

# Bioactive Compounds and Antioxidant Activity of Tomato High Lycopene Content Advanced Breeding Lines

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## ABSTRACT

In Tunisia, tomato (*Lycopersicon esculentum* Mill.) is the main 'vegetable' grown and consumed all year round and is therefore of strategic importance. Tomato fruits are becoming then an important source of natural antioxidants primarily lycopene, phenolics and vitamin C, which are involved in inhibiting reactive oxygen species responsible for many cancer and cardiovascular diseases. Breeding for high nutritional tomato value is becoming an increasingly important aim. In this context, tomato high lycopene content advanced breeding lines (HLT-F51 and HLT-F52) were evaluated for their total carotenoid, lycopene, total phenolics, flavonoids, ascorbic acid, dehydroascorbic acid as well as their hydrophilic and lipophilic antioxidant activities, compared to the variety Rio Grande commonly grown in Tunisia. All tested high lycopene content varieties showed generally satisfying agronomic characteristics. The total carotenoid, lycopene, total phenolics, flavonoids, dehydroascorbic acid, total vitamin C and hydrophilic and lipophilic antioxidant activities in tomato fruit varied significantly between the studied varieties. Compared to the control, the selected line HLT-F51 showed 2.65-, 2.62 and 3.57-fold higher total carotenoid, lycopene and flavonoids, respectively. Also, HLT-F51 showed 2.09 and 2.24-fold higher hydrophilic and lipophilic antioxidant activities respectively. HLT-F52 exhibited particularly higher dehydroascorbic acid and total vitamin C contents compared to the control variety Rio Grande. These results emphasize the promising use of such advanced breeding lines for healthy quality products.

**Keywords:** carotenoids, hydrophilic and lipophilic antioxidant activities, *Lycopersicon esculentum* Mill, phenolics, tomato fruit quality, vitamin C

## INTRODUCTION

In Tunisia, tomato (*Lycopersicon esculentum* Mill.) is the main 'vegetable' grown and consumed and is therefore of strategic importance. In 2007, the tomato crop amounted to 780,000 t, including 558,000 t for processing. Moreover, Tunisia is the main tomato-processing country in Africa and among the world's leaders in terms of per capita annual consumption, with up to 54 kg on a fresh weight basis (Hdider *et al.* 2007).

During the past decades, many tomato varieties were developed with the aim of increasing yield potential, disease tolerance and extending shelf life without enough attention to their level of health-promoting compounds (Dorais *et al.* 2008). Nowadays, the demand for high nutritional quality food is increasing because of the increasing consumer awareness of the relationship between foods and health and the commercial opportunities offered by such products. 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 constituents of the fruit particularly lycopene and  $\beta$ -carotene (Sies and Stahl 1998). These compounds may play an important role in inhibiting reactive oxygen species responsible for many important diseases (Clinton 1998). Besides carotenoids, other antioxidants present in tomatoes, such as phenolic compounds and ascorbic acid, also play an important role in preventing diseases (Robards *et al.* 1999; Karakaya *et al.* 2001). Therefore, maximizing the level of these bioactive compounds in tomato fruit varieties is becoming one of the goals of major importance for tomato breeders (Ata-

nassova *et al.* 2007; Dorais *et al.* 2008).

In order to satisfy the demand of growers, processors and consumers for high nutritive quality food, a large number of new tomato varieties with increased lycopene content (high lycopene content tomato) have been developed by conventional plant breeding techniques. However, it has been reported that the selection of plants which naturally over express genes improving lycopene content may lead to the formation of plant with many undesirable characteristics such as reduced level of other antioxidants such as  $\beta$ -carotene on behalf of lycopene (Sacks and Francis 2001), low fruit size and reduced productivity than in currently available varieties (Atanassova *et al.* 2007). In this context, there is a need to investigate the behaviour of these high lycopene content tomato varieties under various environmental growing conditions. In fact, Dumas *et al.* (2003) and Atanassova *et al.* (2007) reported that lycopene content in tomato fruit vary not only in relation to genotype, but also depends on the environmental factors and agricultural techniques used.

Some researchers have studied new high lycopene content tomato varieties in order to evaluate their quality characteristics. However, few studies have investigated their antioxidant activity (Lenucci *et al.* 2006). In Tunisia, although information on the currently grown tomato varieties is widely available (Hdider *et al.* 2007), little is known about high lycopene content tomato varieties. In an early study, we evaluated a range of high lycopene content tomato varieties and concluded that despite the high lycopene content of some selected varieties, they were characterized by a low fruit size (Ilahy and Hdider 2007), a characteristic generally not appreciated by the Tunisian fresh market consumers (Hdider *et al.* 2007). In addition, some varieties showed both radial and concentric cracking which leaves

the affected tomatoes unmarketable and quickly deteriorating.

Therefore, and based on the facts mentioned above the aim of this study was to evaluate two advanced tomato breeding lines for yield and fruit quality characteristics compared to the variety Rio Grande commonly grown in Tunisia.

## MATERIALS AND METHODS

### Field experiment

The field experiment was carried out at Mannouba support research station in northern Tunisia during the 2008 growing season (March–July). Three tomato varieties were used in this experiment. Two advanced tomato high lycopene content lines (F5 generation), with the assigned names HLT-F51 and HLT-F52, selected by the National Agricultural Research Institute of Tunisia and the open pollinated variety Rio Grande (Petoseed, Saticoy, CA, USA) commonly grown in Tunisia which was used as control. The HLT-F51 and HLT-F52 lines were selected by single seed descendant from accessions obtained from Israeli origin on the basis of larger fruit size and tolerance to cracking. The high-lycopene tomato accessions derive from spontaneous mutant 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). Sowing was carried out in March 2008 in plug-seedling trays. Transplanting in double rows took place on April 2008 at a density of about 33,000 plants ha<sup>-1</sup>. Spacing within rows and between double rows was 0.4 and 1.5 m, respectively. Tomato varieties were grown in three replicated plots. Irrigation was applied using a drip method with 4 L h<sup>-1</sup> drippers placed at 0.4 m intervals along the irrigation line. Drip irrigation may run for 1–2.5 h at a time, after a 1–2 day interval, depending on potential evapotranspiration for research station climate data and crop coefficient. The production method was in accordance with the procedures utilized by the Mannouba support research station and high-yielding Tunisian conventional farmers. It included fertilization with synthetic chemical fertilizers (186 kg N ha<sup>-1</sup>, 129.6 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 440 kg K<sub>2</sub>O ha<sup>-1</sup> and 83.2 kg MgO ha<sup>-1</sup>). Chemical fertilizer solution was added to water irrigation by pump injection once a week. The methods also included weed control with synthetic chemical herbicides and plant pathogen control with synthetic chemical pesticides. Phosalone (350 g L<sup>-1</sup>) and triforine (190 g L<sup>-1</sup>) were utilized to reduce aphids and powdery mildew, respectively. Propargite (570 g L<sup>-1</sup>) and sulfur (800 g L<sup>-1</sup>) were used to reduce/prevent mites. Mancozebe (800 g L<sup>-1</sup>) was applied to prevent mildew. All these pesticides were applied once a cycle.

All varieties 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 on genotype-related variability of field-grown tomatoes.

### Fruit sampling

Tomato fruits were hand harvested once in July 2008 and total yield was recorded. A sample of approximately 2 kg of marketable ripe tomatoes was collected from each variety, weighed and delivered to the laboratory the same day. The tomatoes were cut into small pieces and sequentially homogenized in a mixer. Part of each sample was immediately used for some analyses (°Brix, pH and titratable acidity) and the remaining part was frozen at -20°C and used for lycopene, total phenolics, flavonoids, ascorbic acid, dehydroascorbic acid as well as the hydrophilic and lipophilic antioxidant activities determinations within 1 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 and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were obtained from Sigma-Aldrich, Chemical Co., Milan. Other reagents were of ana-

lytical grade.

### Determination of agronomic characteristics

Soluble solid content was determined in tomato juice using a digital refractometer (Atago PR-100, NSG Precision Cells, Inc., Farmingdale, NY, USA) and expressed in °Brix. pH was measured using an electronic pH meter (WTW, Microprocessor pH Meter, PH 539 Weilheim, Germany). Titratable acidity was estimated by titration at pH 8.1 with 0.1 mol L<sup>-1</sup> sodium hydroxide solution and expressed as % citric acid (National Canners Association 1968).

### Determination of total carotenoid and lycopene content

Total carotenoid and lycopene were extracted with hexane/ethanol/acetone (2: 1: 1, v/v/v) containing butylated hydroxytoluene (BHT) and analysed using a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc., CA, USA) at 450 and 503 nm, respectively as described by Fish *et al.* (2002) and Lee (2001). Total carotenoids were expressed as mg of β-carotene equivalents kg<sup>-1</sup> fresh weight (FW) (mg β-carotene eq kg<sup>-1</sup> FW). A molar extinction coefficient  $\epsilon = 17.2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  was used for lycopene content determination and results were expressed in mg kg<sup>-1</sup> FW.

### Determination of total phenolic content

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method as modified by Eberhardt *et al.* (2000) and Singleton *et al.* (1999). Each sample (0.5 g) was extracted with 10 mL of methanol for 24 h, after which 125 μL of this extract was diluted 1: 5 (v/v) with distilled water. Then 125 μL of the diluted extract was mixed with 500 μL of distilled water in a test tube, 125 μL of Folin–Ciocalteu reagent was added and the mixture was allowed to stand for 3 min. Thereafter, 1.25 mL of 70 g L<sup>-1</sup> sodium carbonate solution was added and the final volume was made up to 3 mL with distilled water. Each sample was allowed to stand for 90 min at room temperature before measurement at 760 nm against a blank in a spectrophotometer (Beckman DU 650). The linear reading of the standard curve was from 0 to 300 μg gallic acid mL<sup>-1</sup>. Results were expressed in mg gallic acid equivalent (GAE) kg<sup>-1</sup> FW.

### Determination of flavonoid content

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<sub>2</sub> was added. After 5 min, 60 μL of 10% AlCl<sub>3</sub> 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 in milligrams of rutin equivalents (RE) kg<sup>-1</sup> FW.

### Determination of ascorbic acid (AsA) and dehydroascorbic acid (DHA)

AsA and DHA contents were determined as reported by Kampfenkel *et al.* (1995) on triplicate samples of the homogenate juice (0.1 g). AsA and DHA were extracted by using 6% metaphosphoric acid and detected at 525 nm in a spectrophotometer (Beckman DU 650) and expressed in mg kg<sup>-1</sup> FW.

### Trolox equivalent antioxidant capacity (TEAC) assay

The measurement of the hydrophilic and lipophilic antioxidant activity was performed using the trolox equivalent antioxidant capacity (TEAC) assay. The antioxidant activity was measured using the ABTS decoloration method (Miller and Rice-Evans 1997). 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). Two different calibration curves were constructed using freshly prepared trolox solutions for HAA and LAA determinations. Values were obtained from three replicates as  $\mu\text{M}$  Trolox 100  $\text{g}^{-1}$  FW.

### Statistical analysis

Effects of variety on yield, physicochemical and nutritional properties of tomato lines 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

### Agronomic characteristics

Tomato plants grown under the experimental conditions were vigorous with excellent foliage cover. The high lycopene content tomato breeding lines were characterized by dark foliage and dark green immature fruit without morphological aberrations. The most important agronomic characteristics of the studied tomato varieties are reported in **Table 1**. The results showed that differences in tomato total yield were significant between the studied tomato varieties ( $P < 0.01$ ). The variety HLT-F52 reached comparable yield to the control Rio Grande. The soluble solids content was significantly different between the studied tomato varieties ( $P < 0.05$ ). HLT-F51 has a comparable value to Rio Grande. With regards to average fruit weight, pH and titratable acidity, the advanced breeding lines showed also comparable values to the control variety Rio Grande. Generally, the tomato high lycopene content advanced breeding lines showed acceptable agronomic characteristics and yield.

### Total carotenoid and lycopene content

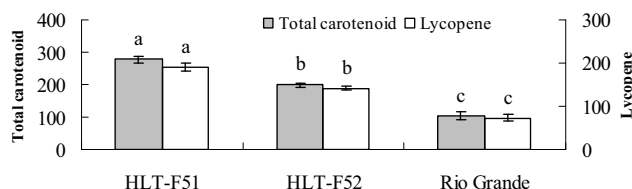
The total carotenoid and lycopene contents of the different tomato varieties are presented in **Fig. 1**. The results showed significant differences among tomato varieties in total carotenoid and lycopene ( $P < 0.01$ ). Total carotenoid values varied from 104.66  $\text{mg kg}^{-1}$  FW in the standard Rio Grande to 277.71  $\text{mg kg}^{-1}$  FW in HLT-F51. HLT-F51 and HLT-F52 were determined to be the richest varieties in carotenoids with a content 2.65- and 1.89-fold higher, respectively compared to the standard Rio Grande. Our values fall within the range reported by Raffo *et al.* (2002) Cookson *et al.* (2003) and recently Lenucci *et al.* (2006) varying from 104 to 299  $\text{mg kg}^{-1}$  FW.

Concerning lycopene, values ranged from 97.01  $\text{mg kg}^{-1}$  FW in Rio Grande to 254.26  $\text{mg kg}^{-1}$  FW in HLT-F51. As for total carotenoids, HLT-F51 and HLT-F52 were found to be also the richest source of lycopene with a content 2.62- and 1.94-fold higher respectively compared to Rio Grande. Our values are comparable with those reported for field-grown tomatoes by Abushita *et al.* (2000), Gomez *et al.* (2001), and Takeoka *et al.* (2001), ranging from 52 to 236  $\text{mg kg}^{-1}$  FW, and confirm that field-grown tomatoes generally present higher levels of lycopene with respect of greenhouse-grown tomatoes, in which it ranges between 1 and 108  $\text{mg kg}^{-1}$  FW (Leonardi *et al.* 2000; Raffo *et al.* 2002). These results are also in line and confirm those of Lenucci *et al.* (2006) who reported that high-pigment tomatoes are characterized by the highest lycopene content compared to the other studied tomato varieties and attained more than 200  $\text{mg kg}^{-1}$  FW. The differences between high-lycopene and ordinary tomato varieties have been attributed to different growing conditions and cultivars (Dumas *et al.* 2003). From the molecular point of view, the considerable lycopene accumulation in high-lycopene tomato varieties can be due to the reduced cycling rate of this molecule to

**Table 1** Agronomic characteristics of the studied tomato varieties

Cultivars	Yield (t/ha)	Average fruit weight (g)	Soluble solids content ( $^{\circ}\text{Brix}$ )	pH	Titratable acidity (%)
HLT-F51	80.61 b	73	5.4 a	4.47	0.44
HLT-F52	96.31 a	73	4.6 b	4.23	0.42
Rio Grande	100.36 a	77	5.2 a	4.35	0.43
Significance	**	ns	**	ns	ns

Signification: \*\* Probability level of 1%; ns: not significant. Values in the same column followed by the same letters do not differ significantly (LSD test,  $P < 0.05$ ).



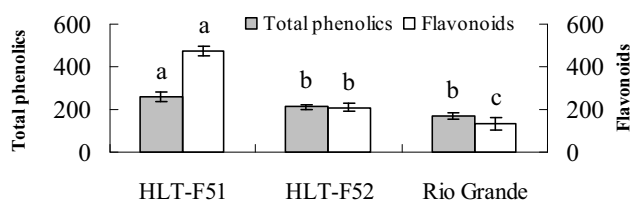
**Fig. 1** Total carotenoid ( $\text{mg } \beta\text{-carotene eq kg}^{-1}$  FW) and lycopene ( $\text{mg kg}^{-1}$  FW) content of the studied tomato varieties. Values are mean  $\pm$  standard error. Values for each variety with the same letters are not significantly different (LSD test,  $P < 0.05$ ).

synthesize carotenes. Thus, these high-lycopene fruits seem exceptional for human nutrition. Consuming one serving of HLT-F52 or HLT-F51 varieties (100 g) provides 75.56 to 101.7% of the recommended daily intake of lycopene compared to only 38.8% for Rio Grande (Rao and Agarwal 1998).

### Total phenolic and flavonoid content

The total phenolic and flavonoid contents of the studied tomato varieties are presented in **Fig. 2**. The results showed significant differences among tomato varieties in total phenolics ( $P < 0.05$ ) and flavonoids ( $P < 0.01$ ). Total phenolic values ranged from 171.0  $\text{mg GAE kg}^{-1}$  FW in Rio Grande to 259.31  $\text{mg GAE kg}^{-1}$  FW in HLT-F51. HLT-F52 variety exhibited similar phenolic level to Rio Grande. Our values are close to those of Toor and Savage (2005) who reported that the pulp total phenolic content mean value (both hydrophilic as well as lipophilic) of three tomato varieties was 150  $\text{mg GAE kg}^{-1}$  FW during the investigation of antioxidant component in different tomato fractions. Even so, Lenucci *et al.* (2006) reported higher pulp total phenolic values in high-pigment tomato varieties. Although genetic control is the primary factor in determining the amount of phenols in fruits and vegetables, variations could also depend on 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 the ripening of tomato fruits as was reported by Buta and Spaulding (1997) and Raffo *et al.* (2002).

Regarding flavonoids, values ranged from 132.59  $\text{mg}$



**Fig. 2** Total phenolics ( $\text{mg GAE kg}^{-1}$  FW) and flavonoids ( $\text{mg RE kg}^{-1}$  FW) content of the studied tomato varieties. Values are mean  $\pm$  standard error. Values for each variety with the same letters are not significantly different (LSD test,  $P < 0.05$ ).

RE kg<sup>-1</sup> FW in Rio Grande to 473.74 mg RE kg<sup>-1</sup> FW in HLT-F51 and were in line with the recent results of Lenucci *et al.* (2006) for high-pigment tomato varieties ranging from 168 to 470 mg RE kg<sup>-1</sup> FW. In our analysis, HLT-F51 and HLT-F52 varieties obtained respectively more than 3.57- and 1.58-fold higher flavonoids compared to Rio Grande. Tomatoes have been considered to be relatively rich source of flavonoids, with an average of 50 mg kg<sup>-1</sup> FW (Stewart *et al.* 2000). In the present study, the standard variety Rio Grande obtained more than 2.65-fold the average tomato fruit flavonoid content reported by Stewart *et al.* (2000). Interestingly, HLT-F51 and HLT-F52 obtained in average respectively 9.47- and 4.02-fold the reported flavonoid average content. Variations can be ascribed to the high lycopene traits. In fact, it has been reported that in red ripe tomato fruits, naturally occurring mutations that increase carotenoid content, including lycopene, are also characterized by a dramatic increase in plastid biogenesis and in the production of other compounds such as vitamin C and flavonoids (Mochizuki and Kamimura 1984). Moreover, it has been reported that field-grown tomato fruits, which receive higher amount of light and UV radiation contain a higher amount of flavonoids (quercetin and kaempferol) in comparison to greenhouse-grown tomato. In our experiment, one serving of HLT-F51 or HLT-F52 fruit provided respectively 189 and 84% of the recommended daily intake of flavonoids compared to 53% for Rio Grande which ascertain the importance of such tomato varieties as dietary flavonoids suppliers (Hertog *et al.* 1992). Although phenolic contents in tomatoes are only moderate compared with those in other vegetables such as onion, their high consumption in the Tunisian diet (Hdider *et al.* 2007) due to their year-round availability and high utility in culinary preparations, makes them a good source of phenols, as reported for Indian and American diets (Vinson *et al.* 1998; George *et al.* 2004). Our results confirmed that variety significantly affects total phenolic contents in tomato, as reported by other authors (Abushita *et al.* 2000; George *et al.* 2004).

### Vitamin C content

The total vitamin C, AsA, and DHA contents of the different tomato varieties are presented in Fig. 3. Except for AsA, the results showed significant differences among tomato varieties in DHA and total vitamin C ( $P < 0.01$ ). DHA, the oxidized form, accounted for between 40 and 68% of the total vitamin C in line with the recent result of Lenucci *et al.* (2006) for high-pigment tomato varieties ranging from 0 to 85%. It is well known that the amount of DHA is strongly influenced by experimental procedure (De Gara 2003). HLT-F52 variety exhibited the highest amount of DHA with 208.66 mg kg<sup>-1</sup> FW. The amount of DHA in HLT-F51 was comparable to Rio Grande. In our experiment, the amounts of AsA and DHA were similar to those reported by Lenucci *et al.* (2006), George *et al.* (2004), Liptay *et al.* (1986) and Souci *et al.* (1994). Regarding total vitamin C, values ranged from 181.16 mg kg<sup>-1</sup> FW in Rio Grande to 308.64 mg kg<sup>-1</sup> FW in HLT-F52. HLT-F51 obtained similar total vitamin C value to Rio Grande. The greatest variation in total vitamin C was 1.7-fold higher observed in HLT-F52 variety. Although the ascorbic acid content for new selected tomato varieties has been only scarcely reported, our result fall within the range reported recently by Lenucci *et al.* (2006) attaining 300 mg kg<sup>-1</sup> FW in different high-pigment tomato varieties. However, higher values, depending on seasons, and ranging from 310 to 710 mg kg<sup>-1</sup> FW were reported by Raffo *et al.* (2006) for the cherry tomato cultivar Naomi grown under greenhouse conditions. Generally, it is widely recognized that field-grown tomato have higher AsA levels (until 258 mg kg<sup>-1</sup> FW) when compared to those produced under shade (155 mg kg<sup>-1</sup> FW). Differences were also observed in the redox state of the system AsA/DHA of the studied tomato varieties. For the reason that many environmental conditions change the redox state of this system, it has been hypothesized that it could function as a sensor

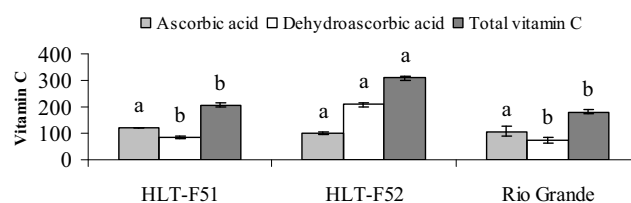


Fig. 3 Ascorbic acid, dehydroascorbic acid and total vitamin C content (mg kg<sup>-1</sup> FW) of the studied tomato varieties. Values are mean  $\pm$  standard error. Values for each variety with the same letters are not significantly different (LSD test,  $P < 0.05$ ).

modulating cellular metabolism and hormone sensitivity in response to exogenous factors (De Gara 2003). This suggests that different tomato varieties can perceive the surrounding environment in a different way, according to their genotype. These varieties seem therefore exceptional for human consumption. In fact, consuming one serving of either HLT-F52 or HLT-F51 varieties provides from 43 to 64% of the recommended daily intake of vitamin C compared to only 37% for the standard variety Rio Grande (Società Italiana di Nutrizione Umana 1998).

### Hydrophilic and lipophilic antioxidant activities TEAC assay

The hydrophilic (HAA) and lipophilic (LAA) antioxidant activities of the different tomato varieties are presented in Fig. 4. The results showed significant differences among tomato varieties in hydrophilic and lipophilic antioxidant activities ( $P < 0.01$ ). HAA values ranged from 129.06  $\mu$ M Trolox 100 g<sup>-1</sup> FW in Rio Grande to 270.91  $\mu$ M Trolox 100 g<sup>-1</sup> FW in HLT-F51. Those findings fill in the range reported by many authors. In fact, Raffo *et al.* (2006) reported recently that the HAA of the cherry tomato variety Naomi ranged between 191 and 420  $\mu$ M Trolox 100 g<sup>-1</sup> FW depending on seasons. Cano *et al.* (2003) reported also that the hydrophilic antioxidant activity attained 218  $\mu$ M Trolox 100 g<sup>-1</sup> FW for the greenhouse grown tomato variety Marmande-Cuarenteno. In our experiment, total antioxidant activity (the sum of both hydrophilic and lipophilic antioxidant activities) varied from 266.37  $\mu$ M Trolox 100 g<sup>-1</sup> FW to 579.39  $\mu$ M Trolox 100 g<sup>-1</sup> FW. The hydrophilic antioxidant activity contributed from 42-49% of the total antioxidant activity.

The HAA values in mature fruits of HLT-F52 and HLT-F51 were 1.28 and 2.09-fold the HAA of the control variety Rio Grande. The hydrophilic antioxidant activity has been attributed to the presence of phenolic compounds, such as caffeic and chlorogenic acid in the methanolic fraction (Gomez *et al.* 2001). After considering data from all tomato varieties, no significant correlation ( $R^2 = -0.076$ ;  $P > 0.05$ ) between TEAC and total vitamin C content was obtained (Table 2). The lack of correlation could be due to the content of phenolics, which may account for most of the TEAC values. In fact, there was a good linear correlation ( $R^2 = 0.823$ ;  $P < 0.01$ ) between flavonoids and TEAC. The total hydrophilic antioxidant activity was certainly correlated

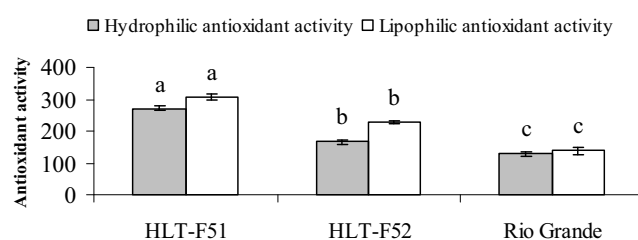


Fig. 4 Hydrophilic and lipophilic antioxidant activities ( $\mu$ M Trolox eq 100 g<sup>-1</sup> FW) of the studied tomato varieties. Values are mean  $\pm$  standard error. Values for each variety with the same letters are not significantly different (LSD test,  $P < 0.05$ ).

**Table 2** Correlation coefficient and related significance between antioxidant compounds and antioxidant activity

Compounds	TEAC assay	
	Corr coeff	P
<b>Hydrophilic fraction</b>		
Ascorbic acid	0.469	ns
Dehydroascorbic acid	0.190	ns
Total vitamin C	-0.076	ns
Total phenolics	0.823	<0.01
Flavonoids	0.977	<0.01
<b>Lipophilic fraction</b>		
Total carotenoids	0.956	<0.01
Lycopene	0.954	<0.01

ns, no significant correlation

with the level of all of the main antioxidants (vitamin C, total flavonoids and phenolic compounds), rather than being the mere sum of their content. Furthermore, it is likely that it 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). The test reaction used for antioxidant activity measurement might be influenced by other compounds involved in complex antioxidant system of tomato fruits such as glutathione and enzymatic components (Jiménez *et al.* 2002).

Regarding LAA, values ranged between 137.31  $\mu\text{M}$  Trolox 100  $\text{g}^{-1}$  FW in Rio Grande to 308.48  $\mu\text{M}$  Trolox 100  $\text{g}^{-1}$  FW in HLT-F51. The lipophilic antioxidant activity values were higher than those obtained by Raffo *et al.* (2006) and Cano *et al.* (2003) ranging between 26 and 88  $\mu\text{M}$  Trolox 100  $\text{g}^{-1}$  FW for different tomato varieties grown under greenhouse conditions. In our experiment, the lipophilic antioxidant activity represented 51-58% of the total antioxidant activity. The higher lipophilic antioxidant activity can be mainly attributed to the large amount of lycopene detected particularly in high lycopene content tomato varieties. In fact, the lipophilic antioxidant activity values in mature fruits of HLT-F52 and HLT-F51 were 1.66 and 2.24-fold higher compared to the standard variety Rio Grande fruits. The lipophilic antioxidant activity has been mainly attributed to the presence of carotenoids particularly lycopene (Martinez-Valverde *et al.* 2002; Raffo *et al.* 2002; Cano *et al.* 2003; George *et al.* 2004). After considering data from all tomato varieties, good and significant correlation between TEAC and total carotenoids ( $R^2 = 0.956$ ;  $P < 0.01$ ) and between TEAC and lycopene content ( $R^2 = 0.954$ ;  $P < 0.01$ ) were obtained. Consequently, the sample with the lowest carotenoid and lycopene content (Rio Grande) showed the lowest lipophilic antioxidant activity and that one with the highest contents (HLT-F51) had the highest activity.

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