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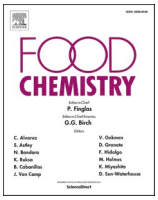

Influence of year, genotype and cultivation system on nutritional values and bioactive compounds in tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Two tomato genotypes were grown in open field by three cultivation systems (one conventional and two distinct organic for mulching) in three years, 2015, 2016 and 2017. Yields, sugars, organic acids, amino acids, ascorbic acid, biothiols, carotenoids and phenols were measured.

Weather conditions largely differed among harvest years, with summer 2016 rainier and less warm, and an opposite summer 2017.

Organic systems had lower yields than conventional one but also, interestingly, lower waste percentages. Furthermore, tilled and no-tilled organic systems provided comparable yields.

With respect to 3-year average, sugars were higher in 2017, acids in 2016 and in organic fruits, and amino acids increased in 2015 and in conventional samples.

A higher glutathione content was found in organic samples, and higher carotenoids in 2017. Phenols increased in 2016, with a higher chlorogenic acid content in organic tomatoes.

Some differences between genotypes were observed, highlighting their different adaptability to growing systems.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated horticultural crops all over the world with more than 180 million tons of harvested fruits for fresh and processing market, on around 5.0 million hectares of cultivated area (FAOSTAT, 2019), with a wide range of cultivars adapted to different cultivation conditions, in different climate zones, in open field as well as in greenhouse. According to the final use of this crop, also for the wide germplasm available, some requirements have been often established, mainly for the shape and the color of the berries, as well as the soluble solids content (4.5 °Bx for round tomato, 8.0 °Bx in cherry tomatoes) and the pH of the juice (minimum 4.5 for processing tomatoes). The main quality indexes of tomato berry were recently reviewed (Paolo et al., 2018), showing that free sugars, organic acids, amino acids and volatiles were indicators of its taste characters, attributing its typical flavor. Moreover, tomato shows a noteworthy nutritional value, due to its significant content of phytochemicals with antioxidant action: ascorbic acid, glutathione and phenols as hydrosoluble ones, and carotenoids, especially lycopene, as the main liposoluble one.

As for tastant compounds, the berries of tomato are typically characterized, besides the well noted soluble sugars and organic acids, by high levels of free amino acids, such as glutamic acid, aspartic acid and glutamine, as well as a cyclized acid derivative of glutamine, namely pyroglutamic acid, known as a marker of tomato processing, but also present in fresh fruits (Paolo et al., 2018).

As for antioxidant compounds, among hydrosoluble ones, glutathione and other biothiols are remarkably present in tomato, with

Abbreviations: AM, artificial mulching; AsA, ascorbic acid; β-CAR, β-carotene; C, cultivation system; CONV, conventional; CYS, cysteine; DHA, dehydroascorbic acid; FBBB, Fast Blue BB; F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; GSH, reduced glutathione; GSSG, oxidized glutathione; NM, natural mulching; ORG, organic; PERBR, "Perbruzzo F1"; RT, retention time(s); SAAB, "SAAB-CRA"; SSC, soluble solids content; *t*-LYC, all-*trans*-lycopene; TPC, total polyphenol content; TTA, total titratable acidity; V, variety; Y, harvesting year.

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respect to other fruit and vegetable products (Demirkol et al., 2004), while, for liposoluble ones, tomato raw fruit is well known as the most important food source of all-*trans*-lycopene (Collins et al., 2006). Moreover, soluble phenolic antioxidants are present in tomato, with chlorogenic acid, quercetin glycosides and naringenin-chalcone as the main identified compounds, especially present in the skin (Tamasi et al., 2019).

The levels of these quality indicators are often subjected to significant variations, especially depending on genetic and environmental factors. Among others, the weather change in different sampling years and the type of cultivation play a special role in the quality variation for this crop (Chassy et al., 2006; Mitchell et al., 2007), showing interesting future perspectives for the yield improvement, for the common opinion that attributes a lower agronomic yield to organic cultivations (Li et al., 2019).

Nowadays, attention is being devoted to the impact of environmental changes on tomato crop, highlighting the big theme of growing management sustainability, studying the influence of the so-called "organic" methodologies, touching multi-disciplinary aspects, suggesting that, with a proper life-cycle assessment approach, the organic production of tomato should be encouraged (He et al., 2016).

The differences in cropping management on growing conditions and nutrient availability for conventional and organic systems affect the plant physiological status (Orsini et al., 2016) with a different exposure to abiotic and biotic stresses. Moreover, these differences influence the balance between growth and differentiation, where the generally accepted theory of carbon/nitrogen balance is a special case (Brandt & Mølgaard, 2001; Rembialkowska, 2007). This theory states that, in a situation of a low nitrogen availability, as can occur in organic systems for the absence of chemical fertilization, the synthesis of carbon compounds with low nitrogen presence is enhanced, so explaining the tendency of a higher content of secondary metabolites in organic crops, compared to conventional ones, as stated by a deep meta-analysis study (Barański et al., 2014). However, conclusive data are still far to be obtained, being influenced by a wide number of added variables, such as cultivated genotypes, sampling time and environmental changes.

Hence, in the present work a specific survey on tomato, within an open-field long-term study on organic horticulture, is presented. It has been performed at the experimental fields of CREA in Monsampolo del Tronto (Ascoli Piceno, Italy), based on a 4-year rotation crop planning (Campanelli & Canali, 2012) and it was focused on production and agronomic aspects with an agro-ecological cultivation approach. In this context, a three-years study on the changes of quality indexes in two tomato cultivars was developed with three different growing methods, one conventional with artificial mulching, and two organic with artificial and natural mulching. The evaluation of agronomic, food and health-related aspects was carried out: marketable and waste yields, dry matter, sugar, acid and amino acid contents, as well as ascorbic acid, thiols, phenols and carotenoids were examined for these points, respectively.

2. Materials and methods

2.1. Cultivation system and plant material.

Two tomato (*Solanum lycopersicum* L.) varieties were chosen: "Perbruzzo F1", a commercial hybrid of Four Sementi seed company (hereafter, PERBR) and "SAAB-CRA" (hereafter, SAAB) a local variety of CREA Research Centre for Vegetable and Ornamental Crops selected with participatory approach by researcher and farmers. These varieties, that belong to the "pear" typology, are of indeterminate growth and are consumed both fresh and processed, especially as puree. They are commonly cultivated in Central-East Italy and were grown in three following years (2015, 2016 and 2017) at two adjacent experimental fields of CREA in Monsampolo del Tronto (42° 53' N, 13° 48' E; 158 m a.s.l.), in the coastal area of the Marche Region, Central Italy.

One field was managed according to organic (ORG) and the other according to conventional (CONV) farming methods. The organically managed field was based on a 4-year crop rotation with different vegetable cash crops, belonging to six botanical families: *Solanaceae*, *Cucurbitaceae*, *Apiaceae*, *Asteraceae*, *Brassicaceae* and *Fabaceae*. Three cover crops were also included in the rotation, belonging to *Poaceae*, *Brassicaceae* and *Fabaceae* (Campanelli & Canali, 2012). The ORG and CONV soils had the following characteristics: loam texture according USDA classification, clay 20%, silt 30%, and sand 50%; pH 7.8. Organic matter contents were 1.1% and 1.6%, for CONV and ORG soils, respectively. The water supplied through drip irrigation and rainfall, respectively, was: 2900 m^3/ha and 1700 m^3/ha, in 2015; 2300 and 2700 m^3/ha, in 2016; 3600 and 570 m^3/ha, in 2017.

In organic management, soils were covered with hairy vetch (*Vicia villosa* R.) up to full flowering stage, and then were artificially or naturally mulched. With artificial mulching (ORG AM), vetch was chopped and soil was tilled, harrowed and then artificially mulched with Mater Bi. With natural mulching (ORG NM), vetch was flattened by roller crimper and soil was simply furrowed (Canali et al., 2013), so lodged vetch acted as natural mulching. In conventional integrated management, vetch was not grown and soil was directly tilled, harrowed and only artificially mulched with Mater Bi (CONV AM) according to local farmer cultivation method. The experiment design was a randomized strip-plot with three replications for both ORG theses, and a randomized block design with three replications for the CONV thesis located in a neighboring field.

After mulching at the end of April-early May, tomato seedlings were transplanted in early May, in coupled rows with a density of 2.5 plants/m^2 (40 plants/plot). Micro-flow dispensing irrigation was performed with a perforated hose.

In the CONV field, the rate of N-P_2O_5-K_2O fertilization was 160-170-200 kg/ha, applied as 1000 kg/ha of granular complex (12-12-17 + 2 MgO + 20 SO_3) before plant transplanting, and the remaining dose provided with fertigation. In the ORG field, N-P_2O_5-K_2O fertilization was applied at the rate of 128-30-13 kg/ha, with 2600 kg/ha of animal pellet (3-0-0) before plant transplanting, 500 kg/ha of organic pellet (7-6-1) based on dried fungal mycelium 20 days after transplanting, and the remaining rate provided with fertigation.

Fruits were harvested in the commercial red stage, in early August, from 24 plants/plot, to avoid border effects. Fruits of the same management system and harvested in the same experimental plot (=replication) were pooled, so that three biological replicates per variety and cultivation were made up, and 18 samples per year were obtained (2 varieties × 3 cultivation systems × 3 replicates).

All the tomato plants from each plot were used to determine the marketable yield and the waste yield, expressed both as fruit number per plant and fruit weight (kg) per plant. For each sample, 10 kg of marketable fruits were randomly chosen for the analyses, immediately stored at 4 °C and sent to CREA laboratories in Milan.

Fruits were washed in running water, dried with paper, and sliced in 4–8 slices, then samples of about 1 kg of slices were randomly made up and rapidly frozen at −50 °C in an air-forced tunnel.

Successively, an aliquot of each sample was stored frozen in polyethylene bags at −20 °C, while another one was freeze dried until constant weight, used to calculate the dry weight (dw) content of the products, expressed in g/100 g fresh weight (fw). The freeze-dried materials were reduced in powder (5–10 mesh) in a food processor at 4–6 °C and stored in dark bottles at −20 °C until analyses.

2.2. Weather monitoring

During the three years of study, the daily mean temperature and the rainfall were registered by a nearby weather station associated with the agro-meteorological national database (https://cma.entecra.it/homePage.htm). The data were processed as monthly mean temperatures and monthly total rainfalls and were compared with the respective

historical averages over thirty years (1971–2000).

2.3. Chemicals and HPLC equipment

Tris(2-carboxyethyl)phosphine and *tert*-butylmethylether were from Acros Organics (Geel, Belgium). Heptane-1-sulfonic acid sodium salt was from Alfa Aesar (Kandel, Germany). Acetonitrile was from VWR-BDH Chem. (Milan, Italy). Other chemicals used for extractions and analyses, and standards of sugars, acids, amino acids, thiols, carotenoids, rutin and chlorogenic acid were purchased from Sigma-Aldrich (Milan, Italy). Reagents were of analytical grade, whereas chromatographic solvents and standards of secondary metabolites were HPLC grade.

Chromatographic analyses were performed by Jasco HPLC systems (Jasco-Europe, Lecco, Italy). For sugars, organic acids, amino acids and thiols, HPLC system was equipped with a PU-1580 pump and a AS-2055 Plus Intelligent sampler, bearing different columns and detectors (Jasco RI930 and Jasco UV1570 for refractive index and UV detection, respectively). For carotenoids and polyphenols, HPLC-DAD system was equipped with a PU-980 pump, a LG-1580–02 ternary gradient unit, a AS-2055 Plus Intelligent sampler and a MD-910 multi-channel detector.

2.4. Common quality parameters

Soluble solids content (SSC), pH, and total titratable acidity (TTA) were measured on tomato freeze-dried powder using 2 g of powder suspended in 25 mL of deionized water. The mixture was stirred, decanted and the supernatants were used for the analyses. SSC was measured using a Multi-Scale refractometer RFM 91 (Bellingham-Stanley Ltd, Tunbridge Wells, UK), and it was expressed as °Bx/100 g dw. TTA and pH were determined with a Dosimat 665 apparatus (Metrohm, Herisau, Switzerland) and TTA, titrated by 0.1 N NaOH to pH 8.1, was expressed as milliequivalent (mEq) NaOH/100 g dw.

2.5. Analysis of simple sugars and organic acids

Sugars and acids were analyzed by HPLC, on an aqueous extract obtained by vortexing freeze dried powder and distilled water (1 g, 10 mL), centrifuging the mixture (10000g, 5 min at 4 °C) and filtrating on 0.45 μm nylon filter.

For soluble sugars (sucrose, glucose and fructose), extracts were analyzed using an Aminex HPX-87C column, 300 × 7.8 mm, 9 μm (Bio-Rad, Hercules, USA), at 85 °C with H_2O as mobile phase (0.6 mL/min) and a refractive index detector. The calibration was performed with commercial standard solutions at known concentrations, with the following retention times (RT): sucrose 7.7 min, glucose 9.4 min, fructose 12.1 min.

For organic acids (citric, malic, succinic, fumaric and *trans*-aconitic), extracts were analyzed using a Repromer H^+ column, 300 × 8 mm, 9 μm (Dr. Maisch, Ammerbuch-Entringen, Germany) at 50 °C, 3 mM H_2SO_4 as mobile phase at a flow of 0.6 mL/min, with UV detection at 214 nm. Solutions of commercial standards at known concentration were used for calibration. In these conditions, RT were: citric 7.6 min, malic 9.1 min, *trans*-aconitic 9.9 min, succinic 11.2 min and fumaric 15.8 min.

Results of sugars and acids were given as g/100 g dw.

2.6. Analysis of amino acids

An HILIC-HPLC separation without pre-column derivatization was used for free amino acids in tomato extracts, according to the method described by Bhandare et al. (2010). Tomato powder (0.5 g) was extracted with10 mL of 0.1 M HCl solution. The injection of the filtered extract was performed at 45 °C at 0.6 mL/min using a Hibar Lichrosorb Si-60 column, 150 × 4 mm, 10 μm (Merck, Darmstadt, Germany), with a mobile phase composed of 85% acetonitrile (99%)/15% aqueous solution containing 0.02 M H_3PO_4 and 3.6 mM KH_2PO_4, and UV detection at 210 nm. Calibration was made with solutions of commercial standards at known concentration.

Aspartic acid, glutamic acid and glutamine were analyzed with this method (RT 11.0 min, 11.8 min and 20.3 min, respectively), whereas the amino acid derivative pyroglutamic acid was analyzed with the organic acids method (see previous paragraph, RT 17.8 min), and results were given as mg/100 g dw.

2.7. Analysis of ascorbic and dehydroascorbic acid

The analysis of ascorbic acid (AsA) was performed by HPLC analysis. AsA was extracted at 4 °C in the dark with 6% metaphosphoric acid, in a ratio 10:1 solvent *vs* powder. After vortexing, the mixture was centrifuged at 25000*g* for 5 min at 4 °C and then filtered on a 0.45 μm filter before injection in the HPLC system. The column was an Inertsil C_{18} ODS-3, 250 × 4.6 mm, 5 μm (GL-Sciences, Tokyo, Japan), flow 0.75 mL/min at 45 °C, with 0.02 M H_3PO_4 as mobile phase, and UV detection at 254 nm. The RT of AsA was 6.2 min. The calibration was performed with commercial standard solutions at known concentrations of AsA, and results were given as mg/100 g dw. Vitamin C, given by the sum of AsA and its oxidized form dehydroascorbic acid (DHA), was obtained by the analysis of the same extract used for AsA, with the adding of a 10% solution of 0.1 M tris(2-carboxyethyl)phosphine dissolved in 0.5 M HCl, and 10 min of reaction at room temperature before HPLC injection, to allow DHA to be reduced to AsA.

2.8. Analysis of thiols

The extracts for thiol analysis were obtained with the same method used for ascorbic acid. The contents of reduced glutathione (GSH), oxidized glutathione (GSSG) and cysteine (CYS) were analyzed by HPLC with coulometric electrochemical detection (ESA mod. 6210, Chelmsford, USA), following the protocol by Yap et al. (2010), with slight modifications. The isocratic elution was carried out using 25 mM monobasic sodium phosphate containing 0.5 mM heptane-1-sulfonic acid sodium salt and 0.25% acetonitrile, adjusting the pH at 2.70 with 85% H_3PO_4. A flow rate of 0.6 mL/min was used with an Inertsil C_{18} ODS-3 column, 250 × 4.6 mm, 5 μm (GL-Sciences, Tokyo, Japan), at 37 °C. GSH and CYS were detected and quantified on the electrodes set at +600 mV, while GSSG at + 900 mV, with RT of 3.0 min, 3.8 min and 10.7 min, respectively. Calibration was made using known concentration of commercial standard compounds in a range between 0.005 and 0.05 mg/mL, and results were expressed as mg/100 g dw.

2.9. Analysis of carotenoids

The main carotenoids in tomato are all-*trans*-lycopene (*t*-LYC) and *β*-carotene (*β*-CAR): they were extracted in darkness at 0–1 °C from 10 g of frozen ground sample using 20 mL of cold hexane/acetone/ethyl acetate (2/1/1, v/v/v) solution added with 1 mg/mL of butylated hydroxytoluene. The mixture was homogenized with Ultra-Turrax for 15 s and cleaned by centrifugation at 5000*g* for 10 min at 2–4 °C. The clean organic layer was filtered on 0.45 μm cellulose filter and analyzed by HPLC-DAD using an Acclaim C_{30} column, 250 × 4.6 mm, 5 μm (Thermo Scientific, Waltham, USA), set at 34 °C, 0.4 mL/min, eluted with a ternary mobile phase composed by MeOH, EtOAc and *tert*-butylmethylether, with detection at 450 and 500 nm. The *β*-CAR (RT 13.4 min) and *t*-LYC (RT 24.5 min) were identified and quantified comparing with calibration curves made with solutions of the respective commercial standards, and results were expressed as mg/100 dw.

2.10. Analysis of polyphenols.

Sample extract for single polyphenol analysis was obtained treating 0.3 g of tomato powder with 8 mL EtOH/HCl 0.06 N, 1/1. The mixture was vortexed and ultrasonicated for 7 min. The supernatant obtained

after centrifugation (8000*g*, 10 min, 4 °C) was filtered on 0.45 μm and used for analysis. Single polyphenols were characterized by HPLC-DAD, performing the separation with an Inertsil C_{18} ODS-3 column, 250 × 4.6 mm, 5 μm (GL-Sciences, Tokyo, Japan) at 35 °C. A binary solvent system was employed, consisting of acetic acid/water (5/95, v/v) as solvent A and acetic acid/water/acetonitrile (5/5/90, v/v/v) as solvent B. The mobile phase gradient was as follows: 0–10 min with 5% solvent B; 10–20 min increase from 5 to 15% solvent B; 20–25 min with 15% B; 25–35 min from 15 to 27% B; 35–45 min with 27% B; 45–50 min from 27 to 40% B; 50–60 min with 40% B; 60–65 min decrease from 40 to 5% B; 65–80 min with 5% B. The flow rate of the mobile phase was 0.6 mL/min, and the injection volume of the sample, 2-fold diluted in solvent A, was 30 μL.

The compounds were identified by comparing with literature and with chromatographic behavior of pure standards using both RT and UV spectra. Commercial chlorogenic acid and rutin were used to quantify hydroxycinnamic and flavonol compounds at 320 nm and 350 nm, respectively. Naringenin chalcone was quantified by comparing with calibration curves of the authentic compound at 350 nm. Results were expressed as mg/100 g dw. Acquisition and analyses of chromatograms were performed by the Jasco ChromNAV software version 1.14.01.

2.11. Analysis of total polyphenol content.

Total polyphenol content (TPC) was measured by spectrophotometric analyses, using both the Folin-Ciocalteu assay and the Fast Blue BB assay, with a Jasco UV–Vis spectrophotometer (V630, Jasco-Europe, Lecco, Italy), on the same extract of single polyphenols.

Folin-Ciocalteu (F-C) analysis was carried out according to Lo Scalzo et al. (2020), with slight modifications. 150 μL of extract was diluted in 3 mL of distilled H_2O, then 1 mL of F-C reagent was added and left for 5 min at room temperature. Two mL of 10% Na_2CO_3 were added to this mixture and left in the dark for 2 h, then absorbance was read at 730 nm.

Fast Blue BB (FBBB) assay was carried out according to Lester et al. (2012), with modifications. Three mL of H_2O were added with 0.15 mL of tomato extract and 0.3 mL of freshly prepared FBBB (4-Amino-2,5-diethoxybenzanilide diazotated zinc double salt) solution 0.1% in H_2O, and further added with 0.3 mL of 5% NaOH. After 90 min in the dark at room temperature, the absorbance was read at 420 nm.

For both assays, calibrations were made against a set of gallic acid standard solutions (0.2–8.0 mg/mL) treated in the same way as samples, and results were expressed as mg gallic acid equivalent (GAE)/100 g dw.

2.12. Statistical analyses

For each biological replicate, each analysis was performed in duplicate on a single extract.

Results were calculated as means of the three biological replicates.

To investigate the effect of the harvesting year (Y), the variety (V) and the cultivation system (C), as well as all their interactions, data were subjected to a three-way analysis of variance (ANOVA), and the means of main factors were compared using Tukey's HSD test, with statistically significant differences accepted for $p < 0.05$. Furthermore, significant differences among samples of different variety and cultivation system were evaluated year by year through one-way ANOVA, comparing the means with the Tukey's test ($p < 0.05$).

Pearson's correlation coefficient was used to determine the correlation among variables, with significant non-zero correlations accepted for $p < 0.05$.

All statistical analyses were performed using the Statgraphics Plus 5.1 software.

3. Results

3.1. Weather monitoring.

Monthly mean temperatures and total rainfall data of the three years of study, covering the months from seedling transplanting to fruit harvesting, are shown in Suppl. Fig. 1, together with the thirty-year averages.

The mean temperatures during the period of tomato growth (Suppl. Fig. 1A) were generally higher or in line than the historical data, except for 2016, and in all years the temperatures of July, the final period of fruit growth before harvesting, were quite high, reaching the maximum in 2015.

The rainy regime (Suppl. Fig. 1B) was rather irregular both among years and compared to the historical rainfall, with values generally higher or lower than the average. April to June months were generally rainy with respect to the historical average, except for June 2017. On the contrary, July and August months were particularly dry, with the noticeable exception of July 2016, very rainy.

The year 2017 stood out for the very dry summer coupled with high temperatures.

3.2. Yield and general quality parameters.

Tomato fruits were harvested at the red stage, on August 3rd, 2nd, and 7th, in 2015, 2016 and 2017 respectively. Total marketable and waste productions were recorded in 2015 and 2016 (Table 1), as in 2017 not all production was harvested. As for main factor means, no statistical differences in yield were evidenced between V whereas, as regard Y, the 2016 season led to a significant decrease of marketable yield ($p < 0.01$) and an associated increase of waste ($p < 0.05$), due to the high rainfall in the ripening phase in July. As regard C, in ORG systems all the external inputs were lower than in CONV system and this may have influenced the yields. In fact, the CONV system resulted in significantly higher marketable and waste yields ($p < 0.001$), both as number and weight of fruits per plant, with respect to both ORG systems, which did not differ between ORG AM and ORG NM. The average decrease of marketable yield in ORG systems with respect to CONV was 38 and 42%, as number or weight of fruit per plant, respectively. However, in ORG systems, a significant lower percentage of waste weight on total weight occurred (7.23% and 4.74%) with respect to CONV (9.72%). It is interesting to note how SAAB variety, selected with a participatory approach with farmers, obtained yields statistically equal to those of the hybrid PERBR.

Average trends as regard year showed interesting divergences examining single factors (Suppl. Table 1), since in rainy 2016 with respect to 2015 no reduction in marketable yield was found in PERBR CONV AM (3.73 *vs* 3.49 kg per plant), and a reduction of waste were found in ORG AM systems, both in absolute value and as a percentage of the total yield (6.2% *vs* 8.2%). Consequently, a significant statistic interaction was found in waste data for Y × C data set (Table 1).

No main factors or interaction statistically affected fruit dry weight and pH values that, in the different V, Y and C, showed very low variations, in the range 5.80–6.01% and 4.43–4.61, respectively (Table 1).

Instead, an influence by Y was found for SSC and TTA. The SSC ranged from 71.2 to 75.9 °Bx dw and, as mean of the main factors, there was a significant increase ($p < 0.05$) from 2015 to 2017 (+6.6%) (Table 1). The TTA, ranging 78.8–101.0 mEq/100 g dw, was statistically higher in 2016 than in 2015 and 2017 ($p < 0.001$), with other differences observed for V and C not significant. The two varieties, however, behaved differently in relation to the year (Y × V, $p < 0.05$), being TTA in 2017 similar or higher for SAAB and lower for PERBR than 2015 (Suppl. Table 1). Noteworthy, in both varieties in 2016 the lower values

Table 1
Main factor means and significance of three-way ANOVA for yields and general quality parameters in the two tomato varieties harvested in three years and three cultivation systems.[a]

	Marketable yield (fr/pl)	Marketable yield (kg/pl)	Waste yield (fr/pl)	Waste yield (kg/pl)	Waste/Total (%, fr/pl) [b]	Waste/Total (%, kg/pl) [b]	Dry weight (% on fw)	SSC (°Bx on dw)	pH	TTA (mEq/ 100 g dw)
YEAR (Y)										
2015	11.43	2.69 a	2.10 b	0.16 b	15.44 b	5.54 b	5.85	71.2 b	4.48	78.8 b
2016	11.10	2.17 b	2.79 a	0.24 a	19.93 a	8.92 a	5.92	73.8 ab	4.43	101.0 a
2017	*n.a.*	*n.a.*	*n.a.*	*n.a.*	*n.a.*	*n.a.*	5.98	75.9 a	4.61	76.4 b
	ns	**	*	*	*	*	ns	*	ns	***
VARIETY (V)										
SAAB-CRA	11.49	2.44	2.32	0.19	16.69	6.94	5.95	73.2	4.55	87.0
PERBRUZZO F1	11.04	2.42	2.57	0.21	18.68	7.52	5.89	74.0	4.46	83.8
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CULTIVATION (C)										
CONV AM	15.08 a	3.38 a	3.27 a	0.34 a	17.82	9.72 a	5.80	74.1	4.43	84.9
ORG AM	9.71 b	2.01 b	2.17 b	0.16 b	18.00	7.23 ab	5.94	73.6	4.58	82.6
ORG NM	9.00 b	1.89 b	1.90 b	0.09 b	17.23	4.74 b	6.01	73.1	4.51	88.8
	***	***	***	***	ns	*	ns	ns	ns	ns
INTERACTIONS										
Y × V	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Y × C	ns	ns	**	**	*	*	ns	ns	ns	ns
V × C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × V × C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a] CONV AM, conventional with artificial mulching; ORG AM, organic with artificial mulching; ORG NM, organic with natural mulching. SSC, soluble solid content; TTA, total titratable acidity; fr/pl, number of fruit per plant; kg/pl, kg of fruit per plant; *n.a.*, not available; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Means followed by different letters in the same column are significantly different according to Tukey's test ($p < 0.05$).

[b] Waste yield (%) values were normalized according to angular transformation before statistical analysis.

Table 2
Main factor means and significance of three-way ANOVA for single and total sugars and organic acids, in the two tomato varieties harvested in three years and three cultivation systems.[a]

	Fructose	Glucose	Sucrose	Total sugars	Citric acid	Succinic acid	Malic acid	Total acids
YEAR (Y)								
2015	24.8 b	21.4 b	0.56 a	46.8 b	6.01 b	2.58 a	1.09 b	9.69
2016	25.5 b	21.3 b	0.77 a	47.6 b	6.89 a	2.13 b	1.24 a	10.26
2017	29.6 a	26.6 a	0.08 b	56.3 a	6.21 b	2.34 b	1.33 a	9.89
	***	***	***	***	**	***	***	ns
VARIETY (V)								
SAAB-CRA	26.8	22.8	0.66 a	50.3	6.07 b	2.46 a	1.16 b	9.69 b
PERBRUZZO F1	26.5	23.4	0.28 b	50.2	6.67 a	2.21 b	1.28 a	10.20 a
	ns	ns	***	ns	**	*	**	*
CULTIVATION (C)								
CONV AM	26.7	23.6	0.46	50.8	6.00 b	2.40	1.19 b	9.59
ORG AM	26.8	22.9	0.47	50.2	6.45 ab	2.38	1.17 b	10.01
ORG NM	26.4	22.7	0.48	49.7	6.66 a	2.27	1.30 a	10.24
	ns	ns	ns	ns	*	ns	*	ns
INTERACTIONS								
Y x V	ns	ns	**	ns	ns	*	**	ns
Y x C	ns	ns	ns	ns	ns	**	**	ns
V x C	ns	ns	ns	ns	ns	ns	ns	ns
Y x V x C	ns	ns	ns	ns	ns	ns	ns	ns

[a] Values are expressed as g/100 g dw. CONV AM, conventional with artificial mulching; ORG AM, organic with artificial mulching; ORG NM, organic with natural mulching. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Means followed by different letters in the same column are significantly different according to Tukey's test ($p < 0.05$).

for TTA were found in ORG AM.

3.3. Simple sugars and organic acids

The quantified sugars were fructose, glucose and sucrose, on average 53%, 46% and 1% respectively of the total sugars, that ranged 46.8–56.3 g/100 g dw in the different samples (Table 2). Despite this large variation, neither the main ones nor the total sugars were significantly affected by V or C influence (only the minority sucrose content was affected by variety), but only the 2017 harvest stood out for the higher contents, about + 20% increase, than 2015 and 2016 ($p < 0.001$), for fructose and glucose, while sucrose was subjected to a significant decrease.

As for organic acids, three main compounds were identified and quantified (Table 2), namely citric acid (the main one, averaging 64% of the total), succinic acid (24%), malic acid (12%). Moreover, minor quantities of fumaric acid (0.04%) and *trans*-aconitic acid (0.02%) were found (Suppl. Table 2). The total content varied between 9.59 and 10.26 g/100 g dw (Table 2), and it was on average statistically higher in PERBR than in SAAB (+5.3%, $p < 0.05$). As for the single acids, these trends were confirmed in the same direction for citric and malic, with significantly higher values in 2016, in PERBR and in ORG NM systems, whereas succinic had opposite contents, with the lower values for the same factors. However, an interaction of Y × V and Y × C affected both malic and succinic acid contents, whereas citric acid data were constant throughout factors and no interaction occurred.

3.4. Amino acids

Three free amino acids and one derivative were identified and quantified, namely glutamic acid (averaging about 43% of the total), glutamine (28%), aspartic acid (17%) and pyroglutamic acid (12%), for a total content varying between 1.8 and 4.4 g/100 g dw in the different theses (Table 3). However, these averaged percentages, as well as single and total contents, were extremely variable according to Y, with a strong decrease in total content from 2015 to 2017 (-59.9%, $p < 0.001$), and with an amino acid profile varying according to Y, since glutamic acid and glutamine together accounted for about four-fifths of the total in 2015 and 2017, whereas in 2016 all the detected amino acids had more similar percentages among them (16–35%). As for V (Table 3), glutamic and aspartic acids were higher in SAAB than PERBR while, as regards C, glutamic acid, glutamine and consequently the total amino acid content were higher in CONV than ORG systems. Anyway, there were different interactions among factors, so many exceptions to these general trends occurred when analyzing the whole theses, for instance above mentioned differences were not seen between V in 2017 and among C in 2016 (Suppl. Table 3).

3.5. Thiols and ascorbic acid

As for thiols, total reduced -SH groups (GSH plus CYS) ranged between 59.4 and 108.1 mg/100 g dw (Table 4). On average, this variability was mainly ascribed to Y, with significantly increasing average content from 2015 to 2017 (+82.0%), due to a strong increase in GSH in 2017 (+101.0%), whereas in 2016 a GSH decrease (–22.5%) with respect to 2015 was observed but counterbalanced by CYS increase (+164.3%). No differences in total -SH groups were observed due to V or C factors. Anyway, since GSH and CYS were significantly and inversely correlated (r = -0.5446, $p < 0.05$, n = 18), the single compounds had different behaviors, and an increase of GSH with a concurrent decrease of CYS was observed from CONV to ORG systems. In particular, GSH content was statistically lower in CONV AM than ORG NM (52.5 and 62.1 mg/100 g dw, respectively, with a 15.5% decrease, $p < 0.01$), with a significant C × Y interaction.

As for vitamin C, its total content (AsA + DHA) in the different tomato samples ranged between 290.3 and 451.3 mg/100 g dw, with the

Table 3
Main factor means and significance of three-way ANOVA for single and total amino acids in the two tomato varieties harvested in three years and three cultivation systems.[a]

	Pyroglutamic acid		Aspartic acid		Glutamic acid		Glutamine		Total amino acids	
YEAR (Y)										
2015	198	c	487	b	2136	a	1604	a	4426	a
2016	508	a	816	a	605	c	379	b	2308	b
2017	256	b	148	c	934	b	439	b	1777	c
	***		***		***		***		***	
VARIETY (V)										
SAAB-GRA	314		535	a	1331	a	774		2954	
PERBRUZZO F1	320		421	b	1146	b	856		2744	
	ns		***		*		ns		ns	
CULTIVATION (C)										
CONV AM	315		483		1480	a	985	a	3262	a
ORG AM	330		484		1120	b	784	b	2718	b
ORG NM	307		465		1124	b	688	b	2584	b
	ns		ns		***		**		***	
INTERACTIONS										
Y × V		***		***		ns		**		**
Y × C		ns		ns		**		*		ns
V × C		ns		ns		ns		ns		ns
Y × V × C		ns		ns		ns		ns		ns

[a] Values are expressed as mg/100 g dw. CONV AM, conventional with artificial mulching; ORG AM, organic with artificial mulching; ORG NM, organic with natural mulching. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Means followed by different letters in the same column are significantly different according to Tukey's test ($p < 0.05$).

Table 4
Main factor means and significance of three-way ANOVA for thiol compounds and ascorbic acid in the two tomato varieties harvested in three years and three cultivation systems.[a]

	GSH		GSSG		CYS		Total -SH		Ascorbic acid		Total vitamin C		DHA (% on total) [b]	
YEAR (Y)														
2015	45.1	b	48.0	a	14.3	b	59.4	c	349.0	b	397.1	b	11.7	a
2016	34.9	c	6.7	b	37.8	a	72.7	b	425.6	a	451.3	a	5.6	b
2017	90.6	a	16.4	b	17.5	b	108.1	a	270.1	c	290.3	c	6.6	b
	***		***		***		***		***		***		**	
VARIETY (V)														
SAAB-CRA	56.0		26.8	a	21.9		77.9		364.9	a	395.2	a	7.7	
PERBRUZZO F1	58.1		19.8	b	24.8		82.9		331.6	b	363.9	b	8.3	
	ns		*		ns		ns		*		**		ns	
CULTIVATION (C)														
CONV AM	52.5	b	22.3		28.2		80.7		359.0		383.8		6.6	
ORG AM	56.3	ab	22.8		22.9		79.2		332.6		373.3		9.9	
ORG NM	62.1	a	24.6		19.3		81.5		353.1		381.6		7.4	
	**		ns		ns		ns		ns		ns		ns	
INTERACTIONS														
Y x V		ns		ns		ns		ns		*		ns		ns
Y x C		**		ns		ns		ns		ns		ns		ns
V x C		ns		ns		ns		ns		ns		ns		ns
Y x V x C		ns		ns		ns		ns		ns		ns		ns

[a] Values are expressed as mg/100 g dw. CONV AM, conventional with artificial mulching; ORG AM, organic with artificial mulching; ORG NM, organic with natural mulching. GSH, reduced glutathione; GSSG, oxidized glutathione; CYS, cysteine; -SH, reduced thiols; DHA, dehydroascorbic acid. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Means followed by different letters in the same column are significantly different according to Tukey's test ($p < 0.05$).
[b] DHA (%) values were normalized according to angular transformation before statistical analysis.

oxidized form DHA representing 5.6–11.7% of the total (Table 4). As already seen for many other compounds, this large variability was mainly related to Y, and significant different values were found in the three years, with an increasing content in 2016 and a subsequent decreasing in 2017 (+13.6% and −26.9% than 2015, respectively, $p < 0.001$). Noticeably, this trend was exactly the opposite than GSH trend as showed in Fig. 1A and Fig. 1B, and in fact both ascorbic acid and total vitamin C were highly inversely correlated to GSH ($r = -0.801$, $p < 0.001$ and $r = -0.866$, $p < 0.001$) and positively correlated to CYS ($r = 0.668$, $p < 0.01$, and $r = 0.572$, $p < 0.01$). However, unlike GSH and most major compounds, vitamin C was significantly different in the two varieties, with an average higher content in the local variety SAAB (+8.6%). Moreover, in a more analytical evaluation year by year, no differences were ascribed to C. As for the percentage of the oxidized form DHA, it was similar in 2016 and 2017 (5.6 and 6.6%, respectively), when the higher and lower values of total vitamin C occurred, whereas a higher average percentage (11.7%) was found in 2015, especially due to the ORG AM systems (Suppl. Table 4).

3.6. Carotenoids and polyphenols.

The sum of single carotenoids ranged between 60.4 and 148.4 mg/100 g dw (Table 5), with *t*-LYC representing the larger part of the total (on average about 92%), and minor quantities of *β*-CAR (ranging 3–16%) (Suppl. Table 5). Both *β*-CAR and *t*-LYC contents were significantly influenced by Y, highlighting an increase in 2017, whereas only *β*-CAR was significantly influenced also by V and C, with higher content in SAAB and in ORG NM, respectively. On the contrary, higher average *t*-LYC and total carotenoid contents were found in CONV than ORG samples, however carotenoids were characterized by a wide variability (Fig. 1C), and this trend was very variable in different years (Suppl. Table 5), for example with opposite behavior in the two varieties in 2015, and with the higher values in ORG AM in 2016.

Four main phenolic compounds were identified and quantified: one hydroxycinnamic acid, namely the chlorogenic acid (CGA), that averaged about 24% of the total, and 3 flavonoids that accounted for the other three-quarters of the total, namely the flavanone precursor naringenin chalcone (NAR-CHA, 44%), and the flavonols rutin (25%) and its derivative rutin-glucoside (7%). Their total content ranged between 62.1 and 128.0 mg/100 g dw (Table 5). A significant higher total content was found in 2016 (+106%) and 2017 (+44%) than 2015, and higher total contents, even if not significant, were observed also in PERBR and in ORG NM (Table 5), with the highest value in PERBR ORG NM 2016 (Fig. 1D). These trends were mainly due to NAR-CHA, fully concordant, whereas CGA showed the same trend only for C factors. On the contrary, flavonols showed just little variation.

A Y × V interaction affected CGA and NAR-CHA, and consequently total contents. In fact, in 2015 both compounds were similar between varieties, whereas in 2016, with the generalized increase, NAR-CHA content in PERBR was much higher than in SAAB, and on the contrary in 2017 both compounds were generally lower in PERBR than in SAAB (Suppl. Table 5). However, statistical differences occurred only among cultivation systems in 2017, with a general higher content of these compounds in ORG (especially NM) than CONV samples.

Furthermore, F-C and FBBB were reciprocally highly correlated ($r = 0.903$, $p < 0.001$), and also with the level of phenols measured by HPLC ($r = 0.725$, $p < 0.01$, and $r = 0.757$, $p < 0.01$), respectively. Their changes were significantly influenced by Y (Table 5), with the lowest values in 2015 and the highest in 2016. As for both V and C factors, they were non influenced, also if an increase can be found in ORG NM samples, in accordance with levels of CGA, NAR-CHA, and total phenols measured by HPLC.

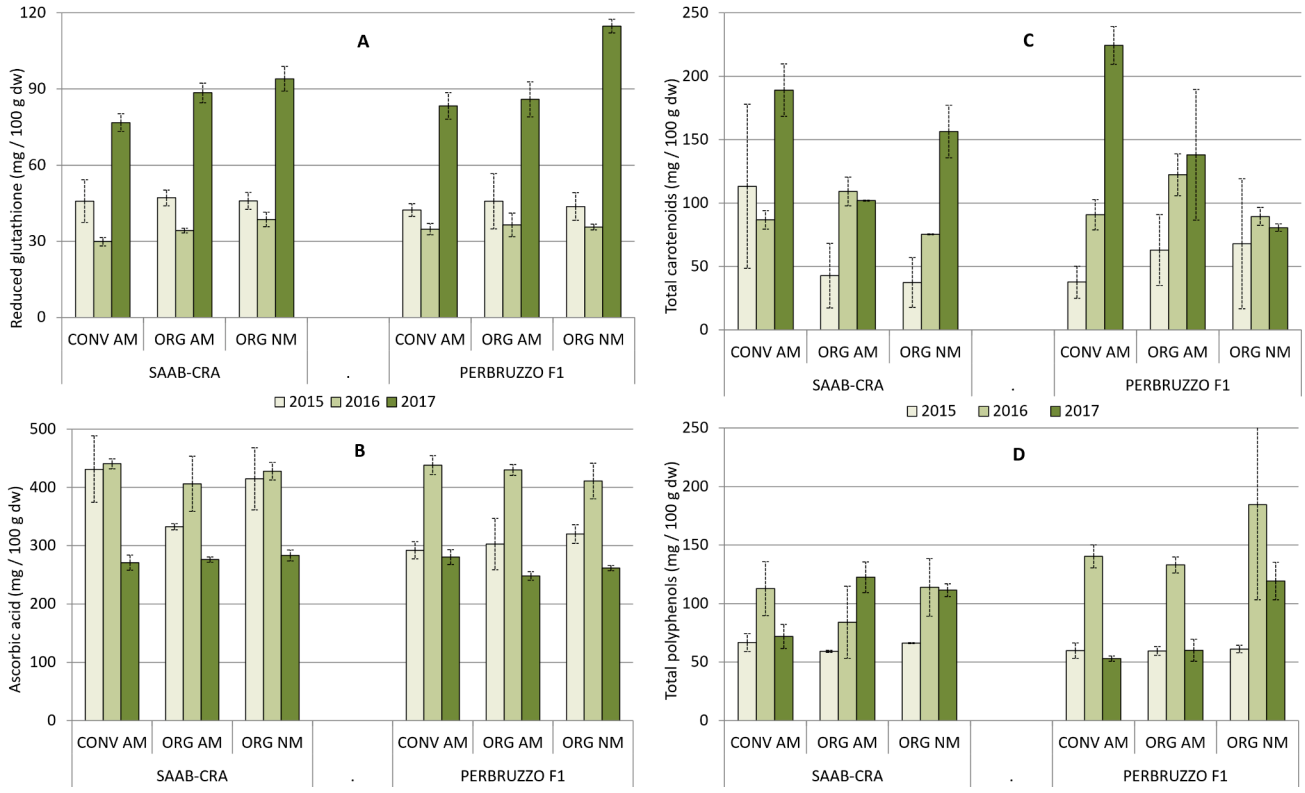

Fig. 1. Reduced glutathione (A), ascorbic acid (B), total carotenoids (C) and total polyphenols (D) in the two tomato varieties, harvested in three years and three cultivation systems (CONV AM, conventional with artificial mulching; ORG AM, organic with artificial mulching; ORG NM, organic with natural mulching). Data are means ± SE, n = 3.

Table 5
Main factor means and significance of three-way ANOVA for single and total carotenoids and polyphenols in the two tomato varieties harvested in three years and three cultivation systems.[a]

	t-Lycopene		*β*-Carotene		Total carotenoids		Chlorogenic acid		Naringenin chalcone		Rutin	Rutin glucoside		Total polyphenols		F-C assay		FBBB assay	
YEAR (Y)																			
2015	56.7	b	3.7	c	60.4	b	16.5	b	16.1	b	23.0	6.6	ab	62.1	b	303.4	b	571.7	c
2016	87.6	b	8.0	b	95.7	b	25.3	a	74.0	a	22.6	6.1	b	128.0	a	482.0	a	1730.0	a
2017	136.7	a	11.7	a	148.4	a	25.2	a	31.8	b	24.6	8.1	a	89.7	b	336.4	b	1006.1	b
	***		***		***		***		***		ns	*		***		***		***	
VARIETY (V)																			
SAAB-CRA	87.2		8.2	a	95.4		24.1	a	34.0		24.5	7.3		89.8		379.1		1115.8	
PERBRUZZO F1	89.4		6.5	b	95.9		20.6	b	47.2		22.4	6.5		96.7		368.8		1089.4	
	ns		**		ns		**		ns		ns	ns		ns		ns		ns	
CULTIVATION (C)																			
CONV AM	105.8		7.5	ab	113.3		19.1	b	37.8		20.6	6.4		84.0		372.5		1080.8	
ORG AM	87.0		6.2	b	93.3		20.2	b	33.0		25.9	7.2		86.3		360.9		1077.9	
ORG NM	72.0		8.3	a	80.3		27.7	a	50.9		23.7	7.1		109.4		388.4		1149.1	
	ns		**		ns		***		ns		ns	ns		ns		ns		ns	
INTERACTIONS																			
Y x V	ns		ns		ns		**		*		ns	ns		*		ns		ns	
Y x C	ns		*		ns		***		ns		ns	ns		ns		ns		ns	
V x C	ns		ns		ns		ns		ns		ns	ns		ns		ns		ns	
Y x V x C	ns		ns		ns		*		ns		ns	ns		ns		ns		ns	

[a] Values are expressed as mg/100 g dw. CONV AM, conventional with artificial mulching; ORG AM, organic with artificial mulching; ORG NM, organic with natural mulching. F-C, Folin-Ciocalteu; FBBB, Fast Blue BB. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. Means followed by different letters in the same column are significantly different according to Tukey's test ($p < 0.05$).

4. Discussion

Generally speaking, CONV systems at present average higher yields than ORG systems, even if a reduced yield gap has been observed in the last years, and strongly depending on crop type and variety (Thorup-Kristensen et al., 2012), as well as to specific site conditions (Juroszek et al., 2008). Results presented here on tomato CONV *vs* ORG yield agree with above findings, however differ from data previously reported in the same experimental field in previous years (Campanelli & Canali, 2012; Migliori et al., 2012) and in the same experiment in different farms (Juroszek et al., 2008), when other tomato varieties yielded similarly in CONV and ORG systems, although showing alike high variability depending on the year and the assayed genotype.

According to Thorup-Kristensen et al. (2012), in cereal grains and

vegetable crops other than tomato no systematic differences in waste percentages were found among crop systems, and Juroszek et al. (2008) in tomato reported waste percentages largely varying according to individual farmers rather than cultivation system. Instead, in present results tomato had on average lower waste percentages as kg per plant in ORG than CONV.

Given yield data gathered over two years, these results are mainly due to the strong weather difference observed. Nevertheless, the data on lower waste in ORG systems are worthy of further study, especially to assess whether the decrease in waste in ORG AM observed in 2016 compared to 2015 is linked to possible lower disease attacks in this system under unfavorable weather conditions, and to a better adaptation promoted by this cultivation system to biotic and abiotic stress, whereas conventional cultivation conditions, with soils poor in organic matter and weeded, could have amplified the condition of water imbalance. It is also important to underline the positive result of the ORG NM thesis, which provided yields comparable to those of ORG AM, against a further reduction of external inputs. The tomato therefore adapts well to the no-tillage technique and allows to reach a higher level of agroecological sustainability without compromising the commercial yield.

Dry weight and pH data fall within the range reported by other authors (Favati et al., 2009) and, agreeing with Hernández Suárez et al. (2008a) for dry matter and Juroszek et al. (2009) for pH, no significant differences due to CONV or ORG regime are also observed. As for SSC and TTA, data expressed on fresh weight are in line with those found in literature (Favati et al., 2009) but, with respect to the cultivation system, soluble solids differ from those reported by Chassy et al. (2006) which, in a similar study, found recurring higher contents in ORG than CONV management, with a significant interaction of the year. On the other hand, no consistent effect by the management system on SSC and TTA has been reported by Juroszek et al. (2009).

Present data of fructose, glucose, citric and malic acid agree with values generally found in literature, whereas succinic acid was found by Loiudice et al. (1995) but not by other authors, who found different acids in relevant quantities (Hernández Suárez et al., 2008b). SSC and sugars were always significantly and positively correlated in all combinations, as expected (data not shown), instead only citric acid was significantly and positively correlated with total acids ($r = 0.887$, $p < 0.001$) and TTA ($r = 0.541$, $p < 0.05$).

The levels of single sugars were mostly influenced by different sampling years, so evidencing a different ripening pattern induced by environmental conditions. In particular, the increase in fructose and glucose and the concomitant decrease in sucrose was evident in 2017, very dry in the months prior to harvest (Suppl. Fig. 1B). An alteration and an increase of sugars accumulation induced by weather conditions in 2017 is evident, enforcing the data of Li et al. (2012), regarding the influence of abiotic stresses on sucrose metabolism, which is also significantly different between the two assayed varieties. Glucose and fructose levels were not influenced by the assayed variety and by the cultivation method, in accordance with previous works (Hernández Suárez et al., 2008a), but not with the study of Halmann (2012), where higher total sugars were found in ORG tomatoes than CONV ones.

The differences in the pattern of organic acids seemed to depend on the different years of sampling. In 2016, in fact, which was characterized by a cold and rainy season, an increase of citric acid and a decrease of succinic acids was observed, as compared to 2015 and 2017. We hypothesize that this apparent inverse relationship between citric and succinic acids can be the result of the different fruit development, as reported by Young et al. (1993). Interestingly, the higher content of citric acid was in ORG, especially in ORG NM, and in PERBR variety. However, and in line with the same authors, sugars were not influenced in a comparable way according to possible different ripening.

The amino acids represent important taste compounds in tomato, as responsible for the typical "umami" sensation of this fruit, mainly due to glutamic acid and glutamine. These compounds strongly increase during tomato ripening (Sorrequieta et al., 2010), and the slower development of tomatoes berries in ORG systems accounted for the low total content of these metabolites, in favor of CONV system of cultivation and in SAAB variety. The higher content in CONV is in full accordance with previous findings (Pieper & Barrett, 2008). These compounds are subjected to the nitrogen availability, and, according to the theory of C/N balance (Brandt & Mølgaard, 2001), it favors the accumulation of amino acid in CONV with respect to ORG systems, where nitrogen availability due to chemical inputs is limited.

The AsA content in organic plants has been well studied and reviewed, highlighting its general increase in ORG with respect to CONV crops, about +25% according to a recent meta-analysis study (Barański et al., 2014). However, in present results, tomato ascorbic acid content was not affected by cultivation system, whereas it was dependent on cultivar (higher in SAAB) and on environmental conditions (higher in 2016), as confirmed by previous data (Martí et al., 2018). There are few literature reports concerning the influence of ORG cultivation on GSH, although a recent work on grape reports an increase in GSH content after foliar applications of organic fertilizers (Gutiérrez-Gamboa et al., 2017).

Biothiols, both in oxidized and reduced forms, are in a deep metabolic relationship with AsA and DHA: they are part of the well-known glutathione-ascorbate or Foyer-Halliwell-Asada cycle, involved in the detoxification processes of Reactive Oxygen Species, especially hydrogen peroxide, produced by plant metabolism. The two reduced forms AsA and GSH are subjected to an enzymatic regeneration from oxidized forms, providing an effective tool to counteract abiotic and biotic stresses; consequently, a high ratio of AsA *vs* DHA and GSH *vs* GSSG ensure in plants a proper antioxidant defense for an optimal physiological status (Fotopoulos et al., 2010). This is evident in the present experiment, where 2016 and 2017 years stressed plants for unfavorable and opposite weather trends, having the same effect on the ratio GSH *vs* GSSG, with higher values, 5.21 and 5.54, compared to 0.94 in 2015. Moreover, the absolute increase of GSH in the most stressed ORG NM system evidently enforces this finding. The levels of AsA did not follow the trend of GSH, with the evident opposite trend over the different years. It is suggested that due to the drastic weather change in 2017, the level of AsA decreased with a concomitant increase of GSH, as a result of a possible alternate activity of reducing enzymes (Lovat et al., 2016).

Carotenoids and phenols are important quality indicators of tomato; their evaluation can also be useful to monitor fruit physiological status. In the present work the sampling year, with the particular trends in 2016 and in 2017, had the greatest influence on these contents, showing significantly higher amounts in 2017 for carotenoids and in 2016 for polyphenols. Instead, the growing system generally had no statistically significant influence, although a clear tendency to an increase in CONV for carotenoids and in ORG NM for phenols was found. Similar results were found by other Authors for carotenoids and for phenols (Chassy et al., 2006; Mitchell et al., 2007; Halmann, 2012).

As for carotenoids content, their levels are enhanced in ripe fruits than unripe ones and show a big seasonal variation (Raffo et al., 2006). Moreover, where ORG samples are subjected to a higher level of stress, their ripeness was lowered and the pigment content decreased, if compared to CONV tomatoes (Migliori et al., 2012). A further confirmation of this phenomenon is also given by the positive influence of nitrogen fertilization on the carotenoid content in vegetable crops, as it is performed in CONV cropping (Brandt & Mølgaard, 2001). On the other hand, the meta-data analysis by Barański et al. (2014), assessed that the comparison for total carotenoid content in ORG *vs* CONV vegetables gave no evident percent change. Specifically, the growing of tomatoes at high temperatures lowered the lycopene content (Gautier et al., 2008), but a further index to be controlled is the ratio lycopene *vs* β-carotene and other carotenoids, that is lowered under stress (Takács et al., 2020). To confirm this, the ratio was clearly higher in 2015 (15.6) than in other two "stressful" years (around 11), and in "stressful" ORG NM it resulted 8.7 *vs* 14 in CONV and ORG AM.

Soluble phenols are very important markers of physiological status, as their accumulation is known to be stimulated or inhibited under several factors. These compounds differ significantly according to years, evidently influenced by climatic conditions. The main phenol measured in tomato berries was NAR-CHA, that largely increased in 2016 (+82%) and mostly influenced their sum, with the same effect (+37%) in 2016, compared to the 3-year average. As regards the variety and the cultivation system, the only significance was found for CGA, 17% higher in SAAB than PERBR, and 45% higher in ORG NM than CONV AM. NAR-CHA was also 35% higher in ORG NM than CONV, although not significant. These levels of increase in ORG *vs* CONV are in line with already published data (Mitchell et al., 2007), while other Authors found higher values in ORG tomatoes, but to a lower extent (Chassy et al., 2006). The different CONV-ORG accumulation of soluble phenols can be due to the growth-differentiation balance (GDB) phenomenon. In fact, phenols are secondary specialized metabolites involved in stress response. Cellular processes can be divided in cell growth and cell differentiation, where differentiation is intended as cell maturation and specialization, including secondary metabolism and increased formation of defense compounds: according to GDB theory, plant activity is a trade-off between these processes, and plants, assessing their resources available in any situation, optimize their metabolism towards growth or differentiation (Brandt & Mølgaard, 2001). A possible further cause of this trend could be related to the particular type of mulching in ORG plants. In this case, in fact, hairy vetch was used, that is a plant known for its allelopathic effect due to the leach of cyanamide (Soltys et al., 2012). Hence, in no-tilled ORG thesis the presence of cyanamide and/or the higher difficulty in root penetration, may in turn have induced an accumulation of secondary metabolites as stress response, that could explain the higher CGA and NAR-CHA content observed in ORG NM tomatoes.

As regards the total phenol contents measured with two spectrophotometric methods (Folin-Ciocalteu and Fast Blue BB), they resulted significantly correlated with total phenols measured by HPLC. These indexes resulted widely increased in 2016 with respect to the 3-year average (+29% and +57%, respectively): data from a study on sweet bell pepper performed in the same environment and in the same years were in strong accordance (Lo Scalzo et al., 2020), highlighting that different growing seasons can strongly influence the content in plant secondary metabolites. Environmental stresses are generally known to positively affect the biosynthesis of secondary metabolites, and increased contents of phenolics and ascorbic are reported under heat and drought stress, as reviewed by Balestrini et al. (2021), in many species and in tomato. Both in the present study on tomato and in Lo Scalzo et al. (2020) on pepper, phenolics and ascorbic showed greater contents in the year with the rainier and less warm summer, thus reinforcing the idea that temperature and water stresses in both directions can act as enhancers of the biosynthesis of these secondary metabolites. Increases of these indexes were found to a lower extent in the comparison ORG NM *vs* CONV, in accordance with Juroszek et al. (2009). Overall, the meta-data study by Barański et al. (2014) gave, as a mean percentage difference, an increase in phenolics of around 25% in ORG *vs* CONV crops. The Authors also distinguished among fruit, vegetables and cereals, and vegetables resulted in a lower percent increase, around 10%. The present work, averaging all the indexes related to single phenolic content and total phenolic indexes, gave a 20% increase for ORG NM with respect to CONV, in line with the data of Barański et al. (2014), while the comparison ORG AM *vs* CONV was much less, with an increase of 3%.

Summarizing, on a 3-year average CONV provided the higher yields, and some quality markers indicate a general higher ripeness (lycopene, amino acids). The year and genotype variability was justified by reciprocal content of some secondary metabolites, such as carotenoids (lycopene *vs* β-carotene), hydrosoluble antioxidants (AsA *vs* GSH), as well as phenols. However, ORG systems provided a lower percentage of waste on total yield, worthy of further study, and a higher quality for some nutritional and bioactive compounds (interestingly, GSH and CGA). Furthermore, no-tilled ORG provided yields comparable to those of tilled ORG, therefore this system seems to be convenient for a greater agro-sustainability in the areas and with varieties suitable for ORG cultivation.

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CRediT authorship contribution statement

Marta Fibiani: Investigation, Formal analysis, Writing – original draft. **Dario Paolo:** Investigation, Writing – review & editing. **Fabrizio Leteo:** Investigation, Writing – review & editing. **Gabriele Campanelli:** Conceptualization, Resources, Supervision, Investigation, Writing – review & editing. **Valentina Picchi:** Investigation, Writing – review & editing. **Giulia Bianchi:** Investigation, Writing – review & editing. **Roberto Lo Scalzo:** Conceptualization, Funding acquisition, Investigation, Writing – original draft, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133090.

References

Balestrini, R., Brunetti, C., Cammareri, M., Caretto, S., Cavallaro, V., Cominelli, E., … Zampieri, E. (2021). Strategies to modulate specialized metabolism in Mediterranean crops: From molecular aspects to field. *International Journal of Molecular Sciences, 22*(6), 2887.

Barański, M., Średnicka-Tober, D., Volakakis, N., Seal, C., Sanderson, R., Stewart, G. B., … Leifert, C. (2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: A systematic literature review and meta-analyses. *British Journal of Nutrition, 112*, 794–811.

Bhandare, P., Madhavan, P., Rao, B. M., & Someswar Rao, N. (2010). Determination of amino acid without derivatization by using HPLC-HILIC column. *Journal of Chemical and Pharmaceutical Research, 2*(22), 372–380.

Brandt, K., & Mølgaard, J. P. (2001). Organic agriculture: Does it enhance or reduce the nutritional value of plant foods? *Journal of the Science of Food and Agriculture, 81*(9), 924–931.

Campanelli, G., & Canali, S. (2012). Crop production and environmental effects in conventional and organic vegetable farming systems: The case of a long term experiment in Mediterranean conditions (Central Italy). *Journal of Sustainable Agriculture, 36*(6), 599–619.

Canali, S., Campanelli, G., Ciaccia, C., Leteo, F., Testani, E., & Montemurro, F. (2013). Conservation tillage strategy based on the roller crimper technology for weed control in Mediterranean vegetable organic cropping systems. *European Journal of Agronomy, 50*, 11–18.

Chassy, A. W., Bui, L., Renaud, E. N., Van Horn, M., & Mitchell, A. E. (2006). Three-year comparison of the content of antioxidant microconstituents and several quality characteristics in organic and conventionally managed tomatoes and bell peppers. *Journal of Agricultural and Food Chemistry, 54*(21), 8244–8252.

Collins, J. K., Perkins-Veazie, P., & Roberts, W. (2006). Lycopene: From plants to humans. *HortScience, 41*(5), 1135–1144.

Demirkol, O., Adams, C., & Ercal, N. (2004). Biologically important thiols in various vegetables and fruits. *Journal of Agricultural and Food Chemistry, 52*(26), 8151–8154.

FAOSTAT (2019). Data, Crops (Production) by regions. http://www.fao.org/faostat/en/#data/QC (accessed January 19, 2021).

Favati, F., Lovelli, S., Galgano, F., Miccolis, V., Di Tommaso, T., & Candido, V. (2009). Processing tomato quality as affected by irrigation scheduling. *Scientia Horticulturae, 122*(4), 562–571.

Fotopoulos, V., Ziogas, V., Tanou, G., & Molassiotis, A. (2010). Involvement of AsA/DHA and GSH/GSSG ratios in gene and protein expression and in the activation of defence mechanisms under abiotic stress conditions. In N. Anjum, M. T. Chan, & S. Umar (Eds.), *Ascorbate-glutathione pathway and stress tolerance in plants* (pp. 265–302). Dordrecht: Springer.

Gautier, H., Diakou-Verdin, V., Bénard, C., Reich, M., Buret, M., Bourgaud, F., … Génard, M. (2008). How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *Journal of Agricultural and Food Chemistry, 56*(4), 1241–1250.

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Gonzalo-Diago, A., Moreno-Simunovic, Y., & Martínez-Gil, A. M. (2017). Effect of different foliar nitrogen applications on the must amino acids and glutathione composition in Cabernet Sauvignon vineyard. *LWT, 75*, 147–154.

Halmann, E. (2012). The influence of organic and conventional cultivation systems on the nutritional value and content of bioactive compounds in selected tomato types. *Journal of the Science of Food and Agriculture, 92*, 2840–2848.

He, X., Qiao, Y., Liu, Y., Dendler, L., Yin, C., & Martin, F. (2016). Environmental impact assessment of organic and conventional tomato production in urban greenhouses of Beijing city, China. *Journal of Cleaner Production, 134*, Part A: 251-258.

Hernández Suárez, M., Rodríguez Rodríguez, E., & Díaz Romero, C. (2008a). Analysis of organic acid content in cultivars of tomato harvested in Tenerife. *European Food Research and Technology, 226*(3), 423–435.

Hernández Suárez, M., Rodríguez Rodríguez, E., & Díaz Romero, C. (2008b). Chemical composition of tomato (*Lycopersicon esculentum*) from Tenerife, the Canary Islands. *Food Chemistry, 106*, 1046–1056.

Juroszek, P., Ledesma, D., Ma, C. H., Yang, R. Y., Lumpkin, H. M., Lin, C. C., … Palada, M. C. (2008). Plant vigour and yields of organically and conventionally grown tomato crops in Taiwan. *Acta Horticulturae, 767*, 257–265.

Juroszek, P., Lumpkin, H. M., Yang, R. Y., Ledesma, D. R., & Ma, C. H. (2009). Fruit quality and bioactive compounds with antioxidant activity of tomatoes grown on-farm: Comparison of organic and conventional management systems. *Journal of Agricultural and Food Chemistry, 57*(4), 1188–1194.

Lester, G. E., Lewers, K. S., Medina, M. B., & Saftner, R. A. (2012). Comparative analysis of strawberry total phenolics via Fast Blue BB vs. Folin-Ciocalteu: Assay interference by ascorbic acid. *Journal of Food Composition and Analysis, 27*(1), 102–107.

Li, M., Peterson, C. A., Tautges, N. E., Scow, K. M., & Gaudin Amélie, C. M. (2019). Yields and resilience outcomes of organic, cover crop, and conventional practices in a Mediterranean climate. *Scientific Reports, 9*, 12283.

Li, Z., Palmer, W. M., Martin, A. P., Wang, R., Rainsford, F., Jin, Y., Patrick, J. W., Yang, Y., & Ruan, Y.-L. (2012). High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. *Journal of Experimental Botany, 63*(3), 1155–1166.

Loiudice, R., Impembo, M., Laratta, B., Villari, G., Voi, A. L., Siviero, P., & Castaldo, D. (1995). Composition of San Marzano tomato varieties. *Food Chemistry, 53*(1), 81–89.

Lo Scalzo, R., Campanelli, G., Paolo, D., Fibiani, M., & Bianchi, G. (2020). Influence of organic cultivation and sampling year on quality indexes of sweet pepper during 3 years of production. *European Food Research and Technology, 246*(6), 1325–1339.

Lovat, C., Nassar, A. M. K., Kubow, S., Xiu-Qing, L.i., & Donnelly, D. J. (2016). Metabolic Biosynthesis of Potato (*Solanum tuberosum* L.) Antioxidants and Implications for Human Health. *Critical Reviews in Food Science and Nutrition, 56*(14), 2278–2303.

Martí, R., Leiva-Brondo, M., Lahoz, I., Campillo, C., Cebolla-Cornejo, J., & Roselló, S. (2018). Polyphenol and l-ascorbic acid content in tomato as influenced by high lycopene genotypes and organic farming at different environments. *Food Chemistry, 239*, 148–156.

Migliori, C., Di Cesare, L. F., Lo Scalzo, R., Campanelli, G., & Ferrari, V. (2012). Effects of organic farming and genotype on alimentary and nutraceutical parameters in tomato fruits. *Journal of the Science of Food and Agriculture, 92*, 2833–2839.

Mitchell, A. E., Yun-Jeong, H., Koh, E., Barrett, D. M., Bryant, D. E., Denison, R. F., & Kaffka, S. (2007). Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *Journal of Agricultural and Food Chemistry, 55*(15), 6154–6159.

Orsini, F., Maggio, A., Rouphael, Y., & De Pascale, S. (2016). "Physiological quality" of organically grown vegetables. *Scientia Horticulturae, 208*, 131–139.

Paolo, D., Bianchi, G., Scalzo, R. L., Morelli, C. F., Rabuffetti, M., & Speranza, G. (2018). The chemistry behind tomato quality. *Natural Product Communications, 13*(9), 1225–1232.

Pieper, J. R., & Barrett, D. M. (2008). Effects of organic and conventional production systems on quality and nutritional parameters of processing tomatoes. *Journal of the Science of Food and Agriculture, 89*(2), 177–194.

Raffo, A., La Malfa, G., Fogliano, V., Maiani, G., & Quaglia, G. (2006). Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1). *Journal of Food Composition Analysis, 19*, 11–19.

Rembialkowska, E. (2007). Review. Quality of plant products from organic agriculture. *Journal of the Science of Food and Agriculture, 87*, 2757–2762.

Soltys, D., Rudzińska-Langwald, A., Gniazdowska, A., Wiśniewska, A., & Bogatek, R. (2012). Inhibition of tomato (*Solanum lycopersicum* L.) root growth by cyanamide is due to altered cell division, phytohormone balance and expansin gene expression. *Planta, 236*, 1629–1638.

Sorrequieta, A., Ferraro, G., Boggio, S. B., & Valle, E. M. (2010). Free amino acid production during tomato fruit ripening: A focus on *l*-glutamate. *Amino Acids, 38*, 1523–1532.

Takács, S., Pék, Z., Csányi, D., Daood, H. G., Szuvandzsiev, P., Palotás, G., & Helyes, L. (2020). Influence of water stress levels on the yield and lycopene content of tomato. *Water, 12*(8), 2165.

Tamasi, G., Pardini, A., Bonechi, C., Donati, A., Pessina, F., Marcolongo, P., Gamberucci, A., Leone, G., Consumi, M., Magnani, A., & Rossi, C. (2019). Characterization of nutraceutical components in tomato pulp, skin and locular gel. *European Food Research and Technology, 245*, 907–918.

Thorup-Kristensen, K., Dresbøll, D. B., & Kristensen, H. L. (2012). Crop yield, root growth, and nutrient dynamics in a conventional and three organic cropping systems with different levels of external inputs and N re-cycling through fertility building crops. *European Journal of Agronomy, 37*(1), 66–82.

Yap, L. P., Sancheti, H., Ybanez, M. D., Garcia, J., Cadenas, E., & Han, D. (2010). Determination of GSH, GSSG, and GSNO using HPLC with electrochemical detection. *Methods Enzymology, 473*, 137–147.

Young, T. E., Juvik, J. A., & Sullivan, J. G. (1993). Accumulation of the components of total solids in ripening fruits of tomato. *Journal of the American Society for Horticultural Science, 118*(2), 286–292.