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Comparison of Nutrient Composition and Antioxidant Activity of Hydroponically Grown Commercial and Traditional Greek Tomato Cultivars

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Abstract: The consumer demand for an enhanced diet intake of antioxidants and bioactive compounds is continuously rising. This work aims to evaluate the fruit nutritional composition and antioxidant activity of five tomato germplasm varieties, alongside a commercial F1 hybrid. Three varieties bear small-fruit (14–40 g), while two varieties and the commercial cultivar yield large fruit (150–300 g). Genotypes under study were cultivated in a greenhouse under the same environmental conditions. Fat, protein, carbohydrate, total phenol, total flavonoid, lycopene, and ascorbic acid contents were assessed at two fruit maturity stages (breaker, red ripe). For both hydrophilic and lipophilic fractions, antioxidant behavior was also evaluated by employing DPPH and FRAP assays. Small-fruit varieties generally possess higher fat and ascorbic acid content, as well as hydrophilic FRAP values as compared to large-fruit ones. In all varieties, lycopene content and lipophilic fraction radical scavenging capacity was considerably higher at red ripe stage. At red ripe stage, all germplasm varieties were clearly and consistently superior in terms of antioxidant activity at the lipophilic fraction owing to enhanced lycopene content. The results emphasize the value of reintroducing germplasm varieties in breeding programs and suggest that local varieties generally encompass high quality features.

Keywords: antioxidant capacity; ascorbic acid; flavonoids; fruit quality; germplasm; lycopene; phenols; *Solanum lycopersicum*

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1. Introduction

The consumption of tomatoes and tomato-based products has been repeatedly associated with the prevention or delay of many diseases. These beneficial effects have been generally attributed to carotenoids. In tomato, lycopene is the major carotenoid (80–90%), and is responsible for the characteristic red fruit color. Ascorbic acid is additionally considered to be an important health-promoting factor. Tomato also contains polyphenols (mainly phenolic acids), which exhibit strong antioxidant properties too. Previous studies showed that tomato antioxidant capacity strongly depends on the cultivar [1].

Although consumers are continuously placing more emphasis on the positive aspects of diet owing to antioxidants, breeding has been massively directed to enhance visual traits and yield. This pressure for selection of improved fruit visual traits and yield might have had unintended adverse impact on fruit health promoting compounds. For instance, the majority of modern tomato cultivars includes a mutation, inactivating the uniform ripening transcription factor [2]. Through this inactivation, a better visual quality and more uniform

ripening are achieved. However, this mutation has also been associated with attenuated carotenoid content. A promising avenue of stimulating the health-promoting profile of tomato is, therefore, to reconsider traditional varieties for quality traits (such as antioxidant properties), which then can be introduced into commercial cultivars.

Tomato is one of the most-produced vegetables around the world, which is consumed fresh and in processed and cooked products, such as dried fruit, sauce, and juice ketchup [3]. It is an excellent source of nutrients and antioxidant compounds, which promote consumer health by reducing the risk of various diseases, including cardiovascular ones [4] and several cancers [5–7]. Vitamins C and E, lycopene, flavonoids, organic acids, and phenolic compounds are the main available bioactive components in tomato fruit [1]. Due to the substantial per capita consumption, tomato consists a major source of antioxidants contributing to the daily intake of a significant amount of the above-mentioned beneficial molecules. Among the most significant bioavailable compounds of tomato fruit is lycopene, which plays important role in prevention of cardiovascular diseases inhibiting the oxidation of LDL cholesterol [8]. In addition, increased lycopene levels in blood have been related with lower risk of prostate and other cancers [9–11]. Studies have shown a direct relationship between plasma or serum lycopene levels and tomato consumption [12,13]. Tomato consists of a major source of ascorbic acid (AsA), tocopherols, and phenols, which play a determinant role in disease prevention.

The elevated levels of phenolic compounds such as flavonoids and hydroxycinnamic acids in tomato fruit are gaining interest due to their apparent multiple effects in several physiological processes, including free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways [14]. The chemical composition of the tomato fruit depends on numerous factors such as genotype, cultivar, maturity, and the environmental conditions [15]. However, consumer acceptance is based on the sensory properties of tomato fruit, which are determined mainly by shape, colour, flavour, and texture [16]. Fruit's flavour is the key factor for consumer's choice, which is directly associated with chemical composition of tomato [17].

In recent years, new commercial varieties (NCV) of tomatoes have been introduced with attractive external attributes such as colour, size, and shape, as well as improved shelf-life and nutritional composition (e.g high lycopene levels). Most commercial tomatoes, especially in northern European countries, are produced in greenhouses that allow better control of agronomic and environmental factors. In Greece, the great majority of intensive hydroponic farms are using NCVs, which are considered as more productive with extended shelf-life under various post-harvest storage conditions. Since NCVs are mainly F1 hybrids, their fruits have relatively stable characteristics, such as size, colour and firmness, but according to consumers, are inferior in flavor and aroma [18,19]. Cultivation of traditional varieties has been inevitably restricted in home gardens. However, in many cases, local consumers prefer these fruits because they are considered tastier and healthier than NCV ones [20].

The aim of the present study was to detect tomato germplasm with enhanced antioxidant profile, which can be further employed in breeding programs. To examine this hypothesis, five Greek tomato germplasm varieties, which were representative of table tomato local varieties and exhibited a range in fruit size, were characterized by evaluatingfruit nutritional composition and antioxidant activity.

2. Materials and Methods

2.1. Chemicals

Folin-Ciocalteu reagent, anthrone, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ [2,4,6-Tris(2-pyridyl)-s-triazine], Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], rutin trihydrate (analytical standard), gallic acid, BHT (2,6-di-tert-butyl-4-methylphenol), 2,6-dichlorophenolindophenol (DCPIP), anhydrous iron(III) chloride, and potassium acetate were purchased from Sigma Aldrich (Steinheim, Germany). Bradford reagent was supplied by Bio-rad. Solvents were purchased from Sigma Aldrich (Steinheim, Germany)

or Alfa Aesar (Thermo Fisher Scientific, Lancashire, United Kingdom), and they were spectrophotometric grade.

2.2. Plant Material and Samples Preparation

Six genotypes of tomato (*Solanum lycopersicum* L.) were used as experimental material in the present study. Five of them are traditional cultivated varieties (landraces) in Greek home gardens, and they are known by local vernacular names, specifically "Short of Kythira", "Long of Kythira", "Chiou", "Authentic of Santorini", "Kaisia", or "Traditional Santorini". The sixth genotype is a widely popular commercial hybrid, F1 Elpida. These fruits are used for fresh consumption, and their characteristics are depicted in Table 1.

Table 1. Description of tomato fruit used for analysis.

Variety	Type	Weight (g)	Shape	Color in Full Ripening
Elpida F1	uniform large round (beef-small beef type)	200–250	spherical	bright red
Short of Kythira	large beefsteake type	250–300	flattened with intense adhesions	light red
Long of Kythira	large beefsteake type	150–200	cordate elongated	light purple
Chiou	cherry	14–19	spherical	light red
Authentic of Santorini	marmande (coctail type)	30–40	flattened	deep red
Kaisia or Traditional of Santorini	cherry	18–20	light flattened, spherical	deep red

The plants were developed hydroponically in a greenhouse of University of Peloponnese (37°03′40.1″ N, 22°03′42.3″ E) and watered with full strength nutrient solution recommended for commercial purposes, as previously described [21]. The fruits of each variety were harvested from 30 plants randomly distributed in the greenhouse. Tomatoes were collected at two developmental stages

(i) Breaker (no more than 10% of the surface was red or pink),
(ii) Red ripe (more than 90% of surface was red).

Tomatoes were visually attributed to the two specific ripening stages. Three biological samples of at least twenty tomatoes were collected from each stage, washed thoroughly with distilled water, and surface dried. They were homogenized using IKA Homogenizer (IKA England LTD, Oxford, UK) and immediately frozen in liquid nitrogen. The homogenized tomatoes were stored at −80 °C until further analysis. The samples used for fats, protein, and hydrocarbon analyses were lyophilized prior the freezing. Lyophilization was also used to determine moisture contents of tomatoes. Ascorbic acid analysis was carried out immediately after homogenization.

2.3. Nutrient Composition

2.3.1. Total Fat

The fat content was determined by Soxhlet extraction of freeze-dried samples with dry ethyl ether according to AOAC official method 920.39 (AOAC, 2005). The results were expressed as mg per 100 g of fresh weight (mg/100 g FW).

2.3.2. Proteins

Lyophilized sample (200 mg) was extracted with 5 mL phosphate buffer (75 mM, pH 7) and then with 5 mL NaOH (1 M). The extracts were combined and used for determination of proteins by Bradford method as follows [22]: 100 μL of extract mixed with 5 mL of diluted Bradford reagent (1:4 v/v) with dd H_2O. After 5 min incubation at room temperature under darkness, the absorption of solution was measured at 525 nm. Bovine serum albumin (BSA)

was used as the standard. The results were expressed as mg per 100 g of fresh weight (mg/100 g FW).

2.3.3. Carbohydrates

Preparation of Extract

The total carbohydrate content of tomatoes was determined by the anthrone method [23]. Five mL HCl (10%) were added to 100 mg of lyophilized tomato, and the mixture was vortexed. Then, it was placed in boiling water bath for 30 min, and it was occasionally agitated. Subsequently, the mixture was neutralized by addition of Na_2CO_3 and centrifuged (6000 rpm, Hermle, Wehigen, Germany) for 5 min. The supernatant was collected, and the precipitate was extracted with 5 mL of water in room temperature and centrifuged (6000 rpm) for 5 min. The supernatants from the two centrifugations were combined and diluted to 200 mL with distilled water.

Anthrone Assay

The extract (500 µL) was mixed with 500 µL distilled water and 4 mL anthrone reagent (0.2%). The solution heated at boiling water bath for 8 min. The absorption was measured at 630 nm using water as blank. Glucose was used as standard for the calibration curve. Total carbohydrate content was expressed as g per 100 g of fresh weight (g/100 g FW)

2.4. Antioxidants Composition

2.4.1. Total Phenols and Flavonoids Content

Preparation of Extract

Frozen samples were warmed to room temperature and shortly homogenized prior to extraction. Sample (2.000 g) was extracted with 2 mL of methanol (75%) and 1 mL HCl (1 M). The mixture was vortexed, and then it was heated at 37 °C for 30 min while it was occasionally agitated. Then, 1 mL of NaOH in 75% methanol was added, and the mixture was vortexed and centrifuged (6000 rpm) for 5 min. The supernatant was collected, and the precipitate was again extracted with 2 mL of acetone (50%). The mixture was centrifuged (6000 rpm) for 5 min. The supernatants from the two centrifugations were combined in a 10 mL volumetric flask and filled to 10 mL with 75% methanol.

Total Phenols Analysis

Total phenols content was measured using the Folin-Ciocalteu method [24]. A mixture of 500 µL extract, 500 µL Folin-Ciocalteu reagent, and 4 mL distilled water was vortexed. After 3 min, 1 mL Na_2CO_3 (20%) was added, and the resulting solution was incubated at room temperature for 2 h under darkness. The absorbance of solution was measured at 725 nm. Blank solution was prepared by replacing tomato extract with methanol (75%). Gallic acid was used as the standard for the calibration curve. The content of total phenols was expressed as mg GAE (gallic acid equivalent) per 100 g of fresh weight (mg GAE/100 g FW).

Flavonoid content was determined according to Silva et al. [25], with some modifications. A mixture of 500 µL extract, 1.5 mL methanol (75%), 100 µL $AlCl_3$ (10%), 100 µL potassium acetate, and 2.8 mL distilled water was incubated for 30 min at room temperature. The absorbance at 415 nm was measured. Blank solution was obtained by replacing tomato extract with methanol (75%). Rutin was used as standard. The flavonoid content was expressed as mg RE (rutin equivalent) per 100 g of fresh weight (mg RE/100 g FW).

2.4.2. Lycopene Analysis

Lycopene was measured according to Rao et al. [26], with some modifications. The frozen samples of tomatoes were allowed to warm at room temperature and homogenized for 30 sec before extraction. The extraction was made in the absence of light. Extraction solvent consisted of a mixture of hexane/methanol/acetone (2/1/1 v/v). Then, 20 mL of extraction solvent and 0.25 g BHT were added to 1.000 g of sample, and the mixture was

vortexed for 10 min. Distilled water (10 mL) was added, and vortex continued for another 2 min. The non-polar phase was separated, and its absorption was measured at 502 nm. Lycopene concentration was calculated using specific extinction coefficient (E 1% 1 cm) 3150 at 502 nm. Lycopene content was expressed as mg carotene per 100 g FW.

2.4.3. Ascorbic acid Analysis

Ascorbic acid determination was performed immediately after sample preparation using DCPIP method [27]. Tomato samples (500 mg) were extracted with 50 mL of 1% metaphosphoric acid for 1 h. The mixture was centrifuged at 6000 rpm at room temperature for 5 min. One mL of the supernatant was added to 9 mL of 0.05 mM DCPIP, and the mixture was vortexed for 15 sec, and then it was measured at 515 nm using water as the blank. The results were expressed as mg ascorbic acid/100 g FW.

2.5. Antioxidant Activity

Antioxidant activity of tomatoes was determined in hydrophilic and lipophilic fractions prepared as follows.

2.5.1. Preparation of Extract

Before extractions the frozen samples were allowed to warm at room temperature and homogenized for 30 s. For preparation of hydrophilic extract, 10 mL distilled water were added to 2.000 g of sample. The mixture was stirred for 2 min, and then it was centrifuged (6000 rpm) for 5 min. The collected supernatant represented the hydrophilic extract.

Lipophilic extraction was obtained as follows [28]: 2.5 mL of methanol were added to 2.000 g of sample, and the mixture was vortexed for 1 min. Subsequently, 2.5 mL Tris pH 7.5 were added, and the resulting mixture was shortly vortexed, and then it was left for 5 min under darkness. Then, 2 mL of chloroform were added. The mixture was vortexed for 2 min and centrifuged (6000 rpm) for 5 min. The phase of chloroform was collected, evaporated under vacuum at room temperature, and the precipitate was reconstituted into 5 mL of 2-propanol. The extraction was performed in the dark. The hydrophilic and lipophilic extracts were used for antioxidant assays.

The antioxidant activity of both hydrophilic and lipophilic fraction of tomatoes was estimated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reduction activity potential) assays, respectively.

2.5.2. DPPH Assay

DPPH radical scavenging capacities of tomato extracts were determined using a known procedure with some modifications [24]. A DPPH working solution (0.1 mM) in methanol was prepared weekly by diluting a methanolic stock solution (0.6 mM). The absorbance of working solution at 517 nm was checked daily. In case of lipophilic extracts, 2-propanol was used for preparation of DPPH stock and working solutions. The DPPH assay was performed by mixing 3.8 mL working solution and 200 μL of hydrophilic or lipophilic extract. After incubation for 30 min at room temperature under darkness, the absorption of solution at 517 nm was measured. A control solution was prepared from 3.8 mL of DPPH working solution and 200 μL of distilled water (or 2-propanol in case of lipophilic extracts), and its absorption (Ac) at 517 nm was measured. Trolox was used as the standard. The percentage scavenging of DPPH radicals was calculated according to the equation

$$\% \text{ DPPH scavenging} = (1 - A_s/A_c) \times 100$$

where A_s = absorption of sample, A_c = absorption of control

The antioxidant activity of the samples was expressed as mg TE (Trolox equivalent) per 100 g of fresh weight (mg TE/100 g FW).

2.5.3. FRAP Assay

The reducing capacity of the samples was determined using the FRAP (ferric reducing antioxidant power) assay according to the Capanoglu et al. [24] procedure, with slight modifications. FRAP reagent was freshly prepared from 1 mL of $FeCl_3$ (0.02 M), 1 mL of TPTZ (0.01 M) in 0.04 M HCl, and 10 mL of acetate buffer (0.3 M, pH 3.6). FRAP reagent (2.9 mL) was mixed with 100 µL of extract, and the solution was incubated for 10 min at 37 °C. Then, it was cooled rapidly, and the absorbance at 593 nm was measured. A standard curve was prepared using Trolox as standard. The results were expressed as mg TE (Trolox equivalent) per 100 g of fresh weight (mg TE/100 g FW).

2.6. Statistical Analyses

Quantitative data are presented as mean values ± standard deviations of three independent measurements. The results were statistically analyzed using SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA) for the analysis of variance [29]. Means were compared with Tukey's test ($p < 0.05$). Correlations were tested by using the Pearson procedure, in which the *p*-value was considered to be significant when lower than 0.05.

3. Results and Discussion

3.1. Nutrition Value

The macronutrient composition of the six tomato genotypes is summarized in Table 2. The moisture content of tomatoes was in the range of 93.8–95.5%, exhibiting non-significant variation regardless of the variety or ripening stage. These results can probably be ascribed to unimpeded nutrients availability in the hydroponic culture. On the other hand, fat content significantly varies among the varieties. Tomatoes are separated in two groups with respect to fat content. Small-fruit varieties, Chiou, Authentic, and Traditional of Santorini, showed the highest fat content. In addition, at the breaker stage, small-fruit varieties exhibited significant higher fat content than in red ripe fruit. On contrary, large-fruit varieties, Eplida F1, Short and Long of Kythira, had lower fat content, which was not significantly different between maturity stages. Eplida F1 hybrid exhibited significant higher fat content than traditional cultivated large-fruit varieties Short of Kythira and Long of Kythira. Although, the fat content of the studied varieties was lower than that determined for Spanish tomato [23], which was comparable to that reported for Portuguese varieties [30].

Total carbohydrates were the most abundant macronutrients of the tomatoes. Their content ranged from 1.01 to 3.04 g/100 g FW. These values were similar to Spanish tomatoes determined by the anthrone method [23]. However, it has been reported [30] that there was significantly higher carbohydrates content for Portuguese tomatoes of various fruit size. The varieties examined for the present study did not exhibit significant differences in carbohydrate content according to the type or the size of fruit. For example, the Long of Kythira (large fruit) and Authentic of Santorini (small fruit) showed a slightly higher carbohydrate content. Whereas, only two varieties, Long of Kythira and Traditional of Santorini, presented significant difference of carbohydrate content between red ripe and breaker stage fruit.

Proteins were determined by Coomassie Brilliant Blue G-250 assay [31]. The cherry type Chiou variety showed the highest protein content, both in red ripe and breaker ripening stages. The other varieties did not reveal variation of protein content based on the type or the size of fruit. Proximate composition of Spanish [23], Portuguese [30], and Korean tomatoes [32] revealed higher protein contents, although, in these studies, the protein contents were estimated by Kjeldahl method. With the exception of Long of Kythira and Authentic of Santorini, protein was significantly ($p < 0.05$) higher at red ripe than breaker stage.

Table 2. Macronutrients and energetic value of six tomato varieties at red ripe and breaker ripening stage.

Variety	Fat (mg/100 g FW)		Total Carbohydrates (g/100 g FW)		Protein (mg/100 g FW)		Energy (Kcal/100 g FW)	
	Red Ripe	Breaker	Red Ripe	Breaker	Red Ripe	Breaker	Red Ripe	Breaker
Elpida F1	47.85 ± 3.90 ^{a}	45.66 ± 5.45 ^{a}	1.80 ± 0.09 ^{ab}	1.84 ± 0.08 ^{a}	255.32 ± 43.68 ^{a*}	120.14 ± 12.02 ^{a*}	7.91 ± 0.44 ^{ab}	7.57 ± 0.26 ^{a}
Short of Kythira	29.15 ± 5.45 ^{a}	23.65 ± 2.33 ^{a}	1.15 ± 0.06 ^{cd}	1.52 ± 0.08 ^{a}	275.99 ± 44.14 ^{a*}	77.76 ± 2.44 ^{b*}	5.68 ± 0.32 ^{c}	6.22 ± 0.31 ^{b}
Long of Kythira	24.20 ± 3.11 ^{a}	38.50 ± 9.34 ^{a}	2.15 ± 0.43 ^{a*}	3.04 ± 0.33 ^{b*}	277.41 ± 59.14 ^{a}	234.90 ± 21.89 ^{c}	9.39 ± 1.47 ^{de*}	12.69 ± 1.23 ^{c*}
Chiou	132.56 ± 10.11 ^{b*}	167.22 ± 7.78 ^{b*}	1.01 ± 0.03 ^{d}	1.03 ± 0.03 ^{c}	596.19 ± 4.01 ^{b*}	394.96 ± 30.53 ^{d*}	10.38 ± 0.42d*	6.95 ± 0.25 ^{ab*}
Authentic of Santorini	99.01 ± 7.80 ^{c*}	176.02 ± 7.78 ^{b*}	2.25 ± 0.40 ^{a}	2.56 ± 0.05 ^{b}	229.24 ± 28.36 ^{a}	199.48 ± 52.09 ^{ac}	10.26 ± 1.52d	11.59 ± 0.35 ^{cd}
Kaisia or Traditional of Santorini	103.96 ± 8.66 ^{c*}	155.12 ± 4.67 ^{b*}	1.56 ± 0.02 ^{bc*}	2.37 ± 0.14 ^{b*}	256.16 ± 12.02 ^{a*}	113.36 ± 9.84 ^{ab*}	7.32 ± 0.62be*	10.62 ± 0.50 ^{d*}

Values in a column followed by the same letter are not significant different ($p < 0.05$) by the Tukey test; an asterisk indicates significant difference of a nutrient content between red ripe and breaker ripening stage of the same variety ($p < 0.05$) by the Tukey test.

The energetic values of the tomatoes were estimated by multiplying the protein, carbohydrate, and fat content by 4.00, 3.75, and 9.00 respectively (AOAC, 1984). The varieties examined in the present study revealed low energy values of 12.69–5.68 Kcal/100 g FW, which verifies that tomatoes are suitable for low caloric diets. The lowest energy was calculated for Sort of Kythira, which exhibited energy as low as 5.68 Kcal/100 g FW in red ripe maturity stage. The calculated energy values were comparable to that reported by Guil-Guerrero and Rebolloso-Fuentes [23], but they were much lower than energy values reported for Portuguese varieties [30].

3.2. Antioxidants Content

The most important bioactive compounds of tomatoes (i.e., ascorbic acid, lycopene, total phenols, and flavonoids) were determined. Ascorbic acid, lycopene, total phenols, and flavonoid content are presented in Table 3.

Table 3. Antioxidants content of six tomato varieties at red ripe and breaker ripening stage.

Variety	Ascorbic acid (mg/100 g FW)		Lycopene (mg/100 g FW)		Total Phenols (mg GAE/100 g FW)		Flavonoids (mg RE/100 g FW)	
	Red Ripe	Breaker	Red Ripe	Breaker	Red Ripe	Breaker	Red Ripe	Breaker
Elpida F1	32.11 ± 0.75 ^{a}	28.49 ± 2.40 ^{a}	2.24 ± 0.22 ^{a*}	1.20 ± 0.05 ^{a*}	34.41 ± 6.42 ^{ab*}	19.08 ± 0.19 ^{a*}	6.90 ± 0.76 ^{a*}	1.25 ± 0.05 ^{a*}
Short of Kythira	29.74 ± 1.47 ^{a*}	36.90 ± 0.38 ^{b*}	6.04 ± 0.35 ^{b*}	1.09 ± 0.09 ^{ab*}	19.01 ± 3.07 ^{c}	14.19 ± 1.09 ^{b}	5.80 ± 0.63 ^{ab*}	2.99 ± 0.59 ^{bc*}
Long of Kythira	36.04 ± 0.56 ^{b*}	39.03 ± 0.20 ^{b*}	5.02 ± 0.08 ^{c*}	0.79 ± 0.02 ^{c*}	17.50 ± 1.08 ^{c*}	13.68 ± 0.19 ^{b*}	4.29 ± 0.50 ^{b*}	1.79 ± 0.12 ^{ab*}
Chiou	41.66 ± 3.35 ^{bc}	40.65 ± 2.11 ^{b}	3.41 ± 0.13 ^{d*}	0.50 ± 0.02 ^{d*}	45.26 ± 7.31 ^{ad}	39.56 ± 4.54 ^{c}	6.96 ± 0.76 ^{a*}	4.11 ± 0.25 ^{cd*}
Authentic of Santorini	54.41 ± 1.04 ^{d*}	61.63 ± 0.61 ^{c*}	4.34 ± 0.02 ^{e*}	1.02 ± 0.03 ^{b*}	27.89 ± 1.97 ^{b*}	21.05 ± 2.31 ^{a*}	5.06 ± 0.21 ^{b}	4.46 ± 0.25 ^{d}
Kaisia or Traditional of Santorini	43.82 ± 4.24 ^{c}	53.06 ± 2.16 ^{d}	7.98 ± 0.03 ^{f*}	3.69 ± 0.09 ^{e*}	47.89 ± 5.74 ^{d*}	23.42 ± 1.49 ^{a*}	6.25 ± 0.54 ^{a}	6.67 ± 1.97 ^{d}

Values in a column followed by the same letter are not significant different ($p < 0.05$) by the Tukey test; an sterisk indicates significant difference of an antioxidant content between red ripe and breaker stage of the same variety ($p < 0.05$) by the Tukey test.

3.2.1. Ascorbic acid (AsA)

AsA is the most important hydrophilic antioxidant of tomatoes. Tomatoes, however, contain moderate amounts of ascorbic acid compared to other vegetables. AsA content ranged from 29.74 to 54.41 mg/100 g FW in red ripe fruit and 28.49 to 61.63 mg/100 g FW in breaker tomatoes. These values are consistent with data reported in the literature [1]. Guil-Guerrero and Rebolloso-Fuentes [23] determined, photometrically, a much higher AsA level for tomatoes grown in Spain. Comparable or slightly higher AsA levels were reported for four typical Portuguese cultivars [33]. Ilahy et al. [34] reported lower AsA content for tomatoes cultivated in Southern Italy. In addition, lower AsA accumulation was determined for tomato cultivars grown in Northern India [35]. AsA levels observed in the varieties of the present study are among the highest values reported in the international literature. The consumption of tomato that contains moderate amounts of ascorbic acid (ca. 20 mg/100 g) contributes to 40% of the recommended dietary allowance (RDA) for AsA [36]. Thus, the consumption of these varieties contributes to sufficient intake the ascorbic acid RDA.

AsA content was investigated at red ripe and breaker ripening stages. The results showed variability during ripening which was dependent on the genotype. Three cultivars (Sort of Kythira, Long of Kythira, and Authentic of Santorini) presented significant higher AsA in breaker than red ripe stage, although, it is considered that ripening increases AsA levels [37]. Our findings are in accordance with previous studies, which showed that, for certain cultivars, AsA content was higher in yellow-orange tomatoes and reduced in mature fruit [34,38]. For the other three varieties, we did not detect significant difference of AsA content between red ripe and breaker maturing stage.

The levels of the AsA were influenced by fruit size. Small fruit varieties (Chiou, Authentic of Santorini, and Traditional of Santorini) showed higher AsA levels than common size ones in both ripening stages. Analogous results were reported for tomato cultivars grown in Romania [39] and in Korea [40]. Both groups observed that small size and cherry varieties accumulated higher AsA levels than common size tomatoes in mature fruit. AsA is mainly accumulated at higher concentrations in the jelly parenchyma and exocarp than in the mesocarp and endocarp, thus small fruit cultivars tend to have higher AsA contents than large fruit varieties.

3.2.2. Lycopene

Lycopene is the major lipophilic antioxidant of tomato and the most abundant carotenoid of tomato fruit. Lycopene content of samples was measured spectroscopically, which produces quite good results with less than 10% difference than the HPLC method [26]. Lycopene content was variety dependent and varied from 2.24 to 7.98 mg/100 g FW for red ripe fruit. The observed lycopene levels are comparable to lycopene levels reported for traditionally cultivated tomatoes in Portugal [30], Spain [29], and Tunisia [41], which were also determined spectroscopically. However, lycopene content of Spanish [23], Romanian [39], and Italian varieties [42] were slightly higher. Lower lycopene content has been reported for cherry tomatoes cultivated in Korea [32]. Quantitative differences are expected, as the levels of lycopene (and carotenoids in general) are dependent on multiple factors (genetic, climatic, and agronomic). The small size fruit cultivar Traditional of Santorini showed the higher lycopene content among all varieties. Figàs, Prohens, Raigón, Fita, García-Martínez, Casanova, Borràs, Plazas, Andújar, and Soler [29] detected higher lycopene levels for small size cherry tomatoes among many local Spanish varieties of various fruit size. Although the small size fruit cultivar Traditional of Santorini showed the higher lycopene content, we did not detect an unequivocal relationship between lycopene values and tomato size. It is worth noticing that four traditional varieties showed higher lycopene than Elpida F1 hybrid. Bhandari et al. [43] reported higher lycopene content for germplasm than commercial cultivars. As expected, red ripe fruit of all varieties showed significantly higher lycopene content than breaker ones (0.50–3.69 mg/100 g FW). It is well known that the

lycopene level gradually increasing during ripening is a characteristic feature of tomato fruit developmental possess [44].

3.2.3. Total Phenolic and Flavonoids Compounds

Total phenols content (TPC) and total flavonoids contents (TFC) of six genotypes are presented in Table 3. The observed TPC at red ripe mature stage ranged from 17.5 to 47.89 mg GAE/100 g FW. Significant genotypic differences were observed for phenols. The large size varieties Short of Kithira and Long of Kythira presented significantly lower TPC (19.01 and 17.50 mg/100 g FW, respectively) in red ripe stage than the other four varieties. On other hand, cherry type cultivars Chiou and Tranditional of Santorini exhibited the higher TPC. This could be attributed to higher surface per volume of small size cherry tomatoes [45]. In accordance, it has been reported that small size tomatoes had the higher TPC among four native and four hybrid tomato genotypes [46]. However, TPC (in red ripe and breaker stage) and fruit size did not exhibit significant correlation ($p > 0.10$), which indicates that genetic control is the primary factor in determining the amount of phenolics. The studied varieties showed similar or relative higher TPC than tomatoes cultivated in Tunisia [34], Spain [29,47,48], or Romania [39]. Higher TPCs have been reported by Kaur et al. [35] and Ilahy et al. [42].

In contrast to the TPC, there was not a clear differentiation of varieties based on TFC. The TFC were in the range of 4.29–6.90 mg RE/100 g FW for red ripe fruit and 1.25–6.67 mg RE/100 g FW for breaker tomatoes. Except Authentic of Santorini and Traditional of Santorini, the other varieties showed significantly higher levels of TFC from breaker to red ripe stage. This is reasonable, since flavonoids accumulate mainly in the exocarp and play an important role in the tomato color development along with carotenoids [49]. Although environmental conditions [50] may be affecting TFC, genetic control is the primary factor in flavonoid biosynthesis. TPC of studied genotypes were comparable with that reported by Vela-Hinojosa et al. [46] and Kavitha et al. [51] for traditional genotypes, as well as commercial and hybrid lines, but were lower than the flavonoid levels reported by Ilahy et al. [42].

3.3. Antioxidant Activity

Hydrophilic antioxidant activity (HAA) and lipophilic antioxidant activity (LAA) of six genotypes were measured by DPPH and FRAP assays, and are shown in Tables 4 and 5, respectively.

Table 4. Hydrophilic antioxidant activity (HAA) of tomatoes at red ripe and breaker ripening stage.

Variety	DPPH (mg TE/100 g FW)		FRAP (mg TE/100 g FW)	
	Red Ripe	**Breaker**	**Red Ripe**	**Breaker**
Elpida F1	27.54 ± 1.59 ^{a}	25.11 ± 2.07 ^{a}	25.41 ± 2.51 ^{a}	23.12 ± 0.53 ^{a}
Short of Kythira	24.92 ± 2.09 ^{a}	21.43 ± 1.05 ^{b}	27.86 ± 0.85 ^{a*}	15.03 ± 1.36 ^{b*}
Long of Kythira	19.14 ± 0.9 ^{b}	15.56 ± 3.10 ^{cb}	26.33 ± 0.88 ^{a}	23.63 ± 0.56 ^{a}
Chiou	33.36 ± 0.71 ^{c}	32.74 ± 0.16 ^{d}	46.38 ± 1.48 ^{b*}	42.77 ± 0.3 ^{c*}
Authentic of Santorini	19.48 ± 1.44 ^{b}	17.86 ± 2.58 ^{cb}	39.64 ± 1.59 ^{c*}	26.54 ± 0.65 ^{a*}
Kaisia or Traditional of Santorini	48.34 ± 1.35 ^{d}	45.65 ± 11.41 ^{e}	56.09 ± 4.94 ^{d}	47.81 ± 10.91 ^{c}

Values in a column followed by the same letter are not significant different ($p < 0.05$) by the Tukey test; Asterisk indicates significant difference of HAA between red ripe and breaker of the same variety ($p < 0.05$) by the Tukey test.

Table 5. Lipophilic antioxidant activity (LAA) of tomatoes at red ripe and breaker ripening stage.

Variety	DPPH (mg TE/100 g FW)		FRAP (mg TE/100 g FW)	
	Red Ripe	Breaker	Red Ripe	Breaker
Elpida F1	1.55 ± 0.04 ^{a*}	0.76 ± 0.18 ^{ab*}	2.45 ± 0.3 ^{a}	1.82 ± 0.68 ^{abc}
Short of Kythira	2.32 ± 0.28 ^{bc*}	0.69 ± 0.05 ^{a*}	5.11 ± 0.31 ^{b*}	0.94 ± 0.05 ^{a*}
Long of Kythira	2.16 ± 0.18 ^{bc*}	0.33 ± 0.02 ^{c*}	4.81 ± 0.03 ^{b*}	0.89 ± 0.03 ^{a*}
Chiou	1.80 ± 0.12 ^{ab*}	0.26 ± 0.08 ^{c*}	3.76 ± 0.21 ^{c*}	1.61 ± 0.18 ^{b*}
Authentic of Santorini	2.25 ± 0.15 ^{c*}	0.89 ± 0.08 ^{ab*}	4.60 ± 0.21 ^{b*}	1.74 ± 0.20 ^{b*}
Kaisia or Traditional of Santorini	3.25 ± 0.28 ^{d*}	1.71 ± 0.16 ^{c*}	5.21 ± 0.67 ^{b*}	2.82 ± 0.77 ^{c*}

Values in a column followed by the same letter are not significant different ($p < 0.05$) by the Tukey test; Asterisk indicates significant difference of HAA between red ripe and breaker of the same variety ($p < 0.05$) by the Tukey test.

3.3.1. Hydrophilic Antioxidant Activity (HAA)

The HAA, estimated by the DPPH assay, was 19.14–48.34 mg TE/100 g FW for red ripe and 15.56–45.65 mg TE/100 g FW for breaker fruit. Cherry varieties Traditional of Santorini and Chiou showed significantly higher HAA than the other four cultivars at both red ripe and breaker ripening stages. This was attributed to the higher TPC and AsA contents of these varieties. Comparable HAA values have been reported for ten tomato F1 hybrids cultivated in Romania [39], while the higher antioxidant activity was provided by cherry type cultivars. In addition, Bhandari and Lee [52] reported higher HAA for cherry than regular type tomatoes.

The HAA, determined by DPPH method, did not change significantly during the ripening process. Non-significant differences of HAA were detected for red ripe and breaker fruit of each variety. Analogue feature has been reported [53] for greenhouse grown tomatoes. Ilahy et al. [42] reported higher HAA for tomatoes at green or orange-green stage. However, Bhandari et al. [43] reported that the antioxidant activities of seven commercial cultivars increased from the breaker to the red ripe stage.

FRAP assay confirmed that cherry type cultivars Traditional of Santorini and Chiou had significantly higher HAA than large fruit varieties. Chiou, Traditional of Santorini, and Authentic of Santorini (small cocktail type fruit) showed at least 30% higher HAA than large size fruit varieties in red ripe stage. In addition, FRAP assay provided significantly higher HAA for red ripe than breaker tomatoes for three varieties. DPPH and FRAP values were not significantly correlated ($p > 0.05$). An analogous feature has been reported early by Ilahy et al. [42] for high-lycopene varieties, as well as by Choi et al. [32] for cherry tomato of various colors. The divergent HAA values obtained from DPPH and FRAP methods could be attributed to a diverse sensibility of the two assays for the hydrophilic antioxidant compounds of tomato. It has been reported that, among ascorbic acid, phenolics, and flavonoids, the most important factor contributing to the FRAP value was the phenolic compounds based on multivariate regression analysis of hydrophilic extracts of different vegetables [54]. In addition, cellular antioxidant components, such as glutathione, interacts in different ways with the assays [55].

3.3.2. Lipophilic Antioxidant Activity (LAA)

LAA was determined by DPPH and FRAP assays for organic extracts of tomatoes. LAA values measured by DPPH ranged from 1.16 to 3.25 mg TE/100 g FW at red ripe stage. FRAP assay produces higher LAA values in the range 1.76–5.21 mg TE/100 g FW. LAA was higher in small fruit cultivars (Chiou, Authentic of Santorini and Traditional of Santorini). In contrast to HAA, the LAA increased significantly during the ripening process. DPPH and FRAP values were about two to four-fold higher in red ripe than

breaker fruit. The increase was attributed to the stimulation of lycopene content, which is the most important lipophilic antioxidant of tomato. Significant correlation between mean DPPH values (r = 0.96, p = 0.002) or FRAP values (r = 0.94, p = 0.006) and lycopene content of red ripe tomatoes was observed. It is well known that the lycopene and antioxidant activity are significantly correlated [56]. However, FRAP assay did not reveal these findings. Although FRAP assay is considered suitable for water soluble antioxidant, we detected an excellent correlation of FRAP values (r = 0.94, p = 0.006) and lycopene. This observation has been reported by other researchers [42]. In addition, it has been reported that, among carotenes, only lycopene is an effective ferric reducing compound [57].

4. Conclusions

Nutrient composition, bioactive compounds, and antioxidant activity were determined for five germplasm and one commercial tomato varieties transitionally cultivated in Greece. Wide genotypic variations were observed in fat, protein, lycopene, ascorbic acid, and total phenolic contents, as well as antioxidant activities. Cherry varieties, Traditional of Santorini, and Chiou showed distinct composition profiles from the remaining cultivars with enhanced ascorbic acid, lycopene, and total phenolic contents and, hence, antioxidant activity. However, all local varieties demonstrated comparable or superior traits than the ones of commercial hybrid. They are rich in antioxidant metabolites, such as ascorbic acid, lycopene, and phenolic compounds. They can be considered as a good source of specific nutrients and antioxidant compounds. The current findings reveal that the consumers should opt for the local tomato varieties not only because they are testier, but also for their nutritional value. Farmers depend upon yearly supply of expensive F1 hybrid seeds, and the nutritional evaluation of germplasm varieties and their superiority provide an alternative source of genetic material. Additionally, in the present report, the plants were grown hydroponically, suggesting that Greek traditional cultured varieties possess antagonistic qualitative traits to commercial F1 hybrids in intensive farming conditions.

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