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Original article

Comparison of on-vine and post-harvest ripening on antioxidant compounds and antioxidant activities of hydroponically grown cherry tomatoes

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Summary

Tomato fruits are considered as an excellent source of hydrophilic and lipophilic antioxidants. It is generally believed that on-vine ripened tomatoes are characterized by superior nutritional value comparing to post-harvest ripened fruits. The objective of this study was to elucidate this belief with respect to antioxidant activity of tomatoes. Hydroponically grown cherry type tomatoes *Solanum lycopersicum* L. var. Chiou were harvested at mature green, breaker and red ripe stages. Tomatoes at breaker stage were post-harvest ripened at room temperature. Total phenols, flavonoids, ascorbic acid, and lycopene content of on-vine and post-harvest ripened tomatoes were estimated. The results suggest that post-harvest ripening reduced total phenols but increased ascorbic acid and lycopene content. Antioxidant activity was evaluated with the use of DPPH, FRAP and ORAC assays. The hydrophilic antioxidant activity (HAA) values depended on the assay. DPPH and ORAC methods showed higher HAA for post-harvest tomatoes but FRAP estimated higher HAA for on-vine ripened fruits. All methods revealed that post-harvest ripened tomatoes exhibited significantly higher lipophilic antioxidant activity (LAA). The findings revealed that post-harvest ripening had contradictory implications to water soluble antioxidants and hence to HAA of tomatoes, while LAA levels due to lycopene accumulation were significantly increased.

Keywords
ascorbic acid, lycopene, post-harvest ripening, tomato, total phenols

Significance of this study

What is already known on this subject?

- There are contradictory findings on the influence of post-harvest ripening on antioxidant capacity of tomatoes.

What are the new findings?

- Post-harvest ripening favors lycopene accumulation and improves the lipophilic antioxidant activity of tomatoes.

What is the expected impact on horticulture?

- The nutritional value of cherry type tomato fruit was altered by post-harvest ripening; both lycopene and ascorbic acid content were significantly increased, while the total phenols were reduced. The results indicated that the fruits collected at the breaker stage and ripened in ambient temperature provide to consumers lycopene and ascorbic acid enriched fruits.

Introduction

Reactive oxygen species could be important causative agents of serious human diseases like coronary heart disease and cancer. Diet rich in fresh fruits and vegetables has been associated with a number of health benefits, including the prevention of chronic diseases (WHO, 2003). Tomato (*Solanum lycopersicum* L.) is one of the most economically important crops and is the leading vegetable worldwide for production and consumption (Frusciante et al., 2000). Tomato fruits are consumed as fresh fruits, paste, sauce, powder and are enjoying a constantly increasing commercial demand. They are an excellent source of human health-related phytonutrients due to the balanced mixture of hydrophilic and lipophilic antioxidants, such us total phenols, vitamins C and E, and carotenoids (Ilahy et al., 2016). Due to high volume per capita consumption, tomato fruits are an exceptional source of antioxidants (Jeffery et al., 2005). Tomato is the most important source of lycopene (Clinton, 1998) and the third source of vitamin C (Willcox et al., 2003) for human nutrition in the USA. Studies strong correlate tomato consumption with reduced risk of some types of cancer, cardiovascular diseases and age-related macular degeneration (Choi et al., 2010).

The antioxidant content of tomato mostly depends on genetic (George et al., 2004), environmental factors (Rosales et al., 2011), cultivar selection (Figàs et al., 2015), cultural practices (Caris-Veyrat et al., 2004) as well as the ripening stage of fruits (Cano et al., 2003). The antioxidant capacity can be also influenced by post-harvest treatments and storage conditions (Vallverdú-Queralt et al., 2011). Ripening of tomato has been well studied with the main objective to extend the fruit shelf-life. Among consumers, there is a general belief that the nutritional value of on-vine ripened tomatoes is superior to that of fruit ripened off the vine. The aim of the present study is to verify if this general belief is justified concerning the antioxidant activity of tomatoes. The effect of postharvest handling of fruits on their nutritional value is crucial because according to agricultural practice, tomatoes are generally harvested at earlier maturity stage before

full ripening and they are off-plant ripened during transportation and storage before consumption. Although, antioxidant capacity of tomatoes received huge scientific interest, according to the best of our knowledge this specific topic has received limited attention (Ozgen et al., 2012; Pék et al., 2010).

Materials and methods

Chemicals

Folin-Ciocalteu reagent, AAPH [2,2′-azobis(2-methylpropionamidine) dihydrochloride], DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ [2,4,6-Tris(2-pyridyl)-s-triazine], Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], rutin trihydrate (analytical standard), gallic acid, BHT (2,6-di-tert-butyl-4-methylphenol), 2,6-dichlorophenolindophenol (DCPIP), anhydrous iron (III) chloride and potassium acetate were purchased from Sigma Aldrich (Steinheim, Germany). Solvents were purchased from Sigma Aldrich (Steinheim, Germany) or Alfa Aesar (Thermo Fisher Scientific, Lancashire, United Kingdom) and they were spectrophotometric grade.

Plant material and samples preparation

Tomatoes (*Solanum lycopersicum* L. var. Chiou), were used in the present study. 'Chiou' is a cherry tomato traditionally grown in Greece. Tomato fruits used for the present analysis were collected from 60 plants developed hydroponically in greenhouses of the University of Peloponnese and watered with full strength nutrient solution as previously described (Darras et al., 2017). 'Chiou' revealed a determinate growth habit and the fruits at the same developmental stage were harvested from plants randomly distributed in the greenhouse. Fruits were simultaneously harvested at three different developmental stages according to maturity classification (Figure 1):

i) Mature green (full-sized green tomatoes);
ii) Breaker (no more than 10% of the surface was red or pink); and
iii) Red ripe (more than 90% of the surface was red) (VineRR).

A fourth sample of tomatoes was post-harvest ripened tomatoes (PhRR). The fruits harvested at breaker stage and stored at room temperature in a well-ventilated growth chamber (temperature 23–25°C, RH 50–60%) until they reached the red ripening stage (in about 5 days).

At least twenty tomatoes were collected for three biological samples from each stage, washed thoroughly with distilled water and surface dried. They were homogenized using IKA Homogenizer (IKA England Ltd., Oxford, United Kingdom), aliquoted and immediately frozen in liquid nitrogen. The samples were stored at -80°C until chemical analysis excluding the samples used for ascorbic acid analysis. Each sample was approximately 0.3 kg and it consisted of comparable sized tomatoes.

Colour measurements

The colour indexes of tomatoes were measured using a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) chromatometer calibrated with a white standard tile. Measurements were taken from three points in the equatorial region of tomatoes and the colour was recorded using the CIELab uniform colour space. Five tomatoes were measured for each stage. Results were expressed as a^*/b^* ratio.

Total phenols and flavonoids content

1. Preparation of extract. Before extraction the frozen samples were allowed to warm at room temperature and homogenized for 30 sec. For determination of total phenols and flavonoids the samples were extracted according to known procedure (García-Alonso et al., 2009) with some modifications. Specifically, 2 mL of methanol 75% and 1 mL HCl 1M were added to 2.000 ± 0.001 g of tomato sample. The mixture was vortexed for 1 min. Then it was heated at 37°C for 30 min while it was vortexed periodically. After heating, 1 mL of NaOH in 75% methanol was added and the mixture was vortexed for 1 min. Subsequently the mixture was centrifuged and the supernatant was collected. To the precipitate 2 mL of acetone 50% was added, the resulting mixture was then vortexed for 1 min and it was centrifuged. The supernatants from the two centrifugations were combined in a 10-mL volumetric flask and filled to 10 mL with 75% methanol.

2. Total phenols analysis. Total phenols content was analyzed using the Folin-Ciocalteu method (Capanoglu et al., 2008). A mixture of 500 µL extract, 500 µL Folin-Ciocalteu reagent and 4 mL distilled water was vortexed. After 3 min 1 mL Na_2CO_3 20% was added and the resulting solution was incubated at room temperature, in the dark, for two hours. Then the absorbance at 725 nm was measured. As blank was used the same mixture replacing extract with methanol 75%. Gallic acid was used as standard. The content of total phenols was expressed as mg gallic acid equivalent (GAE) per kg of fresh weight (mg GAE kg^{-1} FW).

3. Flavonoids analysis. Flavonoids content was determined according to known procedure (Silva et al., 2015) with some modifications. A mixture of 500 µL extract, 1.5 mL methanol

Figure 1. 'Chiou' variety at mature green, breaker and red ripe ripening stages.

75%, 100 µL $AlCl_3$ 10%, 100 µL potassium acetate and 2.8 mL distilled water was incubated for 30 min at room temperature. The absorbance at 415 nm was measured. Rutin was used as standard for the calibration curve. The flavonoids content was expressed as mg rutin equivalent (RE) per kg of fresh weight (mg RE kg^{-1} FW).

Lycopene analysis

Lycopene was measured according to known procedure (Rao et al., 1998) Lycopene concentration was estimated using specific extinction coefficient (E 1% 1 cm) 3150 at 502 nm and it was expressed as mg carotene per kg of fresh weight (mg carotene kg^{-1} FW).

Ascorbic acid analysis

Ascorbic acid determination was performed immediately after sample preparation using DCPIP method (Klein and Perry, 1982). The results were expressed as mg ascorbic acid kg^{-1} FW.

Antioxidant activity

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

1. Preparation of extract. Before extractions the freeze samples were allowed to warm at room temperature and homogenized for 30 sec. For preparation of hydrophilic extract 10 mL distilled water were added to 2.000 ± 0.001 g of sample. The mixture was stirred for 2 min and then it was centrifuged. The collected supernatant was the hydrophilic extract.

Lipophilic extraction was obtained as follows (Shi et al., 2007): 2.5 mL of methanol were added to 2.000 ± 0.001 g of sample and the mixture was vortexed for a minute. Subsequently, 2.5 mL Tris pH 7.5 were added and the resulting mixture was shortly vortexed, left 5 min in the dark. Then 2 mL chloroform were added. The mixture was vortexed for 2 min and centrifuged for 5 min. The phase of chloroform was collected, evaporated under vacuum at room temperature and the precipitate was reconstituted into 5 mL of 2-propanol. The whole procedure took place in the dark. The hydrophilic and lipophilic extracts were used for antioxidant assays.

The antioxidant activity (AA) of both hydrophilic and lipophilic fraction of tomatoes was estimated by DPPH, FRAP and ORAC assay.

2. DPPH assay. DPPH radical scavenging capacities of tomatoes extracts were determined using a known procedure with some modifications (Capanoglu et al., 2008). A DPPH working solution 0.1 mM in methanol was prepared weekly by diluting a methanolic stock solution 0.6 mM. The absorbance of the working solution at 517 nm was checked daily. In case of lipophilic extracts, 2-propanol was used for preparation of DPPH stock and working solutions. The DPPH assay was performed by mixing 3.8 mL working solution and 200 µL of hydrophilic or lipophilic extract. The solution was vortexed for 20 sec and incubated, in dark, for 30 min, at room temperature. The absorption of solution at 517 nm was measured. A control solution was prepared from 3.8 mL of DPPH working solution and 200 µL of distilled water (or 2-propanol in case of lipophilic extracts) and its absorption (A_c) at 517 nm was measured. The % scavenging of DPPH radicals was calculated according to the equation:

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

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

Trolox was used as standard. The antioxidant activity of the samples was expressed as mg Trolox equivalent (TE) per kg of fresh weight (mg TE kg^{-1} FW).

3. FRAP assay. The reducing capacity of the samples was determined using the FRAP (ferric reducing antioxidant power) assay according known procedure (Capanoglu et al., 2008) with slight modifications. FRAP reagent was freshly prepared from 1 mL of $FeCl_3$ 0.02M, 1 mL of TPTZ 0.01M in 0.04M HCl and 10 mL of acetate buffer 0.3M pH 3.6. FRAP reagent (2.9 mL) was mixed with 100 µL of extract and the solution was incubated 10 min at 37°C. Then it was cooled rapidly and the absorbance at 593 nm was measured. A standard curve was prepared using Trolox as standard. The results were expressed as mg Trolox equivalent (TE) per kg of fresh weight (mg TE kg^{-1} FW).

4. ORAC assay. The ORAC (oxygen radical absorbance capacity) assay was conducted according to known method (Dávalos et al., 2004) with some modifications: A solution of 63 nM fluorescein in 75 mM phosphate buffer (pH 7.4) was prepared weekly and it was stored in dark. In addition, stock solution of 515 mM APPH in 75 mM phosphate buffer (pH 7.4) was prepared and stored in refrigerator. 40 µL of tomato extracts were mixed with 1,700 µL of fluorescein solutions and the solution was vortexed and incubated at 37°C for 15 min. Thereafter, 220 µL of APPH solution was added and the resulting solution was immerged in a waterbath at 37°C for 15 sec. Subsequently it was rapidly transferred in a cuvette and the fluorescence was measured (excitation wavelength 485 nm; emission wavelength 528 nm) by means of PerkinElmer LS 55 Fluorescence Spectrophotometer (PerkinElmer, Boston, Mass., USA) equipped with thermostated holder. Fluorescence decay was recorded every 0.2 min for 30 min. A blank was prepared by replacing tomato's extract with 40 µL of phosphate buffer and its fluorescence was measured. The area under fluorescence decay curves was calculated using the FWINLAB software of the spectrophotometer. The net area derived from the difference between the areas under the fluorescein decay curves of the sample and blank was used to estimate the antioxidant capacity of sample. Trolox was used as standard. The standard curve was made by plotting net area versus Trolox concentration. The results were expressed as mg Trolox equivalent (TE) per kg of fresh tomato (mg TE kg^{-1} FW).

Statistical analyses

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

Results and discussion

Materials' characteristics and sampling

Fully ripened tomato fruits of cv. Chiou had an average diameter approximately 3.2 cm. The average fruit weights ranged from 16.65 g (red ripe) to 14.99 g (mature green). These values are within the range of weights and sizes reported in the international literature for cherry tomatoes (Coyago-Cruz et al., 2018; Figàs et al., 2015).

Tomatoes were visually selected and classified to the specific ripening stages (Table 1). Then the colour was precisely assessed using a chromatometer. Post-harvest ripening was monitored again using a chromatometer in order to

Table 1. Ripening stage, abbreviation and physical characteristics of samples.

Sample	Ripening stage	Abbreviation	Weight (g)	a^*/b^*
1	Mature green	MG	14.99±1.75a	-0.53±0.02a
2	Breaker	BR	16.46±2.38a	-0.24±0.04b
3	On-vine red ripe	VineRR	16.65±1.87a	0.79±0.06c
4	Post-harvest red ripe (ripened from breaker stage)	PhRR	16.44±1.90a	0.87±0.07c

Values in a column indicated by the same letter are not significantly different.

assure that vineRR and PhRR samples had comparable color attributes, since the colour of tomatoes is one of the most important quality traits, influencing consumer choice and acceptability. Colour was recorded using the CIELab space and the results were expressed as (a^*/b^*) ratio which represents the red-to-green ratio of colour. This ratio is a simple, significant ripening index and represents the colour index which is better related to colour variation during tomato fruits ripening (Francis and Clydesdale, 1977). The mean (a^*/b^*) values ranged from -0.50 (MG) to 0.79 (VineRR). PhRR tomatoes showed higher (a^*/b^*) values (0.87) than VineRR sample but the difference was not significant. A linear relationship between (a^*/b^*) and lycopene concentration in various tomato genotypes has been reported (Giovanelli et al., 1999).

Antioxidants content

The antioxidant components total phenols, flavonoids, ascorbic acid as well as lycopene were determined in tomato extracts. Figure 2 depicts the variation in content of total phenols, flavonoids, ascorbic acid and lycopene of the four tomato samples. The fruit phenolic levels, varied from 129.6 in MG to 395.6 in BR and finally to 452.6 mg GAE kg^{-1} FW in VineRR tomatoes. Total phenols increased during on-vine ripening, however, no statistically significant differences in BR and VineRR samples were detected. In contrast, PhRR tomatoes showed significantly lower ($p < 0.01$) total phenols content (277.19 mg GAE kg^{-1} FW) than VineRR fruits. The results suggest that post-harvest ripening resulted in an about 39% reduction in total phenols. Total phenolic contents of on-vine and post-harvest ripened red ripe tomatoes were significantly correlated ($r = 0.9999$, $p < 0.05$).

Mean flavonoids content ranged from 19.2 in MG to 148.9 mg RE kg^{-1} FW in BR sample (Figure 2b). Flavonoids content reached a maximum level at breaker stage followed by a decline in red ripe stage, during on-vine ripening. Nevertheless, on-vine ripened red ripe fruits of 'Chiou' variety exhibited higher flavonoids levels (122.5 mg RE kg^{-1} FW) than other cherry tomatoes at full maturity stage as report-

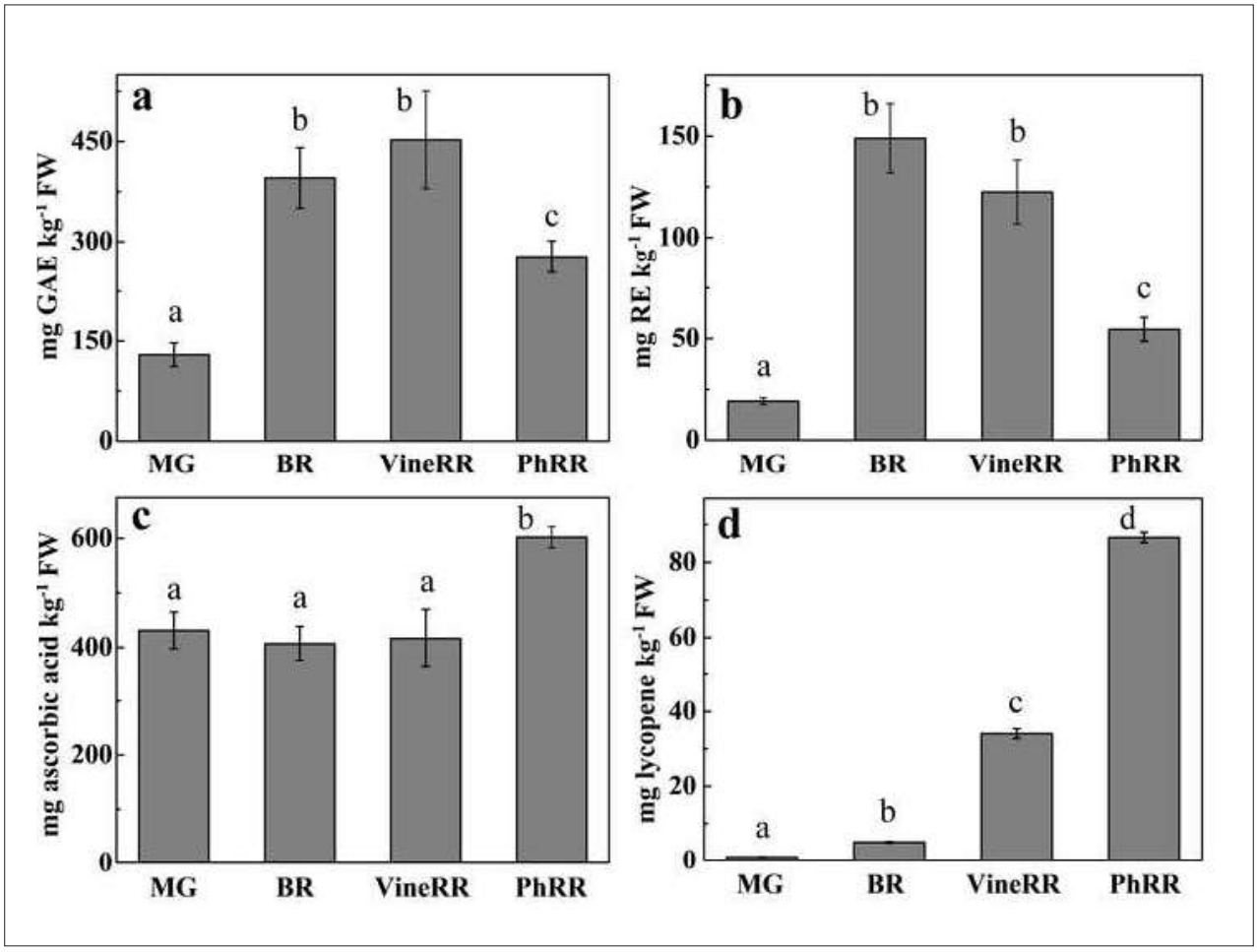

Figure 2. Total phenols (a), flavonoids (b), ascorbic acid (c) and lycopene (d) content of MG, BR VineRR and PhRR tomatoes. Significant differences ($p < 0.05$) among ripening stages are indicated by different letters. Error bars show SD ($n = 3$).

ed by Kavitha et al. (2014). Post-harvest ripening resulted in a sharp decrease in flavonoids content. In comparison to the VineRR fruits (122.5 mg RE kg^{-1} FW) the flavonoid levels were decreased approximately 55% in PhRR fruits (54.5 mg RE kg^{-1} FW). It is worth noticing that during post-harvest ripening the flavonoids levels were reduced at a greater extent than that of total phenols. For the flavonoids levels a relatively high correlation with the ripening stage was found (r = 0.7989–0.9845). In contrast, the accumulation of total phenols in different developmental stages of tomato fruits did not follow such a pattern.

Toor and Savage (2006) reported that the phenolic content during the postharvest life of tomatoes is directly affected by the storage temperature. While at rather low temperatures the increase activity of phenylalanine ammonia lyase (PAL) and hydroxycinnamoyl quinate transferase (HQT) resulted in increased levels of total phenolics, at storage temperature 25°C the phenolic levels decreased significantly. The decrease of phenolic and flavonoid content during the postharvest storage at elevated temperatures is probably the result of the increased activity of polyphenol oxidases that have a temperature optimum at 40°C (Pourcel et al., 2007; Spagna et al., 2005).

Ascorbic acid is an important hydrophilic antioxidant of tomatoes. Mean ascorbic acid content of 'Chiou' variety remained practically unchanged during on-vine ripening, (Figure 2c). Cano et al. (2003) have reported analogous results for greenhouse cultivated tomatoes. Interestingly, 'Chiou' variety exhibited rather high ascorbic acid content comparing to cherry varieties described elsewhere (Kavitha et al., 2014). However, George et al. (2004) reported much higher ascorbic acid content for cherry tomatoes. Post-harvest ripening resulted to a notable increase of the ascorbic acid content. Specifically, PhRR sample presented ascorbic acid content 1.44-fold higher (602.2 mg kg^{-1} FW) than VineRR tomatoes. Toor and Savage (2006) reported that tomatoes harvested at the light-red stage of ripeness showed an increase in ascorbic acid content when stored at 15 or 25°C. These data are in line with previously reported results, where post-harvest stress of low temperature stored cherry tomato fruits significantly increases the expression of genes involved in ascorbic acid biosynthesis and ascorbic acid metabolism (Tsaniklidis et al., 2014).

Lycopene is the major lipophilic antioxidant of tomatoes and the most abundant carotenoid of tomato fruits. It is well known that lycopene levels increase is a feature of the development of tomatoes (Vallverdú-Queralt et al., 2011). Figure 2d illustrates lycopene accumulation levels as function of ripening stage. Lycopene content was significantly increased during on-vine ripening. VineRR tomatoes exhibited 34.1 mg lycopene kg^{-1} FW. Lycopene content of 'Chiou' variety was higher than previously reported results for cherry varieties (Kotíková et al., 2011), but lower than that reported by Coyago-Cruz et al. (2018) and Choi et al. (2014). Lycopene contents of 18–105 mg kg^{-1} FW have been reported for several cherry tomatoes at full maturity stage (Kavitha et al., 2014). Post-harvest ripening promoted the accumulation of lycopene. PhRR tomatoes had 2.5-fold higher lycopene levels (86.6 mg kg^{-1} FW) than VineRR fruits. It has been reported that post-harvest treatment of cherry tomatoes significantly increased lycopene content during storage at ambient temperature (Gharezi et al., 2012). In addition, Javanmardi and Kubota (2006) reported increase of the lycopene content during the initial period of storage at room temperature and suggest that its biosynthesis is accelerated at ambient temperatures.

Interestingly, although VineRR and PhRR samples had comparable ($p > 0.05$) a^*/b^* index, the deference of lycopene content was significant ($p < 0.05$). This indicated that there was not an unequivocal relationship between the colour index and lycopene content. These findings are in accordance with the results of Giovanelli et al. (1999). They concluded that lycopene content was not linearly related to colour changes of on-vine and post-harvest ripened tomatoes.

Antioxidant activity

Hydrophilic antioxidant activity (HAA) and lipophilic antioxidant activity (LAA) were measured by three assays, DPPH, FRAP and ORAC.

1. Hydrophilic antioxidant activity (HAA). Figure 3 shows HAA of tomato extracts determined by the three assays. Antioxidant activities of hydrophilic extracts of tomatoes, determined by DPPH (Figure 3a), were 68.3 mg TE kg^{-1} FW in MG fruits while BR and VineRR fruits showed comparable values of 207.5 and 208.1 mg TE kg^{-1} FW, respectively. This trend was in accordance with fluctuation in total phenols content. However, a significant increase of radical-scavenging ability was observed in PhRR tomatoes (369.2 mg TE kg^{-1} FW), although post-harvest ripening decreased the total phenols content of PhRR sample. This antithesis could be attributed to the substantial increase of ascorbic acid content of PhRR tomatoes. Phenolic compounds and ascorbic acid represent the main water-soluble antioxidants in tomatoes. There was poor correlation between HAA and total phenols contents. In contrast, a relatively high correlation (r = 0.7871) between HAA and ascorbic acid content was observed. Considering the sum of total phenols and ascorbic acid an even higher correlation (r = 0.8552) with HAA was observed.

In contrast to DPPH assay, mean FRAP values (Figure 3b) significantly increased from MG (158.8 mg TE kg^{-1} FW) to vineRR (496.6 mg TE kg^{-1} FW) and then sharply decreased in case of PhRR tomatoes (293.3 mg TE kg^{-1} FW). FRAP values of tomatoes' hydrophilic extracts followed the fluctuation of total phenols content. Hydrophilic FRAP values exhibited significant correlation (r = 0.9953, $p < 0.01$) to total phenols content. This indicates a limited contribution of ascorbic acid to FRAP values. It has been reported that among ascorbic acid, phenolics, and flavonoids, the most important factor contributing to the FRAP value were phenolic compounds based on multivariate regression analysis of hydrophilic extracts of different vegetables (Ji et al., 2011).

ORAC was the third assay employed to evaluate HAA of tomatoes (Figure 3c). ORAC assay provides a direct measure of chain-breaking antioxidant capacity versus peroxyl radicals. Mean ORAC values ranged from 1,034.4 in MG fruits to 1,183.7 mg TE kg^{-1} FW in VineRR tomatoes. On-vine ripening did not significantly affect the ORAC values. PhPP tomatoes exhibited comparable ORAC value (1,200.0 mg TE kg^{-1} FW) to VineRR sample. Therefore, we conclude that post-harvest ripening did not significantly affect HAA. ORAC values exhibited significant correlation (r = 0.9723) with the sum of total phenols and ascorbic acid content.

2. Lipophilic antioxidant activity (LAA). Figure 4 depicts the LAA of tomato extracts determined by DPPH, FRAP and ORAC assay. DPPH assay revealed a significant increase of LAA during on-vine ripening. Mean DPPH values of lipophilic extracts of MG, BR and VineRR fruits were 4.2, 9.2 and 12.1 TE kg^{-1} FW, respectively (Figure 4a). PhRR tomatoes showed significant higher DPPH values (29.5 mg TE kg^{-1} FW) than VineRR sample. Therefore, LAA of post-harvest ripened tomatoes increased 2.4-fold in relation to on-vine ripened

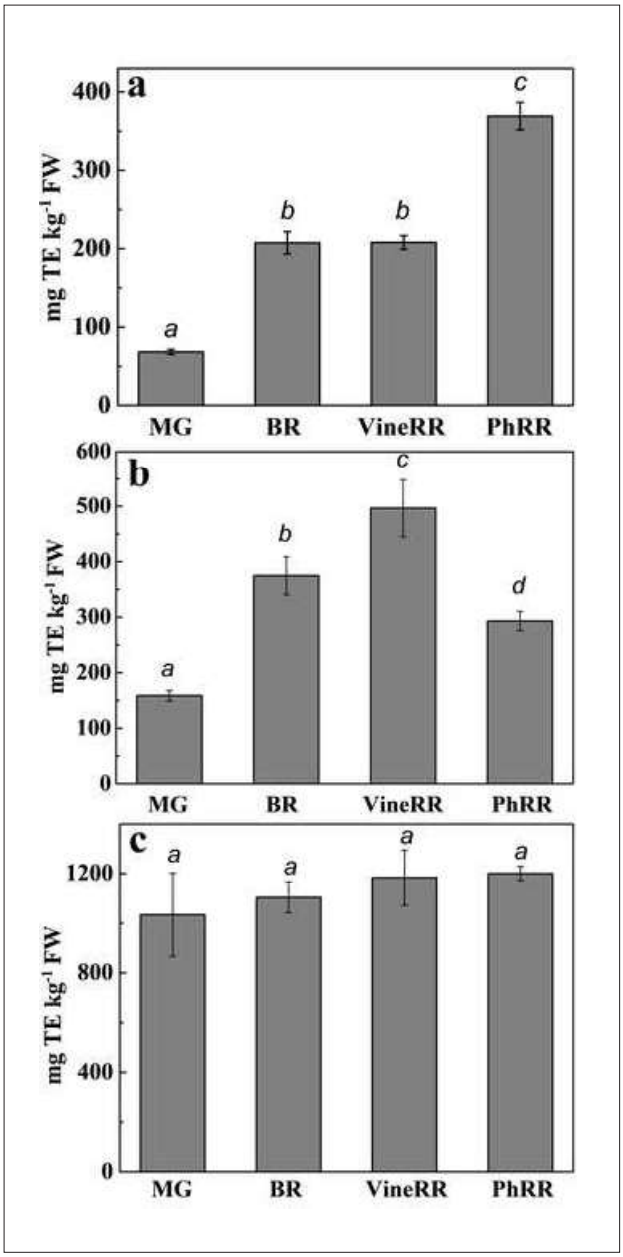

Figure 3. Hydrophilic antioxidant activity of MG, BR VineRR and PhRR tomatoes estimated by DPPH (a), FRAP (b) and ORAC (c) assay. Significant differences ($p < 0.05$) among ripening stages are indicated by different letters. Error bars show SD ($n = 3$).

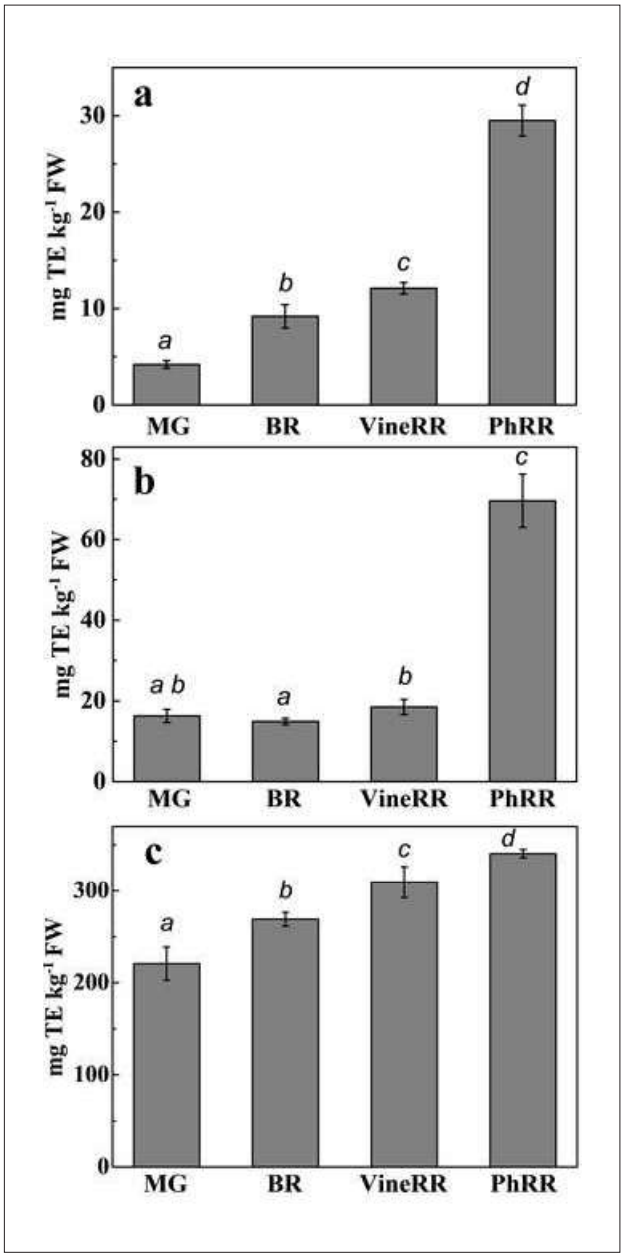

Figure 4. Lipophilic antioxidant activity of MG, BR VineRR and PhRR tomatoes estimated by DPPH (a), FRAP (b) and ORAC (c) assay. Significant differences ($p < 0.05$) among ripening stages in a graph are indicated by different letters. Error bars show SD ($n = 3$).

fruits. This increase was attributed to the stimulation of lycopene content which is the most important lipophilic antioxidant of tomatoes. A high correlation ($r = 0.9133$) between DPPH and lycopene values was observed. Significant correlation between the content of lycopene and antioxidant activity was previously reported (Cano et al., 2003; Ilahy et al., 2011).

FRAP assay reveals that LAA of on-vine ripened tomatoes was not progressively and significantly increased from MG to VineRR fruits (Figure 4b). However, a major increase of LAA of PhRR tomatoes was observed (69.91 mg TE kg^{-1} FW) relative to VineRR sample (18.5 TE kg^{-1} FW). In addition, lipophilic FRAP values where closely correlated to the lycopene values ($r = 0.9443$). This confirms that lycopene is probably the most important component of tomatoes' lipophilic extracts as it affects considerably the ferric reducing antioxidant power. Moreover, it is documented that among carotenes only lycopene is an effective ferric reducing compound (Müller et al., 2011).

ORAC assay showed comparable results to DPPH. Specifically, LAA increased progressively and significantly during on-vine ripening (Figure 4c). ORAC assay verified a significant increase of LAA of post-harvest ripened tomatoes. Specifically, PhRR tomatoes showed ORAC values of 340.4 TE kg^{-1} FW, significantly higher than that of VineRR (309.3 TE kg^{-1} FW). A high correlation ($r = 0.8967$) between mean ORAC and lycopene values was observed. This indicated that lycopene contributed mainly to LAA. Ahmadi et al. (2018) reported that lipophilic ORAC values were not correlated significantly to total carotenoids, which was attributed to other compounds (e.g., vitamin E derivatives) that can affect the antioxidant activity. It has been reported

that lycopene concentration is poorly correlated to ORAC (R^2 = 0.33) (Bangalore et al., 2005). Comparing the three assays, ORAC exhibited lower per cent increment of LAA of post-harvest ripened tomatoes (10%) than DPPH (143%) and FRAP assay (277%). Moreover, ORAC values poorly correlated with DPPH and FRAP values, while DPPH and FRAP values showed a very good correlation (r = 0.9488). Moreover, Soto-Zamora et al. (2005) reported an increase of the major antioxidants during the initial period of storage at 10°C that included ascorbic acid, glutathione, lycopene, and carotenoids.

Conclusion

The implications of on-vine and post-harvest ripening on the main antioxidant compounds (total phenols, flavonoids, ascorbic acid, lycopene) and antioxidant activity of cherry type tomato cv. Chiou was studied. On-vine ripened breaker and red ripe tomatoes exhibited insignificant differences in their phenolics, flavonoid and ascorbic acid content. Consequently, HAA, estimated by DPPH and ORAC were comparable. In contrast, LAA increased significantly during on-vine ripening, which is mainly attributed to lycopene accumulation. Post-harvest ripening showed contradictory implications to water soluble antioxidants, total phenols and ascorbic acid. Total phenols content was lower in the off-vine ripened fruits than in naturally ripened tomatoes, while post-harvest ripening led to the increase of the accumulation of ascorbic acid. HAA of post-harvest ripened fruits depended on the method used for estimation of HAA. DPPH and ORAC revealed a significant increase of HAA which is well correlated with the sum of ascorbic and polyphenol content. FRAP assay was mainly influenced by phenolic content, hence post-harvest ripened tomatoes presented reduced HAA. During post-harvest ripening the accumulation of lycopene increased. Therefore, significantly influenced LAA which was estimated by DPPH, FRAP and ORAC method. The results revealed that post-harvest ripening resulted in the increase of LAA. In conclusion, under our experimental conditions, it was shown that HAA of on-vine ripened breaker and red ripe tomatoes were not significantly different. In contrast, red ripe tomatoes exhibited significantly higher LAA values than those harvested at breaker stage. On the other hand, post-harvest ripening seems to improve HAA values, but also increases LAA of 'Chiou' tomato fruits. These results are important for the post-harvest treatments and the appropriate harvest stage of tomato fruits by the farmers, in view of nutritional value of tomatoes and especially of their antioxidant content.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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References

Ahmadi, L., Hao, X., and Tsao, R. (2018). The effect of greenhouse covering materials on phytochemical composition and antioxidant capacity of tomato cultivars. J. Sci. Food Agric. *98*, 4427–4435. https://doi.org/10.1002/jsfa.8965.

Bangalore, D.V., McGlynn, W., and Scott, D.D. (2005). Effect of β-Cyclodextrin in improving the correlation between lycopene concentration and ORAC values. J. Agric. Food Chem. *53*, 1878–1883. https://doi.org/10.1021/jf048258m.

Cano, A., Acosta, M., and Arnao, M.B. (2003). Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). Postharv. Biol. Technol. *28*, 59–65. https://doi.org/10.1016/S0925-5214(02)00141-2.

Capanoglu, E., Beekwilder, J., Boyacioglu, D., Hall, R., and De Vos, R. (2008). Changes in antioxidant and metabolite profiles during production of tomato paste. J. Agric. Food Chem. *56*, 964–973. https://doi.org/10.1021/jf072990e.

Caris-Veyrat, C., Amiot, M.-J., Tyssandier, V., Grasselly, D., Buret, M., Mikolajczak, M., Guilland, J.-C., Bouteloup-Demange, C., and Borel, P. (2004). 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. https://doi.org/10.1021/jf0346861.

Choi, S.H., Kim, D.-S., Kozukue, N., Kim, H.-J., Nishitani, Y., Mizuno, M., Levin, C.E., and Friedman, M. (2014). Protein, free amino acid, phenolic, β-carotene, and lycopene content, and antioxidative and cancer cell inhibitory effects of 12 greenhouse-grown commercial cherry tomato varieties. J. Food Comp. Anal. *34*, 115–127. https://doi.org/10.1016/j.jfca.2014.03.005.

Choi, S.-H., Lee, S.-H., Kim, H.-J., Lee, I.-S., Kozukue, N., Levin, C.E., and Friedman, M. (2010). Changes in free amino acid, phenolic, chlorophyll, carotenoid, and glycoalkaloid contents in tomatoes during 11 stages of growth and inhibition of cervical and lung human cancer cells by green tomato extracts. J. Agric. Food Chem. *58*, 7547–7556. https://doi.org/10.1021/jf100162j.

Clinton, S.K. (1998). Lycopene: chemistry, biology, and implications for human health and disease. Nutr. Rev. *56*, 35–51. https://doi.org/10.1111/j.1753-4887.1998.tb01691.x.

Coyago-Cruz, E., Corell, M., Moriana, A., Hernanz, D., Benítez-González, A.M., Stinco, C.M., and Meléndez-Martínez, A.J. (2018). Antioxidants (carotenoids and phenolics) profile of cherry tomatoes as influenced by deficit irrigation, ripening and cluster. Food Chem. *240*, 870–884. https://doi.org/10.1016/j.foodchem.2017.08.028.

Darras, A.I., Kotsiras, A., Delis, C., Nifakos, K., Pavlakos, E., and Demopoulos, V. (2017). Reaction of the native Greek tomato varieties 'Chondrokatsari Messinias' and 'Katsari Santorinis' to *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* infection. Hellenic Plant Prot. J. *10*, 70–79. https://doi.org/10.1515/hppj-2017-0008.

Dávalos, A., Gómez-Cordovés, C., and Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. J. Agric. Food Chem. *52*, 48–54. https://doi.org/10.1021/jf0305231.

Figàs, M.R., Prohens, J., Raigón, M.D., Fita, A., García-Martínez, M.D., Casanova, C., Borràs, D., Plazas, M., Andújar, I., and Soler, S. (2015). Characterization of composition traits related to organoleptic and functional quality for the differentiation, selection and enhancement of local varieties of tomato from different cultivar groups. Food Chem. *187*, 517–524. https://doi.org/10.1016/j.foodchem.2015.04.083.

Francis, F.J., and Clydesdale, F.M. (1977). Clydesdale: Food Colorimetry: Theory and Applications. (Westport, Conn.: The Avi Publishing Company, Inc.), 477 pp. https://doi.org/10.1002/food.19770210122.

Frusciante, L., Barone, A., Carputo, D., Ercolano, M.R., Della Rocca, F., and Esposito, S. (2000). Evaluation and use of plant biodiversity for food and pharmaceuticals. Fitoterapia *71*, S66–S72. https://doi.org/10.1016/S0367-326X(00)00175-1.

García-Alonso, F.J., Bravo, S., Casas, J., Pérez-Conesa, D., Jacob, K., and Periago, M.J. (2009). Changes in antioxidant compounds during the shelf life of commercial tomato juices in different packaging materials. J. Agric. Food Chem. *57*, 6815–6822. https://doi.org/10.1021/jf900877c.

George, B., Kaur, C., Khurdiya, D.S., and Kapoor, H.C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chem. *84*, 45–51. https://doi.org/10.1016/S0308-8146(03)00165-1.

Giovanelli, G., Lavelli, V., Peri, C., and Nobili, S. (1999). Variation in antioxidant components of tomato during vine and post-harvest ripening. J. Sci. Food Agric. *79*, 1583–1588. https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1583::AID-JSFA405>3.0.CO;2-J.

Ilahy, R., Hdider, C., Lenucci, M.S., Tlili, I., and Dalessandro, G. (2011). Phytochemical composition and antioxidant activity of high-lycopene tomato (*Solanum lycopersicum* L.) cultivars grown in Southern Italy. Sci. Hortic. *127*, 255–261. https://doi.org/10.1016/j.scienta.2010.10.001.

Ilahy, R., Piro, G., Tlili, I., Riahi, A., Sihem, R., Ouerghi, I., Hdider, C., and Lenucci, M.S. (2016). Fractionate analysis of the phytochemical composition and antioxidant activities in advanced breeding lines of high-lycopene tomatoes. Food & Function *7*, 574–583. https://doi.org/10.1039/C5FO00553A.

Jeffery, E.H., Campbell, J.K., Canene-Adams, K., Zaripheh, S., and Erdman, J.W., Jr. (2005). The tomato as a functional food. J. Nutrition *135*, 1226–1230. https://doi.org/10.1093/jn/135.5.1226.

Ji, L., Wu, J., Gao, W., Wei, J., Yang, J., and Guo, C. (2011). Antioxidant capacity of different fractions of vegetables and correlation with the contents of ascorbic acid, phenolics, and flavonoids. J. Food Sci. *76*, C1257–C1261. https://doi.org/10.1111/j.1750-3841.2011.02402.x.

Kavitha, P., Shivashankara, K.S., Rao, V.K., Sadashiva, A.T., Ravishankar, K.V., and Sathish, G.J. (2014). Genotypic variability for antioxidant and quality parameters among tomato cultivars, hybrids, cherry tomatoes and wild species. J. Sci. Food Agric. *94*, 993–999. https://doi.org/10.1002/jsfa.6359.

Klein, B.P., and Perry, A.K. (1982). Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. J. Food Sci. *47*, 941–945. https://doi.org/10.1111/j.1365-2621.1982.tb12750.x.

Kotíková, Z., Lachman, J., Hejtmánková, A., and Hejtmánková, K. (2011). Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. LWT – Food Sci. Technol. *44*, 1703–1710. https://doi.org/10.1016/j.lwt.2011.03.015.

Müller, L., Fröhlich, K., and Böhm, V. (2011). Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chem. *129*, 139–148. https://doi.org/10.1016/j.foodchem.2011.04.045.

Ozgen, S., Sekerci, S., Korkut, R., and Karabiyik, T. (2012). The tomato debate: Postharvest-ripened or vine ripe has more antioxidant? Hortic., Environm., Biotechnol. *53*, 271–276. https://doi.org/10.1007/s13580-012-0001-y.

Pék, Z., Helyes, L., and Lugasi, A. (2010). Color changes and antioxidant content of vine and postharvest-ripened tomato fruits. HortScience *45*, 466–468. https://doi.org/10.21273/HORTSCI.45.3.466.

Rao, A.V., Waseem, Z., and Agarwal, S. (1998). Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. Food Res. Int. *31*, 737–741. https://doi.org/10.1016/S0963-9969(99)00053-8.

Rosales, M.A., Cervilla, L.M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M.d.M., Blasco, B., Ríos, J.J., Soriano, T., Castilla, N., Romero, L., and Ruiz, J.M. (2011). The effect of environmental conditions on nutritional quality of cherry tomato fruits: evaluation of two experimental Mediterranean greenhouses. J. Sci. Food Agric. *91*, 152–162. https://doi.org/10.1002/jsfa.4166.

Shi, J., Qu, Q., Kakuda, Y., Xue, S.J., Jiang, Y., Koide, S., and Shim, Y.-Y. (2007). Investigation of the antioxidant and synergistic activity of lycopene and other natural antioxidants using LAME and AMVN model systems. J. Food Comp. Anal. *20*, 603–608. https://doi.org/10.1016/j.jfca.2007.03.004.

Silva, L., Pezzini, B., and Soares, L. (2015). Spectrophotometric determination of the total flavonoid content in *Ocimum basilicum* L. (Lamiaceae) leaves. Pharmacognosy Mag. *11*, 96–101. https://doi.org/10.4103/0973-1296.149721.

Toor, R.K., and Savage, G.P. (2006). Changes in major antioxidant components of tomatoes during post-harvest storage. Food Chem. *99*, 724–727. https://doi.org/10.1016/j.foodchem.2005.08.049.

Tsaniklidis, G., Delis, C., Nikoloudakis, N., Katinakis, P., and Aivalakis, G. (2014). Low temperature storage affects the ascorbic acid metabolism of cherry tomato fruits. Plant Physiol. Biochem. *84*, 149–157. https://doi.org/10.1016/j.plaphy.2014.09.009.

Vallverdú-Queralt, A., Arranz, S., Medina-Remón, A., Casals-Ribes, I., and Lamuela-Raventós, R.M. (2011). Changes in phenolic content of tomato products during storage. J. Agric. Food Chem. *59*, 9358–9365. https://doi.org/10.1021/jf202140j.

WHO (2003). Diet, Nutrition and the Prevention of Chronic Diseases. Technical Report Series (149 pp.) (Geneva: World Health Organization).

Willcox, J.K., Catignani, G.L., and Lazarus, S. (2003). Tomatoes and cardiovascular health. AU Critical Rev. Food Sci. Nutr. *43*, 1–18. https://doi.org/10.1080/10408690390826437.

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