Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation

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Abstract

Considering the economic importance of the tomato and its nutritional benefits to human health, a study was made of how two different environmental factors (temperature and overall solar radiation) influence the nutritional quality of cherry tomatoes during the plant full production cycle. *Solanum lycopersicum L.* cv. Naomi plants were grown in an experimental greenhouse. Three fruit samples were taken over the full production period: first sampling at the beginning of harvest (7 January 2004), second at mid-harvest (22 March 2004) and third at harvest end (30 May 2004). Values for temperature and overall accumulated solar radiation peaked at a maximum in the third sampling, without lowering the yield with respect to previous samplings. Regarding the antioxidant activity in the exocarp fraction of the cherry tomato fruits, the results showed that the increase in temperature and solar radiation diminished the lycopene and β-carotene contents in the third sampling, inducing defective pigmentation (sunscald). This occurred simultaneously with an increase in lipid peroxidation during the third sampling, quantified as lipoxygenase activity and malondialdehyde content. Finally, in relation to ascorbate metabolism, the higher temperatures and stronger solar radiation at the third sampling increased the oxidation of reduced ascorbate (AsA) due to intensified ascorbate peroxidase (APX) and ascorbate oxidase (AO) activities and a depression of the enzyme dehydroascorbate reductase (DHAR). In conclusion, the results indicate that despite the oxidation of AsA by APX and AO, the minimal regeneration of the latter, together with the greater lipid peroxidation with increasing temperature and solar radiation in the greenhouse, explained the lower content of antioxidants in the exocarp and therefore the loss of nutritional quality of the cherry tomato fruits grown under these conditions.

Keywords:
antioxidants; ascorbate; cherry tomato; exocarp;lipid peroxidation; solar radiation; temperature

INTRODUCTION

The tomato (*Solanum lycopersicum L.*), according to the FAO, is the second most cultivated vegetable in the world, after the potato, with an annual production of nearly 10^8 t of fresh tomato in 3.7×10^6 ha worldwide, China, the USA and Turkey being the leading producers.[1] In addition to its economic importance, tomato consumption has recently been demonstrated to be beneficial to human health, because of its content of phytochemicals such as lycopene, β-carotene, flavonoids, vitamin C and many essential nutrients.[2] This composition explains the high antioxidant capacity in both fresh and processed tomatoes,[3] associating the fruit with lower rates of certain types of cancer and cardiovascular disease.[4]

Tomatoes are a major source of lycopene, a carotenoid with a notable capacity to eliminate active-oxygen species (AOS), according to Rao *et al.*[5] and Toor and Savage.[6] Lycopene is also responsible for the reddening of the tomato, due to the differentiation of the chloroplasts and chromoplasts, so this carotenoid is very important with regard to the final nutritional and marketable quality of this plant product.[7] Different studies have indicated that the quality of the tomato is strongly correlated with its lycopene content.[8, 9] Another carotenoid present in tomato fruits is β-carotene, although it is of lesser importance than lycopene, given that it constitutes only 7% of the total carotenoid content of the fruit.[10] Flavonoids, such as anthocyanins, are also important as antioxidants, protecting against oxidative stress in plants by acting as a photoprotector that absorbs orange–green light of the visible spectrum and thereby preventing photo-oxidation of chlorophylls.[11]

However, the most effective antioxidant of the different plant products is ascorbic acid or vitamin C.[12] This compound, in addition to being involved directly in eliminating AOS and regenerating vitamin E in plants,[13] participates in cell metabolism and growth control,[14] cell division,[15] expansion of the cell walls[16] and organogenesis.[17] As an antioxidant, ascorbic acid directly eliminates superoxide and also hydroxyl radicals and oxygen singlet radicals and reduces hydrogen peroxide to water by the APX reaction.[18, 19]

One of the physiological processes that can markedly alter or reduce the nutritional quality and the antioxidant activity of the different plant products consumed by humans is oxidative stress. The environmental factors that induce oxidative stress in plants include air pollution (ozone and sulfur dioxide), herbicide/pesticide application, heavy metal contamination, drought, salinity, injuries, UV light, unfavourable temperatures and photo-inhibition from excessive solar radiation.[20, 21] Among these factors, temperature and solar radiation are fundamental in agrosystems of intensive cultivation, such as greenhouses, where most tomatoes are grown.[22, 23] Although few studies have analysed specifically how these environmental variables influence antioxidant activity in tomato fruits, it is known that in other tissues exposure to high temperatures depresses lycopene synthesis and prompts β-carotene degradation due to the presence of AOS.[7, 19, 23] In addition, greater activity of the enzyme lipoxygenase (LOX) under these conditions intensifies lipid peroxidation, provoking cell death and subsequent tissue necrosis.[24, 25] On the other hand, specifically in the exocarp, stronger solar radiation reportedly blocks the accumulation of lycopene, resulting in discoloured zones, commonly known as ‘sunscald’. Although little studied, it is known that sunscald appears in the mature tomato fruits as orange–yellow ring spots surrounding the abscission zone and it is result of reduced lycopene

synthesis or the augmented oxidation of this compound to β-carotene.[7, 26] This condition causes dramatic losses for producers and consumers.[27]

Therefore, considering the economic and nutritional relevance of the tomato crop and foods products, the impact of temperature and solar radiation on the antioxidant activity, lipid peroxidation and ascorbic acid metabolism in cherry tomatoes was examined. The study was focused on the exocarp fraction of the tomato fruit because of its content of antioxidants (such as lycopene), greater than in other fruit fractions (mesocarp and endocarp).[6, 7] The final aim was to define optimal temperature and solar radiation conditions in order to provide the best nutritional quality and marketable yield of cherry tomato fruits.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of cherry tomatoes (*Solanum lycopersicum L.* cv. Naomi) were sown in flat trays (cell size 3 × 3 × 10 cm) filled with peat-lite mixture and kept under greenhouse conditions during 4 weeks. Subsequently (23 September 2003), the seedlings were transplanted to an experimental greenhouse of improved parral type[28, 29] at La Nacla Experimental Station in southern Spain, near the Granada coastline (36°45′N, 3°30′W, altitude 130 m). The plants were grown in Perlite-filled sacks (1.20 m long, 40 L in volume) spaced 0.2 m apart in rows 2 m apart. With nine tomato plants per sack, the planting scheme was 3.21 plants m^{-2}. Other growing conditions (irrigation and fertilization) were as described by Soriano *et al.*[29]

Fruit sampling

The cherry tomato fruits were sampled three times during the crop cycle: 7 January 2004 [85 days after transplanting (dat)], 22 March 2004 (160 dat) and 30 May 2004 (229 dat), corresponding to the beginning, middle and end of the fruit harvesting season, respectively. Fruits were rinsed three times in distilled water after disinfection with non-ionic 1% detergent,[30] then blotted on filter-paper. Fresh matter of the exocarp fraction was used to analyse the parameters described below.

Temperature and solar radiation

Over the full fruit production cycle, the air temperatures were measured with HMP45 probes (Vaisala, Finland) and punctual solar radiation was measured using TSL Delta-T (UK) solarmetric tubes, 900 mm long, randomly set up in different areas of the greenhouse as specified by Soriano *et al.*[29] Air temperature and solar radiation data were quantified in 10-min periods using a datalogger (Campbell Sci CR-10, Spain), recording the average value for three measurements every 30 min.[29]

Pigments

Lycopene and β-carotene from the exocarp fractions were extracted with acetone–*n*-hexane (4:6) and subsequently centrifuged at 3000 × *g* for 5 min at 4 °C. The optical density of the supernatants was measured spectrophotometrically at 663, 645, 505 and 453 nm using acetone–*n*-hexane (4:6) as a blank. Lycopene and β-carotene concentrations were quantified using equations proposed by Nagata and Yamashita[31] as follows:

$$\text{lycopene}(\mu g ml^{-1}) = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

$$\beta\text{-carotene}(\mu g ml^{-1}) = 0.216A_{663} - 1.220A_{645} - 0.304A_{505} + 0.452A_{453}$$

where A_{663}, A_{645}, A_{505} and A_{453} are the absorbances at 663, 645, 505 and 453 nm, respectively. These equations permit the simultaneous determination of lycopene and β-carotene in the presence of chlorophylls.

Anthocyanins were determined according to Lange *et al.*[32] Seedlings were homogenized in propanol–HCl–H_2O (18:1:81) and further extracted in boiling water for 3 min. After centrifugation at 5000 × *g* for 40 min at 4 °C, the absorbance of the supernatant was measured at 535 and 650 nm. The absorbance due to anthocyanins was calculated as $A = A_{535} - A_{650}$.

Lipid peroxidation

For the assay of malondialdehyde (MDA), exocarp fractions were homogenized in 5 mL of 50 mmol L^{-1} buffer solution (containing 0.7 g L^{-1} $NaH_2PO_4 \cdot 2H_2O$ and 16 g L^{-1} $Na_2HPO_4 \cdot 12H_2O$), ground with a mortar and pestle on ice and centrifuged at 20 000 × *g* for 25 min (4 °C). For measurement of MDA content, 4 mL of 200 g L^{-1} trichloroacetic acid containing 5 g L^{-1} thiobarbituric acid was added to 1-mL aliquots of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice-bath. Subsequently, the samples were centrifuged at 10 000 × *g* for 10 min (4 °C) and the absorbance of the supernatant was read at 532 nm. The value for the non-specific absorption at 600 nm was subtracted from the A_{532} reading. The concentration of MDA was calculated using its extinction coefficient of 155 L $mmol^{-1}$ cm^{-1}.[24]

Lipoxygenase enzyme (LOX, EC 1.13.11.12) activity was measured according to Minguez-Mosquera *et al.*[33] using 50 mmol L^{-1} potassium phosphate buffer (pH 6.0) for extraction. Neither the addition of Triton X-100 to improve the solubility of the enzyme nor the addition of dithiothreitol (DTT) to protect SH groups from oxidation improved the assay results. The reaction mixture consisted of 0.2 mL of crude extract and 0.5 mmol L^{-1} linoleic acid in 50 mmol L^{-1} potassium phosphate buffer (pH 6.0). The LOX activity was calculated following the increase in the extinction at 234 nm using an extinction coefficient of 25 000 L mol^{-1} cm^{-1}.[34]

Ascorbate metabolism

The reduced ascorbate (AsA), dehydroascorbate (DHA) and total ascorbate (AsA + DHA) were determined spectrophotometrically following Gossett *et al.*[35] From the same extract, AsA and total ascorbate were assayed. Ascorbate standards of between 0.1 and 1.5 mmol L^{-1} ascorbate in metaphosphoric acid were analysed in the same manner as the extracts. For each sample, DHA was estimated from the difference between total ascorbate and AsA.

The extraction of total H_2O_2 was performed according to a modified version of the method of Rivero *et al.*[36] Hydroperoxides form a specific complex with titanium (Ti^{4+}), which can be measured spectrophotometrically at 415 nm. The concentration of peroxide in the extracts was determined by comparing the absorbance against a calibration curve representing a titanium–H_2O_2 complex from 0.1 to 1 mmol L^{-1}. The hydroperoxides represent the total peroxides.

The ascorbate peroxidase enzyme (APX, EC 1.11.1.11) activity was determined according to Gossett *et al.*[35] by monitoring the decrease in A_{290} of an assay

mixture containing 0.5 mmol L^{-1} ascorbate (extinction coefficient, 2.8 L $mmol^{-1}$ cm^{-1}).

The dehydroascorbate reductase enzyme (DHAR, EC 1.8.5.1) was assayed by tracking the change in absorbance at 265 nm[37] and monodehydroascorbate reductase enzyme (MDHAR, EC 1.6.5.4) by measuring the decrease in absorbance at 340 nm.[38] The DHAR activity was calculated using an extinction coefficient of 7.0 L $mmol^{-1}$ cm^{-1}. The reaction rate was corrected for the non-enzymatic reduction of DHA by reduced glutathione and the contribution to the absorbance by oxidized glutathione was taken into account. For MDHAR the reaction mixture (1 mL) contained 0.4 units ascorbate oxidase, 100 mmol L^{-1} HEPES–KOH (pH 7.6), 2.5 mmol L^{-1} ascorbate, 25 µmol L^{-1} NADPH and up to 100 µL of enzyme extract.[38]

The ascorbate oxidase enzyme activity was measured according to a modified version of the method of García-Pineda *et al.*[39] based on the fact that ascorbate absorbs at 265 nm whereas the oxidation product, dehydroascorbate, does not. The reaction mixture (1 mL) consisted of 0.025 mol L^{-1} citrate/phosphate buffer (pH 5.6), 0.08 mmol L^{-1} L-ascorbic acid, 0.02 mmol L^{-1} neutralized disodium EDTA and 0.15 g L^{-1} bovine serum albumin solution and up to 200 µL of enzyme extract (extinction coefficient = 9246 L mol^{-1} cm^{-1} at 265 nm).

Antioxidant activity

Antioxidant activity was measured in the exocarp fraction using the Ferric Reducing Ability of Plasma (FRAP) assay.[40] The FRAP assay was performed with FRAP reagent, i.e. 1 mmol L^{-1} 2,4,6-tripyridyl-2-triazine (TPTZ) and 20 mmol L^{-1} ferric chloride in 0.25 mol L^{-1} sodium acetate (pH 3.6). An aliquot of 100 µL of tomato extract (10 g L^{-1} in methanol) was added to 2 mL of FRAP reagent and mixed thoroughly. After the mixture had been left at ambient temperature (20 °C) for 5 min, the absorbance at 593 nm was measured. Calibration was against a calibration curve (25–1600 µmol L^{-1} ferrous ion) constructed using freshly prepared ammonium ferrous sulfate.

A test of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging effect was performed according to Hsu *et al.*[41] Aliquots of 0.5 mL of methanolic tomato extract and 2.5 mL of freshly prepared 0.1 mmol L^{-1} DPPH methanolic solutions were thoroughly mixed and kept for 60 min in the dark and cold. The absorbance of the reaction mixture at 517 nm was read with a spectrophotometer. Methanol (0.5 mL), replacing the extract, was used as the blank. The free-radical scavenging effect was calculated as follows:

$$\text{scavenging effect}(\%) = [1 - (A_{517}\text{sample}/A_{517}\text{blank})] \times 100.$$

Statistical analysis

An analysis of variance (ANOVA) was used to assess the significance of treatment means. Differences between the means of the three samplings were compared using the least significant difference (LSD) and Duncan's multiple-range test (DMRT) at the 0.05 probability level. Levels of significance are represented by *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$) and NS (not significant).

RESULTS AND DISCUSSION

Description of temperature and solar radiation and their effects on yield

Over the study period, the values of the maximum air temperatures in the greenhouse increased at the third sampling (229 dat) (Table 1). Nevertheless, the minimum temperatures varied little over the fruit-production cycle (Table 1). At the end of the fruit-production cycle, the greenhouse temperature exceeded the optimal level for tomato growth, which is between 23 and 26 °C,[42] and therefore in our experiments certain metabolic and physiological disorders could have occurred and affected the yield.[43] Another vital environmental variable for the optimal growth and development of greenhouse crops is solar radiation.[26] The results show a progressive increase to a peak during the third sampling at harvest (229 dat) (Table 1).

With respect to yield in kg m^{-2}, the environmental variables studied (temperature and solar radiation) did not notably affect the yield in any samplings (Table 1), as opposed to the results of Adams *et al.*,[22] who reported yield losses in tomato plants when the air temperatures exceeded 26 °C.

Temperature and solar radiation effects on antioxidant contents and lipid peroxidation

As indicated in the Introduction, carotenoids such as β-carotene and especially lycopene are essential for evaluating the nutritional quality of tomato fruit, given their antioxidant activity.[6] In contrast to the yield, both lycopene and β-carotene were affected by changes in air temperature and solar radiation, their contents declining significantly at the third sampling (229 dat, Table 2). Our results agreed with the findings of previous studies on tomato fruits, as in the case of cherry tomatoes, where 32 °C and higher temperatures induced the degradation of lycopene, which peaked in fruits between 17 and 26 °C.[7] The content and synthesis of β-carotene have been demonstrated to diminish from 40 °C upwards.[9] On the other hand, solar radiation has a strong effect on carotenoid content. The exposure of tomato fruits to intense solar radiation overheats the surface, inhibiting carotenoid synthesis and causing discoloration known as sunscald.[7] Therefore, the reduction in the contents of both lycopene and β-carotene in the exocarp fraction of the cherry tomato fruits at 229 dat (Table 2), when the highest temperature and solar radiation values were registered (Table 1), could explain the formation of fruits with defective coloration and sunscald, this physiopathy occurring in 70% of all the fruits. Finally, in agreement with other studies,[6, 7] in our experiments the concentrations of these carotenoids in the mesocarpic and endocarpic fractions were far lower than those present in the exocarp, although these fractions did register a decline in the β-carotene and lycopene concentrations in the third sampling (data not shown).

Another group of compounds that acts as antioxidants and photoprotectors against different types of environmental stresses are anthocyanins.[11] The relationship between the anthocyanin content and air temperature has been analysed recently by Pan *et al.*,[44] who reported a progressive reduction in the content of these flavonoids in fresh fruits at temperatures ranging from 30 to 45 °C. They considered that this explained the appearance of oxidative damage. In our work, the anthocyanin content in the exocarp fraction of the cherry tomatoes

remained constant over the study period (Table 2), regardless of the variations in air temperature and solar radiation recorded in the greenhouse.

Under environmental stress conditions, the generation of AOS is responsible for damage to macromolecules and ultimately to cell structure.[25] The analysis of lipid peroxidation expressed as LOX activity and MDA content indicates the prevalence and activation of AOS in the tissues as a consequence of oxidative stress.[24] Our results indicated that both LOX activity and MDA content peaked in the third sampling, coinciding with the highest temperature and solar radiation values (Table 3). The surge in lipid peroxidation at 229 dat clearly indicates oxidative stress, provoked by the high temperatures and intense solar radiation, which could explain the decreased contents of carotenoids such as lycopene and β-carotene and hence the appearance of sunscald. Moreover, the temperature and solar radiation values reached at 229 dat in the greenhouse caused important reductions in the nutritional quality of the cherry tomato fruits, given that the contents of the antioxidants lycopene and β-carotene declined in the exocarps (Table 2) with a significant rise in lipid peroxidation (Table 3). This, however, did not affect fruit yield over the crop cycle (Table 1). Therefore, given our results, the development of the cherry tomato crop in technologically improved greenhouses capable of maintaining optimal temperatures and solar radiation (in our work represented at 160 dat) could limit the reduction of the carotenoid content and thereby improve quality, particularly at the end of the productive cycle in this crop.

Temperature and solar radiation in relation to ascorbic acid metabolism

In higher plants, ascorbate has greater importance than the antioxidants discussed above. This compound is directly involved in the elimination of AOS through the ascorbate/glutation cycle or the Halliwell–Asada pathway and therefore its metabolism is determinant in the adaptation responses to different types of stress. The main compound in this cycle is AsA, which can inactivate hydrogen peroxide (H_2O_2), generating MDHA by the enzyme APX. This MDHA can either regenerate AsA through the action of MDHAR or else can be transformed into DHA, which is reduced by DHAR to produce AsA.[13, 19] On the other hand, the AsA may be transported towards the apoplast, site of the enzyme ascorbate oxidase (AO), which produces MDHA and DHA. This DHA is then transported to the cytoplasm and is reduced back to AsA via the action of DHAR.[19, 45]

Table 4 gives the values of the different forms of ascorbate and the activities of the different enzymes controlling their oxidation and regeneration to the reduced form. As reflected in this table, the concentration of total ascorbate increased significantly in the third sampling (229 dat) with respect to the previous samplings, since the DHA augmented in the last sampling and the reduced form of AsA remained constant in the three samplings. Data similar to those described above were found for the mesocarpic and endocarpic fractions, although in these the concentrations of the different forms of ascorbate were lower (data not shown). This accumulation of DHA at 229 dat also coincides with peak H_2O_2 concentrations in the exocarp of the fruits (Table 4), which could have been caused by stress, explaining to a certain extent the increase in APX and MDHAR activities, which participated in its detoxification (Table 4). Hence the increased oxidation of AsA at 229 dat is clearly explained by the activities of APX and AO in this sampling (Table 4). In contrast, the regeneration of the AsA from DHA was

inhibited in the third sampling almost certainly because activity controlling this process (DHAR) decreased significantly in this sampling (Table 4). Nevertheless, between the second and third samplings, we detected greater MDHAR activity, which collaborated in the regeneration of AsA at 229 dat (Table 4). The results for the increase in APX and AO activities and therefore DHA accumulation indicate stress conditions in the third sampling, thus inducing the detoxification of AOS.[46] However, the reduction of the regeneration of AsA by the enzymes DHAR and MDHAR at 229 dat could impair the adaptation capacity of the fruits at high temperatures and under strong solar radiation during the third sampling. In summary, the increased oxidation of AsA by APX in the exocarp fraction of the tomato fruits under conditions of environmental stress at 229 dat could constitute an effective response that would prevent serious oxidative damage. This would explain the absence of variations in fruit yield over the study period (Table 1). However, the minimum regeneration of DHA under stress conditions could have caused certain damage, such as the degradation of carotenoids (lycopene and β-carotene) and therefore the appearance of sunscald and diminished nutritional and commercial quality. Finally, despite the minimum reduction of DHA from the enzymes DHAR and MDHAR in the third sampling, the AsA concentrations remained similar in the three samplings (Table 4). Although more research is needed in this respect, under conditions of environmental stress the diminished DHAR and MDHAR activities would trigger an alternative response in the plant, prompting the *de novo* synthesis of AsA from its precursors (D-glucose-6P).[47] In our work, this response would explain that in the third sampling, despite the increased APX activity and the depression of DHAR and MDHAR activities, the AsA levels remained constant.

Finally, the increased capacity of AOS detoxification in the exocarp fraction of the cherry tomato fruits at 229 dat was supported by different tests of antioxidant activity. In Table 5, both the test for antioxidant activity by the FRAP method and the test for the detoxifying effect of AOS showed their highest values in the third sampling, coinciding with the most stressful conditions of temperature and solar radiation in the greenhouse. Many recent studies have related these types of tests to the nutritional quality and health benefits of different agricultural food products.[3, 6] In this study, the use of this relationship would not be appropriate, as the increase in antioxidant activity in the third sampling according to the tested methods would be due primarily to the greater AsA oxidation by APX, as mentioned above, and not to the increase in antioxidant compounds such as lycopene, which proves essential for evaluating the nutritional quality of cherry tomato fruits.

In conclusion, cherry tomato fruits subjected to elevated air temperatures and accumulated solar radiation (Table 1) increased lipid peroxidation and decreased the content of carotenoids (e.g. lycopene) in the exocarp fractions, despite the fact that the oxidation of AsA by APX increased under these conditions. Possibly, the inhibited regeneration of DHA by the enzymes DHAR and MDHAR in the ascorbate/glutation cycle in these experiments proved to be a key step in avoiding the diminished content of antioxidants and therefore in improving or maintaining the nutritional quality of this fruit under experimental conditions of environmental stress.

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Table 1. Maximum and minimum air temperatures and overall accumulated solar radiation values recorded inside the experimental greenhouse and fruit production of cherry tomato in the three different stages of production sampled

	Maximum temperature (°C)	Minimum temperature (°C)	Accumulated solar radiation (MJ m^{-2})	Fruit production (kg m^{-2})
85 dat	27.2b[a]	11.4b	589	1.06a
160 dat	26.7b	12.0b	1232	1.11a
229 dat	35.9a	15.4a	2190	1.06a
P-Value[b]	**	***	***	NS
$LSD_{0.05}$	4.32	1.53	212	0.1

a Values followed by the same letters within each column are not significantly different according to the DMRT at $P \leq 0.05$.

b Levels of significance are represented by $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS, not significant.

Table 2. Content of antioxidant compounds in the exocarp fraction of cherry tomato fruits in three stages of fruit production during the plant growth cycle

	Lycopene (μg g^{-1} fresh weight)	β-Carotene (μg g^{-1} fresh weight)	Anthocyanin (absorbance g^{-1} fresh weight)
85 dat	19.8a[a]	7.37a	0.026a
160 dat	17.1a	7.66a	0.026a
229 dat	12.1b	5.76b	0.023a
P-Value[b]	*	*	NS
$LSD_{0.05}$	4.24	1.24	0.007

a Values followed by the same letters within each column are not significantly different according to the DMRT at $P \leq 0.05$.

b Levels of significance are represented by $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS, not significant.

Table 3. Lipid peroxidation in the exocarp fraction of cherry tomato fruits in three different stages of fruit production during the crop cycle

	MDA (nmol g^{-1} fresh weight)	LOX (U g^{-1} fresh weight)
85 dat	80.7 b[a]	23.2 b
160 dat	76.3 b	24.4 b
229 dat	104.9 a	36.7 a
P-Value[b]	*	**
$LSD_{0.05}$	16.5	6.61

a Values followed by the same letters within each column are not significantly different according to the DMRT at $P \leq 0.05$.

b Levels of significance are represented by $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS, not significant.

Table 4. Parameters of the ascorbic acid metabolism in the exocarp fraction of cherry tomato fruits in three different stages of fruit production period

	AsA_{Total} (mmol g^{-1} fresh weight)	AsA_{Red} (mmol g^{-1} fresh weight)	DHA (mmol g^{-1} fresh weight)	H_2O_2 (µmol g^{-1} fresh weight)	APX (µmol AsA oxidised g^{-1} fresh weight min^{-1})	DHAR (µmol DHA reduced g^{-1} fresh weight min^{-1})	MDHAR (nmol NADH oxidised g−1 fresh weight min^{-1})	AO (nmol AsA oxidised g^{-1} fresh weight min^{-1})
85 dat	2.72b[a]	2.23a	0.49b	60.9b	0.61b	6.26a	85.4a	0.35b
160 dat	3.12ab	2.01a	1.11a	51.1b	0.68ab	1.60b	34.0c	0.31b
229 dat	3.72a	2.17a	1.55a	72.7a	0.78a	1.34b	60.9b	0.43a
P-Value[b]	*	NS	**	**	*	***	*	**
$LSD_{0.05}$	0.9	0.44	0.53	11.2	0.11	1.595	20.8	0.05

a Values followed by the same letters within each column are not significantly different according to the DMRT at $P \leq 0.05$.

b Levels of significance are represented by $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS, not significant.

Table 5. Tests of antioxidant activity in the exocarp fraction of cherry tomato fruits in three different stages of fruit production during the crop cycle

	FRAP assay (μmol g^{-1} fresh weight)	DPPH free-radical scavenging (% g^{-1} fresh weight)
85 dat	30.7 b[a]	42.2 c
160 dat	34.6 b	60.9 b
229 dat	44.6 a	82.6 a
P-Value[b]	**	***
$LSD_{0.05}$	8.27	7.23

a Values followed by the same letters within each column are not significantly different according to the DMRT at $P \leq 0.05$.

b Levels of significance are represented by $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS, not significant.