Food ©2013 Global Science Books

Effect of the Stage of Maturity on the Antioxidant Content and Antioxidant Activity of High-pigment Tomato Cultivars Grown in Italy

Chafik Hdider[1*] • Riadh Ilahy[1] • Imen Tlili[1] • Marcello Salvatore Lenucci[2] • Giuseppe Dalessandro[2]

[1] National Institute of Agronomic Research of Tunisia, 2049 Ariana, Tunisia
[2] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università Del Salento, Via Prov.le Lecce Monteroni, 73100 Lecce, Italy

Corresponding author: * **hdider.chafik@iresa.agrinet.tn**

ABSTRACT

Lycopene has attracted much interest during the last few years because of its antioxidant activity against free radicals, suggesting protective roles in reducing the risk of several chronic diseases. Therefore, tomato cultivars, with increased lycopene content have been developed. However, a detailed assessment of their nutritional value remains scarce in literature. In this study, the effect of the stage of maturity on the antioxidant content and activity of six high-lycopene tomato cultivars ('Lyco 1', 'Lyco 2', 'HLY 02', 'HLY 13', 'HLY 18' and 'Kalvert') and one ordinary ('Donald') was determined. The pattern of change in lycopene and β-carotene was similar in all tomato cultivars, although quantitatively higher in high-lycopene tomatoes. In those cultivars, lycopene and β-carotene were respectively 1.68- to 3.7-fold and 2.11- to 2.48-fold higher during ripening compared to 'Donald'. The lipophilic antioxidant activity was well correlated to the lycopene and β-carotene contents. The pattern of change in total phenolic, flavonoid and total vitamin C was cultivars dependent. At the red ripe stage, 'HLY 13' showed the highest total vitamin C and flavonoid contents. However, 'HLY 02' showed the highest total phenolic content. The hydrophilic antioxidant activity was only well correlated to the phenolic and flavonoid contents.

Keywords: High-lycopene tomato cultivars, lycopene, vitamin C, phenols, ripening stages, antioxidant activity
Abbreviations: AsA, ascorbic acid; **cv(s)**, cultivar(s); **DHA**, dehydroascorbic acid; **GAE**, gallic acid equivalents; **HAA**, hydrophilic antioxidant activities; **LAA**, lipophilic antioxidant activities; **RE**, rutin equivalents

INTRODUCTION

Tomatoes (*Solanum lycopersicon* L.), commonly used in the Mediterranean diet, are a major source of antioxidants and contribute to the daily intake of a significant amount of these molecules. They are consumed fresh or as processed products (canned tomatoes, sauce, juice ketchup, soup) (Hdider *et al*. 2007). The regular ingestion of an adequate amount of fresh tomatoes or tomato products has been inversely correlated to the development of widespread human diseases (Agarwal and Rao 1998). This protective effect has been mainly attributed to the carotenoid constituents of the fruits, particularly lycopene and β-carotene which act as antioxidants in detoxifying free radicals (Stahl and Sies 1996; Clinton 1998). Along with carotenoids, other antioxidant compounds of tomato fruits, such as ascorbic acid and phenolics, play a determinant role in disease prevention (Robards *et al*. 1999; Karakaya *et al*. 2001).

Recently, the attention has been given to antioxidant compounds and antioxidant activity in fruits and vegetables. In fact, their estimation is becoming an important evaluation parameter for the nutritional quality of food and its quantification allows a real evaluation of this nutritional value rather than the analysis of each single antioxidant compound (Lenucci *et al*. 2006; Pellegrini *et al*. 2007). Moreover, it is widely recognized that the protective role of tomato consumption is due to the synergistic effect among the different classes of antioxidants (Amir *et al*. 1999).

It has been reported that the levels of the health-promoting bioactive compounds and of the antioxidant activity of tomato extracts are strongly influenced by several cultural practices and agronomic aspects, particularly the varietals genotype and ripening stage of the fruit (Cano *et al*. 2003; Dumas *et al*. 2003).

A large number of new tomato cultivars, with increased lycopene content (high-lycopene tomato cultivars), have been developed recently by conventional plant breeding techniques. The high-lycopene trait is commonly due to the presence of high-pigment (hp) mutations (*hp-1*, *hp1*^{wm}, *hp-2*, *hp-2*^{j}, *hp-2*^{dg} and recently, *hp-3*) leading to an increase of carotenoids, particularly lycopene, and flavonoid content (Mochizuki and Kamimura, 1984; Mustilli *et al*. 1999; Galpaz *et al*. 2008). A small number of research papers have recently focused on the agronomic characteristics and lycopene content of some of these new cultivars (Macua *et al*. 2007; Cantore *et al*. 2008). However, a detailed assessment of their nutritional quality remains scarce in literature (Lenucci *et al*. 2006; Ilahy *et al*. 2011).

Tomato fruits are harvested at different ripening stages from green-orange (breaking) to a red ripe colour depending on the consumer and market preference. Therefore, the quali-quantitative analysis of different antioxidants, as well as their variation during ripening, is of great relevance both to human health and commercial purposes. Various studies dealing with the effect of ripening stages on tomato antioxidants have been reported. However, the data available related to high-lycopene tomato cultivars is limited. Ilahy *et al*. (2009) focused on the bioactive compounds and antioxidant activity of tomato high-lycopene content advanced breeding lines, namely 'HLT-F51' and 'HLT-F52', selected by the National Agricultural Research Institute of Tunisia. The authors found that these tomato lines showed generally satisfying agronomic characteristics associated to higher antioxidant profile as compared to 'Rio Grande'. Ilahy *et al*. (2010) focused also on lycopene and total phenolic contents in pulp and skin fractions of different high-lycopene tomato

Received: 4 August, 2012. Accepted: 26 September, 2012.

Original Research Paper

varieties and reported that compared to the standard variety 'Rio Grande', 'HLY 18' had 2.7- and 2.1-fold higher pulp and skin lycopene contents respectively. Also 'HLY 18' had 1.6- and 2.6-fold higher pulp and skin total phenolic contents respectively which emphasize the promising use of such varieties for healthy fresh food and value added products. Recently, Ilahy *et al*. (2011) studied the effect of ripening on some high-pigment tomato cultivars grown in Tunisia and found that during ripening, 'HLY 13' and 'HLY 18' exhibited more than 100% higher total carotenoid and lycopene contents compared to the standard 'Rio Grande'. At the red-ripe stage, 'HLY 18' accumulated the highest phenolic and flavonoid contents. However, 'Lyco 2'showed the highest levels of DHA and total vitamin C. Nevertheless, additional studies with other cultivars under different environmental conditions are required to support these results.

In this study, changes in the main bioactive compounds (lycopene, β-carotene, lutein, phenolics, flavonoids, ascorbic acid and dehydroascorbic acid contents) and antioxidant activity (both hydrophilic and lipophilic) of six high-lycopene ('Lyco 1', 'Lyco 2', 'HLY 02', 'HLY 13', 'HLY 18' and 'Kalvert') and ordinary ('Donald') tomato cultivars grown simultaneously in an open-field of the southern Italy were studied during ripening. The correlation of the hydrophilic and lipophilic antioxidant activities to the different classes of antioxidants was also examined.

MATERIALS AND METHODS

Plant culture

The field experiment was carried out in a field in the province of Lecce (southern Italy) during the 2008 growing season (April–July). Seven tomato cultivars were used in these experiments. Six tomato cultivars claimed to be high-lycopene content ('Lyco 1', 'Lyco 2' 'HLY 02', 'HLY 13', 'HLY 18', and 'Kalvert') and the ordinary cultivar 'Donald' currently grown in the south of Italy. 'HLY 02', 'HLY 13', 'HLY 18' and 'Kalvert' cultivars were obtained from COIS' 94 s.r.l. (Belpasso, CT, Italy). Cultivar 'Lyco 1' and 'Lyco 2' was obtained from Hazera Genetics Ltd (Berurim MP Shikmim, Israel). Cultivar 'Donald' was obtained from Nunhems (Nunhems SRL, BO, Italy). Sowing was carried out in alveolar boxes at the beginning of April 2008. One month-old tomato seedlings were transplanted in an open field at a density of about 33,000 plants ha^{-1} and grown to maturity. Spacing within rows and between double rows was 0.4 and 1.5 m, respectively. Standard agronomical techniques were used for plant nutrition and pathogen prevention. Briefly, the field was deep ploughed (60-70 cm) and 1000 kg/ha of a basic organomineral fertilizer (Fertil Agreste Start, Scam) was spread. Post-transplant nitric nutrition with ammonium nitrate (fertilizer Leon, Hydro Agri), 600 kg/ha, was given when required. Propamocarb hydrochloride, a fungicide, Confidor Supra (Bayer), an insecticide, and metallic copper were used for pest control. Drip irrigation may run for 1–2.5 h, at 1-2 day intervals, depending on potential evapo-transpiration, climate data and crop coefficient.

All cultivars under analysis were grown simultaneously in the same field and subjected to identical cultural practices and, of course, environmental conditions in order to minimize the influence of pre- and post-harvest factors as well as agronomic and cultural practices, ripening stage at harvest, and storage conditions on genotype-related variability of field-grown tomatoes (Abushita *et al*. 2000; Dumas *et al*. 2003; George *et al*. 2004).

Tomato harvest and sampling

Tomato fruits were hand harvested randomly from the 20 m rows and from the middle of the plant at different maturity stages. A sample of at least 2 kg of injury-free tomato fruits was harvested from each cultivar, and delivered quickly to the laboratory. Four ripening stages corresponding to the green (fruit surface completely green, varying from light to dark green), green-orange (first appearance of external change in color on not more than 10% of fruit surface), orange-red (over 30% but not more than 60% red) and red-ripe (over 90% red surface; desirable table ripeness) fruit color were visually sorted according to the US color standard for classifying tomato ripeness (USDA 1976). Tomato fruits were cut into small pieces and homogenized in a mixer (Waring Laboratory and Science, Torrington, CT, USA). The obtained samples were frozen at -20°C and used to determine the carotenoids, phenolics, flavonoids and vitamin C contents as well as the hydrophilic and lipophilic antioxidant activity within one week. The reasons for doing this were the significant volume of work involved and the limited shelf life of tomatoes under refrigeration.

Chemicals

2,2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), ascorbic acid, rutin, gallic acid, 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-Dipyridyl, butylated hydroxytoluene (BHT), dithiothreitol (DTT), AlCl3 and N-ethylmaleimide (NEM) were obtained from Sigma-Aldrich, Chemical Co., Milan. Other reagents (acetone, ethanol, hexane, acetonitrile, methanol, HCl, metaphosphoric acid) were of analytical grade and purchased from Carlo Erba (Milan, Italy).

Carotenoid determination

Lycopene, β-carotene and lutein contents were determined on triplicate aliquots of the homogeneous juice (0.5 g) according to the method of Sadler *et al*. (19990) as modified by Perkins-Veazie *et al*. (2001). Carotenoids were extracted with 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol (1:1, v/v). Lycopene, β-carotene and lutein were separated by partition into hexane and directly assayed. A Dionex HPLC (Dionex s.r.l., Milan, Italy) with an AD 25 UV-Vis detector was used, and the separation was performed at 31°C on an Acclaim HPLC column C_{18} (5 μm, 250 × 4.6 mm). The separation was performed by using a linear gradient of acetonitrile (A), hexane (B) and methanol (C) as follow: from 70% A, 7% B, 23% C to 70% A, 4% B, 26% C within 35 min, with a flow rate of 1.5 mL min^{-1}. Concentration of standard solutions was calculated using the molar extinction coefficients 17.2×10^4 for lycopene, 13.9×10^4 for β-carotene and 14.3×10^4 for lutein in hexane. Peaks were detected at 503 nm and results were expressed in mg kg^{-1} FW.

Total phenols determination

Total phenols were extracted as described by Martinez-Valverde *et al*. (2002) on triplicate aliquots of homogenate juice (0.3 g). Briefly, 5 mL of 80% aqueous methanol and 50 μL of 37% HCl were added to each sample. The extraction was performed at 4°C, for 2 h, under constant shaking (300 rpm). Samples were centrifuged at 10000 × *g* for 15 min. The total phenols assay was performed by using the Folin-Ciocalteu reagent as described by Spanos and Wrolstad (1990) triplicate 50 μL aliquots of the supernatant. The absorbance was read at 750 nm using a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc., CA, USA). Results were expressed in mg gallic acid equivalent (GAE) kg^{-1} fresh weight (FW).

Flavonoid determination

The flavonoid content was determined as described by Jia *et al*. (1999) on triplicate aliquots of the homogenous juice (0.3 g). Fifty microliter aliquots of the methanolic extract were used for flavonoids determination. Samples were diluted with distilled water to a final volume of 0.5 mL, and 30 μL of 5% $NaNO_2$ was added. After 5 min, 60 μL of 10% $AlCl_3$ was added and finally 200 μL of 1 M NaOH was added after 6 min. The absorbance was read at 510 nm in a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc., CA, USA) and flavonoid content was expressed as mg rutin equivalent (RE) kg^{-1} FW.

Ascorbic acid and dehydroascorbic acid determination

AsA and DHA contents were determined as repeorted by Kampfenkel *et al*. (1995) on triplicate samples of the homogenate juice (0.1 g). AsA and DHA were extracted by using 6% metaphospho-

Table 1 Lycopene, β-carotene and lutein content in ordinary and high-lycopene tomato cultivars harvested at four different ripening stages. Values within column and ripening stage followed by the same letters do not differ significantly (LSD test, $P < 0.01$).

Cultivar	Ripening stage	Lycopene	β-carotene	Lutein
		mg kg^{-1} FW		
Donald	Green	nd	1.53 ± 0.10 d	Nd
	Green-Orange	9.2 ± 1.1 c	3.54 ± 0.42 c	Nd
	Orange-Red	40.6 ± 2.5 b	4.63 ± 0.16 b	Nd
	Red-Ripe	96.9 ± 3.9 a	5.81 ± 0.10 a	Nd
Lyco1	Green	nd	3.50 ± 0.10 c	0.23 ± 0.01 a
	Green-Orange	25.0 ± 0.5 c	7.90 ± 0.11 b	nd
	Orange-Red	58.7 ± 0.7 b	8.41 ± 0.26 b	nd
	Red-Ripe	122.4 ± 5.9 a	13.01 ± 0.29 a	nd
Lyco 2	Green	nd	3.11 ± 0.10 d	nd
	Green-Orange	48.4 ± 3.5 c	6.73 ± 0.05 c	nd
	Orange-Red	90.7 ± 1.3 b	10.48 ± 0.40 b	nd
	Red-Ripe	132.9 ± 5.8 a	11.57 ± 0.11 a	nd
HLY 02	Green	nd	4.63 ± 0.15 b	nd
	Green-Orange	22.5 ± 0.6 c	7.32 ± 0.08 a	nd
	Orange-Red	64.4 ± 1.1 b	7.37 ± 0.05 a	nd
	Red-Ripe	170.2 ± 2.7 a	7.72 ± 0.17 a	nd
HLY 13	Green	Nd	5.39 ± 0.38 d	0.28 ± 0.02 a
	Green-Orange	44.6 ± 0.7 c	14.24 ± 0.10 c	nd
	Orange-Red	98.3 ± 1.9 b	15.87 ± 0.78 b	nd
	Red-Ripe	177.9 ± 6.1 a	19.88 ± 0.11 a	nd
HLY 18	Green	Nd	4.91 ± 0.50 d	nd
	Green-Orange	43.2 ± 1.0 c	8.51 ± 0.58 c	nd
	Orange-Red	80.4 ± 1.4 b	10.57 ± 0.19 b	nd
	Red-Ripe	232.9 ± 5.9 a	19.37 ± 0.86 a	nd
Kalvert	Green	nd	1.23 ± 0.16 c	nd
	Green-Orange	20.2 ± 0.7 c	5.84 ± 0.20 b	nd
	Orange-Red	66.6 ± 1.1 b	6.10 ± 0.36 b	nd
	Red-Ripe	141.5 ± 5.9 a	8.78 ± 0.16 a	nd

ric acid and detected at 525 nm in a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc.) and expressed in mg kg^{-1} FW**.**

Hydrophilic and lipophilic antioxidant activity determination

The measurement of the hydrophilic and lipophilic antioxidant activity (HAA and LAA) was performed using the Trolox equivalent antioxidant capacity (TEAC) assay. The antioxidant activity was measured using the ABTS decoloration method (Pellegrini *et al*. 2007). Hydrophilic and lipophilic antioxidants were extracted from 0.3 g homogenous juice (three replicates) with 50% methanol or 50% acetone respectively at 4°C under constant shaking (300 rpm) for 12 h. Samples were centrifuged at 10,000 × *g* for 7 min and the different supernatants were recovered and used for antioxidant activity measurements. The antioxidant activities were measured at 734 nm in a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc.). Two different calibration curves were constructed using freshly prepared trolox solutions for HAA and LAA determinations. Values were obtained from three replicates as μM Trolox 100 g^{-1} FW.

Statistical analysis

Effects of variety and ripening stage on the nutritional properties of tomato cultivars were assessed by analysis of variance (ANOVA). When a significant difference was detected, means were compared using the least significant difference (LSD) test ($P < 0.05$). All statistical comparisons were performed using SAS Version 6.1 software (SAS Institute, Cary, NC, USA). Correlations were done using Person's correlation coefficient, $P < 0.05$.

RESULTS AND DISCUSSION

Carotenoid content

The lycopene, β-carotene and lutein contents of the ordinary and high-lycopene tomato cultivars at the four ripening stages corresponding to the green, green-orange, orange-red and red-ripe colour of the fruit are presented in **Table 1**. For all investigated tomato cultivars, the lycopene and β-carotene contents, expressed on a fresh weight (FW) basis, markedly increased during fruit ripening. The values, at each ripening stage, were comparatively much higher in high-lycopene cultivars ('Lyco 1', 'Lyco 2', 'HLY 02', 'HLY 13', 'HLY 18' and 'Kalvert') than the ordinary one ('Donald'). In particular, at the red-ripe stage, lycopene values varied from 96.9 mg kg^{-1} FW in 'Donald' to 232.9 mg kg^{-1} FW in 'HLY 18'. Thus, in high-lycopene tomato cultivars the amount of lycopene was 1.26- to 2.4-fold higher than 'Donald'.

Lycopene was not detected at the green stage, in all cultivars. The accumulation of lycopene was very evident when the tomatoes passed from the green stage to the green-orange stage, commonly referred to as the "breaker" stage. At this stage of maturity 13 to 36% of lycopene was already synthesized in high-lycopene tomato cultivars compared to 9.46% in the fruit of cultivar 'Donald'. This suggests higher patterns of lycopene accumulation in high-lycopene tomato compared to the ordinary cultivar 'Donald' during the ripening process. A similar result was obtained by Lenucci *et al*. (2009) who studied the carotenoid content during tomato fruit ripening in traditional and high-pigment cultivars. They reported that in all of the tomato cultivars investigated ('Donald', 'Incas', 'Kalvert' and 'HLY 18') the amount of lycopene calculated as mg/kg FW increased dramatically from the green to the red stage. The increase was 475-, 465-, 505-, and 488-fold for cultivars 'Donald', 'Incas', 'Kalvert' and 'HLY 18', respectively.

Although lycopene represents the most abundant carotenoid in red-ripe tomatoes, approximately 90 to 96% of the total pigments, we have also measured the amount of β-carotene. Other carotenoids such as α-carotene, phytoene and phytofluene, which have been reported to accumulate during ripening in tomato fruit and to account all together, for less than 6.8% of the total carotenoids (Raffo *et al*. 2002), were not detectable in all analyzed cultivars. β-carotene content showed similar variation to lycopene, in fact, at the red ripe stage, 'HLY 13' and 'HLY 18' exhibited also the highest level of β-carotene (19.84 and 19.37 mg kg^{-1} FW,

respectively) indicating that in these varieties the high amount of lycopene was also associated with a high amount of β-carotene. The differences between high-lycopene and ordinary tomato cultivars have been attributed to different growing conditions and cultivars (Dumas *et al*. 2003; Ilahy *et al*. 2009; 2010). It has been also reported that high-lycopene tomato cultivars derive from spontaneous mutants characterized by deeply pigmented fruits due to their exaggerated light responsiveness with respect to wild-type plants (Mustilli *et al*. 1999; Atanassova *et al*. 2007).

At the green stage, the amount of β-carotene was the lowest in all the studied cultivars. Its level increased markedly between the green stage and the red-ripe stage of tomato ripening. This increase was 3.7-fold in 'Donald' whereas in high-lycopene tomato cultivars, except for 'HLY 02', it was 3.7- to 7.1-fold higher compared to the ordinary tomato cultivar. Similar change tendency was reported by Abushita *et al*. (1997) who found that β-carotene concentration increased in proportion to the advanced ripeness. Recently, Lenucci *et al*. (2009) reported that β-carotene accumulation was cv-dependent since in both 'Donald' and 'Incas' β-carotene content increased during ripening until the red ripe stage however, in 'Kalvert' and 'HLY 18' β-carotene content decreased markedly during ripening. Biacs *et al*. (1987) found that β-carotene approached its maximum level in yellow coloured fruits of the processing cultivar 'Ventura' and then declined. The contrasting results are probably due to the influence of varietals factors on carotenogenesis in tomato fruits.

Lutein was detectable only at the green stage of maturity in 'Lyco 1' and 'HLY 13' cultivars. The loss of lutein during ripe tomato cultivars seems to be due to the progressive loss of functionality of the photosynthetic machinery during the transition from chloroplasts to chromoplasts (Lenucci *et al*. 2009; Ilahy *et al*. 2011). The presence of lutein in young red pepper and orange fruit cultivars has also been demonstrated then disappears during ripening (Hornero-Mendez *et al*. 2000; Rodrigo *et al*. 2004).

In the present study, the amounts of lycopene and β-carotene obtained at the red-ripe stage of the high-lycopene tomato cultivars 'HLY 13' and 'HLY 18' were similar to those of the same cultivars grown in an open-field of the southern Italy (Lenucci *et al*. 2006, 2009). All together, these data confirm that these high-lycopene cultivars synthesize and store a much higher amount of carotenoids, particularly lycopene, and β-carotene compared to ordinary cultivars and exhibit an interestingly stable phenotype regarding lycopene and β-carotene synthesis and storage.

Total phenols and flavonoid content

The total phenol and flavonoid content of the ordinary and high-lycopene tomato cultivars at the four ripening stages corresponding to the green, green-orange, orange-red and red-ripe colour of the fruit are presented in **Table 2**. Except for 'HLY 02' cultivar, the result showed that phenolic content was significantly different between the studied ripening stages ($P < 0.01$). Different patterns of change in phenolic content were observed between the cultivars. In 'HLY 18' a peak in the amount of phenols was reached at the orange-red ripening stage, while the highest phenolic level in 'Kalvert' was obtained in orange-red stage of maturity. Although the determination of phenolic content in high-lycopene tomato cultivar during ripening is scarce, Raffo *et al*. (2002) reported a gradual decline in the concentration of chlorogenic acid, one of the common phenols in tomatoes, during ripening of the greenhouse-grown cherry tomato cultivar 'Naomi' while Helyes and Lugasi (2006) reported that phenolic content of the tomato cultivar 'Lemance' remained unchanged during ripening. Recently, Ilahy *et al*. (2011) reported that different patterns of change in phenolic content were observed between some high-pigment tomato cultivars. In cultivar 'HLY 18' a peak in the amount of phenols was reached at the orange-red ripening stage, while the highest phenolic levels in 'HLY 13' were detected in both green and green-orange stages of ripening. On the contrary, in cultivar 'Rio Grande' the same ripening stages were seen to be those with the lowest phenol content. Although genetic control is the primary factor in determining the amount of phenols in fruits and vegetables, variations could also depend by ripening stages at the moment of harvesting, environmental factors (mainly light and temperature) (Macheix *et al*. 1990; Dumas *et al*. 2003) and analytical methodology. Moreover, the often contradictory results could be attributed to different pattern of changes in different classes of phenolic during tomato fruit ripening as was reported by Buta and Spaulding (1997) and Raffo *et al*. (2002).

Table 2 Total phenolics and flavonoids in ordinary and high-lycopene tomato cultivars harvested at four different ripening stages. Values within column and ripening stage followed by the same letters do not differ significantly (LSD test, $P < 0.01$).

Cultivar	Ripening stage	Phenolics (mg GAE kg^{-1} FW)	Flavonoids (mg RE kg^{-1} FW)
Donald	Green	293.6 ± 2.84b c	155.6 ± 10.84 a
	Green-Orange	341.1 ± 11.35 a	172.8 ± 26.00 a
	Orange-Red	315.5 ± 14.75 ab	125.8 ± 9.82 a
	Red-Ripe	280.0 ± 4.18 c	146.1 ± 13.24 a
Lyco 1	Green	465.6 ± 17.74 a	335.8 ± 25.94 c
	Green-Orange	498.0 ± 33.45 a	435.0 ± 25.10 b
	Orange-Red	305.9 ± 24.76 b	286.7 ± 20.00 d
	Red-Ripe	105.8 ± 3.80 c	480.6 ± 2.00 a
Lyco 2	Green	314.8 ± 15.44 a	253.1 ± 3.27 b
	Green-Orange	200.3 ± 11.10 b	590.6 ± 6.95 a
	Orange-Red	278.0 ± 10.11 a	562.6 ± 19.10 a
	Red-Ripe	188.4 ± 26.99 b	105.6 ± 2.46 c
HLY 02	Green	358.4 ± 48.11 a	258.6 ± 0.27 a
	Green-Orange	396.3 ± 9.69 a	220.8 ± 6.66 b
	Orange-Red	348.5 ± 6.50 a	232.8 ± 19.72 ab
	Red-Ripe	394.5 ± 34.82 a	239.4 ± 3.08 ab
HLY 13	Green	439.7 ± 36.52 a	353.1 ± 3.41 c
	Green-Orange	475.9 ± 13.67 a	510.6 ± 9.74 a
	Orange-Red	404.5 ± 14.12 a	426.1 ± 3.19 b
	Red-Ripe	181.0 ± 2.48 b	511.9 ± 23.62 a
HLY 18	Green	544.2 ± 40.72 b	392.8 ± 13.01 c
	Green-Orange	318.8 ± 17.73 c	525.0 ± 10.48 a
	Orange-Red	760.8 ± 12.82 a	461.1 ± 12.25 b
	Red-Ripe	222.7 ± 2.78 b	498.1 ± 9.72 ab
Kalvert	Green	523.9 ± 12.64 b	259.2 ± 8.90 b
	Green-Orange	877.2 ± 63.56 a	341.1 ± 22.63 a
	Orange-Red	255.1 ± 8.82 c	323.1 ± 7.63 a
	Red-Ripe	146.6 ± 12.62 d	301.9 ± 8.11 ab

As for phenolics, the averaged results showed that flavonoid content was significantly different between studied ripening stages except for 'HLY 02' and 'Donald' ($P <$ 0 .01). The pattern of change in flavonoid was different in all high-lycopene tomato cultivars. In 'Lyco 1' a peack in the amount of flavonoids was reached at the red-ripe stage, while the lowest levels in 'Lyco 2' was detected at the same ripening stage. It should be underlined that differences between high-lycopene and ordinary tomato cultivars were evident at all the studied ripening stages. In fact, when averaged across cultivars, their flavonoid level was 2.00-, 2.20-, 3.03- and 2.43-fold higher at the green, green-orange, orange-red and red-ripe stages, respectively compared to the cultivar 'Donald'. This is mainly due to genotypic differences, in fact, it has been reported, that naturally occurring mutations leading to increased carotenoid content, particularly lycopene, are also characterized by a dramatic increase in plastid biogenesis and in the production of many other compounds such as vitamin C and flavonoids (Mochizuki and Kamimura 1984). Although different patterns of change in flavonoid were observed by Ilahy *et al*. (2011), high-lycopene tomato had stored higher flavonoid levels during all the studied maturity stages compared to the cultivar 'Rio Grande'.

Table 3 Ascorbic acid (AsA), dehydroascorbic acid (DHA) and total vitami C (AsA+DHA) in ordinary and high-lycopene tomato cultivars harvested at four different ripening stages. Values within column and ripening stage followed by the same letters do not differ significantly (LSD test, $P < 0.01$).

Cultivar	Ripening stage	AsA	DHA	Total vitamin C
			mg kg^{-1} FW	
Donald	Green	60.3 ± 2.48 d	139.9 ± 9.36 ab	200.2 ± 6.87 d
	Green-Orange	182.5 ± 7.86 a	152.6 ± 17.83 a	335.1 ± 10.90 a
	Orange-Red	151.1 ± 3.75 b	116.0 ± 10.50 ab	266.5 ± 7.88 b
	Red-Ripe	119.1 ± 6.10 c	108.8 ± 11.20 b	227.9 ± 5.11 c
Lyco 1	Green	61.7 ± 3.26 d	169.2 ± 1.30 a	230.9 ± 1.23 c
	Green-Orange	188.9 ± 1.59 a	85.0 ± 12.61 c	273.9 ± 11.62 a
	Orange-Red	158.5 ± 4.79 b	86.6 ± 6.17 c	245.1 ± 3.16 bc
	Red-Ripe	124.7 ± 5.29 c	144.0 ± 1.12 b	268.7 ± 4.76 ab
Lyco 2	Green	56.3 ± 2.21 d	220.4 ± 11.75 a	276.7 ± 10.61 bc
	Green-Orange	172.3 ± 7.34 a	128.5 ± 4.23 b	300.8 ± 4.94 ab
	Orange-Red	147.9 ± 7.26 b	106.7 ± 8.94 b	254.6 ± 5.12 c
	Red-Ripe	107.5 ± 2.69 c	204.5 ± 7.14 a	312.0 ± 8.79 a
HLY 02	Green	48.8 ± 3.00 b	236.2 ± 10.79 a	284.8 ± 9.69 a
	Green-Orange	135.8 ± 9.08 a	131.6 ± 16.29 b	267.4 ± 8.02 a
	Orange-Red	130.2 ± .68 a	72.2 ± 2.51 c	202.4 ± 5.31 b
	Red-Ripe	117.8 ± 7.43 a	163.1 ± 1.43 b	280.9 ± 6.46 a
HLY 13	Green	74.3 ± 1.50 c	141.3 ± 1.52 b	215.6 ± 1.89 c
	Green-Orange	157.1 ± 5.01 ab	155.9 ± 8.54 b	313.0 ± 9.23 b
	Orange-Red	142.0 ± 9.64 b	148.5 ± 3.26 b	290.3 ± 9.16 b
	Red-Ripe	163.7 ± 4.09 a	189.0 ± 5.49 a	352.8 ± 9.89 a
HLY 18	Green	75.9 ± 5.40 c	155.4 ± 14.08 a	231.3 ± 8.83 c
	Green-Orange	218.3 ± 0.82 a	108.7 ± 10.46 b	326.7 ± 10.17 a
	Orange-Red	198.8 ± 9.16 a	90.3 ± 6.29 b	289.1 ± 7.28 b
	Red-Ripe	128.3 ± 9.68 b	176.7 ± 4.69 a	305.0 ± 5.16 ab
Kalvert	Green	57.8 ± 0.94 c	88.4 ± 12.18 a	146.2 ± 13.06 b
	Green-Orange	180.6 ± 4.19 a	68.9 ± 3.78 a	249.5 ± 6.03 a
	Orange-Red	150.8 ± 10.32 a	82.0 ± 8.59 a	232.8 ± 2.86 a
	Red-Ripe	169.8 ± 9.35 ab	66.0 ± 7.53 a	235.7 ± 1.89 a

Vitamin C content

The Ascorbic acid (AsA), dehydroascorbic acid (DHA) and total vitamin C (AsA + DHA) contents of the investigated tomato cultivars at the four different ripening stages are presented in **Table 3**. The result showed that AsA, DHA and total vitamin C levels were significantly different between the studied ripening stages ($P < 0.01$). Such variability in the total vitamin C content during ripening was genotype dependant, in fact different patterns of change were evidenced for the studied tomato cultivar. The highest amount of total vitamin C was recorded at the red-ripe ripening stage in 'HLY 13' (352.8 mg kg^{-1} FW) but at the green-orange stage for 'Donald' and at the green-orange, orange-red and red-ripe for 'Kalvert', 'HLY 18', and 'Lyco 1'. This is also confirmed by the conflicting results reported by Raffo *et al*. (2002) and Abushita *et al*. (1997) on the variability of AsA, DHA and total vitamin C content during ripening of greenhouse grown 'Naomi' and 'Floriset' cultivars. Nevertheless, the discrepancy could be attributed to environmental and agronomical factors (Abushita *et al*. 1997; Dumas *et al*. 2003). In addition, the variation can be ascribed to the antioxidant function of ascorbic acid in ripening cells which absorb high amount of oxygen as a result of increasing rate of cell respiration (Tünk *et al*. 1993). Similar results were obtained recently by Ilahy *et al*. (2011), who evidenced different patterns of change in vitamin C for the studied tomato cultivar during ripening.

During the ripening of 'HLY 13', the AsA levels contributed for between 34 and 50% to the total vitamin C whereas the DHA contributed for between 50 and 66%. Therefore, we considered that the determination of both DHA and AsA contents in tomato fruits at each ripening stage is of great importance since it leads to a real estimation of the total vitamin C content in tomato fruits rather than the simple estimation of the ascorbic acid content.

It is valuable to underline that, at the red-ripe stage of maturity all high-lycopene tomato cultivars, except for 'Kalvert', exhibited a higher amount of total vitamin C particularly 'HLY 13', 'Lyco 2' and 'HLY 18' obtained 1.54-, 1.36- and 1.33-fold higher respectively compared to cultivar 'Donald'. These results provide further evidences that the spontaneous mutations which determines the character high-lycopene, determine, in some of the new selected cultivars exhibiting such phenotype, a dramatic increase in the production of many important antioxidant such as vitamin C as previously reported by Mochizuki and Kamimura (1984), Mustilli *et al*. (1999), Lenucci *et al*. (2006), and Ilahy *et al*. (2010, 2011).

Hydrophilic and lipophilic antioxidant activity

Results for hydrophilic and lipophilic antioxidant activities (HAA and LAA) determined by the TEAC assay in ordinary and high-lycopene tomato cultivars at the four different ripening stages are presented in **Table 4**. For all high-lycopene tomato cultivars, the highest HAA value was estimated in tomato fruits at the green-orange and orange-red stage of ripening and the lowest value was recorded at the green and red-ripe stage. The HAA in the ordinary variety 'Donald' was unaffected by the ripening process. Raffo *et al*. (2002) reported a similar trend for the hydrophilic antioxidant activity during fruit maturation of the cherry tomato cultivar 'Naomi', grown under greenhouse conditions. Similarly, Ilahy *et al*. (2011) reported that for all the studied tomato cultivars, the highest HAA value was estimated in tomato fruits at the green stage of ripening and the lowest value was recorded at the red-ripe stage. Thus, the HAA exhibited a significant decrease during fruit ripening. This decrease was from 217 to 129 µM Trolox100 g^{-1} FW for the standard cultivar 'Rio Grande' and from 401 to 166 µM Trolox 100 g^{-1} FW for high lycopene tomato cultivars. However, Cano *et al*. (2003) found that the hydrophilic antioxidant activity remained practically unchanged during ripening (ranging from 195 to 218 µM Trolox100 g^{-1} FW) of the greenhouse grown tomato cultivar 'Marmande-Cuarenteno'.

In general, simultaneously to the reported decrease of HAA from the green-orange ripening stage (except for 'Donald'), an increase of LAA was evident in all tomato

Table 4 Antioxidant activity in ordinary and high-lycopene tomato cultivars harvested at four different ripening stages. Values within column and ripening stage followed by the same letters do not differ significantly (LSD test, $P < 0.01$).

Cultivar	Ripening stage	TEAC assay (μM Trolox 100 g^{-1} FW)	
		Hydrophilic	Lipophilic
Donald	Green	414.2 ± 36.74 a	96.7 ± 0.13 d
	Green-Orange	407.6 ± 28.67 a	117.0 ± 0.1 c
	Orange-Red	401.0 ± 61.52 a	126.9 ± 0.11 b
	Red-Ripe	405.8 ± 16.52 a	133.5 ± 1.72 a
Lyco 1	Green	522.6 ± 8.53 c	336.7 ± 3.49 ab
	Green-Orange	694.4 ± 5.71 a	316.1 ± 5.10 b
	Orange-Red	660.4 ± 5.69 b	329.6 ± 1.19 ab
	Red-Ripe	519.1 ± 10.70 c	348.8 ± 11.11 a
Lyco 2	Green	572.5 ± 1.70 c	330.1 ± 2.74 d
	Green-Orange	740.8 ± 8.91 a	389.9 ± 4.74 c
	Orange-Red	687.6 ± 15.76 b	429.1 ± 10.09 b
	Red-Ripe	554.4 ± 16.91 c	540.1 ± 13.81 a
HLY 02	Green	514.6 ± 0.63 b	336.5 ± 3.74 b
	Green-Orange	641.7 ± 15.46 a	339.0 ± 11.51 b
	Orange-Red	656.0 ± 7.27 a	345.5 ± 3.55 ab
	Red-Ripe	518.9 ± 3.96 b	368.5 ± 5.28 a
HLY 13	Green	627.1 ± 14.11 b	387.0 ± 14.25 b
	Green-Orange	730.2 ± 8.30 a	393.9 ± 3.45 b
	Orange-Red	734.7 ± 10.12 a	406.1 ± 5.20 b
	Red-Ripe	505.5 ± 5.68 c	470.2 ± 7.17 a
HLY 18	Green	586.8 ± 4.18 c	369.2 ± 9.80 d
	Green-Orange	793.6 ± 10.26 a	418.0 ± 6.62 c
	Orange-Red	697.0 ± 5.43 b	440.9 ± 11.01 b
	Red-Ripe	572.1± 11.52 c	479.5 ± 5.12 a
Kalvert	Green	563.4 ± 2.86 b	317.9 ± 11.29 d
	Green-Orange	752.4 ± 19.88 a	372.2 ± 1.81 c
	Orange-Red	746.3 ± 6.70 a	431.1 ± 3.78 b
	Red-Ripe	489.4 ± 9.19 c	488.6 ± 4.71 a

cultivars (except for 'Lyco 1' and 'HLY 02' varieties) as ripening process proceeds. This increase was in average 30% in red-ripe tomato fruits compared to the green stage. Our results are in agreement with those of many authors (Raffo *et al*. 2002; Cano *et al*. 2003) who found variations ranging from 15 to 88 μM Trolox 100 g^{-1} FW in LAA during ripening of different greenhouse grown tomato variety. In addition, our results are in agreement with those reported recently by Ilahy *et al*. (2011), who evidenced an increase of LAA in all tomato cultivars as ripening process proceeds. This increase was in average slightly higher than 50% in red-ripe tomato fruits compared to the green stage when LAA were measured with the TEAC assay and approximately 91% when the FRAP method was used. The increase in LAA was from 94.4 to 137 μM Trolox 100 g^{-1} FW for 'Rio Grande' and from 156 to 309 μM Trolox 100 g^{-1} FW during ripening of different high-pigment tomato cultivars.

It is valuable to underline that at the red-ripe stage of ripening both HAA and LAA values of all high-lycopene tomato cultivars were respectively 1.20- to 1.40- and 2.61- to 4.04-fold higher compared to red-ripe 'Donald' fruits. This fact could be considered as an indication of the superior antioxidant composition of such tomato cultivars which suit the consumer requirement for nutritive and healthy foods.

Many authors have studied correlations between bioactive compounds and antioxidant activities in numerous fruits and vegetables particularly tomatoes (Cano *et al*. 2003; Lenucci *et al*. 2006; Ilahy *et al*. 2011). However, little information is known concerning these types of correlations in high-lycopene tomato cultivars. In the present study, after considering data from all tomato cultivars (**Table 5**), no significant correlation between HAA TEAC values and total vitamin C ($r = 0.109$, $P > 0.05$) was obtained, whereas they well correlated with AsA ($r = 0.414$, $P < 0.01$), DHA contents ($r = -0.315$, $P < 0.01$) and both phenolics ($r = 0.346$, $P < 0.01$) and flavonoids ($r = 0.594$, $P < 0.01$). Although, it is likely that HAA depends upon synergistic effects among all hydrophilic antioxidants and their interaction with other constituents of the fraction (Diplock *et al*. 1998; Lenucci *et al*. 2006; Ilahy *et al*. 2010, 2011), this differential presence/lack of correlation between HAA values obtained with the different classes of hydrophilic antioxidants, could be due to a higher sensibility of the TEAC assays for such classes of compounds. Moreover, the test reaction used for antioxidant activity measurement might be differentially influenced by other compounds involved in complex antioxidant system of tomato fruits such as glutathione and enzymatic components (Jiménez *et al*. 2002; Lenucci *et al*. 2009; Ilahy *et al*. 2011).

Table 5 Correlation coefficient and related significance between antioxidant content and antioxidant activity.

Compounds	TEAC assay	
	r	P
Hydrophilic fraction		
Ascorbic acid	0.414	<0.01
Dehydroascorbic acid	-0.315	<0.01
Total vitamin C	0.109	Ns
Total phenolics	0.346	<0.01
Flavonoids	0.594	<0.01
Lipophilic fraction		
Lycopene	0.426	<0.01
β-carotene	0.567	<0.01
Lutein	0.038	ns[a]

[a]No significant correlation

The lipophilic antioxidant activity of tomato fruits has been mainly attributed to the presence of carotenoids particularly lycopene (Mochizuki and Kamimura 1984; Martinez-Valverde *et al*. 2002; Raffo *et al*. 2002; George *et al*. 2004; Lenucci *et al*. 2006; Ilahy *et al*. 2010, 2011). After considering data from all tomato cultivars, good and significant correlations between TEAC values and both lycopene ($r = 0.426$, $P < 0.01$) and β-carotene ($r = 0.567$, $P < 0.01$) contents were obtained whereas Lutein did not correlate with LAA TEAC values due to its loss during the ripening process. It should be underlined that to the approximate 5- and 3.5-fold increase in lycopene and β-carotene contents respectively (averaged across varieties) of the red-ripe tomato fruits compared to the green stage of ripeness, does not correspond a likewise high increase of LAA. This could be probably due to the presence of other lipophilic antioxidant compounds, such as chlorophylls (Endo *et al*. 1985), in the tomato fruits which could account for most of the LAA at the green stage. Chlorophylls decrease during tomato ripening being substituted by carotenoids, mainly lycopene; this change of pattern of lipophilic antioxidants could partially compensate the variation of LAA of ripening tomato fruits. In addition, the high LAA at the early stages of ripening can be attributed to different tocopherol analogues present in high amount in tomato fruits (Abushita *et al*. 1997; Raffo *et al*. 2002).

CONCLUSIONS

Although limited biological variability (just one year sampling) has been taken into account in this study, it gives a general but defined idea of the trends of antioxidant molecules during tomato ripening. This study confirmed that high-lycopene tomato cultivars had considerably higher levels of bioactive compounds. The stage of maturity affected tomato fruit antioxidant properties and this effect varied not only between different tomato typology but also among tomato varieties from the same type. The use of high-lycopene tomato fruit harvested at the appropriate ripening stage for fresh consumption and processing purposes will hopefully contribute towards increasing the intake of antioxidants.

ACKNOWLEDGEMENTS

The authors are grateful to thank Prof. Gabriela Piro for laboratory facilities, for all of Anna Montefusco, Gian Pietro Disansebastiano, Daniela Pacoda, Sergio Brizio for technical assistance and for Guaetano carrozzo for technical assistance in tomato plant cultivation.

REFERENCES

Abushita AA, Daood HG, Biacs PA (2000) Change in carotenoid and antioxidant vitamins in tomato as a function of varietals and technological factors. *Journal of Agricultural Food and Chemistry* **48**, 2075-2081

Abushita AA, Hebshi EA, Daood HG, Biacs PA (1997) Determination of vitamin and antioxidant in tomatoes. *Food Chemistry* **60 (2)**, 207-212

Agarwal S, Rao AV (1998) Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. *Lipids* **33**, 981-984

Amir H, Karas M, Giat J, Danilenko M, Levy R, Yermiahu T, Levy J, Sharoni Y (1999) Lycopene and 1,25-dihydroxyvitamin D_3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutrition and Cancer* **33**, 105-112

Atanassova B, Stoeva-Popova P, Balacheva E (2007) Cumulating useful traits in processing tomato. *Acta Horticulturae (ISHS)* **758**, 27-36

Biacs PA, Daood HG, Czinkotai B, Hajdú F, Kisskutz N (1987) Effect of Titavit treatments on the dynamics of tomato fruit ripeness. *Acta Horticulturae* **220**, 433-438

Buta JG, Spaulding DW (1997) Endogenous levels of phenolics in tomato fruit during growth and maturation. *Journal of Plant Growth Regulation* **16**, 43-46

Cano A, Acosta M, Arnao MB (2003) Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). *Postharvest Biology and Technology* **28 (1)**, 59-65

Cantore V, Boari F, Vanadia S, Pace B, Depalma E, Leo L, Zacheo G (2008) Evaluation of yield and qualitative parameters of high lycopene tomato cultivars. *Acta Horticulturae (ISHS)* **789**, 173-180

Clinton SK (1998) Lycopene: chemistry, biology, and implications for human health and disease. *Nutrition Reviews* **56**, 35-51

Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M (1998) Functional food science and defence against reactive oxygen species. *British Journal of Nutrition* **80**, S77-S112

Dumas Y, Dadomo M, Lucca GD, Grolier P, Di Lucca G (2003) Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *Journal of the Science of Food and Agriculture* **83**, 369-382

Endo Y, Usuki R, Kaneda T (1985) Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects. *Journal of the American Oil Chemists' Society* **62**, 1375-1378

Galpaz N, Wang Q, Menda N, Zamir D, Hirschberg J (2008) Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. *Plant Journal* **53**, 717-730

George B, Kaur C, Khurdiya DS, Kapoor HC (2004) Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. *Food Chemistry* **84 (1)**, 45-51

Hdider C, Guezel I, Arfaoui K (2007) Agronomic and qualitative evaluation of processing tomato cultivars in Tunisia. *Acta Horticulturae (ISHS)* **758**, 281-286

Helyes L, Lugasi A (2006) Formation of certain compounds having technological and nutritional importance in tomato fruit during maturation. *Acta Alimentaria* **35 (2)**, 183-193

Hornero-Méndez D, Gómez-Ladrón de Guevara R, Mínguez-Mosquera MI (2000) Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. *Journal of Agricultural Food and Chemistry* **48**, 3857-3864

Ilahy R, Hdider C, Lenucci MS, Tlili I, Dalessandro G (2011) Antioxidant activity and bioactive compound changes during fruit ripening of high lycopene tomato cultivars. *Journal of Food Composition and Analysis* **24 (4-5)**, 588-595

Ilahy R, Hdider C, Tlili I (2009) Bioactive compounds and antioxidant activity of tomato high lycopene content advanced breeding lines. In: Daami-Remadi M (Ed) *Tunisian Plant Science and Biotechnology I. The African Journal of Plant Science and Biotechnology* **3 (Special Issue 1)**, 1-6

Ilahy R, Hdider C, Tlili I (2010) Assessing agronomic characteristics lycopene and total phenolic contents in pulp and skin factions of different tomato varieties. In: Daami-Remadi M (Ed) *Tunisian Plant Science and Biotechnology II. The African Journal of Plant Science and Biotechnology* **4 (SI 2)**, 64-67

Jia Z-S, Tang M-C, Wu J-M (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **64 (4)**, 555-559

Jiménez A, Creissen G, Kular B, Firmin J, Robindon S, Verhoyen M, Mullineaux P (2002) Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta* **214**, 751-758

Kampfenkel K, Van Montagu M, Inzé D (1995) Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Analytical Biochemistry* **225**, 165-167

Karakaya S, El-SN, Tas AA (2001) Antioxidant activity of some foods containing phenolic compounds. *International Journal of Food Science and Nutrition* **52**, 501-508

Lenucci MS, Cacciopola A, Durante M, Serrone L, De Caroli M, Piro G, Dalessandro G (2009) Carotenoid content during tomato (*Solanum lycopersicum* L.) fruit in traditional and high-pigment cultivars. *Italian Journal of Food Science* **21 (4)**, 461-472

Lenucci MS, Cacciopola A, Durante M, Serrone L, Piro G, Dalessandro G (2007) Carotenoid content in ripe raw and processed (sauce) berries of high pigment tomato hybrids. *Acta Horticulturae (ISHS)* **758**, 173-179

Lenucci MS, Cadinu D, Taurino M, Piro G, Dalessandro G (2006) Antioxidant composition in cherry and high-pigment tomato cultivars. *Journal of Agricultural Food and Chemistry* **54**, 2606-2613

Macheix JJ, Fleurient A, Billot J (1990) Phenolic compounds in fruit processing. In: *Fruit Phenolics*, CRC Press, Boca Raton, FL, pp 295-342

Macua JI, Lahoz I, Garnica J, Santos A, Armendariz R (2007) The influence of planting time on the lycopene content of commercial tomato varieties for industry from the Ebro valley. *Acta Horticulturae (ISHS)* **758**, 315-319

Martinez-Valverde I, Periago MJ, Provan G, Chesson A (2002) Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicon esculentum*). *Journal of the Science of Food and Agriculture* **82**, 323-330

Mochizuki T, Kamimura S (1984) Inheritance of vitamin C content and its relation to other characters in crosses between hp and og varieties of tomatoes. In: *EUCARPIA. Tomato Working Group. A New in Tomato Breeding*, Wageningen, The Netherlands, pp 8-13

Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C (1999) Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. *Plant Cell* **11**, 145-157

Pellegrini N, Colombi B, Salvatore S, Brenna OV, Galaverna G, Del Río D, Bianchi M, Bennet RN, Brigenthi F (2007) Evaluation of antioxidant capacity of some fruit and vegetable foods: Efficiency of extraction of a sequence of solvents. *Journal of the Science of Food and Agriculture* **87 (1)**, 103-111

Perkins-Veazie P, Collins JK, Pair SD, Roberts W (2001) Lycopene content differs among red-fleshed watermelon cultivars. *Journal of the Science of Food and Agriculture* **81**, 983-987

Raffo A, Cherubino L, Vincenzo F, Ambrozino P, Salucci M, Gennaro L, Bugianesi R, Giuffrida F, Quaglia G (2002) Nutritional value of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1) harvested at different ripening stages. *Journal of Agricultural Food and Chemistry* **50**, 6550-6556

Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W (1999) Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry* **66**, 401-436

Rodrigo MJ, Marcos JF, Zacarias L (2004) Biochemical and molecular analysis of carotenoid biosynthesis in flavedo of orange (*Citrus sinensis* L.) during fruit development and maturation. *Journal of Agricultural Food and Chemistry* **52**, 6724-6731

Sadler G, Davis J, Dezman D (1990) Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *Journal of Food Science* **55**, 1460-1461

Spanos GA, Wrolstad RE (1990) Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. *Journal of Agricultural Food and Chemistry* **38**, 1565-1571

Stahl W, Sies H (1996) Lycopene: A biologically important carotenoid for humans. *Archives of Biochemistry and Biophysics* **336**, 1-9

Tünk R, Seniz V, Özdemir N, Süzen MA (1993) Changes in chlorophyll, carotenoid and lycopene content of tomatoes in relation to temperature. *Acta Horticulturae (ISHS)* **398**, 856-862

United States Department of Agriculture, USDA (1976) United States Standards for Grade of Fresh Tomatoes. Agricultural Marketing Service, USDA, Washington DC. Available online: www.ams.usda.gov