Phytochemical composition and antioxidant activity of high-lycopene tomato (Solanum lycopersicum L.) cultivars grown in Southern Italy

Riadh Ilahya, Chafik Hdiderb,∗, Marcello S. Lenuccic, Imen Tiliia,b, Giuseppe Dallessandroc

a Department of Biology, Faculty of Sciences, Bizerte, Zarzouna 7021 Bizerte, Tunisia
b Laboratory of Biotechnology and Plant Physiology, National Agricultural Research Institute of Tunisia, Tunis, Rue Hédi Karray 2049 Ariana, Tunisia
c Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy

ABSTRACT

In this study, the antioxidant components and of six high-lycopene (Lyco 1, Lyco 2, HLY 02, HLY 13, HLY 18 and Kalvert) and one ordinary (Donald) tomato cultivars (cvs) grown simultaneously in an open-field of the Southern Italy were investigated. Lycopene, β-carotene, lutein, total phenols, flavonoids, ascorbic acid (AsA), dehydroascorbic acid (DHA) and total vitamin C (AsA + DHA) contents, as well as hydrophilic and lipophilic antioxidant activities (HAA and LAA) were determined. Significant differences were detected among tomato cvs in all studied antioxidant components, as well as in the antioxidant activity of their hydrophilic and lipophilic fractions. High-lycopene tomato cvs showed higher lycopene, β-carotene, HAA and LAA when compared to cv Donald. Cv HLY 18 showed the highest lycopene and β-carotene content with 232.9 mg/kg fresh weight (fw) and 19.4 mg/kg fw, respectively. Except for Kalvert, high-lycopene tomato cvs also obtained higher total vitamin C levels, with cv HLY 13 top ranking with an average of 352.8 mg/kg fw in cv Lyco 2 and was significantly correlated to lycopene (r = 0.60; p < 0.01). Although these data require confirmation over a longer period of time, this investigation suggests a promising use of the high-lycopene tomato cvs for the production of tomatoes with higher nutritional quality.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Tomatoes (Solanum lycopersicum 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 such as canned tomatoes, sauce, juice ketchup and soup (Lenucci et al., 2006). The regular consumption of fresh tomato or tomato products has been inversely correlated to the development of widespread human diseases when taken daily in adequate amounts (Rao and Agarwal, 1998). This protective effect has been mainly attributed to the carotenoid content of the fruit particularly lycopene and β-carotene (Clinton, 1998; Sies and Stahl, 1998). These compounds may play an important role in inhibiting reactive oxygen species responsible of many important diseases (Clinton, 1998). Along with carotenoids, other antioxidant compounds present in tomato, including ascorbic acid (AsA), tocopherols and phenols, play a determinant role in disease prevention (Robards et al., 1999; Karakaya et al., 2001).

The demand for high nutritional quality food is increasing because of (a) the commercial opportunities offered by such products due to their visual and functional properties, (b) increasing consumer awareness of the relationship between food and health and (c) the widespread industrial use for nutrient supplementation, pharmaceutical purposes, food additives and animal feeds. In this context, a large number of new tomato cultivars (cvs) with increased levels of lycopene (high-lycopene cvs) have been recently developed by conventional plant breeding techniques in order to satisfy the demand of growers, processors and consumers for high nutritive quality food. However, it has been reported that the selection of high-lycopene tomatoes may lead to the formation of plant with poor horticultural performances such as reduced seed germination, stem fragility, limited fruit size and reduced productivity (Atanassova et al., 2007) as well as decreased levels of other antioxidants such as β-carotene to compromise the increase in lycopene (Sacks and Francis, 2001), compared to currently available cvs. Therefore, there is a need to investigate the behaviour of these high-lycopene tomato cvs under various envi-

doi:10.1016/j.scienta.2010.10.001
ronmental growing conditions. Dumas et al. (2003) and Atanassova et al. (2007) reported that the antioxidant contents in tomato fruit vary in relation to genotype, but also depend on environmental and agronomic factors.

The quasi-quantitative characterization of many chemicals (carotenoids, phenols, flavonoids, organic acids, minerals, etc.) of tomato fruits, as well as their antioxidant properties are well defined, and comparisons are available among a large number of cvs at different ripening stages (Stewart et al., 2000; Cano et al., 2003; Lenucci et al., 2009). However, studies on the characterization and quantification of phytochemicals and antioxidant properties of high-lycopene tomato cvs are very limited. Previously, Armendariz et al. (2006) and Cantore et al. (2008) studied the agronomic characteristics and lycopene content of some of these new tomato cvs. Furthermore, Lenucci et al. (2006) focused on the antioxidant composition of some high-pigment tomato cvs concluding that they have a high antioxidant potential. However, using the FRAP assay method, an excessively low lipophilic antioxidant activity (LAA) was detected in comparison with the high content of lipophilic antioxidants. It has been reported that the evaluation of LAA with this method is not reliable because of the inability of carotenoids to reduce ferric chloride (George et al., 2004). Additional studies using other analytical methods for the assessment of antioxidant capacity in high-pigment tomato cvs are required.

In this study, the contents of the main phytochemicals (lycopene, β-carotene, lutein, phenols, flavonoids, AsA and dehydroascorbic acid (DHA)) and the antioxidant activities (both hydrophilic and lipophilic) of six high-lycopene (Lyc 1, Lyc 2, HLY 02, HLY 13, HLY 18 and Kalvert) and one ordinary (Donald) tomato cvs grown simultaneously in an open-field of the Southern Italy were studied. The correlation of hydrophilic antioxidant activity (HAA) and LAA with the different classes of antioxidants was also examined.

2. Materials and methods

2.1. Plant culture

The field experiments were carried out in a field in the province of Lecce (Southern Italy) during the 2008 growing season (April–August). Seven tomato cvs with a determinant growth habit were used in these experiments: six tomato cvs claimed to be high-lycopene (Lyc 1, Lyc 2, HLY 02, HLY 13, HLY 18 and Kalvert) and one ordinary cv (Donald). Cvs HLY 02, HLY 13, HLY 18 and Kalvert seeds were from COIS’ 94 S.r.l. (Belpasso, CT, Italy). Cvs Lyc 1 and Lyc 2 seeds were from Hazera Genetics Ltd (Berurim MP Shikmim, Israel) and cv Donald seeds were 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 with a spacing of approximately 50 cm within the row and 100 cm between rows matching a density of about 20,000 plants/ha and grown to maturity. About 1500 plants per cultivar were arranged in 15 consecutive rows of 100 individuals for a total cultivated area of roughly 0.5 ha. The experimental design was a randomised complete block with three blocks (replicates). Standard agronomical techniques were used for plant nutrition and pathogen prevention as described by Lenucci et al. (2006). Drip irrigation ran for 1–2.5 h, at 1–2 day intervals, depending on potential evapo-transpiration, climate data and crop coefficient.

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

2.2. Fruit sampling

Tomato fruits were hand harvested randomly from the rows and from the middle of each plant at the red-ripe stage. A sample of at least 2 kg of visually selected injury free red-ripe tomato fruits was harvested from each cv, and delivered quickly to the laboratory. Sampling was repeated three times during the last three months of the growing season. Tomato fruits were washed, cut into small pieces and ice-cold homogenized in a mixer (Waring Laboratory & Science, Torrington, CT, US). The obtained homogenates were immediately frozen at −20 °C and used to determine the carotenoids, total phenols, flavonoids, AsA and DHA contents, as well as the HAA and LAA within less than one week, in order to minimize the depletion of nutrients that inevitably occurs even during frozen homogenate storage (Phillips et al., 2010).

2.3. Analytical procedures

2.3.1. Determination of carotenoid contents

Lycopene, β-carotene and lutein contents were determined on triplicate aliquots of the homogeneous suspension (0.5 g) according to the method of Sadler et al. (1990) as modified by Perkins-Veazie et al. (2001). Carotenoids were extracted with 0.05% (w/v) butylated hydroxyltoluene (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 C18 (5μm, 250 mm × 4.6 mm). The separation was performed using a linear gradient of acetonitrile (A), hexane (B) and methanol (C) as follows: 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. Concentration of standard solutions was calculated using the molar extinction coefficients 17.2 × 104 for lycopene, 13.9 × 104 for β-carotene and 14.3 × 104 for lutein in hexane. Peaks were detected at 503 nm and results were expressed in mg/kg fresh weight (fw).

2.3.2. Determination of total phenols

Total phenols were extracted as described by Martinez-Valverde et al. (2002) on triplicate aliquots of the homogenate (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 10,000 g for 15 min. The total phenols assay was performed using the Folin–Cioclauet reagent as described by Spanos and Wrolstad (1990) on 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 fw.

2.3.3. Determination of flavonoid content

The flavonoid content was determined as described by Zhishen et al. (1999) on triplicate aliquots of the homogenous suspension (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% NaNO2 was added. After 5 min, 60 μL of 10% AlCl3 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) and flavonoid content was expressed as mg rutin equivalent (RE)/kg fw.
2.3.4. Determination of AsA and DHA contents

AsA and DHA contents were determined as reported by Kampfenkel et al. (1995) on triplicate samples of the homogeneous suspension (0.1 g). AsA and DHA were extracted using 6% metaphosphoric acid and detected at 525 nm in a spectrophotometer (Beckman DU 650) and expressed in mg/kg fw.

2.3.5. Hydrophilic and lipophilic antioxidant activity assay

The measurement of HAA and LAA was performed using the trolox equivalent antioxidant capacity (TEAC) assay. The antioxidant activity was measured using the ABTS decolorization method (Pellegrini et al., 2007). The TEAC assay is standardly used for antioxidant activity assessment of fruit and vegetables, its numerous advantages consist in reproducibility, simplicity, and a good estimate of the antioxidant activity of pure compounds and complex matrices (Thaipong et al., 2006; Pellegrini et al., 2007). Hydrophilic and lipophilic antioxidants were extracted from 0.3 g homogeneous suspension (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). Two different calibration curves were constructed using freshly prepared trolox solutions for HAA and LAA determinations. Values were expressed as μM Trolox/100 g fw.

2.4. Statistical analysis

Variations in the nutritional properties of tomato cvs 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 Pearson’s correlation coefficient (r).

3. Results

3.1. Plant growth and development

Tomato plants grown under the reported meteorological (Fig. 1) and experimental conditions were vigorous with excellent foliage cover. All that claimed to be high-lycopene tomato cvs were characterized by dark foliage and dark green immature fruit without morphological aberrations. In general, all the tested high-lycopene tomato cvs showed higher productivity (ranging from a minimum of ∼110 t/ha for Lyco 1 and Lyco 2 to a maximum of ∼150 t/ha for HLY 13) than the reference cv Donald (∼94 t/ha), as far as the season 2008 is concerned.

3.2. Lycopene and β-carotene content

Lycopene and β-carotene contents of the ordinary and high-lycopene tomato cvs are reported in Figs. 2 and 3, respectively. Lycopene and β-carotene contents were significantly different among the studied tomato cvs (p < 0.05). Lycopene ranged from 96.9 mg/kg fw in cv Donald to 232.9 mg/kg fw in cv HLY 18, whereas β-carotene ranged from 5.8 mg/kg fw to 19.4 mg/kg fw in the same cvs accounting for between 4.3% and 10% of the total carotenoids. All high-lycopene tomato cvs showed higher lycopene and β-carotene contents when compared to cv Donald.

3.3. Total phenol and flavonoid content

Total phenol and flavonoid contents (Fig. 4) were significantly different between the studied tomato cvs (p < 0.05). Total phenols ranged from 105.8 mg GAE/kg fw in cv Lyco 1 to 394.5 mg GAE/kg
3.4. Vitamin C content

AsA, DHA and total vitamin C (AsA + DHA) contents (Fig. 5) were significantly different between studied tomato cvs (p < 0.05). Total vitamin C content ranged from 227.9 mg/kg fw in cv Donald to 352.8 mg/kg fw in cv HLY 13. Except for cv Kalvert, a significantly higher total vitamin C content was detected in all high-lycopene tomato cvs compared to cv Donald. DHA accounted for between 28.0% and 65.5% of the total vitamin C content. Kalvert showed a high AsA content (169.8 mg/kg fw) but exhibited the lowest DHA content (96.0 mg/kg fw). On the contrary, the cv Lyco 2 was characterized by a moderate AsA content (105.8 mg/kg fw) but the highest DHA content (96.0 mg/kg fw).

3.5. Hydrophilic and lipophilic antioxidant activity

HAA and LAA values determined by the TEAC assay in ordinary and high-lycopene tomato cvs are shown in Fig. 6. HAA and LAA were significantly different between studied tomato cvs (p < 0.05). HAA values ranged from 405.8 μM Trolox/100 g fw in cv Donald to 572.1 μM Trolox/100 g fw in cv HLY 18. Cv Lyco 2 had similar HAA value to cv HLY 18. Total antioxidant activity (HAA + LAA) varied from 539.3 to 1094.5 μM Trolox/100 g fw. HAA contributed from 50% to 75.2% to the total antioxidant activity. A variation between 1.2- and 1.4-fold was found in the HAA of high-lycopene tomato cvs compared to the ordinary cv Donald. Regarding LAA, values ranged from 133.5 μM Trolox/100 g fw in cv Donald to 540.1 μM Trolox/100 g fw in cv Lyco 2. HLY 13, HLY 18 and Kalvert cvs obtained similar LAA values with respectively 470.2, 479.5 and 488.6 μM Trolox/100 g fw. A variation between 2.7- and 4.0-fold was found in LAA of high-lycopene tomato cvs compared to cv Donald.
lycopene than those grown under a greenhouse (Leonardi et al., 2006). These high-lycopene tomato fruits seem to be a massive production of lycopene precursors in ripening tomato (Fraser et al., 2009). This high lycopene accumulation in the tomato cv Donald is due to the reduced lycopene content (>200 mg/kg fw). The differences in lycopene content between ordinary and high-lycopene tomato cvs are mainly due to genotypic factors. The considerable lycopene accumulation in high-lycopene tomato cvs can be, in fact, due to the reduced lycopene content caused by high-lycopene tomato cvs that high-pigment tomatoes are characterized by a very high content of carotenoids (Hertog et al., 1992; George et al., 2004; Dumas et al., 2003). While genetic control is the primary factor in determining phenols in fruits and vegetables, their level may be affected by environmental conditions, such as light and temperature (Macheix et al., 1992; George et al., 2004; Dumas et al., 2003).

This is in agreement with the well recognized fact that LAA of tomato cvs. Considering data from all tomato cvs, significant correlations between LAA values and both lycopene (r = 0.53; p < 0.01) and ß-carotene (r = 0.56; p < 0.01) content were obtained (Table 1). This is in agreement with the well recognized fact that LAA of tomato fruits provides up to 40% of the recommended daily intake of vitamin A compared to only 15% assumed consuming the same amount of the ordinary cv Donald (Società Italiana di Nutrizione Umana, 1996). Regarding LAA, the obtained values were considerably higher compared to those reported by many authors (Cano et al., 2003; Raffo et al., 2002, 2006) for greenhouse-grown tomato cvs ranging from 26 to 88 µM Trolox/100 g fw. As expected, due to the high amount of detected lipophilic antioxidants, all high-lycopene tomato cvs showed a higher LAA than the ordinary cv Donald (2.7- to 4.0-fold higher). Recently, Lenucci et al. (2006), using the FRAP assay method, found an excessively low LAA in some high-pigment tomato cvs in comparison with the high amount of detected lipophilic antioxidant. This is probably due to the inability of carotenoids to reduce ferric chloride in the FRAP assay (George et al., 2004; Lenucci et al., 2006). The results obtained in this study showed that the TEAC assay appears to be more efficient for assessing the antioxidant activity of high-lycopene tomato fruits. Considering that most of the lycopene is associated with the lipophilic fraction in tomato fruits (Sharma and Le Maguer, 1996), a real estimation of the antioxidant activity of high-lycopene tomatoes strongly depends on the real estimation of the antioxidant activity in the lipophilic fraction. It insures not only an evaluation of this nutritional value but also the possible synergistic and/or antagonistic effects among bioactive compounds taken together in determining the antioxidant activity of such extracts (Pinilla et al., 2005; Lenucci et al., 2006; Pellegrini et al., 2007). In fact, Pastori et al. (1998) reported that lycopene synergizes with other natural compounds such as α-tocopherol and 1,25-dihydroxy-vitamin D3, in inhibiting prostate carcinoma cell proliferation.

Several authors have studied correlations between bioactive compounds and antioxidant activities in numerous fruits and vegetables particularly tomatoes. However, little information is known concerning these types of correlations in high lycopene content tomato cvs. Considering data from all tomato cvs, significant correlations between LAA values and both lycopene (r = 0.53; p < 0.01) and ß-carotene (r = 0.56; p < 0.01) content were obtained (Table 1). This is in agreement with the well recognized fact that LAA of tomato fruits was mainly attributed to the presence of carotenoids particularly lycopene (Martinez-Valverde et al., 2002; Raffo et al., 2002; Cano et al., 2003; Toor and Savage, 2005).

For total phenol content, values were close to the range reported by George et al. (2004) and Toor and Savage (2005) ranging from 74.1 to 217 mg GAE/kg fw and confirmed that genotype significantly affects total phenol content in tomato, as reported by many authors (Hertog et al., 1992; George et al., 2004; Dumas et al., 2003). While genetic control is the primary factor in determining phenols in fruits and vegetables, their level may be affected by environmental conditions, such as light and temperature (Macheix et al., 1992) and analytical methodology. In tomato fruits, flavonoids rep-
resent the major component of the total phenol content (Toor and Savage, 2005). In this study, the flavonoid content in HLY 18, HLY 13, Lyco 1 and Kalvet cvs were 2.1- to 3.5-fold higher than in cv Donald. These values were similar to those reported by Lenucci et al. (2006) for high-pigment tomato cvs ranging from 168 to 470 mg RE/kg fw. Variations can be ascribed to the high-lycopene traits. In fact, it has been reported that in high-pigment mutants the increase in carotenoid content, was accompanied by a dramatic raise in plastid biogenesis and in the synthesis of other compounds such as flavonoids and vitamin C (Mochizuki and Kaminura, 1984; Cookson et al., 2003; Bino et al., 2005; Kolotilin et al., 2007). Moreover, it has been reported that field-grown tomato fruits, which receive a higher amount of light and UV radiation contain a higher amount of flavonoids (quercetin and kaempferol) in comparison to greenhouse-grown tomato. Although tomato phenol content is moderate compared with other vegetables such as onion, their high consumption in the Mediterranean diet makes them a good source of phenols as also reported for Indian and American diets (Vinson et al., 1998; George et al., 2004).

In terms of AsA, DHA and total vitamin C, values were similar to that reported by Liptay et al. (1986), George et al. (2004) and Lenucci et al. (2006). However, higher values, ranging from 310 to 710 mg/kg fw in dependence on harvesting season, were reported by Raffo et al. (2006). Generally, it is widely recognized that field-grown tomato have higher AsA levels (up to 258 mg/kg fw) when compared to those produced under shade (155 mg/kg fw). In this study, all high-lycopene tomato cvs, with the exception of Kalvert, showed a higher amount of total vitamin C (particularly HLY 13, Lyco 2 and HLY 18) than cv Donald. These results provide further evidence that the high-lycopene phenotype, determine, in some of the new cvs, a dramatic increase in the production of many important antioxidants, such as vitamin C, as previously reported by Mochizuki and Kaminura (1984) and Mustilli et al. (1999).

In terms of vitamin C, the high-lycopene tomato cvs appear beneficial for human nutrition, consuming one serving of Lyco 2, HLY 13 and HLY 18 tomato fruits provides from 52% to 60% of the recommended daily intake of vitamin C at full ripeness compared to 39% for the ordinary cv Donald (Società Italiana di Nutrizione Umana, 1996).

It has been hypothesized that variations in the redox state of the system AsA/DHA could function as a sensor modulating cellular metabolism and hormone sensitivity in response to exogenous factors (De Gara, 2003). Differences among cvs were observed in the ratio AsA/DHA suggesting that the perception of the surrounding environment may be diverse in relation to genotype. Concerning HAA, values were 1.2- to 1.4-fold higher in high-lycopene tomato cvs than in cv Donald. Our results confirm those reported by Lenucci et al. (2006) who, using the FRAP assay, found that high-pigment tomato had high HAA values. The HAA values of the cv Donald were in the range usually reported for ordinary tomato cvs. This high HAA can be attributed to the influence of different hydrophilic antioxidants. In fact, in spite the low phenol and flavonoid contents, cv Lyco 2 had higher AsA (r = 0.61; p < 0.01) and total vitamin C content (r = 0.60; p < 0.01) (Table 1). The HAA was certainl correlated with the levels of all the main antioxidants (vitamin C, flavonoids and total phenols), but it was not the simple sum of their content. It can also depend on synergistic effect among all hydrophilic antioxidants and their interactions with other constituents of the fraction (Lenucci et al., 2006; Jiménez et al., 2002).

5. Conclusion

In this study, the antioxidant capability of various high-lycopene tomato cvs was investigated and compared to that of the ordinary cv Donald. High-lycopene tomato cvs had a considerable higher level of carotenoids, particularly lycopene, in comparison to the ordinary cv Donald. This was, also, correlated with their high LAA. On the other hand, except for Kalvert, high-lycopene tomato cvs had higher flavonoid and vitamin C contents compared to cv Donald. Nevertheless, only DHA and total vitamin C correlated with their HAA. The results showed that most of the high-lycopene tomato cvs, particularly HLY 18, seem to be promising cvs with improved bioactive compounds and antioxidant capacity. However, further studies on the effect of agricultural practices and environmental conditions on high-lycopene tomato cvs are required.

Acknowledgement

The authors wish to thank Prof. Gabriela Piro for laboratory facilities, Dr. Gaetano Carrozzo for technical assistance in tomato plant cultivation, Dr. Silvana Di Sabatino and Mr. Gennaro Rispoli for supplying the meteorological data.

References


