.4	b	63.2	a
	(*E,E*)-pseudoionone	5.33	d	39.8	b	31.7	c	49.4	a

ALC = alcohols; Cac = carbonyl compounds; PHE = phenolic derivates; HeC = heterocyclic compounds; TER = terpenes; Different letters between different time-points indicate significant differences according to Tukey's test ($p \leq 0.05$). T0 = harvest; T40, T80, T120 = 40, 80, 120 days postharvest.

It is interesting to note that this increase in volatile fractions in the tomato counteracts the decrease in the soluble sugars and sweetness index, thus giving the "piennolo" tomato its particular flavor that is so sought after by consumers. This further adds to appeal of this tomato variety, which is available in winter, a very long time from the harvest.

Regarding the carotenoids, which are the most representative antioxidant compounds in tomatoes, *cis*-isomers of lycopene did not show any significant differences up until the end of storage (Table 3).

Table 3. Postharvest time-course of lycopene (and its isomers) and β-carotene in "Vesuviano" tomatoes under "natural" storage conditions (concentrations are reported as mg 100 g^{-1} dw).

Treatments	Lycopene								β-Carotene mg 100 g^{-1} dw		β-Carotene vs. Total Lycopene (%)	
	cis Isomers mg 100g^{-1} dw		All-*trans* + 5 *cis* Isomers mg 100 g^{-1} dw		Total Lycopene mg 100g^{-1} dw		% *cis* vs. all-*trans*					
T0	6.49	a	79.7	c	86.2	c	7.53	a	11.5	a	13.3	a
T40	6.60	a	98.5	bc	105	bc	6.69	ab	12.3	a	11.7	a
T80	7.14	a	114	ab	121	ab	6.23	ab	15.2	a	12.5	a
T120	7.26	a	123	a	131	a	5.53	b	16.7	a	12.7	a
Average	6.87		104		111		6.60		13.9		12.5	
p-value	N.S.		*		*		*		N.S.		N.S.	

T0 = harvest; T40 = 40 days postharvest; T80 = 80 days postharvest; T120 = 120 days postharvest. NS, *, nonsignificant or significant at $p \leq 0.05$. Different letters within each column indicate significant differences according to Tuckey's test ($p \leq 0.05$).

For the all-*trans* + 5-*cis* isomers, a significantly higher amount was found at T80-T120 as compared with T0, and a similar behavior was also observed for total lycopene content, as the sum of all detected isomers. With regard to the ratio of *cis* to all-*trans* isomers, the first was equal to 6.6% (on average) of *all-trans* isomers, with a slight decrease during storage. With regard to β-carotene, its concentration was significantly less than 20% in comparison with total lycopene, which averaged 12.5%, with weak variation up until T120. These data indicate an optimal ripening pattern up until the end of the "natural" storage period of "piennolo" tomatoes. Finally, from the harvest to the end of storage, total lycopene exhibited an increase of + 52.2%, while no significant variation over time was detected for β-carotene content, although a slight increasing trend was noted during storage.

4. Discussion

Superior quality attributes that are maintained over extended storage periods characterize several LSL tomato landraces ("piennolo" or "Vesuviano", "Penjar", "pizzutello", "Regina", etc.) originating in the Mediterranean area [19–24].

The primary barrier to transpirational water loss for a tomato fruit is the cuticle; thus, its composition significantly affects storability [45,46]. Indeed, the delayed fruit deterioration (dfd) tomato is a mutant with an unusual cuticle composition, and, as compared with other tomato genotypes, it has far longer shelf-life [45]. The extended shelf-life of the *dfd* mutant is greater than 4 months, which is close to the value indicated for "Penjar" and "Vesuviano" tomatoes [23,35].

Interestingly, Caiazzo et al. [47] demonstrated that the wax content of the "Vesuviano" fruit cuticle was higher than that of other tomatoes with a shorter storability. Moreover, the authors reported that the fruit cuticle of "piennolo", which varies among different biotypes, was thicker and heavier than a shorter shelf-life tomato (Ailsa Craig).

The DM content measured using fresh "Vesuviano" tomatoes (T0) was comparable to values reported by Ruggieri et al. [5], while they were lower than the results from Migliori et al. [35] (10.8 g 100 g^{-1} fw). In our research, this quality attribute decreased up until T120, which is in disagreement with Migliori et al. [35] who did not observe any significant variations during the "natural" storage period. Conversely, Manzo et al. [48], when comparing the main physico-chemical traits of "piennolo" tomato at harvest and after 6 mounts of storage at room temperature, reported an increase in total soluble solids (°Brix) and DM content.

For MAL, CITR, and TAC, our results were very similar to those of Migliori et al. [35]; Contrarily, the same authors detected higher GLU, FRU, and SS contents in comparison to this research. These comparisons are very notable because they concern the same "Vesuviano" tomato biotype (PV-10 ISCI); thus, the differences in concentrations were solely due to extrinsic and environmental factors, such as weather trends during ripening, irrigation regimes, and fertilization practices [49–52]. Moreover, the amounts of SS and CITR in the

fresh fruit were in agreement with results of Fratianni et al. [29] and Ercolano et al. [53], who analyzed other "Vesuviano" biotypes. These variations in sugar contents, independent of the analyzed genotype, with significant variations according to environmental and cropping conditions, were also evidenced in previous work [37] on different tomato varieties, highlighting the importance of these findings in the context of possible future environmental changes and their influence on the quality of horticultural crops.

Considering the postharvest decreases in SS, CITR, and TAC, and the increases in the GLU/FRU ratio, our findings were in accordance with previous research by Migliori et al. [35]. The increasing GLU/FRU ratio trend indicated a higher decrease in GLU than FRU, which indicates that the first sugar underwent a more remarkable catabolism than the second ones [54]; these phenomena were in agreement with the results from the "Penjar" tomato [55].

The opposite trends in MAL and CITR contents (from T0 to T80) were in agreement with the results of Migliori et al. (2017) [35]; furthermore, Missio et al. [55] supposed that the increased MAL amount in stored "Penjar" tomatoes could be related to the changes in the ethylene content, which is under the genetic control of the alcobaça (*alc*) allele that is found in this LSL landrace [56,57]. A similar explanation could also be formulated for the "Vesuviano" tomato; however, the presence of this genetic mutation is uncertain in the biotype studied herein. Finally, it is interesting to note that water availability during cultivation alters the cuticle structure or permeability, and hence alters fruit transpiration rates; thus, LSL tomato traits are greatly affected by the irrigation regime [58]. This remarkable finding could explain the contrasting results reported in different studies on postharvest changes in the DM and SS of "Vesuviano" tomatoes. Furthermore, the temperature and humidity time-course profiles in the storage period greatly affect the postharvest metabolism of tomatoes [59] and represent an added variable to be considered in the quality evaluation of "piennolo" tomatoes during their shelf-life.

Regarding volatile substances, more than 400 molecules have been detected in tomatoes and tomato products [60]; nevertheless, only a limited number of compounds play a significant role in tomato flavor [11,61–64].

This quality attribute is influenced by several internal (genetic background) and external factors (cultivation practices, pedoclimatic conditions, time and method of storage, etc.) [51]. A noticeable genetic variability at the intra-landrace level [3,21] could explain the variability in VOC content in the "Vesuviano" landrace reported by different authors [35,48]. However, the similar contents of different volatiles substances found in this work were consistent with those of Lisanti et al. [32], who analyzed the aromatic profile of the same "Vesuviano" tomato biotype (PV-10 ISCI).

To our knowledge, there are few studies on "Vesuviano" tomatoes that investigate the time-course of the VOC content under prolonged storage. Manzo et al. [48] reported a decrease in heptanal and an increase in (*E*)-2-hexenal and hexanal levels in an unknown "piennolo" biotype stored for 6 months. The change in the latter compound in respect to the end of storage was in agreement with our results, as well as those of Migliori et al. [35]. Hexanal, which is derived from the oxidative degradation of linoleic acid, represents one of the major volatile aldehydes in tomatoes [60] and is characterized by a fresh grass odor. Regarding (*E*)-2-hexanal, in our work, we noted no increase after T40; contrarily, Manzo et al. [48] reported the highest level of compound at 6 months of storage. Interestingly, the present data were in general accordance with Klee and Giovannoni [65] and Wang et al. [66], who found a strong increase in C6 aldehydes upon tomato ripening, confirming the physiological behavior, with similar trends between commercial tomato cultivars and LSL ones.

Regarding alcohol compounds (Table 2), increases in (*Z*)-3-hexen-1-ol and 1-hexanol contents (the latter, imparting a mint flavor to tomatoes) were found up until T80, after which their concentrations were below to the detection threshold. This decreasing trend was in agreement with the results of Manzo et al. [48]. The most abundant alcohol compound, 1-butanol-3-methyl, reached the maximum concentration of 213 μg/100 g^{-1} dw at T80;

similarly, a concentration of 170 μg kg^{-1} fw was reached during full ripening of FL47 tomato cv [66], while higher amounts (303–3285 μg kg^{-1} fw) were found in four common tomato varieties by Li et al. [67].

Among the carbonyl compounds, 6-methyl-5-hepten-2-one, which has a tomato-like flavor, musty aroma, and fruity taste [68], was the most abundant VOC in the present work, increasing up until T120 and reaching 769 μg 100 g^{-1} dw (Table 2). The concentrations of this apocarotenoid compound in the VOC profile of fresh tomatoes was similar to those found by other authors [66,69], who reported the highest amount of 6-methyl-5-hepten-2-one at the fully red ripening stage of the FL47 tomato line. Furthermore, Tandon et al. [70] associated the loss of flavor in modern tomato varieties with much lower amounts of this VOC compound (21–53 μg kg^{-1} fw) [67]. Considering that 6-methyl-5-hepten-2-one is a precursor to carotenoids [71], the detected increases could be explained by the accumulation of lycopene content up until the end of storage (T120), which resembled a similar pattern to the physiological tomato ripening process [72]. With regard to the phenolic compounds, methyl salicylate, characterized by a minty, spicy odor [61], is associated with tomato fruit responses to abiotic stress and its synthesis is related to the phenylalanine catabolism pathway [73]. Lisanti et al. [32] detected a lower concentration of this compound in fresh "Vesuviano" tomato fruit as compared with its commercial homologue: the "Principe Borghese" variety. To our knowledge, our results represent the first report on the time-course of methyl salicylate over such a prolonged storage period. In the present research, the higher amount of methyl salicylate was detected at T120 (144 μmol 100 g^{-1} dw) (Table 2), with a relevant increase being noted throughout the storage period. As reported by Li et al. [67], very low contents ($\leq$18 μg kg^{-1} fw) were found in modern tomato cultivars.

According to Lisanti et al., [32] a low level of β-ionone was always found at harvesting; furthermore, increases in the *Z*- and *E*- stereoisomer were also detected up until T120 in our experiments. Considering the very low odor (a characteristic floral odorous note) threshold of this apocarotenoid ketone (0.007 ppb, as reported by Buttery et al. [61]), this molecule could contribute to the "Vesuviano" odor profile up until the end of the "natural storage" period. 2-Isobutylthiazole, which is derived from amino acid metabolism, is characterized by a tomato leaf odor and several authors suggested it as a key-component of the tomato aroma [68,74]. A great amount of this heterocyclic sulphur compound was detected in "Vesuviano" tomato fruits at harvest, and this result is in agreement with those of Lisanti et al. [32]; furthermore, the increases in 2-isobutylthiazole up until T80 (527 μg 100 g^{-1} dw, Table 2) resembled the postharvest trend detected by Migliori et al. [35], which analyzed the same "piennolo" biotype (PV-ISCI 10). Other authors found much lower amounts of 2-isobutylthiazole in common tomato varieties, ranging from 0.30 to 13.0 μg kg^{-1} fw [67]. Research to improve the organoleptic quality of tomato cultivars leads to higher concentrations of this metabolite (27.4–62.4 μg kg^{-1} fw), though never reaching the concentrations found in "Vesuviano" tomato [69].

With regard to the antioxidant quality of the "piennolo" landrace, the lycopene content in fresh tomatoes was higher than that reported by Ruggieri et al. [5], while our results were comparable to the findings of Migliori et al. [35]. Furthermore, considering the lycopene content based on dry matter, our results were quite similar to those reported by different authors [33,48,75]. Moreover, it was interesting to note the similar *cis*-isomers/*trans*-isomers ratio in our experiment and that of Fattore et al. [75], which was performed under undefined experimental field conditions. In other research, the lycopene amounts in fresh fruits varied as a result of differing biotypes; thus, the values reported by Fratianni et al. [29] can reach twice those of the PV- ISCI 10 biotype. Moreover, the strong effects that external factors (pedoclimatic condition, agronomic management, etc.) have on the synthesis of secondary metabolites in tomatoes (carotenoids, flavonoids, ascorbic acid, etc.) are widely known, as reviewed by Dorais et al. [18] and Dumas et al. [76]. Definitively, the wide ranges of lycopene contents as reported by Collins et al. [77] in fully red tomato germplasm (10–150 mg kg^{-1} fw) largely agreed with the concentrations found in the present work.

To date, there are few studies investigating changes in the carotenoids content in the LSL tomato genotype available in the literature [24,35,48]. Indeed, biochemical studies [78–80] are generally concerned the modern tomato varieties with a shorter shelf-life (not exceeding 20 days on average).

The ratio of β-carotene vs. total lycopene (Table 3) was within the ranges reported in other investigations, which include a great number of tomato varieties (5–10-fold higher lycopene than β-carotene content) [80,81]. Furthermore, the same authors highlighted a decrease in the β-carotene/lycopene ratio during ripening.

The fruits of the "Vesuviano" landrace were harvested at the full ripening stage, as was demonstrated by levels of measured carotenoids; furthermore, these postharvest increases could be related to the air temperature during storage, as was suggested by Toor and Savage [79] when investigating changes in major antioxidant components in tomatoes (cv Tradiro) stored for 10 days at 7, 15, and 25 °C. Nevertheless, lycopene is degraded through isomerization and oxidation phenomena as a result of processing and storage [17]. As can be seen in the present data, a good quantity of *cis*-isomers was retained, as compared to *all*-trans lycopene, up until T80, highlighting the good functional quality of "Vesuviano" tomato fruits during prolonged storage at room temperature.

5. Conclusions

This study regarding the relevant quality indexes of "piennolo" tomatoes stored for 120 days led to four main interesting observations: (a) a strong water retention capacity of the tomato fruit was noted, as demonstrated by slight changes in DM; (b) a postharvest fruit metabolic process was observed, as evidenced by the decreasing sugars contents and the related sweetness index; (c) organic acidity was retained throughout storage, which produced stable sugars/acids ratios; (d) there was a relevant increase in certain volatile compounds and carotenoids. These issues represent an important added value for this traditional tomato variety.

The "Vesuviano" is a tomato landrace well adapted to its typical cultivation area and the low cultural input required (irrigation and fertilization) considerably limit the production costs for farmers.

Moreover, the "naturally extended" shelf-life of "piennolo" tomatoes, which does not require fungicides or a controlled atmosphere, further increases the environmental and economic sustainability of this genetic resource. Thus, consumers are willing to pay even higher prices for this healthy and tasty product, especially during the winter months, than they do for low-quality commercial tomatoes.

As a result of the high market demand, the cultivated area of "piennolo" is expanding rapidly and this limits the risk of genetic erosion of this important and valuable tomato landrace, which is now being increasingly appreciated in other European countries.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su132111885/s1, Figure S1: Typical hung-shape conservation "al piennolo" of "Vesuviano" tomatoes at room temperature.

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