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Functional, Flavor and Visual Traits of Hydroponically Produced Tomato Fruit in Relation to Substrate, Plant Training System and Harvesting Time

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Abstract: Currently, a great portion of tomatoes is produced by soilless cultivation systems and the substrate selection among the various materials is one of the most important factors affecting yield and quality traits. On the other hand, grafting has been successfully used in soilless systems to ensure long-term cultivation. However, due to the high cost of grafted seedlings, plant training systems are sought. Given the fact that most literature refers to studies intended to mainly reveal production differences among treatments and the quality aspect was secondary, the present study was focused on the evaluation of tomato fruit functionality, flavor and visual traits. Tomato plants cv 'Beef Bang F1' were cultivated in a glasshouse hydroponic culture in four substrates: rockwool slabs, perlite in sacks, pumice in sacks and pumice in 9 L pots. The type of cultivated plants used were self-rooted or grafted onto 'Defensor' trained in single and double stems. Tomato fruit were harvested three times during the season (6 June, 31 July, 6 November). The fruit quality was measured based on visual (average fruit mass, and Minolta color values), flavor (dry mass, soluble solids content, titratable acidity, pH, flesh firmness) as well as functional traits (total phenolic content, ascorbic acid, lycopene, β-carotene, total carotenoid content and antioxidant capacity). Harvest time was the most important factor followed in many of these cases by the substrate (flavor and functional traits), as well as in certain cases by the plant grafting/training (flavor traits and antioxidants) or by both in some flavor traits and antioxidants. Correlation of color values with lycopene, though significant, was weak. Each individual harvest time revealed the rise in different parameters. Pumice, whether used in pot or in sack, enhanced the visual and flavor attributes the most, self-rooted plants and mid-summer harvest resulted in the highest tomato fruit quality.

Keywords: vegetables; fruit; quality; antioxidants

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1. Introduction

Tomato fruit is considered the most popular vegetable among consumers worldwide because of its availability throughout the year and its richness in beneficial for human health phytochemicals such as carotenoids (lycopene and β-carotene), vitamin C and phenolics, all possessing antioxidant capacity [1–6]. Tomato fruit antioxidants have been reported to contribute to protection of free radicals which are considered as risk factors in the development of cancer and cardiovascular diseases [7]; based on fresh tomato fruit antioxidant potential, they have been proposed to be termed a "functional food" [8,9] and therefore the nutritional quality traits can be referred to as functional.

Tomato fruit phytochemical content is affected by different biotic and abiotic factors, such as cultivar, production system, plant grafting and training techniques, harvest ma-

turity and the environmental conditions before and after harvesting [10–13]. Currently, a great portion of tomatoes is produced by soilless cultivation systems which ensures high yields and quality production. The soilless production system is known to provide a soilborne disease-free environment as well as proper nutrition-irrigation according to the needs of the plants. In soilless production systems the substrate type is considered among the most important factors affecting yield and quality of greenhouse grown vegetables [14]. Soilless substrates [15] include several inert inorganic or organic materials [16] of which the major proportion is rockwool, due to its stable structure, high water holding capacity and moderate porosity [17,18] and peat for its desirable physicochemical and biological properties for plant growth [19,20]. Lately, coconut coir, an environmentally friendly material with stable physicochemical and biological properties, has also been increasingly used as a cultivation substrate in horticultural production [21]. Further, the utilization of perlite and pumice have been reported for the hydroponic cultivation of tomatoes [22–24], as well as vermiculite and peat and their mixtures [25]. Additionally, comparable substrates consisting of mixtures of pumice and peat (85%:15%, *v*/*v*), perlite and peat (85%:15%, *v*/*v*), sepiolite and perlite (80%:20%, *v*/*v*), sepiolite and leonardite (97%:3%, *v*/*v*), peat and composted bark (66.6%:33.4%, *v*/*v*) or rock wool [26–28], as well as mixtures of fresh white spruce and fir sawdust (40%:60%, *v*/*v*), or white spruce and fir shavings (40%:60%, *v*/*v*) have also been reported [28]. Sedaghat et al. [29] reported superior quality of tomato fruit containing 7.31% dry matter content and 4.74 brix was produced from plants grown in a media of a mixture 50% coco-peat + 50% perlite. In general, the substrate selection among the various materials is one of the most important factors affecting plant growth and development in the greenhouse and influencing vegetable quality [14].

Vegetable grafting has been successfully used in soil and soilless systems to control soilborne diseases and root-knot nematodes [30,31], adverse effects of salinity [32], nutrient and heavy metals [33] as well as temperature [34]. Djidonou et al. [13] reported that grafting with interspecific hybrid rootstocks could be an effective horticultural technique for enhancing fruit yield of tomato plants without affecting fruit quality components, though they observed some reduction in ascorbic acid content. Khah et al. [35] reported no significant differences in titratable acidity or soluble solids content in grafted and control plants. On the other hand, increased levels of lycopene, β-carotene, vitamin C and antioxidant activity in tomato fruit have been reported by grafting [10,36]. These findings that sometimes appear contradictory are largely due to the complexity of the biochemical processes that determine the synthesis of these various compounds that define the tomato fruit flavor and functional quality.

Tomato fruit harvested at the red stage of maturity may differ in flavor quality attributes such as total soluble solid content (TSS) and titratable acidity. The ratio of these two attributes largely determines the eating quality/taste and defines the level of fruit flavor [37,38]. TSS is measured by refractometer and includes total carbohydrates (glucose, fructose), non-volatile organic acids (citric and malic) and minor components such as phenols, amino acids, ascorbic acid and inorganic salts [39]. Tomato fruit maturation, among other factors, is a physiological process leading to ripening; it induces changes in fruit size and mass, coloration, texture and sensory and aroma development as well as other compositional changes [40,41]. Thus, lycopene (and carotenoids) which is responsible for the red coloration of the fruit is the basis as well as the main indicator of the maturation of tomato fruit. On the other hand, vitamin C (ascorbic and dehydroascorbic acid) and phenolic compounds, although they may increase, they show little overall change during tomato ripening [42,43]. Helyes and Lugasi [43] found that lycopene and total antioxidant capacity increased during maturation, while polyphenol content remained almost the same.

The effect of genetic and environmental factors on the quality characteristics of tomato fruits has been studied in detail [3,6,8,16,38,41]. Given the fact that most literature refers to experimental designs mainly intended to reveal production differences among treatments and the quality aspect is secondary, the present study was focused on the evaluation of tomato fruit quality *per se*. Thus, the aim of this study was to investigate the quality of

tomato fruit produced following the implementation of a soilless system under protection using inorganic substrates (rockwool, perlite and pumice), as well as different plant types regarding grafting (self-rooted and grafted) and to training (single or double stem) during 10-month (February–November) cultivation. To investigate the quality of tomato fruit, changes in fruit visual quality (average Minolta color values and fruit mass), flavor quality (dry mass, soluble solids content, titratable acidity, pH and flesh firmness) as well as functional quality traits (total phenolic content, ascorbic acid, lycopene, β-carotene, total carotenoid content and ta antioxidant capacity) were determined.

2. Materials and Methods

2.1. Experimental Design and Fruit Sampling

Experimentation was performed at Agris S.A, located at Kleidi Imathia, Greece, during the 2018 season (1 February–30 November). Three plant types of the indeterminate tomato cultivar 'Beef Bang F1' were compared including (1) self-rooted 'Beef Bang F1', (2) 'Beef Bang F1' grafted onto 'Defensor' trained in single stem and (3) 'Beef Bang F1' grafted onto 'Defensor' trained in double stem. All plants were cultivated in a glasshouse of total area 1000 m^2 in four substrates: rockwool slabs, perlite in sacks, pumice in sacks and pumice in 9 L pots. Tomato cultivar 'Beef Bang F1' grafted onto 'Defensor' proved to be the best in comparative performance of various cultivars grafted in 7 different rootstocks, grown in an open hydroponic system with rockwool substrate in the same environmental conditions during the previous two years (unpublished data).

Fruits at the red ripe stage of ripeness were harvested at three harvest times: at 6 June, 31 July and 6 November and brought to the Lab of Vegetable Crops of the Aristotle University of Thessaloniki. The fruit were then graded and marketable fruit, after weighing, were kept separately (per treatment per replication) and were used to evaluate the fruit quality attributes. More specifically, at each harvest, 12 of these fruits per treatment (three replicates per treatment, four fruit per replication) and each fruit was measured separately for weight, color and firmness.

2.2. Measurement of Fruit Skin Color

Skin color was measured at two diametrically opposite spots at the equator of the fruit using a colorimeter (Minolta CR-400, Minolta, Osaka, Japan), equipped with an 8-mm measuring head and a C illuminant (6774 K). The colorimeter was calibrated using the manufacturer's standard white plate. Color changes were quantified in the L*, a* and b* color space. Hue angle ($h^\circ = 180 + \tan^{-1}(b^*/a^*)$ and chroma values ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated from a* and b* values. L* refers to lightness, ranging from 0 = black to 100 = white; hue angle (h°) value is defined as a color wheel, with red-purple color at an angle of 0°, yellow color at 90°, bluish-green color at 180° and blue color at 270°, and chroma (C*) represents color saturation, which varies from dull (low values) to vivid (high values) [44].

2.3. Firmness

Fruit firmness was measured at two diametrically opposite spots at the equator of the fruit using a penetrometer Chatillon (John Chatillon and Sons, New Gardens, NY, USA) bearing a tip of 3.2 mm in diameter and 9.5 mm in length.

2.4. Sample Preparation

The fruits were stored at −20 °C until determination of the main qualitative and nutritional ingredients. The samples were then left to thaw and were sliced and homogenized in a Waring blender for 1 min. Freshly homogenized samples were used for measurements of ascorbic acid content, pH, titratable acidity (TA), total soluble solids (TSS) and dry matter (DM). Additional homogenized samples were placed in solvent and stored at −20 °C and extracts were later used to quantify the levels of carotenoids (lycopene and β-carotene), total soluble phenolics and total antioxidant capacity (TAC), as described below.

2.4.1. Determination of Ascorbic Acid Content

For the determination of ascorbic acid content, 30 g of homogenized tissue were homogenized again in a Waring blender with 50 mL of 1% oxalic acid for 15 s and were double filtered using filter paper. Ascorbic acid content was then determined for the extract using a handheld reflectometer RQflex 10 (Merck KGaA, Darmstadt, Germany) with ascorbic acid test strips.

2.4.2. Determination of Dry Matter, Ph, Titratable Acidity, and Total Soluble Solids

Dry matter (DM) was measured after drying 50 g of homogenized tissue at 70 °C for 72 h. pH was measured in 50 mL of filtered extract, obtained by mixing 10 g of homogenized tissue with 100 mL of deionized water in a blender. The solution was titrated with 0.01 N NaOH to pH 8.2 and the titratable acidity (TA) was expressed as % of citric acid. Total soluble solids (TSS) were measured in filtered juice of the homogenized tissue using an Atago PR-1 handheld refractometer (Atago Co. Ltd., Tokyo, Japan).

2.4.3. Determination of Carotenoid Compounds

Lycopene (LYC), β-carotene (β-CAR) and total carotenoid (TCC) content were determined using the methods of Lichtenhaler and Wellburn [45] and D'Souza et al. [46]. One gram of homogenized tissue was mixed with 10 mL of 100% acetone and was stored overnight at −20 °C. The samples were then centrifuged at 10,000 rpm for 10 min at 20 °C. The supernatant of each sample was filtered through Whatman No. 1 filters and 10 mL of 100% acetone was added to each precipitate and stirred in a vortex. The supernatant was then added to each previous sample and 100% acetone was added to 25 mL final volume. The absorbance of the samples was measured at 450, 470, 503, 645 and 662 nm using a spectrophotometer Thermospectronic Helios a (Thermo Fisher Scientific, Waltham, MA, USA).

For the individual determination of the pigments, the following equations were used:

$$\text{Lycopene } (\mu g/g) = (3.521 \times \text{Abs503} - 0.587 \times \text{Abs450}) \times V/W \quad (1)$$

$$\beta\text{-carotene } (\mu g/g) = (4.367 \times \text{Abs450} - 2.947 \times \text{Abs503}) \times V/W \quad (2)$$

$$\text{Total carotenoids } (\mu g/g) = (1000 \times \text{Abs470} \times V/W) - ((2.27 \times \text{Chl a}) - (81.4 \times \text{Chl b}))227 \quad (3)$$

where: Abs = absorbance, V = extract volume and W = weight of homogenized tissue.

2.4.4. Determination of Total Phenolics Content and Total Antioxidant Capacity

For the determination of total soluble phenolic compounds (TPC) and total antioxidant capacity (TAC), 5 g of homogenized tissue were mixed with 25 mL 80% methanol and filtered through a Whatman No. 1 filter.

The total soluble phenols were determined photometrically according to the method of Scalbert et al. [47], using a standard curve of gallic acid. Specifically, 2.5 mL 10% Folin–Ciocalteu and 2 mL sodium carbonate (75 g/L) were added in 0.5 mL quantities of the extract over a period of 30 s for 8 min. The samples were then placed in a water bath at 50 °C for 5 min. After they cooled at room temperature, the absorbance was measured at 760 nm using a spectrophotometer (Thermospectronic Helios a). The standard curve was made by measuring the absorbance of known concentrations of gallic acid solutions (8–80 mg/mL) and the data were expressed as mg of gallic acid equivalents (GAE) per g of fresh weight.

Total antioxidant capacity was determined according to the method of Brand-Williams et al. [48]. From the above extract, 200 µL were used, in which 2800 µL of a solution of 100 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 95% methanol were added in a test tube. The samples were vortexed and kept for 1 h in the dark. The absorbance was then measured at 517 nm using a Thermospectronic Helios a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The standard curve was made by measuring the absorbance of

known concentrations of ascorbic acid solutions (0–0.1 mg/mL). The neutralizing capacity of the free radicals was expressed as mg of ascorbic acid equivalents (AEAC) per 100 g of fresh weight.

2.5. Statistical Analyses

For this experiment a complete randomized factorial design (3 × 4 × 3) was used, with three replicates per treatment. The factors were harvest time, substrate and plant type. Data were subjected to analysis of variance (ANOVA) using the statistical program SPSS v.25 and Microsoft Excel. The means were separated with Duncan's new multiple range test ($p < 0.05$). The effect size of each factor was evaluated using η^2 (eta squared) calculated as follows: η^2 = SS factor/SS total, where SS = sum of squares. Additional analysis of variance and mean separation was done in each harvest time and is also presented in the results.

3. Results

Analysis of variance indicated that visual (Table 1), flavor (Table 2) and functional traits (Table 3) were predominantly influenced by the harvest date factor, since most of the variation accounted for this factor; a visual trait (L*) or some flavor ones, such as DM and pH were highly influenced (η^2 = 48.1−44.8%) and some others, such as AFM and a*/b*, or TSS and FF, moderately (η^2 = 16.5−12.3%) (Tables 1 and 2). However, functional traits (TPC, TAC, AA, β-CAR, LYC and TCC) were all highly influenced (η^2 = 40.9−19.7) (Table 3). The substrate factor influenced TSS, TSS/TA, FF and functional traits except β-CAR, but the variation accounted for was between 19.0 and 15.3% for TSS and TAC, respectively, while the variation accounted for other parameters was below 10.5%. The significant total variation accounted for by the plant type factor was ~5% for AFM and DM and between 9.1 and 33.3 for functional traits. As expected, significant interactions were also observed; the most important and significant interaction was exhibited by harvest time × substrate. For this reason, analyses of variance were respectively performed for each harvest time separately (Tables 4–12).

Table 1. Analysis of variance for average visual quality traits (fruit mass: AFM, luminance: L*, chroma: C*, hue, a*/b*) of tomato fruit harvested at three harvest dates (6 June, 31 July and 6 November). The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		AFM		L*		C*		Hue		a*/b*	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Harvest time (H)	2	16.5	***	44.8	***	24.1	***	14.7	***	14.4	***
Substrate (S)	3	0.8		2.7		1.2		1.9		1.9	
Plant type (P)	2	5.0	*	1.2		0.6		3.3		3.4	
H × S	6	8.5		1.9		6.4		4.5		4.9	
H × P	4	4.3		0.6		3.9		4.3		4.2	
H × S × P	12	15.1		2.5		7.1		5.2		5.3	
S × P	6	1.7		6.4		3.9		3.5		4.0	
Error	72										
Harvest time											
6 June		230.3 ± 2.89 a		41.7 ± 0.11 b		33.2 ± 0.21 a		53.0 ± 0.31 a		0.76 ± 0.008 b	
31 July		202.9 ± 3.28 b		42.1 ± 0.20 b		33.5 ± 0.25 a		51.1 ± 0.40 b		0.81 ± 0.011 a	
6 November		223.4 ± 6.14 a		43.7 ± 0.18 a		31.8 ± 0.23 b		51.1 ± 0.39 b		0.81 ± 0.011 a	
Substrate											
Rockwool		222.9 ± 7.01 a		42.5 ± 0.27 a		33.1 ± 0.38 a		51.7 ± 0.39 a		0.79 ± 0.011 a	
Perlite		216.6 ± 5.10 a		42.7 ± 0.25 a		32.8 ± 0.26 a		52.3 ± 0.47 a		0.78 ± 0.013 a	
Pumice in sacks		218.7 ± 5.03 a		42.6 ± 0.29 a		32.9 ± 0.19 a		51.6 ± 0.57 a		0.80 ± 0.016 a	
Pumice in pots		217.2 ± 5.02 a		42.1 ± 0.20 a		32.6 ± 0.35 a		51.4 ± 0.38 a		0.80 ± 0.011 a	
Grafting/Training											
Self rooted		227.8 ± 5.59 a		42.7 ± 0.22 a		33.0 ± 0.27 a		52.3 ± 0.41 a		0.78 ± 0.011 a	
Grafted/single stem		212.9 ± 4.50 b		42.4 ± 0.21 a		32.8 ± 0.25 a		51.3 ± 0.40 a		0.80 ± 0.011 a	
Grafted/double stem		215.9 ± 3.95 b		42.4 ± 0.23 a		32.7 ± 0.27 a		51.5 ± 0.38 a		0.80 ± 0.010 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. * and *** significant at 0.05 and 0.001 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 2. Analysis of variance for average flavor quality traits (dry mass: DM, total soluble solids: TSS, titratable acidity: TA, flesh firmness: FF) of tomato fruit harvested at three harvest dates (6 June, 31 July and 6 November). The fruit was obtained from self-rooted 'Beef Bang F1' 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		DM		TSS		pH		TA		TSS/TA		FF	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Harvest time (H)	2	48.1	***	11.7	***	45.6	***	25.0	***	10.6	***	12.3	***
Substrate (S)	3	0.5		15.3	***	3.3		8.3		10.5	**	6.8	*
Plant type (P)	2	5.4	***	4.1		0.9		0.0		3.2		14.2	***
H × S	6	7.2	**	7.6		5.7		0.0		7.5		7.0	
H × P	4	1.1		1.1		1.3		0.0		0.8		3.3	
H × S × P	12	6.1		7.4		4.6		8.3		11.1		9.8	
S × P	6	5.5	*	1.0		3.7		8.3		7.1		2.2	
Error	72												
Harvest time													
6 June		5.0 ± 0.05 a		4.7 ± 0.06 a		4.02 ± 0.019 c		0.07 ± 0.001 a		65.0 ± 1.23 b		1.02 ± 0.014 a	
31 July		5.1 ± 0.05 a		4.4 ± 0.06 b		4.27 ± 0.019 a		0.06 ± 0.002 b		70.4 ± 1.60 a		0.91 ± 0.022 b	
6 November		4.5 ± 0.06 b		4.3 ± 0.07 b		4.17 ± 0.019 b		0.06 ± 0.001 b		72.4 ± 1.67 a		1.02 ± 0.029 a	
Substrate													
Rockwool		4.8 ± 0.07 a		4.3 ± 0.05 c		4.19 ± 0.034 a		0.07 ± 0.003 a		64.3 ± 1.90 b		0.92 ± 0.029 b	
Perlite		4.9 ± 0.10 a		4.4 ± 0.08 bc		4.12 ± 0.031 b		0.06 ± 0.001 a		69.1 ± 1.45 a		1.01 ± 0.029 a	
Pumice in sacks		4.8 ± 0.07 a		4.5 ± 0.07 ab		4.18 ± 0.032 ab		0.06 ± 0.002 a		72.0 ± 2.09 a		1.02 ± 0.031 a	
Pumice in pots		4.9 ± 0.09 a		4.7 ± 0.09 a		4.13 ± 0.019 ab		0.07 ± 0.002 a		71.7 ± 1.56 a		0.98 ± 0.019 ab	
Grafting/Training													
Self rooted		4.9 ± 0.07 a		4.6 ± 0.06 a		4.15 ± 0.022 a		0.07 ± 0.001 a		70.7 ± 1.22 a		1.03 ± 0.021 a	
Grafted/single stem		5.0 ± 0.07 a		4.4 ± 0.06 ab		4.14 ± 0.028 a		0.07 ± 0.001 a		70.2 ± 1.68 a		1.01 ± 0.023 a	
Grafted/double stem		4.7 ± 0.07 b		4.4 ± 0.07 b		4.17 ± 0.028 a		0.07 ± 0.002 a		66.9 ± 1.80 a		0.90 ± 0.024 b	

DF: degrees of freedom, η^2: eta squared, *P*: probability. *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 3. Analysis of variance for average functional quality traits (total phenolic content: TPC, ascorbic acid: AA, lycopene: LYC, beta carotene: β-CAR, total carotenoid content: TCC, total antioxidant capacity: TAC) of tomato fruit harvested at three harvest dates (6 June, 31 July and 6 November). The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		TPC		AA		LYC		β-CAR		TCC		TAC	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Harvest time (H)	2	40.9	***	25.0	***	31.5	***	19.7	***	32.9	***	40.2	***
Substrate (S)	3	4.5	*	4.1	*	4.5	**	3.1		4.2	**	19.0	***
Plant type (P)	2	0.2		0.7		0.4		0.5		0.5		3.6	***
H × S	6	9.1	***	29.9	***	33.3	***	24.9	***	31.9	***	14.4	***
H × P	4	0.0		1.5		0.9		1.1		0.7		3.5	***
H × S × P	12	9.1		8.4	*	4.9		10.5		4.5		5.2	**
S × P	6	4.5	*	4.1		2.1		3.4		2.7		1.6	
Error	72												
Harvest time													
6 June		0.12 ± 0.002 a		93 ± 2.3 b		26.8 ± 0.93 a		10.6 ± 0.26 a		43.5 ± 1.46 b		5.7 ± 0.23 b	
31 July		0.12 ± 0.002 a		102 ± 3.3 a		27.9 ± 1.48 a		11.2 ± 0.40 a		48.8 ± 2.49 a		7.2 ± 0.40 a	
6 November		0.10 ± 0.002 b		80 ± 2.2 c		18.1 ± 0.73 b		9.0 ± 0.23 b		30.9 ± 1.22 c		4.0 ± 0.09 c	
Substrate													
Rockwool		0.11 ± 0.002 ab		93 ± 4.7 ab		22.3 ± 1.59 b		9.6 ± 0.40 b		38.7 ± 2.69 b		4.9 ± 0.20 c	
Perlite		0.11 ± 0.002 b		87 ± 2.7 b		26.9 ± 1.87 a		10.6 ± 0.46 a		45.5 ± 3.30 a		4.9 ± 0.20 c	
Pumice in sacks		0.11 ± 0.002 ab		90 ± 2.8 b		24.1 ± 1.37 b		10.3 ± 0.37 ab		40.9 ± 2.04 b		5.5 ± 0.25 b	
Pumice in pots		0.12 ± 0.004 a		97 ± 3.1 a		23.8 ± 1.07 b		10.4 ± 0.34 ab		39.2 ± 1.72 b		7.1 ± 0.62 a	

Table 3. *Cont.*

		TPC		AA		LYC		β-CAR		TCC		TAC	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Grafting/Training													
Self rooted		0.11 ± 0.003 a		92 ± 3.1 a		23.8 ± 1.11 a		10.0 ± 0.32 a		40.2 ± 1.82 a		6.1 ± 0.43 a	
Grafted/single stem		0.11 ± 0.002 a		89 ± 3.1 a		24.9 ± 1.41 a		10.4 ± 0.36 a		42.3 ± 2.37 a		5.2 ± 0.26 b	
Grafted/double stem		0.11 ± 0.002 a		93 ± 2.8 a		24.0 ± 1.42 a		10.3 ± 0.35 a		40.7 ± 2.39 a		5.4 ± 0.31 b	

DF: degrees of freedom, η^2: eta squared, *P*: probability. *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 4. Analysis of variance for average visual quality traits (fruit mass: AFM, luminance: L*, chroma: C*, hue, a*/b*) of tomato fruit harvested on 6 June. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1 grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		AFM		L*		C*		Hue		a*/b*	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	31.8	**	2.8		1.4		5.4		4.7	
Plant type (P)	2	15.6	*	7.1		13.1		4.5		4.6	
S × P	6	16.2		28.5		24.5		11.1		11.9	
Error	35										
Substrate											
Rockwool		231.6 ± 5.56 a		41.6 ± 0.18 a		33.3 ± 0.62 a		52.5 ± 0.68 a		0.77 ± 0.018 a	
Perlite		214.7 ± 5.58 b		41.8 ± 0.20 a		33.1 ± 0.31 a		53.5 ± 0.55 a		0.74 ± 0.014 a	
Pumice in sacks		233.8 ± 5.38 a		41.7 ± 0.33 a		33.1 ± 0.40 a		53.3 ± 0.78 a		0.75 ± 0.021 a	
Pumice in pots		241.0 ± 2.97 a		41.6 ± 0.09 a		33.4 ± 0.35 a		52.7 ± 0.42 a		0.76 ± 0.011 a	
Grafting/Training											
Self rooted		238.7 ± 3.62 a		41.9 ± 0.21 a		33.6 ± 0.35 a		53.3 ± 0.57 a		0.75 ± 0.016 a	
Grafted/single stem		230.1 ± 6.14 ab		41.5 ± 0.14 a		33.5 ± 0.37 a		52.5 ± 0.48 a		0.77 ± 0.013 a	
Grafted/double stem		222.1 ± 4.08 b		41.6 ± 0.19 a		32.6 ± 0.33 a		53.3 ± 0.54 a		0.75 ± 0.014 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. * and ** significant at 0.05 and 0.01 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 5. Analysis of variance for average visual quality traits (fruit mass: AFM, luminance: L*, chroma: C*, hue, a*/b*) of tomato fruit harvested on 31 July. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		AFM		L*		C*		Hue		a*/b*	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	10.3		7.1		1.6		9.8		10.6	
Plant type (P)	2	0.9		3.3		2.5		0.2		0.6	
S × P	6	27.8		11.2		17.0		12.2		13.7	
Error	35										
Substrate											
Rockwool		211.2 ± 5.64 a		42.4 ± 0.57 a		33.5 ± 0.80 a		51.8 ± 0.53 a		0.79 ± 0.015 a	
Perlite		196.7 ± 8.49 a		42.2 ± 0.36 a		33.6 ± 0.40 a		51.4 ± 0.64 a		0.80 ± 0.018 a	
Pumice in sacks		206.5 ± 7.16 a		42.1 ± 0.37 a		33.2 ± 0.28 a		51.3 ± 1.14 a		0.81 ± 0.032 a	
Pumice in pots		197.0 ± 3.66 a		41.6 ± 0.24 a		33.8 ± 0.55 a		49.9 ± 0.68 a		0.84 ± 0.020 a	
Grafting/Training											
Self rooted		203.6 ± 5.60 a		42.3 ± 0.39 a		33.9 ± 0.37 a		51.1 ± 0.80 a		0.81 ± 0.022 a	
Grafted/single stem		200.2 ± 5.98 a		42.2 ± 0.34 a		33.4 ± 0.36 a		51.2 ± 0.82 a		0.81 ± 0.023 a	
Grafted/double stem		204.8 ± 5.86 a		41.8 ± 0.32 a		33.4 ± 0.57 a		50.9 ± 0.41 a		0.81 ± 0.012 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 6. Analysis of variance for average visual quality traits (fruit mass: AFM, luminance: L*, chroma: C*, hue, a*/b*) of tomato fruit harvested on 6 November. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		AFM		L*		C*		Hue		a*/b*	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	6.9		11.7		27.7	*	6.6		6.8	
Plant type (P)	2	12.9		1.8		4.0		20.0	*	19.4	*
S × P	6	18.8		17.8		3.0		7.5		7.5	
Error	35										
Substrate											
Rockwool		226.0 ± 19.71 a		43.5 ± 0.37 a		32.5 ± 0.54 a		50.8 ± 0.73 a		0.82 ± 0.021 a	
Perlite		238.3 ± 6.48 a		44.0 ± 0.33 a		31.6 ± 0.35 ab		51.9 ± 1.07 a		0.79 ± 0.029 a	
Pumice in sacks		215.8 ± 10.88 a		44.1 ± 0.39 a		32.3 ± 0.23 a		50.3 ± 0.81 a		0.83 ± 0.024 a	
Pumice in pots		213.5 ± 10.01 a		43.3 ± 0.28 a		30.7 ± 0.43 b		51.5 ± 0.49 a		0.80 ± 0.014 a	
Grafting/Training											
Self rooted		241.0 ± 13.19 a		43.9 ± 0.22 a		31.5 ± 0.38 a		52.6 ± 0.61 a		0.77 ± 0.016 b	
Grafted/single stem		208.4 ± 8.73 a		43.5 ± 0.33 a		31.6 ± 0.39 a		50.3 ± 0.66 b		0.83 ± 0.019 a	
Grafted/double stem		220.8 ± 8.99 a		43.7 ± 0.36 a		32.2 ± 0.42 a		50.4 ± 0.63 b		0.83 ± 0.018 a	

DF: degrees of freedom, η^2: eta squared, *P*: Probability. * significant at 0.05 level. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 7. Analysis of variance for average flavor quality traits (dry mass: DM, total soluble solids: TSS, titratable acidity: TA, flesh firmness: FF) of tomato fruit harvested on 6 June. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		DM		TSS		pH		TA		TSS/TA		FF	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	8.8		26.7	*	23.5		1.1		16.5		4.9	
Plant type (P)	2	10.5		9.9		5.2		0.1		13.5	*	12.8	
S × P	6	17.6		10.7		6.1		0.1		24.5		13.9	
Error	35												
Substrate													
Rockwool		4.9 ± 0.05 a		4.5 ± 0.07 b		4.02 ± 0.028 a		0.07 ± 0.004 a		61.9 ± 2.87 a		1.00 ± 0.021 a	
Perlite		5.0 ± 0.11 a		4.7 ± 0.15 b		3.93 ± 0.020 a		0.07 ± 0.002 a		65.0 ± 1.46 a		1.02 ± 0.029 a	
Pumice in sacks		5.1 ± 0.14 a		4.6 ± 0.11 b		4.06 ± 0.048 a		0.07 ± 0.001 a		63.4 ± 1.60 a		1.05 ± 0.033 a	
Pumice in pots		5.1 ± 0.11 a		5.0 ± 0.09 a		4.07 ± 0.037 a		0.07 ± 0.002 a		69.8 ± 3.05 a		1.00 ± 0.034 a	
Grafting/Training													
Self rooted		5.1 ± 0.11 a		4.8 ± 0.07 a		4.05 ± 0.038 a		0.07 ± 0.001 a		66.3 ± 1.33 ab		1.05 ± 0.023 a	
Grafted/single stem		5.1 ± 0.10 a		4.7 ± 0.12 a		3.99 ± 0.029 a		0.07 ± 0.001 a		67.5 ± 2.48 a		1.02 ± 0.025 a	
Grafted/double stem		4.9 ± 0.04 b		4.6 ± 0.11 a		4.03 ± 0.032 a		0.08 ± 0.003 a		61.3 ± 2.15 b		0.98 ± 0.025 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. * significant at 0.05 level. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 8. Analysis of variance for average flavor quality traits (dry mass: DM, total soluble solids: TSS, titratable acidity: TA, flesh firmness: FF) of tomato fruit harvested on 31 July. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		DM		TSS		pH		TA		TSS/TA		FF	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	24.4	**	57.7	***	17.1		0.0		27.5	*	15.9	***
Plant type (P)	2	2.5		4.2		4.6		0.0		2.4		43.6	***
S × P	6	38.4	**	10.6		10.8		25.0		16.7		22.4	**
Error	35												

Table 8. *Cont.*

		DM		TSS		pH		TA		TSS/TA		FF	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate													
Rockwool		5.1 ± 0.08 a		4.2 ± 0.06 b		4.35 ± 0.007 a		0.07 ± 0.004 a		65.1 ± 4.05 b		0.83 ± 0.067 c	
Perlite		5.3 ± 0.09 a		4.1 ± 0.06 b		4.25 ± 0.002 a		0.06 ± 0.002 a		66.3 ± 1.98 b		0.92 ± 0.025 ab	
Pumice in sacks		4.9 ± 0.06 b		4.6 ± 0.10 a		4.27 ± 0.001 a		0.06 ± 0.001 a		77.4 ± 2.95 a		0.90 ± 0.029 b	
Pumice in pots		5.2 ± 0.2 a		4.8 ± 0.11 a		4.22 ± 0.002 a		0.07 ± 0.002 a		72.7 ± 1.81 ab		0.97 ± 0.034 a	
Grafting/Training													
Self rooted		5.2 ± 0.10 a		4.5 ± 0.12 a		4.24 ± 0.001 a		0.06 ± 0.001 a		72.1 ± 1.29 a		0.95 ± 0.022 a	
Grafted/single stem		5.2 ± 0.10 a		4.4 ± 0.10 a		4.28 ± 0.002 a		0.06 ± 0.001 a		70.6 ± 3.34 a		0.99 ± 0.022 a	
Grafted/double stem		5.1 ± 0.06 a		4.5 ± 0.10 a		4.30 ± 0.005 a		0.07 ± 0.003 a		68.5 ± 3.30 a		0.79 ± 0.040 b	

DF: degrees of freedom, η^2: eta squared, *P*: Probability. *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 9. Analysis of variance for average flavor quality traits (dry mass: DM, total soluble solids: TSS, titratable acidity: TA, flesh firmness: FF) of tomato fruit harvested on 6 November. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		DM		TSS		pH		TA		TSS/TA		FF	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	12.1		1.6		9.9		0.0		15.5		18.0	
Plant type (P)	2	22.3	*	4.4		2.7		1.1		1.3		8.9	
S × P	6	13.3		7.8		27.7		0.0		23.1		9.0	
Error	35												
Substrate													
Rockwool		4.5 ± 0.11 a		4.3 ± 0.12 a		4.19 ± 0.044 a		0.07 ± 0.003 a		65.8 ± 3.10 a		0.93 ± 0.039 a	
Perlite		4.3 ± 0.09 a		4.3 ± 0.12 a		4.17 ± 0.022 a		0.06 ± 0.002 a		75.9 ± 2.39 a		1.09 ± 0.069 a	
Pumice in sacks		4.6 ± 0.13 a		4.4 ± 0.16 a		4.19 ± 0.059 a		0.06 ± 0.003 a		75.2 ± 4.16 a		1.09 ± 0.073 a	
Pumice in pots		4.4 ± 0.10 a		4.4 ± 0.18 a		4.10 ± 0.012 a		0.06 ± 0.002 a		72.6 ± 3.23 a		0.95 ± 0.035 a	
Grafting/Training													
Self rooted		4.5 ± 0.07 ab		4.5 ± 0.11 a		4.15 ± 0.026 a		0.06 ± 0.002 a		73.7 ± 2.83 a		1.07 ± 0.050 a	
Grafted/single stem		4.6 ± 0.08 a		4.3 ± 0.09 a		4.16 ± 0.040 a		0.06 ± 0.002 a		72.6 ± 2.91 a		1.03 ± 0.063 a	
Grafted/double stem		4.2 ± 0.10 b		4.2 ± 0.16 a		4.19 ± 0.037 a		0.06 ± 0.002 a		70.8 ± 3.31 a		0.94 ± 0.036 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. * significant at 0.05 level. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 10. Analysis of variance for average functional quality traits (total phenolic content: TPC, ascorbic acid: AA, lycopene: LYC, beta carotene: β-CAR, total carotenoid content: TCC, total antioxidant capacity: TAC) of tomato fruit harvested on 6 June. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		TPC		AA		LYC		β-CAR		TCC		TAC	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	20.0	*	25.1	*	41.8	***	27.9	*	34.2	**	48.8	***
Plant type (P)	2	0.1		1.5		0.1		1.2		0.1		8.2	
S × P	6	0.1		22.2		12.5		17.5		13.4		6.7	
Error	35												
Substrate													
Rockwool		0.12 ± 0.003 a		82 ± 4.0 b		21.8 ± 1.59 c		9.7 ± 0.44 b		36.7 ± 2.69 b		5.3 ± 0.26 b	
Perlite		0.11 ± 0.003 b		100 ± 3.3 a		25.6 ± 1.35 bc		9.8 ± 0.42 b		42.2 ± 3.30 b		4.8 ± 0.24 b	
Pumice in sacks		0.12 ± 0.002 a		96 ± 4.8 a		31.4 ± 1.79 a		11.4 ± 0.62 a		50.8 ± 2.04 a		5.4 ± 0.19 b	
Pumice in pots		0.13 ± 0.005 a		94 ± 4.3 a		28.5 ± 1.12 ab		11.3 ± 0.36 a		44.4 ± 1.72 ab		7.3 ± 0.56 a	

Table 10. *Cont.*

		TPC		AA		LYC		β-CAR		TCC		TAC	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Grafting/Training													
Self rooted		0.12 ± 0.003 a		91 ± 3.4 a		26.6 ± 1.42 a		10.3 ± 0.48 a		40.2 ± 1.82 a		6.2 ± 0.37 a	
Grafted/single stem		0.12 ± 0.004 a		94 ± 5.2 a		26.9 ± 1.95 a		10.7 ± 0.53 a		42.3 ± 2.37 a		5.6 ± 0.50 a	
Grafted/double stem		0.12 ± 0.002 a		95 ± 3.2 a		26.8 ± 1.56 a		10.6 ± 0.38 a		40.7 ± 2.39 a		5.3 ± 0.26 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 11. Analysis of variance for average functional quality traits (total phenolic content: TPC, ascorbic acid: AA, lycopene: LYC, beta carotene: β-CAR, total carotenoid content: TCC, total antioxidant capacity: TAC) of tomato fruit harvested on 31 July. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		TPC		AA		LYC		β-CAR		TCC		TAC	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	33.1	***	68.0	***	65.6	***	37.4	***	65.1	***	58.9	***
Plant type (P)	2	0.0		4.5	*	2.3		2.6		2.1		13.3	***
S × P	6	38.2	***	12.8	*	9.9		21.4		10.4		12.8	*
Error	35												
Substrate													
Rockwool		0.11 ± 0.001 b		124 ± 3.2 a		30.2 ± 2.28 b		11.3 ± 0.64 b		52.7 ± 3.78 b		5.6 ± 0.19 c	
Perlite		0.12 ± 0.002 b		84 ± 4.0 c		38.1 ± 1.85 a		13.3 ± 0.65 a		65.8 ± 3.20 a		5.9 ± 0.30 bc	
Pumice in sacks		0.11 ± 0.002 b		89 ± 2.6 c		18.8 ± 1.53 d		9.2 ± 0.66 c		33.6 ± 2.63 d		6.9 ± 0.37 b	
Pumice in pots		0.13 ± 0.006 a		110 ± 5.3 b		24.7 ± 1.50 c		10.9 ± 0.71 bc		43.1 ± 2.55 c		10.2 ± 0.94 a	
Grafting/Training													
Self rooted		0.12 ± 0.005 a		105 ± 5.9 a		26.2 ± 2.09 a		10.6 ± 0.66 a		45.9 ± 3.47 a		8.2 ± 0.90 a	
Grafted/single stem		0.12 ± 0.002 a		96 ± 6.3 b		29.4 ± 2.77 a		11.5 ± 0.71 a		51.2 ± 4.69 a		6.1 ± 0.37 c	
Grafted/double stem		0.12 ± 0.002 a		104 ± 5.1 a		28.2 ± 2.90 a		11.4 ± 0.74 a		49.2 ± 4.90 a		7.1 ± 0.61 b	

DF: degrees of freedom, η^2: eta squared, *P*: probability. * and *** significant at 0.05 and 0.001 levels, respectively. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

Table 12. Analysis of variance for average functional quality traits (total phenolic content: TPC, ascorbic acid: AA, lycopene: LYC, beta carotene: β-CAR, total carotenoid content: TCC, total antioxidant capacity: TAC) of tomato fruit harvested on 6 November. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

		TPC		AA		LYC		β-CAR		TCC		TAC	
Source of Variability	**DF**	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***
Substrate (S)	3	0.0		11.8		34.3	**	36.3	**	35.3	**	34.5	**
Plant type (P)	2	1.3		0.6		2.8		0.9		1.8		1.2	
S × P	6	0.0		19.9		7.9		5.4		6.4		14.2	
Error	35												
Substrate													
Rockwool		0.10 ± 0.003 a		74 ± 3.5 a		15.1 ± 1.59 b		8.0 ± 0.49 b		26.9 ± 2.67 b		3.7 ± 0.07 b	
Perlite		0.10 ± 0.002 a		78 ± 3.6 a		17.1 ± 0.85 b		8.8 ± 0.34 b		28.6 ± 1.27 b		4.0 ± 0.16 ab	
Pumice in sacks		0.11 ± 0.003 a		83 ± 6.0 a		22.0 ± 1.41 a		10.3 ± 0.42 a		38.1 ± 2.22 a		4.3 ± 0.14 a	
Pumice in pots		0.10 ± 0.002 a		85 ± 2.3 a		18.2 ± 0.98 ab		8.9 ± 0.29 b		30.0 ± 1.85 b		3.8 ± 0.07 b	
Grafting/Training													
Self rooted		0.10 ± 0.002 a		81 ± 4.2 a		18.7 ± 1.30 a		9.2 ± 0.5 a		31.4 ± 2.12 a		4.0 ± 0.13 a	
Grafted/single stem		0.10 ± 0.002 a		79 ± 3.2 a		18.5 ± 1.24 a		8.9 ± 0.36 a		31.7 ± 2.01 a		4.0 ± 0.13 a	
Grafted/double stem		0.10 ± 0.002 a		80 ± 36 a		17.1 ± 1.34 a		8.9 ± 0.44 a		29.5 ± 2.37 a		3.9 ± 0.11 a	

DF: degrees of freedom, η^2: eta squared, *P*: probability. ** significant at 0.01 level. Different letters following values within each factor and column, indicate significantly different values at 0.05 level according to Duncan's multiple range test.

3.1. Visual Quality

Tomato fruit were harvested on the basis of red coloration. Fruit color parameters, lightness (L*), chroma (c*) and hue as well as a*/b*, all showed a similar trend, however, significantly different effects were observed only by harvest time (Table 1). Lightness (L*) values were highest for the fruit of the November harvest (43.7) followed by the July and June fruit which did not differ between them significantly. Fruit chroma values were, on the other hand, the lowest for fruit of the November harvest (31.8). Hue and a*/b* values were the highest (53.0) and the lowest (0.76), respectively, on fruit of the June harvest.

Average fruit mass (AFM) was 230.3, 202.9 and 223.4 g for the June, July and November harvests, corresponding to thrushes 3–4, 10–11 and 21–22, respectively (Table 1), whereas, AFM was significantly higher in self-rooted plants (227.8 g) compared to grafted ones (212.9 and 215.9 g). There were virtually no differences in AFM levels within different substrates. However, analysis of variance of the June harvest (Table 4) indicated that fruit obtained from plants grown on perlite or from grafted-double stemmed plants had the lowest AFM while in the July (Table 5) or November harvests (Table 6) there was no difference between treatments. AFM showed significant ($p < 0.05$) but weak ($r \leq 0.30$) correlations with hue and a*/b* (Table 13).

Table 13. Correlation coefficients between average visual (luminance: L*, chroma: C*, hue, a*/b*, fruit mass: AFM), flavor (dry mass: DM, total soluble solids: TSS, titratable acidity: TA, flesh firmness: FF) and functional quality traits (total phenolic content: TPC, ascorbic acid: AA, lycopene: LYC, beta carotene: β-CAR, total carotenoid content: TCC, total antioxidant capacity: TAC) of tomato fruit over three harvest dates. The fruit was obtained from self-rooted 'Beef Bang F1', 'Beef Bang F1' grafted onto 'Defensor', trained in single or double stem and grown hydroponically in four substrates (rockwool slabs, perlite in sacks, pumice in sacks and pumice in pots) in a glasshouse.

	L*	C*	Hue	a*/b*	AFM	DM	TSS	pH	TA	TSS/TA	FF	TPC	AA	LYC	β-CAR	TCC
L*	1.00															
C*		1.00														
Hue	0.28 **		1.00													
a*/b*	−0.28 **		−1.00 ***	1.00												
AFM			0.30 **	−0.29 **	1.00											
DM	−0.48 ***	0.37 ***				1.00										
TSS	−0.23 *					0.26 **	1.00									
pH			−0.23 *	0.22 *	−0.25 *		−0.22 *	1.00								
TA	−0.29 **	0.23 *	0.20 *	−0.20 *		0.29 **			1.00							
TSS/TA						−0.23 *	0.23 *		−0.74 ***	1.00						
FF			0.28 **	−0.27 **	0.22 *			−0.30 **			1.00					
TPC	−0.48 ***	0.43 ***				0.65 ***	0.38 ***		0.35 ***	−0.21 *		1.00				
AA	−0.33 ***	0.23 *				0.50 ***	0.32 ***					0.50 ***	1.00			
LYC	−0.39 ***	0.28 **				0.61 ***				−0.21 *		0.35 ***	0.28 *	1.00		
β-CAR	−0.28 **	0.24 *				0.56 ***						0.38 ***	0.23 *	0.91 ***	1.00	
TCC	−0.37 ***	0.29 **				0.63 ***				−0.21 *		0.34 ***	0.29 **	0.99 ***	0.90 ***	1.00
TAC	−0.43 ***	0.40 ***				0.51 ***	0.44 ***					0.71 ***	0.51 ***	0.22 *	0.25 *	0.23 *

*, ** and *** correlation is significant at the 0.05, 0.01 and 0.001 levels, respectively.

3.2. Flavor Quality

Fruit dry mass (DM) was significantly different among harvest times or plant types. Average DM content was 5.0–5.1% for June and July harvest times and only 4.5% for the November harvest (Table 2). Again, fruit from grafted-double stemmed plants had the lowest DM (4.7%) of all plant types (4.9–5.0%). However, there was no difference observed in DM between substrates (Table 1) except across the harvest times (Tables 7–9). A significant interaction of substrate × plant type or substrate × harvest time was also observed in the case of DM (Table 2), but it was of minimal practical significance. Fruit obtained from grafted-double stemmed plants showed the lowest DM in all harvests (Tables 7–9). Correlations of fruit DM were significant with most of the fruit attributes except for hue, a*/b*, pH and FF (Table 13).

Harvest time and substrate had a significant effect on TSS (Table 2). SSC decreased with harvest time; the June harvest had the highest average SSC (4.7%) while the November harvest the lowest (4.3%). Significantly higher SSC was observed in fruit obtained from self-rooted, among plant types, as well as from plants grown on pumice, among substrates (Table 2). SSC correlated significantly with L* color attribute, pH, TSS/TA, TPC, AA and TAC (Table 13).

There was a significant effect of harvest time on pH and TA (Table 2). Highest values were observed in the July harvest for pH (4.27) and in the June harvest for TA (0.07) (Table 2). The ratio TSS/TA followed a similar trend to that of TSS between fruit from different harvest times.

Flesh firmness (FF), on the other hand, showed a different trend (Table 2) and it was significantly affected by all three factors; fruit obtained from plants at mid harvest had the lowest FF value of all treatments (0.91 kg). Grafted-double stemmed plants or grown on rockwool also showed minimal FF values of 0.90 or 0.92 kg, respectively, among plant types and substrates. FF showed a weak but significant correlation with color attributes hue and a*/b* as well as with AFM and pH (Table 13).

3.3. Functional Quality

Hydrophilic antioxidants (TPC and AA) were significantly affected by harvest time and substrate factors and some of their interactions between them or with the plant type (Table 3). AA was maximum in the July harvest (102 mg/kg) and minimum in the late harvest (80 mg/kg) and this correlated with antioxidant capacity (TAC). Levels of TPC were very low and minimum amounts were detected in the November harvest (0.10 mg/kg). AA and TPC also showed a trend to be higher in fruit grown in pumice or in rockwool compared to perlite, and TAC was also found to be higher in self-rooted plants compared to other plant types.

Lipophilic antioxidants (TCC and LYC) were also significantly affected by harvest time and substrate factors as well as their interaction, while β-CAR was affected only by harvest time and its interaction with the plant type (Table 3). All values were maximum in the July harvest and minimum in the November harvest and all correlated with TAC similarly to water soluble antioxidants. A trend of increased β-CAR, LYC and TCC was observed in fruit obtained from plants grown on perlite compared to other substrates. TAC showed significant correlations with most traits examined (Table 13); correlations were strong and with TPC (r = 0.71, $p < 0.0005$), moderately strong with DM (r = 0.51, $p < 0.0005$) and weak with SSC, L*, C* and the other nutritional traits.

4. Discussion

The vibrant red color of fresh tomato fruit is considered as the first of the appearance attributes that on one hand, indicates harvest maturity (ripening stage) and fruit quality, and on the other, influences consumer preference and inspires consumers to purchase and consume them. To determine red coloration, the Minolta chromameter L*, a* and b* values are currently used; the red maturity/ripeness stage corresponds to an a*/b* value of >0.95 [49] and values in the range of 0.6–0.95 correspond to a light red color according to the USDA [50] color classification. In this study, however, all treatments had a*/b* values between 0.76 and 0.81 and therefore all fruit could be characterized as light red.

Color is a major quality characteristic in virtually all fruits and vegetables and uniformity of color within tomatoes is a principal requirement of the E.U. quality standards for this crop [51]. To compare the quality of hydroponic tomato fruit among different substrate and plant type treatments, one would expect that color-based harvesting would be a safe criterion for harvesting of uniform red ripe (colored) fruit. However, this was proved not to be the case in this study. Harvesting of red fruit is performed by pickers, based on the perceived visual red tomato color, directly or following a comparison of tomato fruit color to reference chart color; subtle differences in visual color are difficult to be identified by the human eye, and thus, the fruit which were visually harvested at red ripe stage, upon transfer to the lab and measured using the Minolta L*, C*, hue and a∗/b∗ values, stage of ripeness changed towards light red. Such a deviation was evident among treatments and particularly among harvest times (Table 1). Minolta L*, a* and b* color readings in hydroponic tomatoes have been correlated with red color [52,53] and associated with lycopene content [54]. The lowest Minolta a∗/b∗ values among all harvest times (0.76) were observed in the June harvest, however, they did not correspond to lower

fruit LYC or β-CAR content (Table 1). This might indicate a seasonal effect on differential distribution of lycopene in epidermal and pulp fruit tissues, since Minolta a∗/b∗ values are measured in the fruit surface (epidermis) and lycopene content in fruit flesh (the ratio of epidermis to flesh is very low).

Furthermore, correlations of red color attributes (L*, C*, hue and a∗/b∗) with other fruit visual (AFM), flavor (DM, TSS, TA, pH) or functional quality traits (TPC, AA, β-CAR, LYC), including TAC, appeared very weak although significant (Table 5); i.e., the fruit of the November harvest showed a value a*/b* of 0.81, similar to those of the July harvest, but these fruit had the lowest DM, TPC, AA, TAC β-CAR and LYC content of all harvest times (Table 1). Lycopene, β-carotene and other carotenoids produced during ripening of tomato fruit are deposited in chloroplasts which are progressively transformed into chromoplasts, while chlorophyll is degraded or metabolized [55]. Lycopene synthesis is favored at temperatures between 16 and 21 °C and inhibited at temperatures above 30 °C, particularly 10 days before harvesting at the red stage [52]. However, the climatic and ripening factors might be different for synthesis of AA and TPC. Hernandez et al. [56] reported considerable seasonal variations in the levels of fruit flavor and functional traits due to inherent variability of individual tomato metabolisms and the dependence of ripening on climatic conditions. They also pointed out that the cultivation method had little influence on the concentration of these parameters [57]. Thus, a*/b* values did not significantly show any significant and strong correlation with the functional or flavor traits; however, a*/b* values correlated with C*, but this was because both a* and b* participate in its calculation (Table 13).

Further, in this study, the plant type did not affect the carotenoid content of tomato fruit (lycopene or β-carotene). However, other studies reported an increase in lycopene or β-carotene in fruit of grafted tomato [36,58]. Increased levels of AA content were observed in fruit of the second harvest (Table 1) although Di Gioia et al. [59] reported a decrease during harvest time. Furthermore, AA differed only in fruit of the plants grown in pumice in pots (Table 3). Grafted tomato plants were reported to produce fruit with decreased [60], or with unaffected [13], total phenolics and ascorbic acid; in another study, however, vitamin C was increased in fruit from grafted plants [61]. In any case, such changes of AA (or TPC) are considered minimal in the nutritional contribution of fresh tomato fruit since tomatoes are listed among the sources of high vitamin C fruit.

In respect to substrate, both TPC and AA content were highest in pumice in pots compared to other substrates (Table 3). Tzortzakis and Economakis [22] reported that hydroponic cultivation in pumice resulted in a remarkable early yield and increased carotenoids and ascorbic acid.

The differences in fruit nutritional traits (AA, TPC and LYC) might be reflecting the changes in light conditions during growth of tomato plants. It is known that tomato fruit exposure to full sunlight promoted synthesis of hydrophilic phytochemicals (TPC, AA), while the synthesis of lipophilics, such as LYC and β-CAR, took place in fruit shaded by foliage. On the other hand, the spectral quality of light, which changes during harvest time, also depends on the cultivar [62,63]. In consequence, there is a harvest-time (seasonal) effect on LYC and antioxidant levels in fruit [64,65]. Tomato fruit quality is also related to the presence of antioxidant capacity which in turn reflects both the hydrophilic and lipophilic phytochemicals found in red ripe tomato fruit. TAC was maximized in fruit of the July harvest and in plants gown in pumice in pots (Table 1), and similarly for both harvest times in June and July (Tables 10 and 11).

Cultivation on different substrates resulted in significant differences for DM and pH values on expanded clay compared to rockwool or perlite [66]. In another study [22], cultivation in pumice resulted in a remarkable increase in TSS, due to lower yield, given that the TSS content of the fruits was inversely related to the fruit yield. However, in our study no significant differences in yield were observed between pumice (in pot or sack) and rockwool, both of which were superior to perlite (data not shown). Therefore, the induced improvement in some of the visual and flavor attributes by cultivation in pumice could

be attributed to the optimization of physicochemical characteristics in the environment of root growth in pumice. Having a high porosity, pumice always contains a sufficient amount of air, even immediately after the supply of the nutrient solution, ensuring very good drainage and improved ventilation and in combination with the satisfactory water retention is an ideal means of growing vegetables. Moreover, its insulating properties ensure small temperature fluctuations in the root environment, thus favoring the nutrition and growth of plants as well as nutritional traits of the fruits, especially during the summer.

Slight decreases in fruit DM and TSS were observed for fruit harvested from grafted double stemmed plants compared to the self-rooted ones (Table 1). Turhan et al. [67], in a greenhouse study, reported that fruit dry matter decreased by 0.4–0.6% in three different tomato cultivars grafted onto 'Beaufort' and 'Arnold' rootstocks in comparison with non-grafted plants. Similar results were obtained by Schwarz et al. [68], who also reported a slight (0.4%) decrease of fruit dry mass of grafted 'Piccolino' tomatoes compared to the control. Turhan et al. [67] also reported that dry matter reductions were accompanied by 9.5–10.5% decreases in TSS. TSS increased in the fruits harvested from the grafted single stemmed and double stemmed plants which resulted in significant DM increase [61]. This was confirmed by the weak but significant correlations of fruit DM with SSC (Table 13). Even though grafting was reported to enhance tomato AFM [13,67], this was not the case in this study. Probably, in cultivation under optimal conditions, such as soilless culture, any obstacle in plant function may restrict quality in terms of AFM, DM and TSS (Table 1). TSS represents approximately 75% of the total DM and they are comprised primarily of the reducing sugars and of 10–15% of citric and malic acids, being the major constituents, which assure the nutritional value of tomatoes [69]. Hobson [70] reported that high dry matter or low water content of the tomato affect fruit taste positively because the major components of tomato taste, especially sugars and acids, are more concentrated. High fruit TSS and TA lead to preference of these fruit by consumers [55]. Many studies have shown that tomato flavor is related to the balance between total sugars and organic acids (sugars:acids ratio) in the fruit [71]. Lately, the enhanced flavor aspect of tomatoes was related to increasing amino acid content [72]. In the present study, the TSS:TA ratio of fruit followed the trend of TSS. Overall, there was no significant differences in pH and TA due to the factors examined [21,59] except for harvest time (Table 1).

Fruit obtained from the July harvest had lower FF value compared to the June and November harvests (Table 1). However, lower firmness has been recorded in spring compared to fall harvests by Anza et al. [73] and Kasampalis et al. [41]. This may reflect differences in plant growth [74] as well as in light and temperature between seasons. Regarding plant grafting/training, the grafted double stem plants had less firm fruit compared to those from self-rooted or single-stemmed, although this is not confirmed in another case [61].

5. Conclusions

Overall, comparative analysis of tomato quality in response to harvest time, substrate and plant type (grafting) using indeterminate tomato 'Beef Bang F1' revealed important effects on visual, flavor and functional fruit quality attributes. Visual harvesting at the red stage, particularly during different harvest times, might deviate from the stage of ripeness intended to be harvested at when these fruits are measured with Minolta chromameter.

The harvest time was observed as the most important factor for most parameters measured followed in many of these cases by the substrate (TSS, pH, TSS/TA, FF and all the nutritional attributes), in certain cases by the plant type (AFM, DM, TSS/TA, FF, and TAC) or by both in some of them (TSS, FF, TAC). Each individual harvest time revealed the rise in different parameters in interaction with either the substrate (June and July harvests) on DM, TSS and FF as well as on nutritional traits, or the grafting/training; July harvest on some nutritional traits and late harvest on DM.

Pumice, whether used in pot or in sack, enhanced most of the visual and flavor attributes.

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