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Assessing Agronomic Characteristics, Lycopene and Total Phenolic Contents in Pulp and Skin Fractions of Different Tomato Varieties

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ABSTRACT

Considering the lycopene antioxidant activity against free radicals responsible for many actual important diseases, new tomato varieties with increased lycopene content have recently been developed. Besides some agronomic characteristics, we evaluated five tomato varieties for their lycopene and total phenolic contents in both pulp and skin fractions. All tested varieties generally showed satisfying agronomic characteristics. The lycopene and phenolic contents in fruit pulp and skin varied significantly between the studied varieties. Compared to 'Rio Grande', the variety '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. These results are the first data on skin lycopene and phenolic levels of tomato varieties with high lycopene content and emphasize the promising use of such varieties for healthy quality products and by-products.

Keywords: fruit quality, lycopene, phenolics, processing tomato, pulp, skin, *Solanum lycopersicon* L

INTRODUCTION

In Tunisia, tomato (*Solanum lycopersicon* L.) is the main vegetable grown and consumed. Tunisia is also the main tomato-processing country on the African continent and between the world's leaders in terms of per capita consumption with up to 54 kg, on a fresh weight basis (Hdider *et al.* 2007).

Lycopene, the pigment responsible for the red colour of tomato fruits, accounts for more than 80% the total tomato carotenoids in fully red ripe fruit (Nguyen and Schwartz 2000). This pigment has strong antioxidant activity and exhibits the highest physical quenching rate constant with singlet oxygen (Di Mascio *et al*. 1989; Stahl and Sies 1996; Agarwal and Rao 2000). Therefore, great interest has been focused on lycopene due to its preventive activity against several actual important pathologies such as cardiovascular heart disease (Rao and Rao 2007), hepatic fibrogenesis (Kitade *et al*. 2002), solar light induced erythema (Stahl and Sies 2002) and many other chronic disease states (Carrapeiro *et al.* 2007). Therefore growers, processors and consumers are interested in the lycopene content of tomato varieties. The increase in lycopene content is of particular importance in processing tomato varieties in order to compensate for the loss of antioxidant components due to chemical, physical and biological factors (Lenucci *et al*. 2006, 2007).

In order to satisfy the demand of growers, processors and consumers of highly 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, the selection of plants which naturally over express genes improving lycopene content may lead to the formation of plants with many undesirable characteristics such as reduced levels of other antioxidants such as β-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 tomato varieties with high lycopene content under various environmental growing conditions. In fact, Dumas *et al*. (2003) and Atanassova *et al.* (2007) reported that lycopene content in tomato fruit varies not only in relation to genotype, but also depends on environmental factors and agricultural techniques used.

Various studies recently focused on the evaluation of yield and qualitative parameters of tomato varieties with high lycopene content (Armendariz *et al*. 2006; Lenucci *et al*. 2006; Lenucci *et al*. 2007; Macua *et al*. 2007; Cantore *et al*. 2008; Ilahy *et al*. 2009). However, in all these studies, lycopene content was measured mainly in whole tomato fruits or processed tomato products without separating pulp and skin fractions. Tomato skin has received a great deal of interest because of its exceptional lycopene content (Alwandawi *et al*. 1985; George *et al*. 2004; Toor and Savage 2005; Ilahy and Hdider 2007).

Due to its high consumption, tomato has also gained the attention as a source of phenolics (Lenucci *et al*. 2006; Ilahy *et al*. 2009). Because of their structure, phenolic compounds are very efficient scavengers of peroxyl radicals (Aruoma 1999; Halliwell and Gutteridge 1999). Phenolic compounds are increasingly attracting attention for their antioxidant, anti-inflammatory and antimicrobial properties and their likely beneficial effect on the prevention of cancer and other serious human disorders (Hollman *et al*. 1996; Le Marchand 2002; Tripoli *et al*. 2007). However, little is known about phenolic levels in tomato varieties and particularly in the skin fraction.

Therefore, and based on the reasons above mentioned, the aim of this study was to investigate the behaviour of dif-

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ferent tomato varieties cultivated in Tunisia with a particular focus on lycopene and phenolic content on both tomato pulp and skin fractions.

MATERIALS AND METHODS

Plant culture

The experiment was carried out at the Mannouba support station located in the north of Tunis Tunisia. Five tomato varieties were used in this experiment. Four tomato varieties had high lycopene content ('Lyco 1', Lyco 2', 'HLY 13' and 'HLY 18') (Lenucci *et al.* 2006; Ilahy *et al.* 2007) and one ordinary tomato variety, commonly used in Tunisia, the open pollinated variety 'Rio Grande' (Petoseed, Saticoy, CA, USA). 'HLY 13' and 'HLY 18' varieties are not yet commercialised and were obtained from COIS' 94 s.r.l. Italy. 'Lyco 1' and 'Lyco 2' were obtained from Hazera Genetics. Seeds were sown in plug-seedling trays (Makrolon multi UV IQ-Relax Generation, Bayer Sheet, Europe Darmstadt, Germany·) and transplanting in double rows with a planting density of about 33, 000 plants/ha. Tomato varieties were grown in four replicated plots. For each variety, plots were 20 m long. Ferti-irrigation was adjusted to the needs of the plants at different ripening stages. 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^{-1}, 129.6 kg P_2O_5 ha^{-1}, 440 kg K_2O ha^{-1} and 83.2 kg MgO ha^{-1}). Chemical fertilizer solution was added to water irrigation by pump injection once a week. The conventional methods also included weed control with synthetic chemical herbicides and plant pathogen control with synthetic chemical pesticides. Phosalone (350 g L^{-1}) and triforine (190 g L^{-1}) were utilized to reduce aphids and powdery mildew respectively. Propargite (570 g L^{-1}) and sulfur (800 g L^{-1}) were used to reduce/ prevent mites. Mancozebe (800 g L^{-1}) was applied to prevent mildew. All these pesticides were applied once a cycle.

Fruit sampling

Tomato fruits were hand harvested once and total yield was recorded. A sample of approximately 2 kg of injury-free and uniformly ripe tomato fruit samples was collected from each variety, weighed and delivered quickly to the laboratory. The red ripe stage was selected according to the USDA tomato classes (over 90% red; desirable table ripeness). Tomato pulp and skin were separated using a scalpel according to D'Souza *et al.* (1992). Tomato pulp was cut into small pieces and sequentially homogenized in a mixer. Part of the sample was immediately used for two analyses (°Brix, pH and titratable acidity). The other part, together with the separated skin fraction, were frozen at -20°C and used for lycopene and total phenolic determination within one week.

Determination of agronomic characteristics

Soluble solids (SS) content of the tested varieties was expressed by the °Brix of fresh juice. The measurement was taken by placing a drop of filtered juice on the prism of a digital refractometer with automatic temperature compensation (Atago PR-100 NSG Precision Cells, Inc., Framingdale, NY). The pH of the obtained juice from mixed fruit was assessed with a pH meter (WTW, Microprocessor pH Meter, PH 539, Weilheim, Germany). Titrable acidity (TA) was estimated after titration at a pH of 8.1 with a sodium hydroxide solution (0.1 M) and results were expressed as a percentage of citric acid.

Lycopene content determination

Pulp and skin lycopene extraction and determination were conducted as described by Fish *et al.* (2002) and D'Souza *et al.* (1992). The method uses a mixture of hexane-ethanol-acetone in a 2: 1: 1 proportion containing 0.05% butylated hydroxytoluene (BHT). For the skin, lycopene was extracted on a rotary shaker at 150 rpm for 1 h. During extraction, some precautions, like working in a room with reduced luminosity and wrapping glass materials with aluminium to avoid lycopene loss by photo-oxidation, were taken. Absorbance of the hexane extract was measured at 503 nm with a spectrophotometer Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments 152 Ltd., Cambridge, UK). Lycopene molar extinction $\varepsilon = 17.2\ 10^4\ M^{-1}\ cm^{-1}$ was used to determine lycopene content and results were expressed as milligrams per kilogram of fresh weight (FW).

Total phenolic content determination

Total phenolic content was analysed spectrophotometrically using the modified Folin-Ciocalteu method (Singleton *et al.* 1999; Eberhardt *et al.* 2000). Each sample (2 g) was extracted with 10 ml methanol for 24 h. 125 µl of the diluted extract was mixed with 500 µl distilled water in a test tube followed by the addition of 125 µl of Folin-Ciocalteu reagent (Carlo Erba Milan, Italy) and allowed to stand for 3 min. Then, 1250 µl of 7% 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 and measured at 760 nm against the blank on a spectrophotometer (CECIL CE 2501). The linear reading of a standard curve was from 0 to 300 µg of gallic acid per ml. Results were expressed as milligrams gallic acid equivalent (GAE) per 100 g of FW of tomato fruit.

Statistical analysis

The experimental design was a randomised complete block with five treatments (varieties) and four blocks. Analysis of variance was performed according to the General Linear Models (GLM) procedure developed by SAS (SAS Inst., v. 6.1, Cary, N.C., U.S.A.). The LSD test was applied to establish significant differences between means with a 95% confidence level.

RESULTS AND DISCUSSION

Agronomic characteristics

Plants obtained from the experiment were vigorous with excellent foliage cover. All the tomato varieties claimed to have high lycopene content were characterized by dark foliage and dark green immature fruit without morphological aberrations. The most important agronomic characteristics of the studied varieties are shown in **Table 1**. The yield of the various varieties were high and the average total yield obtained for the experiment was 137.78 t/ha. This relatively high yield is partially due to the performance of the tested varieties and to the excellent growth condition encountered during the experiment. The results showed that differences in tomato total yield were significant between the studied varieties ($P < 0.01$). 'HLY 13' had the highest yield and 'HLY 18' had comparable yield to the control 'Rio Grande'. Regarding average fruit weight, 'HLY 18' produced relatively square and medium-sized fruit. 'HLY 13' has long and large fruit. 'Lyco 1' fruits were ovate and medium in size. 'Lyco 2' produced large, ovate fruits. The control 'Rio Grande' produced large, ovate fruits suitable for both fresh

Table 1 Agronomic characteristics of the studied high-lycopene and ordinary tomato varieties

Cultivars	Yield (t/ha)	Average fruit weight (g)	Soluble solids content (°Brix)	pH	Titratable acidity (%)
Lyco 1	128.96 c	75 bc	5.50	4.80 a	0.36 bc
Lyco 2	116.31 d	80 b	5.87	4.68 b	0.39 a
HLY 13	157.70 a	93 a	6.80	4.86 a	0.35 c
HLY 18	140.61 b	70 c	6.10	4.88 a	0.38 ab
Rio Grande	145.36 b	81 b	5.87	4.84 a	0.37 ab
Significance	**	**	Ns	**	**

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

market and processing. The SS content was not significantly different between the studied tomato varieties ($P > 0.05$). Concerning pH and TA, significant differences were also recorded between varieties ($P < 0.01$). 'Lyco 2' had the highest percentage of titratable acids.

Generally, all the tested tomato varieties showed acceptable agronomic characteristics and high productivity. However, some fruits of 'HLY 13' showed both radial and concentric cracking which makes the tomatoes unmarketable and deteriorate quickly.

Lycopene content in tomato pulp and skin

The lycopene content of tomato pulp varieties is shown in **Fig. 1**. Lycopene contents were significantly different between studied tomato varieties ($P < 0.05$). All the studied varieties with claimed high lycopene content showed higher lycopene values than 'Rio Grande'. In particular, 'HLY 18' and 'Lyco 1' had 240.69 and 237.05 mg/kg FW, respectively which is 2.6 and 2.4-fold, respectively higher than the value obtained for 'Rio Grande'. These results are in agreement and confirm those found by Lenucci *et al*. (2006) who, using whole tomato fruit, reported that 'HLY 18' is among cultivars characterized by the highest lycopene content compared to other studied tomato varieties and attained more than 200 mg/kg FW. Our results are also consistent with those of Macua *et al*. (2007) who reported that 'Ha-3518', marketed for its high lycopene content, can reach high lycopene content values ranging from 170.9 to 223 mg/kg FW depending on the season, and which were much higher than the control variety. Very high lycopene content values ranging from 97 to 255 mg/kg FW were also recently reported for different tomato varieties grown in Tunisia (Ilahy *et al*. 2009).

The lycopene content of tomato skin varieties is shown in **Fig. 2**. Values obtained were higher than tomato pulp lycopene content and ranged between 436.63 and 915.21 mg/kg FW. These results are in agreement with those of Binoy *et al*. (2004) and Ilahy and Hdider (2007) who reported that tomato skin contains many fold higher lycopene than tomato pulp. Also, our results are consistent with those of George *et al*. (2004) who reported that on average, skin lycopene content is 2.5-fold higher than pulp lycopene content. Lycopene content was significantly different in the tomato skin of the studied varieties ($P < 0.05$). As for tomato pulp, 'HLY 18' and 'Lyco 1' showed the highest skin lycopene content values with 915.21 and 838.43 mg/kg FW, respectively. Compared to 'Rio Grande', the skin lycopene content values obtained were 2.1 and 1.9-fold higher, respectively. To our knowledge, this is the first study that determined lycopene content of the skin fraction of tomato varieties with high lycopene content and showed that these varieties also have high skin lycopene content. Therefore, the consumption of tomato fruit with high lycopene content along with their skin, as recommended by Toor and Savage (2005), can contribute to attain maximum health benefit. Otherwise, the skin fraction of these tomato varieties with high lycopene content could be used as a value-added ingredient in other food products. In fact, the tomato skin fraction can be used to increase the consistency of tomato products as suggested by Del Valle *et al*. (2002). Besides, tomato skin can also be used to extract different components with high nutritional and economic value. Such exploitation of valuable components from the skin of tomato varieties with high lycopene content could contribute to obtain new value-added products and provide extra income for these tomato plants.

Total phenolic content in tomato pulp and skin

The total phenolic content of the studied tomato pulp varieties are shown in **Fig. 3**. Values ranged from 16.66 to 24.77 mg GAE/100 g FW. These values are close to those of Toor and Savage (2005) who reported that the mean total phenolic content of pulp (hydrophilic and lipophilic) of

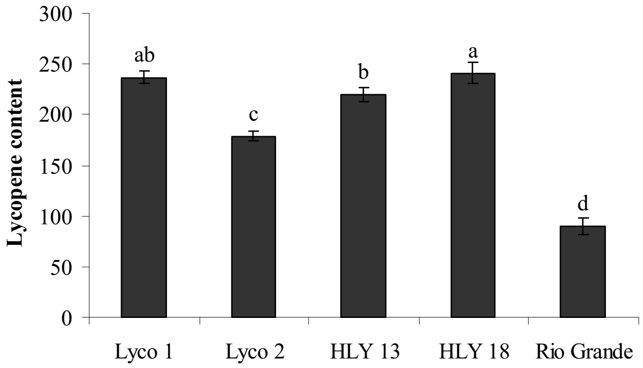

Fig. 1 Pulp lycopene content of the studied tomato varieties. Data are means of four replicates ± standard error. Bars with the same letters are not significantly different (LSD test, $P < 0.05$).

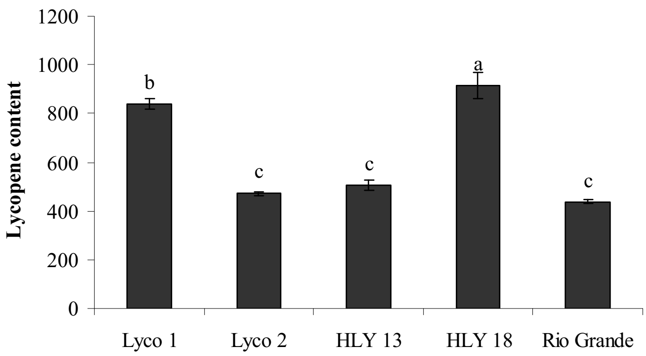

Fig. 2 Skin lycopene content of the studied tomato varieties. Data are means of four replicates ± standard error. Bars with the same letters are not significantly different (LSD test, $P < 0.05$).

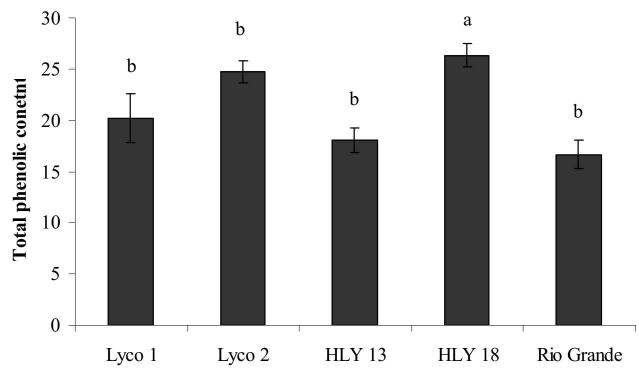

Fig. 3 Pulp total phenolic content of the studied tomato varieties. Data are means of four replicates ± standard error. Bars with the same letters are not significantly different (LSD test, $P < 0.05$).

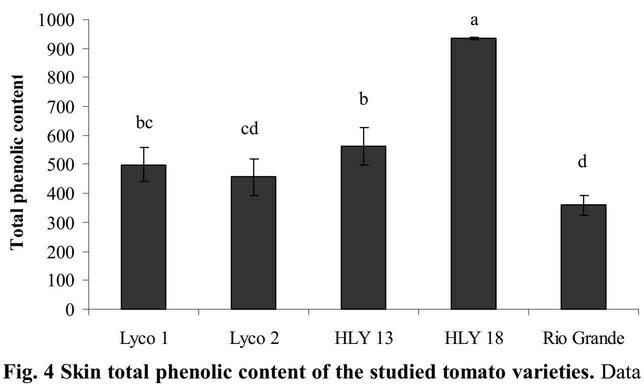

Fig. 4 Skin total phenolic content of the studied tomato varieties. Data are means of four replicates ± standard error. Bars with the same letters are not significantly different (LSD test, $P < 0.05$).

three tomato varieties was 15 mg GAE/100 g FW during a study on antioxidant components in different tomato fractions. Our values were also close to the range described by

George *et al*. (2004) who reported that pulp total phenolic contents varied between 9.2 and 27 mg catechin/100 g FW. Values of pulp total phenolic content were significantly different between the studied tomato varieties ($P < 0.05$). Nevertheless, 'HLY 18' was the only variety showing significantly higher phenolic levels than other studied tomato varieties. Its total phenolic content value was 1.6-fold higher than 'Rio Grande'. Little is known about total phenolic levels of tomato varieties with high lycopene content. Even so, Lenucci *et al*. (2006) reported higher pulp total phenolic values in tomato varieties with high lycopene content. Ilahy *et al*. (2009) reported a variation between 17.0 and 26 mg GAE/100 g FW in different advanced breeding lines of tomato with high lycopene content. This differing result may be due to environmental conditions (Macheix *et al*. 1990) and analytical methodology.

The total phenolic content of the studied tomato fruit skin varieties are shown in **Fig. 4**. Values were higher than those obtained in tomato pulp and ranged between 358.78 to 936.20 mg GAE/100 g FW. Determination of phenolic content in the skin of high lycopene tomato fruit varieties is still lacking. Nevertheless, our results are in agreement with those of George *et al*. (2004) who found that skin of open field-grown tomato contains many times higher phenolics than tomato pulp. Similarly, Toor and Savage (2005) reported that the skin fraction of greenhouse-grown tomato contains the highest phenolic content compared to other tomato fruit fractions. The high level of phenolic compounds in tomato skin is due to their potential role in protection against ultraviolet radiation, to act as attractants in fruit dispersal and as defence chemicals against pathogens and predators (Strack 1997). In term of values, we obtained higher total phenolic content than Toor and Savage (2005) reaching 34.7 mg GAE/100 g FW and by George *et al*. (2004) ranging from 10 to 40 mg catechin equivalent/100 g FW. This variation can be explained by genotypic differences and a large number of external factors such as agrotechnical processes, climatic conditions and analytical methodology. The results obtained in this study also show that skin total phenolic content was significantly different between the studied tomato varieties ($P < 0.01$). Except for 'Lyco 2', all the other tomato varieties with high lycopene content had higher levels of skin phenolics than 'Rio Grande'. The highest value was recorded for 'HLY 18' with 2.6-fold the value recorded for 'Rio Grande'.

CONCLUDING REMARKS

The studied tomato varieties claimed to be high-lycopene content in particular 'HLY 18' are generally a rich source of antioxidants mainly lycopene and phenolics. Such varieties can be considered as nutritious varieties and recommended for both processing and fresh market use despite of the medium fruit size of 'HLY 18', a characteristic generally not appreciated by the Tunisian fresh market consumers. Moreover, 'HLY 18' and 'Lyco 1' varieties are characterized by high skin lycopene content that can offer an added value in new products and for animal feeding providing an extra economical benefit.

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