Research Article

Received: 20 May 2011 Revised: 16 September 2011 Accepted: 21 September 2011 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.4720

# Organically grown tomato (*Lycopersicon esculentum* Mill.): bioactive compounds in the fruit and infection with *Phytophthora infestans*

**Afrah E Mohammed,[a] Inga Smit,[a] Elke Pawelzik,[a] Anna J Keutgen[b] and Bernd Horneburg[c]***

## Abstract

**BACKGROUND: Tomato fruits are characterized by a good nutritional profile, including different bioactive compounds such as carotenoids, phenolic compounds and ascorbic acid. The objective of this study was to analyze the content of bioactive compounds in the fruit and the infection by *Phytophthora infestans* of 28 tomato genotypes from organic outdoor production. The relationship between bioactive compounds in the fruit and infection with *P. infestans* was estimated. Field experiments were carried out in 2004 and 2005 at two locations in central Germany.**

**RESULTS: Significant variation among genotypes, locations and years was observed for the content of lycopene, ascorbic acid, total phenolic compounds, antioxidant capacity and the infection level of *P. infestans*. Antioxidant capacity seemed to be influenced mainly by the phenolics and was highest in small fruits, which were less infected with *P. infestans*.**

**CONCLUSION: The large genetic variation among tomato genotypes for the content of bioactive compounds in their fruit allows for selection gains. None of the investigated bioactive compounds can be recommended for the indirect selection for increased field resistance against *P. infestans*.**


**Keywords:** tomato; ascorbic acid; lycopene; phenolics; organic agriculture; *Phytophthora infestans*

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the most important vegetable in the world, with a global production of more than 130 million tonnes of fresh fruit in 2008. China, the USA and India are the main producers.[1] However, tomato fruit production is restricted by fungal infestations in the field. One of the most serious diseases is late blight caused by *Phytophthora infestans* (an oomycete),[2] which is found in nearly all tomato-producing areas of the world and can cause complete crop loss.[3] Under favourable environmental conditions (i.e. temperatures between 15 and 23 °C, coupled with high humidity and prolonged periods of leaf wetness), the disease can spread very quickly, with only 3–4 days between infection and sporulation. The foliage is killed prematurely; infected fruits are unmarketable and may rot during storage.[4] However, the excessive use of fungicides, their irregular application as well as possible side effects might cause serious environmental problems as well as causing toxicity to living organisms.[5] Recently, much attention has been focused on organic farming to avoid the frequent use of agrochemicals that may result, amongst other problems, in environmental pollution.[6] However, pests and diseases are the most serious problems in organic farming and, since the application of chemical pesticides is not allowed, organically grown plants have to defend themselves against pathogens to ensure their survival, growth and development.

The experiment presented here is part of the Organic Outdoor Tomato Project, which was started in 2003 as a participatory selection and breeding programme in Germany. A screening based on 3500 accessions identified cultivars for improving amateur gardening.[7] The best parent cultivars were chosen to develop breeding strategies in the breeding programme.[8] The first open-pollinated cultivars resulting from the Organic Outdoor Tomato Project were released in 2010.

Tomatoes are an important provider of dietary antioxidants in human nutrition; for example, the daily intake of antioxidative capacity per capita in the USA for tomatoes is half that provided by potatoes but higher than that provided by other vegetables (e.g. cabbage or bell peppers).[9] Because of this importance, the main purpose of the current investigation was to study the level of bioactive compounds, ascorbic acid and antioxidant capacity in organic outdoor cultivation depending on genotype, location and year. Additionally, we wanted to identify any possible relationships

* *Correspondence to: Bernd Horneburg, Department of Crop Sciences, Göttingen University, 37075 Göttingen, Germany. E-mail: bhorneb@gwdg.de*

a *Department of Crop Sciences, Göttingen University, 37075 Göttingen, Germany*

b *Department of Food Technology, University of Technology and Life Sciences, 85-225 Bydgoszcz, Poland*

c *Department of Crop Sciences, Göttingen University, 37075 Göttingen, Germany*

between the concentrations of bioactive compounds in the fruit and the plant's susceptibility to *P. infestans*.

The production of reactive oxygen species (ROS) is one of the earliest cellular responses following successful pathogen recognition.[10] ROS (such as the superoxide anion, hydrogen peroxide and hydroxide radical) have the ability to trigger a defence response after pathogenic recognition with a direct cytotoxic effect on the invading pathogen. However, their accumulation causes oxidative cell damage through actions such as lipid peroxidation associated with membrane destruction, protein inactivation and DNA mutation.[11]

Ascorbic acid, the carotenoids and phenolic compounds are able to interact with ROS.[12] Both the carotenoids and polyphenols belong to the pathogenic defence system in the plant cell.[13] The response of polyphenols to pathogenic recognition is characterized by an early and rapid accumulation of phenolics at the infection site, resulting in an effective inhibition of pathogen penetration.[14] One carotenoid, the pigment lycopene, is the main carotenoid in tomatoes and it accumulates in high concentrations in mature red fruits. It is this compound which has been connected to the fact that tomato consumption is associated with a reduced risk of chronic diseases like cancer and heart diseases.[15] According to Arnao *et al.*,[16] its antioxidative capacity is 1.16 times higher than that of $\beta$-carotene and 2.9 times higher than the antioxidant capacity of vitamin C (ascorbic acid). In addition to lycopene, several phenolics have been recognized among the compounds acting as antioxidants in tomatoes.

Mainly caffeic acid, coumaric acid and caffeoylquinic acid have been identified as phenolic acids in all tomato fruit tissues. The flavonoids detected in the fruit skin have been mainly found to be quercetin, rutin, kaempferol, naringenin and naringenin chalcone.[17] Some phenolic compounds (especially quercetin) show a comparable or even higher antioxidative potential than ascorbic acid.[18]

In addition to the biotic influences described above, abiotic factors like climate and fertilization may influence the content of bioactive compounds in tomato fruits. Phenolic accumulation increases due to high light intensity to protect the fruits against harmful UV light.[19] Toor *et al.*[11] came to the assumption that organic fertilization led to an increase in the C/N ratio and in certain non-N-containing compounds such as phenolics, terpenoids and ascorbic acid. This is most probably due indirectly to a lower leaf production and a higher sunlight influence, as well as to a lower N-consuming protein metabolism rate. Nevertheless, these authors could not validate their assumption for lycopene. Studies comparing organic and conventional farming systems could demonstrate a comparable or even higher content of phenolic compounds, ascorbic acid and lycopene in tomatoes grown under organic farming management, which may be attributable to the use of organic fertilizers.[20–21]

## EXPERIMENTAL

### Plant materials

Twenty-eight tomato genotypes from commercial sources, a gene bank, non-governmental organization (NGO) private seed savers and the University of Göttingen's organic outdoor tomato breeding programme were investigated (Table 1). The genotypes represented a wide range of fruit weights and different colours.

### Cultivation procedure and experimental layout

The tomato seeds were sown in a greenhouse on 18 March 2004 and 31 March 2005. The average air temperature was 20 °C during the day and 15 °C during the night. The photoperiod was 14 h with additional plant light. The seeds were sown in an organic substrate (Kleeschulte Topfsubstrat ohne Torf organisch, Rüthen, Germany) in small pots (4 cm × 4 cm × 7 cm).

The plants were transferred on 4 April 2004 and 25 April 2005, to larger pots (11 cm × 11 cm × 15 cm) and placed in a greenhouse with no additional light. The temperature was adjusted so that about 50% of the genotypes had open flowers when the plants were transferred to the experimental locations (Table 2). The organic field trials were established at Ellingerode (Hessen) and Schönhagen (Thuringia) in central Germany. A subgroup of 12 genotypes was planted in 2004, and all 28 genotypes under consideration were planted in 2005. The genotypes were planted in a randomized block design with two replications and two plants per plot. Typical to the research area, the plants were grown as staked tomatoes and pruned to one main shoot.

### Evaluation of fruit infection by *P. infestans*

*Phytophthora infestans* infections were scored according to Horneburg and Becker[22] at intervals of 5–17 days during the periods of infection. Score 1 = no fruits infected, 3 = up to 25% of the fruits with typical dark spots, 5 = up to 50% of the fruits with dark spots, 7 = up to 75% of the fruits with dark spots and 9 = all fruits infected.

For all the experiments, the infection rate was calculated as the area under the disease progressive curve (AUDPC), using the following formula according to Kranz:[23]

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{x_{i+1} + x_i}{2} \right) (t_{i+1} - t_i)$$

where $x_i$ = score at time $i$, $t_i$ = day of the $i$th observation and $n$ = number of scores. Lower AUDPCs indicate a higher level of field resistance.

### Sample collection and quality measurements

Healthy and mature fruits were harvested from both locations on 2 September 2004 and 14 September 2005. The sample size included a minimum of four fruits per plant, equalling a minimum of eight fruits per plot and 16 per location. The collected samples were stored at −18 °C for further analysis. Three samples of each field replication were used for the quality assays.

Except for ascorbic acid determination, the samples for bioactive compound measurements were freeze-dried (Epsilon 2–40, Christ, Osterode, Germany) for 4 days, milled in a speedy mixer (Speedy Pro Plus F7247010F, Krups, Ecully, France) and milled to a fine powder in a ball-milling machine for further analysis.

The ascorbic acid in fresh fruits was measured by titration against 0.21% 2,6-dichlorophenolindophenol (DIP) dye according to Albrecht,[24] after extraction of 5 g fresh sample in 5% metaphosphoric acid. The lycopene content was determined from the freeze-dried material extracted with hexane–methanol–acetone (2 : 1 : 1), containing 2.5% butylated hydroxyl toluene (BHT) according to George *et al.*[25] The measurements were performed by spectrophotometer at 502 nm against the extraction solution as blank.

The total phenolic compounds content was determined with Folin–Ciocalteu phenol reagent by spectrophotometry. Tomato

  

**Table 1.** Origin, fruit colour and weight of 28 tomato genotypes grown at Ellingerode (2004 [in bold] and 2005) and Schönhagen (2005). The genotypes are listed according to increasing fruit weight

| Genotype | Code | Origin | Fruit colour | Fruit weight (*g*)[1] |
|---|---|---|---|---|
| S 030a, | SO | Gerhard Bohl | yellow | 3.4 |
| Celsior, | CE | Dreschflegel | red | 13.0 |
| Resi, | RG | Gerhard Bohl | red | 14.7 |
| Cuban Pink, | CP | Gerhard Bohl | red-violet | 16.1 |
| Cerise gelb | CG | Dreschflegel | yellow | 17.1 |
| Philovita F1 | PH | De Ruiter | red | 19.7 |
| **Cerise rot** | **CR** | Dreschflegel | red | 25.0 |
| Celsior x Matina F6 | CM | cross developed in the project | red | 29.0 |
| Golden Currant x Matina F4 | GM | cross developed in the project | yellow | 30.1 |
| **LYC 2469** | **L9** | IPK Gatersleben gene bank | red | 30.3 |
| **LYC 2466** | **L6** | IPK Gatersleben gene bank | red | 42.6 |
| Rosa Roma | RR | Jürgen Koch | red | 51.2 |
| **Matina** | **MA** | Dreschflegel | red | 56.5 |
| **Lämpchen** | **LM** | Gerhard Bohl | yellow | 58.3 |
| **Ostravske Rane** | **OR** | IPK Gatersleben gene bank | red | 59.8 |
| Goldene Königin | GK | Saatgut Quedlinburg | yellow | 63.5 |
| **Zuchtstamm 4** | **Z4** | Spieß/Matthes | red | 64.1 |
| Baumtomate | BT | Zollinger | red | 69.6 |
| Pfirsichhäutige | PU | Arche Noah | red | 71.0 |
| **Hybrid-2 Tarasenko** | **HT** | Gerhard Bohl | red | 73.0 |
| Harzfeuer F1 | HF | Bruno Nebelung | red | 76.1 |
| Zuchtstamm 22 | Z2 | Spieß/Matthes | red | 86.2 |
| **Z 21** | **Z1** | Gerhard Bohl | red | 97.8 |
| **Vitella F1** | **VF** | Bruno Nebelung | red | 104.4 |
| Phantasia F1 | PS | De Ruiter | red | 104.7 |
| **Ferline F1** | **FF** | Thompson and Morgan | red | 129.2 |
| **Paprikaförmige** | **PO** | Dreschflegel | pink-red | 203.3 |
| Schlesische Himbeer | SH | Arche Noah | red | 220.0 |

[1] Average, Ellingerode and Schönhagen 2005.

**Table 2.** Locations, pre-crops, fertilization and planting dates for the years 2004 and 2005

| | Schönhagen 51° 19′ 60″ N, 10° 1′ 0″ E Altitude 300 m | Ellingerode 51° 19′ 60″ N, 9° 49′ 0″ E Altitude 140 m, southern slope | |
|---|---|---|---|
| | 2005 | 2004 | 2005 |
| Pre-crops | *Malva verticillata* | Oats | Crimson clover |
| Fertilization | 200 dt ha$^{-1}$ composted cow manure, October 2004 | 180 dt ha$^{-1}$ composted sheep manure, November 2003 | 150 dt ha$^{-1}$ composted sheep manure, Autumn 2004 |
| Soil | Deep loamy clay | Shallow sandy loam | Shallow sandy loam |
| Planting date | 25 May | 19 May | 25 May |

extract was prepared from 1 g freeze-dried tomato powder in 5 mL of 82% ethanol. A calibration curve was performed with gallic acid with 1 mL of 0.5 mol L$^{-1}$ sodium hydroxide solution and 100 µL Folin–Ciocalteu reagent. The absorbance was measured at 765 nm and calculated as gallic acid equivalents (mg GAE 100 g$^{-1}$ DM) according to Singleton and Rossi.[26]

The antioxidant capacity was determined by the ferric reducing antioxidant potential (FRAP) assay,[27] which uses iron for oxidation. The FRAP assay was done with FRAP reagent: 20 mL 2,4,6-tripyridyl-2-triazine (TPTZ) and 20 mmol L$^{-1}$ ferric chloride in 200 mL sodium acetate buffer pH 3.6, and 24 mL distilled water. 100 µL tomato extract (0.5 g/10 mL in extraction solution) was added to 1 mL FRAP reagent and mixed thoroughly. After incubation for 4 min at 37 °C in a water bath, the absorbance at 593 nm was measured against a water blank. A calibration curve with a wide range of ferrous ion concentrations (50–1000 µmol L$^{-1}$) was produced with freshly prepared ammonium ferrous sulfate. The FRAP values were obtained by comparing the absorption changes in the test mixture with those obtained with an increasing concentration of $Fe^{3+}$ and expressed as mmol $Fe^{2+}$ equivalents kg$^{-1}$ of fresh sample. Three replicates for each sample of the field replicates were performed for each of the laboratory analyses.

### Reagents and standard solutions

Folin–Ciocalteu's phenol reagent (analytical grade), DIP dye (analytical grade), butylated hydroxyl toluene (BHT), sodium hydroxide (analytical grade) and sodium acetate × $3H_2O$ (99.5%, analytical grade) were obtained from Merck (Darmstadt, Germany). Gallic acid monohydrate (98%), metaphosphoric acid (analytical grade), hexane (99.0%, analytical grade), methanol (99.9%, analytical grade), acetone (99.8%, analytical grade), methanol (99.9%, analytical grade), glacial acetic acid (100%, extra pure), concentrated HCl (37%, extra pure), 2,4,6-tri[2-pyridyl]-*s*-triazine (TPTZ, 98.5%, analytical grade), ferric chloride (97.0%, analytical grade) and ferrous sulfate (99.0%, analytical grade) were obtained from Carl Roth (Karlsruhe, Germany).

### Statistical analysis

Normal distribution and the evaluation of the main effects and interaction of factors were tested using a two-way analysis of variance with the program Sigma Stat (statistical software version 2.0, SPSS Inc., Chicago, IL, USA). The Tukey test was employed to determine the differences between means at a 5% significance level using JMP8 (Start Statistics, 3rd edition, SAS Institute, Inc., Cary, NC, USA). Correlations among parameters were evaluated with Pearson's test. The graphics were prepared with Sigma Plot (statistical software version 10, SPSS Inc.).

## RESULTS AND DISCUSSION

Significant variations in the content of fruit bioactive compounds were observed among the investigated genotypes (Table 3). The individual compounds, their relationship to fruit weight and fruit infection by *P. infestans*, and the overall correlations are presented in this section. The influence of location and year is discussed. All the data are based on fresh matter to reflect the possible influence of tomato genotypes on human nutrition.

For all the investigated traits, both new (crosses and hybrid varieties except for Harzfeuer F1) and old genotypes (old commercial varieties and heritage varieties) were among the top- and the bottom-ranking genotypes. No general trend could be observed, however, for the formation of fruit bioactive compounds in old *versus* new genotypes. In contrast, Murphy *et al.*[28] demonstrated an increased nutrient uptake in old wheat in comparison to new varieties. Quality investigations on old varieties are becoming important as interest in these crops increases. The revival of heirloom tomatoes in farmers' markets and home gardens has been reported by Jordan.[29] Indeed, some of the non-commercial genotypes investigated in this study should be grown as they have excellent fruit quality. That such traits are inheritable was indicated by one cross, the Celsior × Matina F6, which exceeded both its parents in lycopene content and antioxidant capacity. In contrast, this cross contained fewer total phenolic compounds than both its parents and an intermediate amount of ascorbic acid. However, none of these differences were significant.

### Lycopene

Significant differences in lycopene content were detected among the genotypes (Table 3). Lycopene is the pigment principally responsible for the characteristic deep-red colour of mature tomato fruit and tomato products.[30] The lowest lycopene concentration was detected in the yellow-coloured genotypes Goldene Königin (24.8 mg $kg^{-1}$), Lämpchen, Cerise gelb and Celsior × Matina. Surprisingly, however, the yellow S 030a (47.5) had a higher lycopene level than Philovita F1 (45.5) with red fruit. As expected, the highest lycopene concentrations were obtained in the red-fruited genotypes such as Zuchtstamm 4 (104.5), Ferline F1, Hybrid-2 Tarasenko, Celsior × Matina F6, Zuchtstamm 22 and Resi. The obtained data are in agreement with Toor and Savage[31] for greenhouse-grown tomatoes and in the same range as that found in field-grown cherry tomatoes by Lenucci *et al.*[32]

The tomato is an important source of lycopene. A recommended average daily dietary lycopene intake level of 25.2 mg per day was computed from the lycopene contents of different food products consumed by the Canadian population.[33] According to this estimation, the results from our study indicate that the consumption of 100 g fresh tomato (mean of the tested genotypes) might provide the human body with 27% of the recommended daily intake of lycopene, while the highest lycopene-containing genotype Zuchtstamm and the cross Celsior × Matina F6 could provide 41% and 35%, respectively.

### Ascorbic acid

Large variations in ascorbic acid content were observed among genotypes (Table 3). The highest ranked was S 030a (272.5 mg $kg^{-1}$), followed by Harzfeuer F1, Vitella F1 and Ostravske Rane. The lowest contents were observed in Paprikaförmige (163.5), Ferline F1 and Pfirsichhäutige. Generally, the values obtained in this study were in agreement with George *et al.*[25] for field-grown tomatoes in India. Chassy *et al.*[34] found ascorbic acid contents in the range of the current experiment and these authors demonstrated a significant increase in ascorbic acid in one genotype grown in an organic farming system.

The recommended daily uptake of ascorbic acid according to the US National Research Council was estimated to be 60 mg per day for healthy, non-smoking adults.[35] According to this estimation, the consumption of about 100 g fresh tomatoes (mean of the tested genotypes) might provide the body with 35% of the recommended daily uptake of ascorbic acid, while the highest ascorbic acid-containing genotype S030a could provide 46%.

### Total phenolic compounds

Cuban Pink had the highest content of total phenolic compounds (980.8 mg $kg^{-1}$; Table 3). The red-violet colour of this genotype is probably derived from anthocyanins, which belong to the flavonoids; however, anthocyanins do not belong to the main group of flavonoids in tomatoes.[17,36]

Next in ranking were Philovita F1, Matina, Schlesische Himbeer and Celsior. The total phenolic compounds obtained in this study were lower than those found by Lenucci *et al.*[32] in field-grown cherry tomatoes and higher than those found by Chun *et al.*[9] in tomato fruits from local US supermarkets. The total phenolic compounds were higher in the current study than in the investigations done by Chassy *et al.*[34] The reason why our values differ from those of Chun *et al.*[9], Chassy *et al.*[34] and Lenucci *et al.*[32] may be the farming system (in particular, the different C/N ratios of the fertilizers), different extraction methods or the different tomato genotypes used.

Chassy *et al.*[34] investigated the influence of organic and conventional farming systems on phenolic compounds in tomato fruits and could demonstrate a significant increase in quercetin and kaempferol in fruits from organic farming, but the authors could not distinguish between cropping systems with respect to the total phenolic content of the fruit.

  

**Table 3.** Fruit lycopene, ascorbic acid, total phenolic compounds, antioxidant capacity and infection by *P. infestans* for 28 tomato genotypes grown at Ellingerode and Schönhagen in 2005. The genotypes are arranged according to mean fruit weight

| Genotype | Lycopene (mg kg$^{-1}$ fresh weight) | Ascorbic acid (mg kg$^{-1}$ fresh weight) | Total phenolic compounds (mg kg$^{-1}$ fresh weight) | Antioxidant capacity (mmol $Fe^{2+}$ kg$^{-1}$ fresh weight) | Infection level (AUDPC)[a] |
|---|---|---|---|---|---|
| S 030a | 47.5 ± 24.6efghi | 272.5 ± 60.1a | 376.6 ± 71.4bcde | 40.8 ± 8.1cd | 61.2 ± 10.9ab |
| Celsior | 57.8 ± 19.4cdefghi | 182.3 ± 33.8cde | 699.1 ± 104.2abcd | 45.8 ± 8.2abcd | 79.3 ± 9.1abcd |
| Resi | 83.9 ± 19.8abcd | 193.3 ± 40.7cde | 648.6 ± 198.2abcde | 58.6 ± 8.2ab | 48.8 ± 4.9a |
| Cuban Pink | 66.6 ± 12.3bcdefg | 224.4 ± 35.4abcd | 980.8 ± 575.0a | 53.8 ± 12.8abcd | 79.6 ± 36.7bcde |
| Cerise gelb | 29.1 ± 8.5hi | 207.8 ± 15.3bcde | 706.3 ± 166.6abcd | 48.9 ± 9.3abcd | 66.8 ± 13.9abc |
| Philovita F1 | 45.5 ± 10.7fghi | 230.8 ± 59.4abc | 774.6 ± 391.4ab | 53.8 ± 8.4abcd | 66.3 ± 15.9ab |
| Cerise rot | 78.3 ± 13.3abcdef | 181.8 ± 11.1cde | 563.3 ± 232.8abcde | 41.8 ± 5.4bcd | 102.3 ± 17.6efghi |
| Celsior × Matina | 87.7 ± 10.1abc | 208.5 ± 31.6bcde | 429.6 ± 140.3bcde | 53.9 ± 6.8abcd | 111.1 ± 13.5efghij |
| Golden Currant × Matina | 32.6 ± 16.8ghi | 179.1 ± 3.7cde | 539.5 ± 71.1bcde | 48.5 ± 13.8abcd | 128.4 ± 26.6ghijkl |
| LYC 2469 | 64.8 ± 16.8bcdefg | 203.3 ± 10.0bcde | 565.8 ± 207.9abcde | 57.8 ± 12.7abc | 96.4 ± 27.0defg |
| LYC 2466 | 74.0 ± 21.6abcdef | 196.8 ± 23.4cde | 431.0 ± 119.2bcde | 59.7 ± 11.6a | 150.7 ± 55.4ghijkl |
| Rosa Roma | 71.1 ± 11.3abcdef | 199.8 ± 15.6bcde | 368.5 ± 58.7bcde | 47.1 ± 9.4abcd | 119.5 ± 39.0efghijk |
| Matina | 79.4 ± 27.3abcdef | 222.9 ± 29.2abcd | 747.2 ± 418.4abc | 44.5 ± 10.7abcd | 179.3 ± 20.4hijkl |
| Lämpchen | 32.9 ± 7.5ghi | 185.0 ± 7.2cde | 340.8 ± 126.1cde | 44.2 ± 9.6abcd | 139.9 ± 30.9ghijkl |
| Ostravske Rane | 79.5 ± 15.5abcdef | 235.8 ± 58.2abc | 549.7 ± 93.3bcde | 43.5 ± 6.3abcd | 237.8 ± 51.7kl |
| Goldene Königin | 24.8 ± 10.1i | 223.0 ± 6.0abcd | 562.2 ± 175.0abcde | 37.4 ± 13.5d | 212.6 ± 36.9hijkl |
| Zuchtstamm 4 | 104.5 ± 39.1a | 192.8 ± 4.7cde | 464.5 ± 150.5bcde | 53.4 ± 16.3abcd | 99.9 ± 26.6efgh |
| Baumtomate | 80.2 ± 22.4abcde | 195.5 ± 38.0cde | 323.7 ± 154.2de | 37.3 ± 7.4d | 129.3 ± 39.5ghijkl |
| Phantasia F1 | 61.4 ± 13.3cdefgh | 219.3 ± 17.6abcde | 419.4 ± 87.8bcde | 41.4 ± 7.5bcd | 87.7 ± 30.0cdefg |
| Hybrid-2 Tarasenko | 91.3 ± 13.5abc | 225.1 ± 49.0abcd | 360.1 ± 39.3bcde | 39.2 ± 3.1d | 119.4 ± 37.9efghijk |
| Harzfeuer F1 | 53.3 ± 11.0defghi | 256.0 ± 17.2ab | 370.2 ± 122.4bcde | 45.8 ± 9.8abcd | 244.5 ± 24.6l |
| Zuchtstamm 22 | 86.9 ± 26.5abcd | 200.3 ± 17.5bcde | 486.8 ± 251.0bcde | 44.4 ± 6.8abcd | 115.1 ± 26.3efghij |
| Z 21 | 64.5 ± 8.4bcdefg | 220.8 ± 32.0abcde | 560.3 ± 331.6abcde | 39.5 ± 4.5d | 86.3 ± 23.4bcdef |
| Pfirsichhäutige | 79.9 ± 11.0abcde | 177.4 ± 8.6cde | 512.2 ± 73.7bcde | 47.6 ± 7.7abcd | 213.5 ± 36.2ijkl |
| Vitella F1 | 69.2 ± 8.9bcdef | 234.6 ± 28.9abc | 285.6 ± 101.1de | 39.0 ± 4.9d | 220.5 ± 36.9jkl |
| Ferline F1 | 94.4 ± 7.4ab | 171.8 ± 20.3de | 404.2 ± 172.5bcde | 48.5 ± 8.3abcd | 125.5 ± 42.8fghijk |
| Paprikaförmige | 76.5 ± 11.4abcdef | 163.5 ± 18.2de | 252.3 ± 82.2e | 38.5 ± 6.7d | 151.6 ± 46.1hijkl |
| Schlesische Himbeer | 58.0 ± 34.3cdefghi | 216.1 ± 40.8abcde | 699.7 ± 409.0abcd | 45.3 ± 9.6abcd | 176.1 ± 63.1hijkl |
| Mean | 66.9 | 207.8 | 515.1 | 46.4 | 130.3 |

Data are expressed as mean ± SD. Different letters within a column indicate significant differences in the Tukey test; $\alpha = 5\%$.
[a] Area under the disease progressive curve.

Recent data concerning the recommended daily uptake of total phenolic compounds are, to our knowledge, not available. However, Chun *et al*.[9] calculated that in the USA an average daily intake of 129.4 mg total phenols was provided by vegetables. Tomatoes with an average serving size of 103 g per day per person were the second most important contributors (24.4 mg per day per person) of total phenolics after potatoes.[9] Considering these data, the consumption of 132 g fresh Cuban Pink fruit would generate a similar amount of total phenolics as the average daily per capita intake in the USA.

## Antioxidant capacity

Antioxidant capacity varied greatly among the genotypes (Table 3). LYC 2466 had the highest antioxidant capacity (59.7 mmol $Fe^{2+}$ kg$^{-1}$), followed by Resi and LYC 2469. The lowest contents were found in the genotypes bearing larger fruit: Baumtomate (37.3), Goldene Königin, Paprikaförmige and Vitella F1. According to Chun *et al*.,[9] tomato fruits provide an antioxidative potential (calculated as ascorbic acid equivalents) which is in the medium range of the vegetables tested by these authors. To our knowledge, no recommended daily intake has been given.

## Correlation of traits

Differences in genotype susceptibility to disease might be accredited to variations in physiological attributes such as the accumulation of secondary metabolites.[37] The correlations given in Table 4, however, do not indicate any direct influence of the content of fruit bioactive compounds on late blight fruit infection. A lack of correlations might be due to the significant effects of location and location × genotype interaction (Table 5).

The total phenolic compounds were positively correlated ($R = 0.40$) with the antioxidant capacity (Table 4 and Fig. 1). This correlation was expected since phenolic compounds are an important part of antioxidant capacity and they have a higher antioxidative potential than ascorbic acid.[18] According to George *et al*.,[25] lipid-soluble carotenoids such as lycopene do not contribute to the antioxidant capacity determined by the FRAP assay. This is in agreement with our results, which showed no correlation between lycopene content and antioxidative capacity (Table 4).

A negative correlation ($R = -0.42$) between the antioxidant capacity and fruit weight was observed (Fig. 2). The same was also detected by George *et al*.[25] and Lenucci *et al*.,[32] who stated that cherry tomatoes with a low fruit weight represent a better source

**Table 4.** Correlation coefficients of fruit bioactive compounds, fruit weight and late blight fruit infection of 28 tomato genotypes from Ellingerode and Schönhagen 2005 ($n = 28$)

| | Ascorbic acid | Total phenolic compounds | Antioxidant capacity | Fruit weight | Fruit infection |
|---|---|---|---|---|---|
| Lycopene | −0.25 | −0.21 | 0.14 | 0.19 | −0.003 |
| Ascorbic acid | | 0.09 | −0.17 | −0.17 | 0.14 |
| Total phenolic compounds | | | 0.40* | −0.33 | −0.29 |
| Antioxidant capacity | | | | −0.42* | −0.33 |
| Fruit weight | | | | | 0.41* |

Significant at * $P \leq 0.05$.

**Table 5.** Effect of location, genotype and their interaction on fruit weight, bioactive compounds and late blight infection at Ellingerode and Schönhagen 2005

| Parameter | Ellingerode Mean ± SD | Schönhagen Mean ± SD | Location DF = 1 VC | Genotype DF = 27 VC | Location × genotype DF = 27 VC | Error DF = 168 VC |
|---|---|---|---|---|---|---|
| Fruit weight | 55.8 ± 47.1 | 74.5 ± 60.8 | 169.6*** | 2.689.4*** | 88.4*** | 253.5 |
| Lycopene | 63.3 ± 25.4 | 71.8 ± 26.2 | 23.9*** | 221.2*** | 306.5*** | 152.5 |
| Ascorbic acid | 220.3 ± 32.4 | 195.3 ± 40.1 | 271.3*** | 80.4*** | 1.130.0*** | 175.3 |
| Total phenolic compounds | 445.9 ± 178.5 | 584.2 ± 321.5 | 7.788*** | 4.261*** | 44.613*** | 20.056 |
| Antioxidant capacity | 47.9 ± 11.5 | 43.5 ± 10.2 | 7.6*** | 10.5*** | 53.1*** | 56.9 |
| Fruit infection | 145.2 ± 68.4 | 115.4 ± 53.7 | 416.2*** | 2.738.2*** | 624.2*** | 512.7 |

VC, variance component; DF, degrees of freedom.
Statistically significant at *** $P \leq 0.001$.

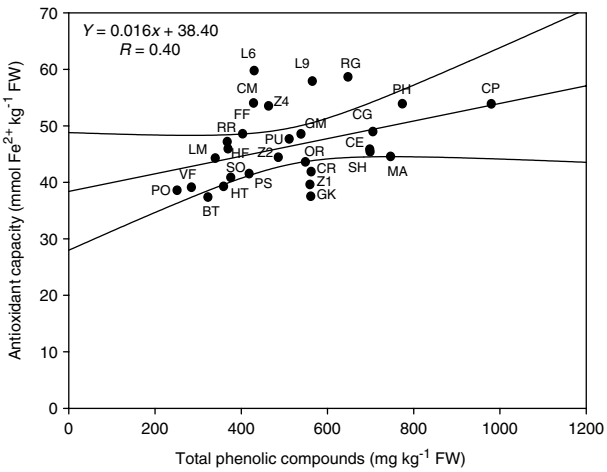


**Figure 1.** Relationship between antioxidant capacity and total phenolic compounds with 99% confidence interval. The data show the mean values of genotypes ($n = 28$) from two locations; for genotype abbreviations see Table 1.

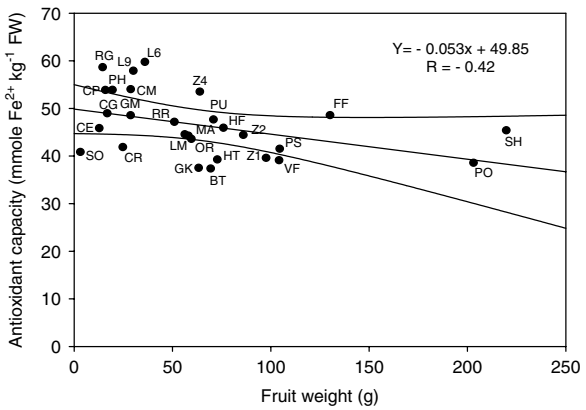


**Figure 2.** Relationship between fruit weight and antioxidant capacity with 99% confidence interval. The data show the mean values of genotypes ($n = 28$) from two locations; for genotype abbreviations see Table 1.

for antioxidants in the human diet than fruits of a larger size. A correlation between antioxidant capacity and infection level was not found, but other researchers have observed that an increase in the antioxidant capacity of plants is expected to increase their tolerance to pathogens.[38]

Table 4 and Fig. 3 demonstrate that the level of fruit infection was correlated with fruit weight. Genotypes far above the confidence interval are not suitable for organic outdoor tomato production. They include traditional varieties like Harzfeuer F1, Ostravske Rane, Goldene Königin and Pfirsichhäutige, as well as the recently released Vitella F1. This result is in agreement with Jinsin,[39] who stated that small tomato fruit had a higher resistance to disease. This could be related to the higher fruit skin/pulp ratio in small tomato genotypes, since phenolic compounds are concentrated in the skin and may act as defence compounds there.[40] Furthermore, large fruit size and weight are frequently associated with cracking,[41] which could be an easy way for pathogens to penetrate into the fruit.

Interesting for breeding and production are the genotypes with a low infection level in combination with a high nutritional value. The small-fruited tomato genotypes like Philovita F1, Cuban pink and Resi have gained these properties and therefore seem to be most interesting for breeding (Fig. 3 and Table 3). Also the

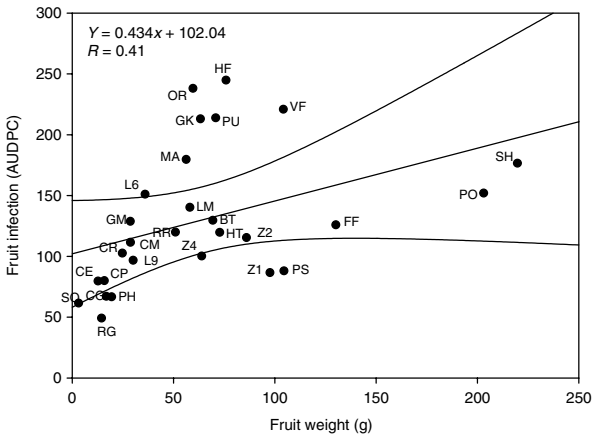


**Figure 3.** Relationship between infection level and fruit weight with 99% confidence interval. The data show the mean values of genotypes ($n = 28$) from two locations; for genotype abbreviations see Table 1.

small-fruited crosses Celsior × Matina F6 and Golden Currant × Matina F4 resulting from the breeding programme showed good nutritional properties and increased resistance compared to their parent Matina. Even though SO3Oa had a low infection level and had fruits with the highest ascorbic acid content, it was found to be very poor in phenolic content and antioxidative capacity. This may imply that a high fruit phenolic content is not solely responsible for preventing fungal infection; indeed, other secondary plant compounds (e.g. alkaloids)[42] might be responsible.

Only two genotypes with a middling fruit weight – Z 21 and Phantasia F1 – showed sufficient field resistance against *P. infestans*; however, their nutritional quality was in the middle and low range. The two genotypes with the highest fruit weight – Paprikaförmige and Schlesische Himbeer – had a high susceptibility to *P. infestans* and only Schlesische Himbeer had an adequate nutritional quality comparable to that of the small-fruited genotypes.

### Effect of the environment

All the experiments were conducted under low-input conditions. The amount of fertilizer applied to the research plots was far less than the amount recommended for commercial tomato cropping. The fields were not irrigated, even during drought periods. Because the soil nutrient levels and water capacity were not determined, a detailed factorial analysis could not be done although they will be carried out in future projects.

The location had a significant effect on all the investigated traits (Table 5). On the poorer soil at Ellingerode, the fruits were smaller, contained less lycopene and total phenolic compounds than at Schönhagen, but they had a higher content of ascorbic acid and a higher antioxidant capacity.

The interactions of location and genotype were the most important variance components for all the fruit bioactive compounds. The influence of the location was stronger than the influence of the genotype for ascorbic acid and total phenolic compounds. Previous studies have also come to the same result.[43] In contrast, the variance in lycopene content and antioxidant capacity depended more on genotype than location. The genotype was also the most important variance component for the traits fruit weight and late blight fruit infection.

The year had a significant effect on all traits (Table 6): the fruits were larger in 2004 than in 2005, but their lycopene content and antioxidant capacity were lower. The opposite was observed for ascorbic acid and total phenolic compounds. The most important variance component for ascorbic acid and total phenolic compounds was the interaction of year and genotype. For all the traits apart from fruit weight, the influence of the year was stronger than the influence of the genotype.

## CONCLUSIONS

The quantities of the evaluated secondary plant compounds and ascorbic acid observed in this study were comparable to the contents found in greenhouse- and field-grown tomatoes from various previous investigations. In particular, the amount of phenolics provided by the tested genotypes may be a good source for antioxidants in human nutrition. The genetic variation among tomato genotypes for the content of fruit bioactive compounds and their susceptibility to *P. infestans* is large, allowing for possible selection gains. No general trend was observed, however, for the formation of fruit bioactive compounds in old *versus* new genotypes. The independent selection for a high content of lycopene, ascorbic acid or total phenolic compounds seems possible due to the lack of correlations between these compounds. The positive correlation of fruit weight and fruit infection by late blight, however, is a severe restriction to breeding programmes. None of the investigated fruit bioactive compounds can be recommended for the indirect selection for increased field resistance against *P. infestans*.

All the traits were significantly influenced by the environment; therefore, particularly, the contents of ascorbic acid and total phenolic compounds need to be analysed over a number of

**Table 6.** Effect of year, genotype and their interaction on fruit weight, bioactive compounds and late blight infection 2004 and 2005 at Ellingerode

| Parameter | 2004 Mean ± SD | 2005 Mean ± SD | Year DF = 1 VC | Genotype DF = 11 VC | Year × genotype DF = 11 VC | Error DF = 72 VC |
|---|---|---|---|---|---|---|
| Fruit weight | 76.7 ± 65.7 | 67.4 ± 49 | 27.1* | 2.955.3*** | 58.3 | 545.4 |
| Lycopene | 41.5 ± 18.7 | 68.1 ± 19.1 | 341.3*** | 124.3*** | 133.1*** | 114.9 |
| Ascorbic acid | 258.5 ± 50 | 219.4 ± 35 | 632.3*** | 185.1*** | 1.520.6*** | 265.9 |
| Total phenolic compounds | 550.6 ± 146.5 | 398.2 ± 190.2 | 10.044*** | 0 | 15.250*** | 14.667 |
| Antioxidant capacity | 25.2 ± 10.3 | 46.6 ± 11.2 | 227.9*** | 56.5*** | 0 | 64.3 |
| Fruit infection | 85.8 ± 39.4 | 158.0 ± 59.5 | 2.545.4*** | 1.499.2*** | 663*** | 524.3 |

VC, variance component; DF, degree of freedom; 0 indicates negative estimates.
Statistically significant at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

different environments (years and locations) to characterize tomato genotypes. Antioxidant capacity seemed to be mainly influenced by the phenolic compounds. In future, the contribution of single phenolic compounds to the antioxidant capacity and their relation to *P. infestans* infection should be studied.

## REFERENCES


1 FAOSTAT, [Online]. Available: http://faostat.fao.org/site/567/DesktopDefault.aspx?#ancor [14 January 2011].
2 Wisler GC and Duffus JE, A century of plant virus management in the Salinas Valley of California 'East of Eden'. *Virus Res* **71**:161–169 (2000).
3 Foolad MR, Merk HL and Ashrafi H, Genetics, genomics and breeding of Late Blight and Early Blight resistance in tomato. *Crit Rev Plant Sci* **27**:75–107 (2008).
4 Tamm L, Smit AB, Hospers M, Janssens SRM, Buurma JS, Mølgaard JP, *et al*, Assessment of the socio-economic impact of Late Blight and state-of-the-art management in European organic potato production systems (2004). [Online]. Available: http://www.orgprints.org/2936 [14 January 2011].
5 Tort N, Öztürk I and Güvensen A, Effects of some fungicides on pollen morphology and anatomy of tomato (*Lycopersicon esculentum* L.). *Pak J Bot* **37**:23–30 (2005).
6 Lampkin N, *Organic Farming*. Farming Press, Ipswich, UK (1990).
7 Horneburg B, Tomaten im Freiland, Die Suche nach *Phytophthora*-toleranten Sorten für den ökologischen Anbau. *Vortr Pflanz* **70**:99–104 (2006).
8 Horneburg B and Becker HC, Does regional organic screening and breeding make sense? Experimental evidence from organic outdoor tomato breeding, in *Cultivating the Future Based on Science*. Vol. 1: *Organic Crop Production. Proceedings of the Second Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR)*, 18–20 June 2008, Modena, Italy, ed. by Neuhoff D, Halberg N, Alföldi T, Lockeretz W, Thommen A, Rasmussen IA, *et al*. ISOFAR, Bonn, pp. 670–673 (2008).
9 Chun OK, Kim D-O, Smith N, Schroeder D, Han JT and Lee CY, Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *J Sci Food Agric* **85**:1715–1724 (2005).
10 El-Khallal SM, Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid and salicylic acid). 2: Changes in the antioxidant enzymes, phenolic compounds and pathogen-related proteins. *Aust J Basic Appl Sci* **1**:717–732 (2007).
11 Toor RK, Savage GP and Heeb A, Influence of different types of fertilizers on the major antioxidant components of tomatoes. *J Food Comp Anal* **19**:20–27 (2006).
12 Davey MW, Van Montagu M, Inze D, Sanmartin M, Kanellis A, Smirnoff N, *et al*, Plant l-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* **80**:825–860 (2000).
13 Baranski R, Baranska M and Schulz H, Changes in carotenoid content and distribution in living plant tissue can be observed and mapped *in-situ* using NIR-FT-Raman spectroscopy. *Planta* **222**:448–457 (2005).
14 Chérif M, Sadafi N, Benhamou N, Hajlaoui MR, Boubaker A and Tirilly Y, Ultrastructure and cytochemistry of *in-vitro* interactions of the antagonistic bacteria *Bacillus cereus* X16 and *B. thuringiensis* 55T with *Fusarium roseum* var. sambucinum. *J Plant Pathol* **84**:83–93 (2002).
15 Rao AV and Agarwall S, Role of antioxidant lycopene in cancer and heart disease. *J Am Coll Nutr* **19**:563–569 (2000).
16 Arnao MB, Cano A and Acosta M, The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem* **73**:239–244 (2001).
17 Moco S, Capanoglu E, Tikunov Y, Bino R, Boyacioglu D, Hall R, *et al*, Tissue specialization at the metabolite level is perceived during the development of tomato fruit. *J Exp Bot* **58**:4131–4146 (2007).
18 Pulido R, Bravo L and Saura-Calixto F, Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric Food Chem* **48**:3396–3402 (2000).
19 Luthria DL, Mukhopadhyaya S and Krizek DT, Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *J Food Comp Anal* **19**:771–777 (2006).
20 Caris-Veyrat C, Phe Amoit ML, Tyssandier V, Grasselly D, Buret M, Mikolajczak M, *et al*, Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *J Agric Food Chem* **52**:6503–6509 (2004).
21 Mitchell AE, Hong YJ, Koh E, Barrett DM, Bryant DE, Ford Denison R, *et al*, Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *J Agric Food Chem* **55**:6154–6159 (2007).
22 Horneburg B and Becker HC, Selection for *Phytophthora* field resistance in the $F_2$ generation of organic outdoor tomatoes. *Euphytica* **180**:357–367 (2011).
23 Kranz J, *Epidemiologie der Pflanzenkrankheiten*. Ulmer Verlag, Stuttgart (1996).
24 Albrecht JA, Ascorbic acid and retention in lettuce. *J Food Qual* **16**:311–316 (1993).
25 George B, Kaur C, Khurdiya DS and Kapoor HC, Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. *Food Chem* **84**:45–51 (2004).
26 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–158 (1965).
27 Benzie IFF and Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal Biochem* **239**:70–76 (1996).
28 Murphy KM, Reeves PR and Jones SS, Relationship between yield and mineral nutrient concentration in historical and modern spring wheat cultivars. *Euphytica* **163**:381–390 (2008).
29 Jordan J, The heirloom tomato as cultural object: investigating taste and space. *Sociol Ruralis* **47**:20–41 (2007).
30 Shi J and Maguer ML, Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit Rev Biotechnol* **20**:293–334 (2000).
31 Toor RK and Savage GP, Antioxidant activity in different fraction of tomatoes. *Food Res Int* **38**:487–494 (2005).
32 Lenucci MS, Cadinu D, Taurino GP and Dalessandro G, Antioxidant composition in cherry and high-pigment tomato cultivars. *J Agric Food Chem* **54**:2606–2613 (2006).
33 Rao AV, Waseem Z and Agarwal S, Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. *Food Res Int* **31**:737–741 (1998).
34 Chassy AW, Bui L, Renaud ENC, Van Horn M and Mitchell AE, Three-year comparison of the content of antioxidant microconstituents and several quality characteristics in organic and conventionally managed tomatoes and bell peppers. *J Agric Food Chem* **54**:8244–8252 (2006).
35 *National Research Council Recommended Dietary Allowances*. National Academy Press, Washington, DC (1989).
36 Butelli E, Titta L, Giorgio M, Mock HP, Matros A, Peterek S, *et al*, Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat Biotechnol* **26**:1301–1308 (2008).
37 Cowan MM, Plant products as antimicrobial agent. *Clin Microbiol Rev* **12**:564–582 (1999).
38 Barna B, Fodor J, Pogany M and Kiraly Z, Role of reactive oxygen species and antioxidants in plant disease resistance. *Pest Manage Sci* **59**:459–464 (2003).
39 Jinsin Y, Evaluation of cherry tomato inbred lines for multiple disease resistance. AVRDC Report, Tainan, Taiwan (1999).
40 Crozier A, Clifford MN and Ashihara H, *Plant Secondary Metabolites, Occurrence, Structure and Role in the Human Diet*. Blackwell, Oxford (2006).
41 Jones JB, *Tomato Plant Culture: in the Field, Greenhouse, and Home Garden*. CRC Press, Boca Raton, FL (2008).
42 Friedman M, Tomato glycoalkaloids: role in the plant and in the diet. *J Agric Food Chem* **50**:5751–5780 (2002).
43 Dumas Y, Dadomo M, Di Lucca G and Grolier P, Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J Sci Food Agric* **83**:369–382 (2003).


  