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Original research article

Nutritional value of tomatoes (*Solanum lycopersicum* L.) grown in greenhouse by different agronomic techniquesDaniela Erba^{a,*}, M. Cristina Casiraghi^a, Albert Ribas-Agustí^b, Rafaela Cáceres^c, Oriol Marfà^c, Massimo Castellari^b^a Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Celoria, 2, Milano 20133, Italy^b Institute for Food and Agricultural Research and Technology (IRTA), Granja Camps i Armet, Monells 17121, Girona, Spain^c Biosystems Engineering and Agronomy (IRTA), Ctra. De Cabrils s/n, Cabrils 08348, Barcelona, Spain

A B S T R A C T

The relevance of agronomic practices on the nutritional quality greenhouse-grown tomatoes has been recognized. We investigated the influence of (1) cultivar: two local (Pera-Girona and Montserrat) and one commercial (Caramba) varieties; (2) nitrogen dose in nutrient solution (low vs. standard N dose); (3) treatment for plant disease control (sulfur vs. Milsana[®]) and (4) ripeness (orange vs. full-red color) on levels of carotenoids, phenolic compounds, ascorbic acid and minerals of fruits. Carotenoids and ascorbate were mainly influenced by variety and ripening stage, while N dose slightly affected minerals in fruits; treatments against plant diseases exerted only negligible effects on measured compounds. Local tomato varieties appear more promising as food source of carotenoids, mainly lycopene, and of hydroxycinnamates, such as 5-caffeoylquinic acid and caffeoylquinic derivatives, than commercial variety (total carotenoids: 67.43 mg kg⁻¹ fw vs. 56.34 mg kg⁻¹ fw of Pera-Girona vs. Caramba and total hydroxycinnamates: 90.87 mg kg⁻¹ fw vs. 37.90 mg kg⁻¹ fw of Montserrat vs. Caramba, at full-red color). Tomato variety and harvest maturity of fruit were the main factors affecting nutritional value of tomatoes, while Milsana[®] treatment did not result in evident nutritional benefits. However, the use of this elicitor might be appropriate considering the increasing environmentally friendly attitudes of consumers.

Keywords:

Tomato
Carotenoids
Phenolic compounds
Minerals
Trace elements
Ascorbic acid
Food composition
Food analysis
Horticulture and nutritional content

1. Introduction

Tomato is one of the major food crops worldwide, representing the second highest produced and consumed vegetable in western countries (Willcox et al., 2003). Together with its derived products, tomatoes are one of the major food sources of carotenoids, providing an estimated 80% of daily intake of lycopene, and of folate, ascorbic acid, flavonoids, α -tocopherol and potassium in the western diet (Bramley, 2000; Willcox et al., 2003). Several epidemiological studies have underlined the beneficial effect of tomato consumption in the prevention of chronic diseases such as cancer and cardiovascular disease (Klipstein-Grobush et al., 2000; Giovannucci et al., 2002). This effect has been attributed mainly to the antioxidant activity of tomato phytochemicals, in particular lycopene, a very efficient radical quencher capable to fight reactive oxygen species and thus avoid cell injury (Riso et al., 2004);

however, several other mechanisms of the healthy action of carotenoids have been suggested (Krinsky and Johnson, 2005). In addition, tomatoes contain many other compounds, such as vitamin C and phenolics, whose synergistic effects on human disease prevention should be considered (Willcox et al., 2003).

Agronomic practices have been recognized as a critical factor in determining the nutritional quality of crops (Barrett et al., 2007). Levels of bioactive food compounds in fresh tomatoes can be affected by many pre- and postharvest factors such as cultivar, ripening stage at harvest and agricultural techniques (Dumas et al., 2003). For instance, tomato cultivars significantly influence carotenoid expression and phenolic compound levels, and partially affect ascorbic acid content (Abushita et al., 2000; Slimestad and Verheul, 2009); ripening stage at harvest exerts a relevant positive effect on carotenoid levels, and specifically affects the phenolic compounds content (Gautier et al., 2008), while concentrations of vitamin C and some phenolic compounds tend to be positively affected by low N fertilization (Simonne et al., 2007; Benard et al., 2009).

Most commercial tomatoes, especially in northern European countries, are produced in greenhouses that allow better control of agronomic and environmental factors. Notwithstanding, green-

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house-grown crops are often affected by powdery mildew whose infection may result in considerable economical damage and low fruit quality, and require the use of fungicides (Konstantinidou-Doltsinis et al., 2006). The growing attention of consumers toward environmentally friendly primary production has led to a search for alternative methods to control plant disease. Among these, Milsana[®] – the commercial name given to the extract from leaves of giant knotweed (*Reynoutria sachalinensis* (F: Schmidt) Nakai) – has been reported to have a good protective effect against powdery mildew on cucumber as an inducer of plant defense systems (Daayf et al., 2000; Fofana et al., 2002) and on tomato (Konstantinidou-Doltsinis et al., 2006), but the effect of Milsana[®] treatment on nutritional value has scarcely been investigated (Daayf et al., 2000).

The objectives of this study were to investigate the influence of four agronomic variables such as (1) tomato varieties (one commercial and two local), (2) level of nitrogen in nutrient solution (low vs. standard), (3) treatment against plant diseases (sulfur vs. Milsana[®]) and (4) ripeness at harvest (green-orange vs. red fruits), and their reciprocal interactions, on nutritional quality of tomatoes evaluated as contents of carotenoids, ascorbic acid, phenolic compounds and minerals of fruit.

2. Materials and methods

2.1. Tomato samples

The experiment was performed in a 140 m² multi-span greenhouse covered with plastic film. The greenhouse was located at IRTA Centre of Cabriels (Cabriels, Barcelona, Spain, 41°25' N, 2°23' E, altitude 85 m).

Tomato (*Solanum lycopersicum* L.) seedlings obtained from a commercial nursery were cultivated in 60-L perlite bags. The particle size of perlite was between 0.1 and 5 mm. Each bag contained three plants. The experiment lasted for five months (from March to July 2008). Plants were fertilized by fertigation and the nutrient solution was applied with a drip irrigation system. Pollinating wasps (*Bombus terrestris* L.) were introduced at 50% opened flowers of the first truss.

A full factorial design was performed and three agronomic treatments were carried out, including: three tomato varieties were cultivated: Pera Girona and Montserrat (both locals) and Caramba (commercial variety); two nitrogen (N) dose in nutrient solution: standard (9.50 meq NO₃⁻ L⁻¹) and low (5.31 meq NO₃⁻ L⁻¹). The reduced N dose was considered because of environmental concerns on nitrate leaching and the associated pollution (Thompson et al., 2013). Two foliar fungicide/elicitor treatments against fungi-related tomato diseases: sulfur, as a traditional approach, and an innovative approach using the elicitor Milsana[®] (Biofa, Germany). The elicitor was applied at a dose of 3 mL L⁻¹ and wettable sulfur (80%) was applied at a dose of 2.5 g L⁻¹. The first treatment applications were carried out on the 9th May 2008 and further foliar treatments were performed every week. Pollinators were removed from the greenhouse before treatment application and returned two days after spraying.

The sampling of the tomato fruits for nutritional analysis started on 9 June 2008 and was finished on 8 July 2008. Fruits sampled for analysis were harvested from 2nd, 3rd or 4th truss. Tomatoes of each plant in the experiment were collected at two ripening stages: stage 5 (green-orange) and stage 10 (red) according to the French Colour Code Guide (CTIFL, France). These ripeness stages were chosen to represent tomatoes for fresh consumption (stage 5) and for processing (stage 10). Therefore, the ripeness degree of the tomato was including as a source of variation, thus resulting in 192 tomato samples to be analyzed (3 varieties × 2 N dose × 2 plant disease treatments × 2 ripeness

stages × 8 replicates). After harvesting, fruits were washed with tap and de-ionized water, refrigerated at 4 °C for 2 h, vacuum packed (0.0115 MPa) in side-sealed aluminum bags, stored at -80 °C and sent to laboratories for analysis. Results are expressed as mg kg⁻¹ fresh weight.

2.2. Analysis of carotenoids

Carotenoid contents were assessed in accordance to the method of Khachik et al. (1992) with slight modification, as previously reported (Ribas-Agustí et al., 2013). Briefly, after an exhaustive extraction with tetrahydrofuran of mixed samples (about 12 g or 6 g for stages 5 and 10, respectively), carotenoids residue was dissolved and injected in HPLC system equipped with a C18 reverse phase column (Vydac 201 TP 54, 25 172 cm, 5 μm, Grace, Sint-Niklaas, Belgium) at 22 °C, and Photodiode Array Detector (Waters model 2996, Milford, MA, USA). Identification of the peaks was carried out by comparison of UV-vis spectra and retention times of eluted compounds with pure standards (lycopene purity ≥ 90%, β-carotene ≥ 95%, lutein ≥ 70% from Sigma-Aldrich, Munich, Germany; phytoene ≥ 98% and phytofluene ≥ 95% from Carote Nature, Lupingen, Switzerland). The percentage recovery of carotenoids was determined by adding a known amount of internal standard (β-apo-8'-carotenal, Sigma-Aldrich, Munich, Germany) to each sample before extraction; recovery of this internal standard was greater than 90% for all extractions.

2.3. Analysis of minerals and trace elements

Approximately 100 g of homogenized tomato sample was lyophilized in a freeze-drier laboratory equipment (Edwards Freeze Drier Pirani 1001, IVT, Milano, Italy). Then, about 0.2 g of freeze-dried sample was acid digested in microwave oven (Ethos TC, Milestone, Italy) and clear solution brought to the final volume with bi-distilled water. These solutions were analyzed in an air/acetylene flame spectrometry (Perkin-Elmer Analyst 800, Waltham, MA, USA), following the instrumental condition recommended for each mineral (Ca 422.7 nm, Mg 285.2 nm, Cu 324.8 nm, Zn 213.9 nm, K 766.5 nm, Na 589.0 nm, Fe 248.3 nm). Phosphorus was measured by a colorimetric method by using of vanadate-molybdate reagent (Fiske and Subbarow, 1925).

2.4. Analysis of total ascorbic acid

The analysis of total ascorbic acid was based on the method of Lopez et al. (2005) with minor adaptations: 1 g of ground tomato was blended in 9 g of 750 mM HPO₃-1.0 mM EDTA (Sigma-Aldrich, Madrid, Spain). After gentle stirring for 5 min at 4 °C, samples were centrifuged at 10,000 × g for 10 min at 4 °C (Beckman Coulter, Brea, CA, USA) and an aliquot of the supernatant (500 μL) was mixed with 300 μL 13.0 mM DTT-200 mM Na₂PO₄ pH 7 buffer (Sigma-Aldrich, Madrid, Spain) and 150 μL 2.5 mM K₂HPO₄ (Sigma-Aldrich, Madrid, Spain). Reaction was kept in the darkness at room temperature for 10 min and then stopped by addition of 300 μL 2.0 M H₃PO₄. Samples were diluted 1:10 (v/v) in mobile phase (0.01 mM H₃PO₄) and filtered through 0.2 μm nylon membrane.

The samples (5 μL) were injected into a HPLC Agilent 1100 system equipped with a quaternary pump, ASL, and DAD detector (Agilent Technologies, Palo Alto, CA, USA). Separation was performed in a 3.0 mm id × 150 mm Zorbax SBAq (Agilent Technologies, Palo Alto, CA, USA) at a flow rate of 0.45 ml min⁻¹ and the quantification was done at 244 nm according to a standard curve of ascorbic acid (0-340 μM ascorbic acid, purity ≥ 98%, Sigma-Aldrich, Madrid, Spain).

2.5. Analysis of phenolic compounds

Phenolic acids and flavonoids were analyzed according to the method described by Ribas-Agustí et al. (2012). Briefly, 20.0 g of cold methanol were added to 5.0 g of frozen minced tomato and the mixture was sonicated (42 kHz) for 15 min in an ice-water bath (J. P. Selecta, Abrera, Spain). Samples were centrifuged at $17,400 \times g$ for 10 min at 4 °C (Beckman Coulter, Brea, CA, USA) and aliquots of 7.0 g of the methanolic extract were evaporated until dryness under N₂ flux. Samples were dissolved in 1 mL mobile phase A (water–methanol–phosphoric acid 94.6:5.0:0.4 v/v/v, pH 2.5) and filtered through 0.2 μm PTFE membrane and injected (80 μL) into the chromatographic system. Separation and quantification was performed in a Waters 1525 HPLC-DAD system equipped with 4.6 mm id × 150 mm RP₁₈ BEH column (Waters, Milford, MA, USA), using a linear gradient (30% B to 55% B in 45 min at 1.1 mL min⁻¹ flow rate) of mobile phase B (methanol–phosphoric acid 99.98:0.02 v/v, pH 2.5) and mobile phase A. The DAD acquired in the range of 210–450 nm and peaks were identified according to retention times and UV spectra of pure commercial standards: 5-caffeoylquinic acid (Sigma–Aldrich, Madrid, Spain), quercetin-3-O-rutinoside (Sigma–Aldrich, Madrid, Spain), kaempferol-3-O-rutinoside (Extrasynthèse, Genay, France), naringenin (Sigma–Aldrich, Madrid, Spain) and naringenin chalcone (Apin, Abingdon, UK).

UHPLC-DAD-MS/MS experiments were also carried out to support the tentative identification of dicaffeoylquinic acids I and II, tricaffeoylquinic acid and quercetin trisaccharide. The system consisted of an Acquity UPLC (Waters, Milford, MA, USA) with DAD set at the 210–150 nm range and a triple quadrupole mass spectrometer with electrospray ionization (ESI-TQD). The separation was carried out using an Acquity Shield RP₁₈ BEH column (1.0 mm id × 150 mm) and a linear gradient elution from 100% mobile phase A (water–acetonitrile–formic acid 94.9:5.0:0.1 v/v/v) to 28% mobile phase B (water–acetonitrile–formic acid 39.9:60.0:0.1, v/v/v) in 25 min, at a flow rate of 0.130 ml min⁻¹. The ESI-TQD operated in negative mode and experiments were performed in scan mode (MS) and product ion scan mode (MS/MS), obtaining the *m/z*⁻ values of parent ions and their fragmentation patterns.

Levels of carotenoids in tomatoes expressed as mg kg⁻¹ fw in relation to agronomic procedures (means of 8 replicates).

Variety	Ripeness	Elicitor	N dose	Lutein	Lycopene	β-Carotene	Phytoene	Phytofluene	Sum of carotenoids
Caramba	5	Sulfur	Low	2.47	3.24	2.62	0.64	0.08	9.05
			Standard	2.88	3.61	3.77	0.80	0.13	11.19
	10	Sulfur	Low	2.09	2.83	3.34	0.54	0.03	8.83
			Standard	1.70	4.31	3.14	0.85	0.09	10.09
		Milsana	Low	1.62	48.63	7.66	6.69	1.29	65.89
			Standard	1.81	34.49	6.53	7.80	1.71	52.34
Montserrat	5	Sulfur	Low	4.51	9.99	5.79	2.37	0.31	22.97
			Standard	3.57	8.35	5.38	2.10	0.27	19.67
	10	Sulfur	Low	2.72	6.12	5.10	1.24	0.24	15.42
			Standard	3.59	6.89	4.05	1.48	0.19	16.20
		Milsana	Low	2.79	37.06	5.35	6.44	0.65	52.29
			Standard	2.08	44.04	6.31	8.61	1.01	62.05
Pera-Girona	5	Sulfur	Low	2.48	11.69	2.88	3.04	0.43	20.52
			Standard	2.00	10.06	2.68	2.41	0.41	17.56
	10	Sulfur	Low	2.28	10.04	3.00	2.40	0.45	18.17
			Standard	2.99	9.73	3.78	1.72	0.30	18.52
		Milsana	Low	1.40	59.58	4.24	12.08	2.09	79.39
			Standard	1.71	65.78	5.10	12.05	1.60	86.24
Milsana	Low	2.44	33.95	3.54	7.21	1.25	48.39		
	Standard	2.09	40.26	4.31	7.94	1.14	55.74		

Quantification was made by external calibration with commercial pure standards when available, or as equivalents of the most similar standard (5-caffeoylquinic acid for dicaffeoylquinic acids I and II and tricaffeoylquinic acid; quercetin-3-O-rutinoside for quercetin trisaccharide). All solvents were of HPLC grade (J.T. Baker, Deventer, The Netherlands). Limits of detection (LOD) and quantitation (LOQ) were calculated on the basis of the calibration curves as the concentration corresponding to signal to noise (*s/n*) of 3 and 10, respectively.

2.6. Statistical analysis

The data were subjected to MANOVA using the General Linear Model procedure in SPSS (release 18) to identify significant treatment effects and interactions: the agronomic parameters (variety, ripening stage, elicitor and N dose) were set as independent variables and contents of nutritional compounds (carotenoids, minerals, vitamin C and phenolic compounds) as dependent variables. If significant effects were found (*p* < 0.05), differences between variables were checked by LSD post hoc test.

3. Results and discussion

3.1. Carotenoid levels

Several studies have investigated the carotenoid levels in raw tomatoes of different varieties, reporting a high range of contents (Khachik et al., 1992; Hart and Scott, 1995). The concentrations of lutein, β-carotene, lycopene, phytoene and phytofluene in tomatoes here reported are in line with data of literature, despite comparison is often hard because of the great influence of agronomic factors such as varieties and harvest time (Table 1). As expected, ripening process, linked to a noticeable change in fruit pigmentation, increased carotenoid levels (*p* < 0.001): on the average, lycopene was 6-times (42.69 mg kg⁻¹ fw vs. 7.23 mg kg⁻¹ fw), β-carotene 1.5-times (5.63 mg kg⁻¹ fw vs. 3.8 mg kg⁻¹ fw), phytoene 5-times (7.77 mg kg⁻¹ fw vs. 1.63 mg kg⁻¹ fw) and phytofluene 6-times (1.29 mg kg⁻¹ fw vs. 0.2 mg kg⁻¹ fw) higher in ripening stage 10 with respect to 5. Only lutein concentration showed an opposite trend decreasing its concentration in fruit along with ripening

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Table 2
 Values of *F* and significance for MANOVA of agronomic factors effects (variety, ripeness, elicitor and N dose and their main interactions) on lutein, β -carotene, lycopene, phytoene and phytofluene contents.

	df	Lutein	β -Carotene	Lycopene	Phytoene	Phytofluene	Sum of carotenoids
Variety V	2	18.988 c	32.007 c	5.868 b	12.885 c	7.815 c	5.023 b
Ripeness R	1	29.552 c	87.796 c	275.275 c	220.088 c	177.223 c	279.038 c
Elicitor E	1	1.754	1.338	8.266 b	12.018 c	2.527	9.708 b
N-dose N	1	0.189	0.751	1.160	2.547	1.273	1.462
V \times R	2	1.387	12.296 c	1.857	2.498	7.440 c	2.371
V \times E	2	5.943 b	0.567	2.962	1.894	2.081	2.163
R \times E	1	2.084	0.320	5.461 a	4.822 a	0.803	5.016 a
V \times N	2	0.925	0.957	1.058	1.106	3.140 a	0.759
R \times N	1	0.495	0.664	1.346	3.722	2.093	1.735

a = $p \leq 0.05$; b = $p \leq 0.01$; c = $p \leq 0.001$.

process. This result, in accordance with the study of Fraser et al. (1994), could be due to inhibition of enzymes catalyzing xanthophylls synthesis in chromoplasts.

In addition to fruit ripeness, tomato variety exerted a significant effects on carotenoid levels as well as the interaction between those two factors (Table 2) that significantly affected β -carotene and phytofluene ($p < 0.001$). In particular, total carotenoid levels in Pera-Girona variety at the ripening stage 10 resulted significantly higher than the other varieties at the same ripeness ($p < 0.05$) (67.43 mg kg⁻¹ fw Pera-Girona vs. 54.31 and 56.34 for Montserrat and Caramba, respectively), while a trend toward higher levels of total carotenoids at ripening stage 5 in local varieties than in commercial one was found ($p = 0.06$) (Fig. 1) (18.69 and 18.46 vs. 9.78 for Pera-Girona and Montserrat vs. Caramba, respectively). These findings underline the relevance of the agronomic choices to achieve vegetable production with high nutritional value for human nutrition.

The increasing environmentally friendly behavior of consumers leads to the need for testing new approaches against powdery mildew that could be used as alternatives to fungicides and as a valid control measure in low input/organic tomato production system. In the present study, efficacy of Milsana[®] was compared to sulfur treatment as plant disease controller, with the expectation to improve or at least to maintain the nutritional value of fruits. The effect of these treatments was significant (Table 2), however Milsana[®] negatively influenced levels of total carotenoids (33.3 mg kg⁻¹ Milsana[®] vs. 41.6 sulfur; $p < 0.01$, expressed as mean level) and specifically decreased levels of lycopene (21.7 mg kg⁻¹ Milsana[®] vs. 28.0 sulfur, $p < 0.01$) and phytoene (4.0 mg kg⁻¹ Milsana[®] vs. 5.4 sulfur, $p < 0.001$). Finally, in

accordance with Benard et al. (2009), no significant effect was observed in relation to nitrogen level of nutrient solutions.

3.2. Ascorbic acid and polyphenol levels

The ingestion of a serving size (~200 g) of fresh tomatoes provides about 30% and 36% of the recommended dietary allowances (RDA) for vitamin C for men and women, respectively. Levels of ascorbic acid found in the present study are consistent with some data reported in literature (Gautier et al., 2008), but higher than others (Anza et al., 2006; Mohammed et al., 2011). This disagreement could be due to different experimental variables such as tomato varieties and/or climatic conditions. Vitamin C content was greatly influenced by varieties (Table 3): in ascending order Pera Girona (274.9 mg kg⁻¹ fw, expressed as mean level) < Montserrat (298.4 mg kg⁻¹ fw) < Caramba (341.5 mg kg⁻¹ fw). Significant effect was also exerted by ripeness and level of N in nutritional solution (Table 4), although they were negatively correlated to vitamin C (ripeness 5: 316.6 mg kg⁻¹ fw vs. ripeness 10: 293.3 mg kg⁻¹ fw; low N-dose: 309.9 vs. standard N-dose: 300.1 mg kg⁻¹ fw, $p < 0.05$, as mean of all data). Consistent with our results, other studies also found slight increase in the ascorbate values when N levels were lowered in the nutrient solution (Dumas et al., 2003; Benard et al., 2009); this is probably attributable to a higher foliage development with high N supply, which reduced light exposure to fruits and therefore could influence negatively the ascorbate levels. The effect of ripeness needs close examination: it is generally considered that vitamin C increases with ripening process in tomato fruit (Dumas et al., 2003; Gautier et al., 2008), but different behaviors have been reported regarding maturity stages. The maximum concentration of ascorbic acid was found in fruits that turned yellow-orange, while red-overred fruits show lower levels (Abushita et al., 1997; Ilahy et al., 2011), in accordance with our results. This trend has been related to the antioxidant function of vitamin C when the ripening cells absorb high amount of oxygen as a result of increasing rate of respiration. No effect of treatments for plant diseases was found on vitamin C levels.

The most abundant phenolic compounds in our samples were 5-caffeoylquinic acid (5-CaQA) followed by caffeoylquinic acids derivatives (diCaQA I and II; and triCaQA) (Table 3). 5-Caffeoylquinic acid has been detected as the main phenolic compound in some varieties, e.g. Daniella and cherry (Gautier et al., 2008; Vallverdú-Queralt et al., 2013), but not in others (Slimestad and Verheul, 2009; Barros et al., 2012), underlining the great influence of cultivars on this variable. Variety significantly affected phenolic levels (Table 4) ($p < 0.001$): Montserrat contained the highest amount of the above cited compounds (82.2, 3.1, 13.2 and 6.92 mg kg⁻¹ fw, respectively, expressed as mean level), and Caramba was particularly rich in quercetin derivatives (Q-Tris: 10.93; Q-Rut: 13.04 mg kg⁻¹ fw) and naringenin chalcone

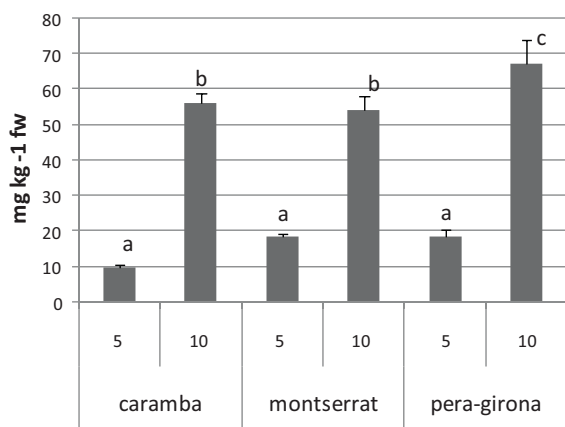


Fig. 1. Levels of total carotenoids (mg kg⁻¹ fresh weight) in relation to varieties and ripening stages (mean \pm SEM, $n = 32$). Data marked with different letters are significantly different ($p < 0.05$).

Table 3
 Levels of vitamin C and major phenolics in tomatoes expressed as mg kg⁻¹ fw in relation to the agronomic procedures (means of 8 replicates).

Variety	Ripeness	Elicitor	N dose	Vitamin C (mg kg ⁻¹)	5-CaQA ^a (mg kg ⁻¹)	DiCaQA I (mg kg ⁻¹)	DiCaQA II (mg kg ⁻¹)	TriCaQA (mg kg ⁻¹)	Q-Tris (mg kg ⁻¹)	Q-Rut (mg kg ⁻¹)	K-Rut (mg kg ⁻¹)	Nar (mg kg ⁻¹)	Nar chalc (mg kg ⁻¹)	
Caramba	5	Sulfur	Low	368.17	62.34	1.78	4.58	1.81	12.72	16.98	0.34	0.27	8.01	
			Standard	341.80	62.72	1.87	5.42	2.02	11.65	21.04	0.40	0.49	15.69	
		Milsana	Low	371.92	62.33	1.72	4.64	1.83	10.93	16.29	0.32	0.32	10.12	
			Standard	316.71	62.20	1.55	4.17	1.58	9.51	11.69	0.20	0.21	6.05	
		10	Sulfur	Low	362.93	27.39	2.12	4.10	2.17	11.51	10.40	0.21	0.29	6.95
				Standard	332.60	28.62	2.01	4.10	2.16	10.98	10.69	0.22	0.28	6.94
	Milsana		Low	312.28	29.97	2.29	4.40	2.58	10.30	9.20	0.19	0.21	6.98	
			Standard	326.00	31.03	2.15	4.37	2.15	9.89	8.04	0.16	0.39	8.50	
	Montserrat	5	Sulfur	Low	312.62	90.58	2.31	9.53	3.75	2.29	1.70	<LOQ	<LOQ	<LOQ
				Standard	311.85	107.16	3.57	15.19	7.02	2.44	2.32	<LOQ	<LOQ	<LOQ
			Milsana	Low	307.67	95.67	2.68	11.78	4.77	3.45	2.22	<LOQ	<LOQ	<LOQ
				Standard	322.45	105.65	2.71	12.93	5.40	3.41	2.22	<LOQ	<LOQ	<LOQ
10			Sulfur	Low	281.68	74.98	3.82	16.28	9.85	1.13	2.04	<LOQ	<LOQ	<LOQ
				Standard	271.88	46.21	2.77	8.78	5.11	<LOQ ^b	0.83	<LOQ	<LOQ	<LOQ
		Milsana	Low	293.87	65.26	3.44	14.57	9.18	0.79	1.34	<LOQ	<LOQ	<LOQ	
			Standard	285.08	72.37	3.77	16.82	10.26	1.42	2.23	<LOQ	<LOQ	<LOQ	
Pera-Girona		5	Sulfur	Low	282.51	69.09	1.81	7.82	3.12	3.23	3.30	0.15	<LOQ	<LOQ
				Standard	277.61	68.38	2.12	9.80	4.15	2.37	1.76	<LOQ	<LOQ	<LOQ
			Milsana	Low	308.34	76.36	1.54	7.21	2.40	3.17	3.88	0.16	<LOQ	<LOQ
				Standard	277.49	55.78	1.61	5.84	2.49	1.28	1.21	<LOQ	<LOQ	<LOQ
	10		Sulfur	Low	267.00	45.37	2.88	10.69	7.43	2.43	4.38	0.23	<LOQ	<LOQ
				Standard	268.94	37.29	2.11	7.63	4.12	0.65	0.90	<LOQ	<LOQ	<LOQ
		Milsana	Low	249.29	51.94	2.69	10.14	5.82	2.08	3.74	0.23	<LOQ	0.09	
			Standard	268.65	39.27	2.13	7.22	3.76	<LOQ	0.86	<LOQ	<LOQ	<LOQ	

^a 5-CaQA=5-caffeoylquinic acid (chlorogenic acid); DiCaQA I=dicaffeoylquinic acid I; DiCaQA II=dicaffeoylquinic acid II; TriCaQA=tricaffeoylquinic acid; Q-Tris=quercetin trisaccharide; Q-Rut=quercetin-3-O-rutinoside; K-Rut=kaempferol-3-O-rutinoside; Nar=naringenin; Nar Chalc=naringenin chalcone.

^b LOQ. Q-Tris = 0.076 mg kg⁻¹; K-Rut = 0.055 mg kg⁻¹; Nar = 0.025 mg kg⁻¹; Nar chalc = 0.090 mg kg⁻¹.

(Nar chalc: 8.66 mg kg⁻¹ fw), while containing detectable amounts also of kaempferol-3-O-rutinoside (K-rut: 0.26 mg kg⁻¹ fw) and naringenin (Nar: 0.31 mg kg⁻¹ fw). The majority of phenols were influenced by ripening stage: green-orange stage had high levels of Q-Rut, Nar chalc and 5-CaQA that decreased with ripeness process (ripeness 5: 76.5 mg kg⁻¹ fw vs. ripeness 10: 45.8 mg kg⁻¹ fw; $p < 0.05$) in contrast to caffeoylquinic acid derivatives that increased in red fruits, in accordance with data reported in another study (Gautier et al., 2008). In the present study, we observed a trend toward increases of phenolic compounds in tomato treated with low N-dose in nutrient solution, although it resulted significant only for K-Rut and 5-CaQA in Pera-Girona variety (Table 4). A similar slight effect of low nitrogen supply on phenolics was also found in other studies (Stewart et al., 2001; Benard et al., 2009). On the contrary, more important effect of low N dose was found on phenolic compounds in tomato leaves, in which low N supply has been reported to induce enzymes of phenolic synthesis pathway (Bongue-Bartlsman and Phillips, 1995). The difference, linked to the plant tissue, is probable due to the highly protected structure of fruit. Negligible effect on

Table 4
 Values of F and significance for MANOVA of agronomic factors effects (variety, ripeness, elicitor and N dose and their main interactions) on major phenolic compounds.

	df	Vitamin C	5-CaQA ^a	DiCaQA I	DiCaQA II	TriCaQA	Q-Tris	Q-Rut	K-Rut	Nar	Nar chalc
Variety V	2	100.60 c	127.11 c	51.16 c	151.45 c	72.21 c	127.10 c	156.24 c	65.18 c	83.82 c	278.61 c
Ripeness R	1	36.61 c	237.44 c	44.29 c	4.51 a	30.84 c	1.19	19.43 c	1.28	1.83	6.45 a
Elicitor E	1	0.77	0.06	0.01	0.00	0.25	3.20	4.21 a	2.86	0.00	1.97
N-dose N	1	6.43 a	3.95 a	1.26	0.38	0.67	0.37	3.04	13.73 c	3.24	1.59
V × R	2	1.18	3.88 a	0.07	2.67	2.88	2.70	15.28 c	6.44 b	0.53	6.50 b
V × E	2	4.67 b	0.05	1.83	4.66 a	1.98	4.20 a	4.49 a	1.50	1.46	2.12
R × E	1	1.66	5.17 a	7.54 b	5.79 a	6.21 a	0.93	0.67	0.11	0.07	5.02 a
V × N	2	3.71 a	2.13	2.04	1.51	2.39	1.75	2.00	13.51 c	0.94	1.52
R × N	1	3.73 a	1.95	17.13 c	15.20 c	18.62 c	0.79	0.33	0.82	0.28	0.30
E × N	1	0.27	0.19	0.68	0.04	0.12	0.70	1.97	0.23	0.07	6.21 a

a = $p \leq 0.05$; b = $p \leq 0.01$; c = $p \leq 0.001$.

^a 5-CaQA=5-caffeoylquinic acid (chlorogenic acid); DiCaQA I=dicaffeoylquinic acid I; DiCaQA II=dicaffeoylquinic acid II; TriCaQA=tricaffeoylquinic acid; Q-Tris=quercetin trisaccharide; Q-Rut=quercetin-3-O-rutinoside; K-Rut=kaempferol-3-O-rutinoside; Nar=naringenin; Nar chalc=naringenin chalcone.

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Table 5
 Levels of minerals in tomatoes expressed as mg kg⁻¹ fw in relation to agronomic procedures (means of 8 replicates).

Variety	Ripeness	Elicitor	N dose	Fe	Ca	Mg	Cu	Zn	K	Na	P
Caramba	5	Sulfur	Low	3.43	38.58	97.3	1.27	1.31	2370	65.9	132.9
			Standard	4.51	39.81	111.0	1.41	1.73	2465	85.3	175.7
	10	Milsana	Low	4.70	34.23	112.6	1.28	1.80	2494	53.6	156.1
			Standard	4.79	38.65	95.2	1.39	1.35	2309	44.5	132.9
		Sulfur	Low	3.63	56.28	98.1	1.12	1.16	2284	38.8	118.6
			Standard	4.38	57.09	94.9	1.03	1.14	1838	50.8	117.6
Montserrat	5	Sulfur	Low	5.37	45.19	93.8	1.65	1.78	2400	59.7	162.8
			Standard	5.42	41.23	87.8	2.26	1.72	2135	61.5	133.8
	10	Milsana	Low	4.55	37.74	90.2	1.66	1.76	2358	74.8	151.6
			Standard	4.99	43.59	90.3	1.95	1.55	2249	55.0	122.6
		Sulfur	Low	4.44	49.93	89.8	1.29	1.57	2029	47.6	145.8
			Standard	5.94	59.04	105.6	1.49	1.47	2352	47.2	150.8
Pera-Girona	5	Sulfur	Low	4.69	44.93	109.7	1.55	1.55	2426	40.9	131.4
			Standard	4.61	51.84	107.6	1.16	1.23	2222	53.3	107.1
	10	Milsana	Low	4.25	43.19	96.5	1.09	1.23	2236	42.8	104.8
			Standard	3.64	51.56	129.9	1.34	1.84	2968	77.0	169.0
		Sulfur	Low	4.01	61.93	101.1	0.99	1.06	2030	24.2	117.1
			Standard	5.93	65.95	120.1	1.41	1.33	2393	47.8	132.7
Milsana	Low	4.46	43.98	111.1	0.94	1.33	2595	45.3	111.0		
	Standard	4.09	53.13	108.2	1.47	1.28	2423	54.4	127.0		

Table 6
 Values of *F* and significance for MANOVA of agronomic factors effects (variety, ripeness, elicitor and N dose and their main interactions) on mineral contents.

	df	Fe	Ca	Mg	Cu	Zn	K	Na	P
Variety V	2	13.977 c	2.668	13.516 c	6.905 c	12.722 c	2.944	4.991 b	5.211 b
Ripeness R	1	0.437	29.956 c	0.092	9.711 b	19.454 c	7.417 b	27.863 c	4.127 a
Elicitor E	1	1.130	5.146 a	0.001	0.650	0.983	3.600	0.156	0.123
N-dose N	1	12.048 c	5.643 a	4.740 a	4.141 a	0.013	0.196	5.399 a	0.624
V × E	2	6.973 c	0.852	0.096	0.109	0.395	1.839	13.396 c	0.488
V × N	2	1.052	0.363	1.504	0.126	1.864	1.453	10.893 c	3.611 a
E × N	1	7.135 b	0.978	0.024	0.269	0.462	0.650	3.833	0.256
V × R	2	1.022	2.577	2.165	1.944	2.302	1.590	2.507	4.663 a
R × E	1	0.084	1.709	0.202	0.049	0.157	0.065	2.430	0.024
R × N	1	5.171 a	0.401	0.691	0.101	0.008	0.084	0.007	0.550

a = $p \leq 0.05$; b = $p \leq 0.01$; c = $p \leq 0.001$.

conditions largely affect mineral levels: once again, variety and ripening stage appear as the more important factors, although to a minor extent than for carotenoids (Table 6). Average data show that Montserrat cultivar contains higher levels of trace elements and P than the other two cultivars suggesting a direct correlation between these elements, further supported by the analysis of Pearson's coefficient (Table 7). Accordingly, red fruits displayed lower levels of P, but also of Cu and Zn, with respect to orange fruit ($p < 0.05$). Notwithstanding, the interaction between variety and ripeness exerted a significant effect only on P level (Table 6). Minerals were also influenced by N dose in nutrient solutions: standard N dose significantly increased fruit mean contents of Fe with respect to low N

Table 7
 Pearson's correlation coefficient and significant levels among levels of analyzed minerals ($n = 196$).

	Ca	Mg	Cu	Zn	K	Na	P
Fe	0.256 c	0.437 c	0.456 c	0.514 c	0.360 c	0.192 b	0.337 c
Ca		0.369 c	0.105	0.018	0.171 a	-0.077	-0.003
Mg			0.231 c	0.474 c	0.814 c	0.374 c	0.458 c
Cu				0.420 c	0.290 c	0.280 c	0.181 a
Zn					0.660 c	0.669 c	0.662 c
K						0.539 c	0.578 c
Na							0.583 c

a = $p \leq 0.05$; b = $p \leq 0.01$; c = $p \leq 0.001$.

dose (4.9 mg kg⁻¹ fw vs. 4.4 mg kg⁻¹ fw, respectively, $p < 0.001$), Ca (51.2 vs. 45.9, $p < 0.05$) and Mg (104.4 vs. 98.4, $p < 0.05$). Treatments for controlling plant diseases significantly affected only Ca concentration ($p < 0.05$).

As above mentioned, Pearson's correlation coefficients among analyzed minerals in tomatoes show in particular that P was positively correlated with all the other minerals, with the only exclusion of Ca, and that levels of trace minerals, such as Fe, Cu and Zn, were significantly correlated each other (Table 7).

4. Conclusion

All crop management factors evaluated in the present study affected levels of nutritional compounds to some degree, sometimes with negative results in relation to specific compounds as in the case of ripeness with respect to phenolics. In addition to the effect of a single agronomic parameter, often their interaction affects the nutrient contents of tomatoes. Varieties and ripening stage were the most important factors affecting carotenoids, minerals, vitamin C and phenolic compounds. Low N dose in fertilizer solution seems to exert a positive effect on vitamin C and some phenolics, while the use of elicitor Milsana[®] does not appear to be a suitable strategy to positively influence the nutritional quality of tomato. Nevertheless, Milsana[®] should be considered as a factor for effective control of powdery mildew in greenhouse-grown tomatoes in order to

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improve the sustainability of agricultural practices and limit environmental pollution or farmer risks.

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