*The African Journal of Plant Science and Biotechnology* ©2010 Global Science Books

# Phytochemical Composition and Antioxidant Activity of Selected Watermelon Varieties Grown in Tunisia

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## ABSTRACT

Interest in bioactive compounds with antioxidant capacity and potential health benefits in watermelon is increasing but data on this topic remains scarce in the literature. Therefore, the variability of antioxidant component and antioxidant activity of six watermelon varieties (four commercial varieties 'Aramis', 'Crimson Sweet', 'Dumara', 'Giza', and two new selections 'P503' and 'P403' developed by the National Agricultural Research Institute of Tunisia) were determined. All varieties were grown simultaneously in the same field and subjected to identical cultural practices in order to minimise the effects of environmental conditions and maximize those related to genotype. Significant differences were found in lycopene, vitamin C and phenolic contents between watermelon varieties. 'Giza' showed the highest analyzed antioxidants levels. 'P503' had the highest lycopene and flavonoid contents. 'Aramis' showed the highest hydrophilic antioxidant activity (239.90 µM Trolox 100 $g^{-1}$ FW). However, the highest lipophilic antioxidant activity was detected in 'Crimson Sweet' (283.97 µM Trolox 100 $g^{-1}$ FW). The present study demonstrates that the amount of assayed antioxidant molecules and the hydrophilic and lipophilic antioxidant activities were influenced by genotype, which emphasizes the need to evaluate watermelon biodiversity in order to improve its nutritional value.

**Keywords:** *Citrullus lanatus*, flavonoids, lycopene, phenolics, vitamin C

## INTRODUCTION

Watermelon (*Citrullus lanatus* Thunb. Mansfeld) is largely consumed as a refreshing summer fruit all over the Mediterranean basin and particularly in Tunisia. Watermelon fruits contain a wide range of dietary antioxidant molecules such as carotenoids, phenolics, vitamins (A, B, C and E) and many important amino acids (citrulline and arginine) (Perkins-Veazie 2002; Perkins-Veazie *et al*. 2007). Phytochemical compounds, particularly lycopene, vitamin C, flavonoids and other phenolics, have attracted much interest because of their antioxidant activity against free radicals, suggesting protective roles in reducing the risk of certain types of cancers, cardiovascular diseases and age-related degenerative pathologies (Rice-Evans *et al*. 1996; Sies and Stahl 1998; Giovanucci 1999; Robards *et al*. 1999; Karakaya *et al*. 2001; Edwards *et al*. 2003; Rao 2006).

The demand for high-quality health products 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. Therefore, the content of different classes of antioxidants and the antioxidant activity are important parameters in the qualitative evaluation of watermelon fruits.

Identification and quantification of bioactive compounds and antioxidant properties of fruits and vegetables, especially tomatoes are well defined (Stewart *et al*. 2000; Cano *et al*. 2003; Lenucci *et al*. 2006; Ilahy and hdider 2007; Ilahy *et al*. 2009). However studies on characterisation and quantification of phytochemical and antioxidant properties of watermelon are very limited. Previously, Lescovar *et al*. (2004), Perkins-Veazie *et al*. (2006) and Perkins-Veazie and Davis (2007) emphasized the importance of genotype when assessing the lycopene, soluble solids and ascorbic acid contents in watermelons but these studies focused on specific cultivars or genotypes with limited physicochemical and antioxidant properties. In our previous studies, we highlighted genotype variability in the lycopene and total phenolic content of different watermelon cultivars grown in Tunisia without determining their antioxidant activity (Tlili *et al.* 2009). In fact the determination of antioxidant activity allows a real evaluation of the nutritional quality of food rather than the analysis of each single antioxidant compound (Pellegrini *et al*. 2007). In addition, it has been reported that the levels of health-promoting bioactive compounds and the antioxidant activity of fruits and vegetables are strongly influenced by genotypic differences and external factors such as agro-technical process, environmental conditions, ripening degree, and harvest and postharvest manipulations (Waterman and Mole 1994; Abushita *et al*. 2000; Dumas *et al.* 2003).

Therefore, and based on the facts mentioned above, the aim of this study was to evaluate six selected watermelon cultivars grown in Tunisia for their phytochemical compounds (lycopene, total phenolic, flavonoids, ascorbic acid and dehydroascorbic acid) as well as their hydrophilic and lipophilic antioxidant activity.

## MATERIALS AND METHODS

### Chemicals and reagents

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic 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 analytical grade.

Received: 1 April, 2010. Accepted: 14 May, 2010.

Original Research Paper

## Field experiment

The experiment was conducted in 2008 at the Research and Experimental Station of Teboulba, Monastir, Tunisia. A total of six watermelon varieties, including four commercial varieties considered important in Tunisia and two new selections 'P503' and 'P403', selected by the National Agricultural Research Institute of Tunisia, were used in this experiment. The commercial varieties were 'Crimson Sweet' (Clause), 'Dumara' (Nunhems), 'Aramis' (Nunhems) and 'Giza' (Egyptian variety selected and improved by the National Agricultural Research Institute of Tunisia). Sowing was carried out in plug-seedling trays. Watermelons were transplanted into a sandy soil with an in-row spacing of 125 cm and a between-row spacing of 150 cm. Four blocks were used with 10 plants per variety. All cultivars under analysis were subjected to identical cultural practices as described by Tlili *et al*. (2009) and, of course, environmental conditions in order to minimize the influence of pre- and postharvest factors.

Ripe watermelons were harvested in June. Field ripeness was judged by various methods including tendril browning, yellowing of the ground spot, and loss of surface gloss and by a thumping sound which changes from a metallic ringing when immature to a soft hallow sound at maturity. Watermelons were selected randomly from the different blocks. Four ripe fruits were harvested per block per variety. All fruits were transported carefully to the laboratory for analysis to avoid internal bruising.

## Fruit tissue sampling

Fruit were cut longitudinally from stem end to blossom end through the ground spot, and tissue samples were taken from heart area. Soluble solids content (°Brix) was immediately determined on the juice obtained from mixed tissue fruit. For further analysis, about 250 g of flesh without seeds per fruit was collected, wrapped with aluminum, placed into plastic bags and placed quickly at -80°C.

## Analytical determinations

### *Soluble solids content determination*

Soluble solids content in watermelon (°Brix) was measured by cutting a wedge of flesh from the entire heart area and the juice was squeezed into a digital refractometer (Atago PR-100, NSG Precision Cells, Inc, Farming dale, NY) calibrated with a 10% sucrose solution. Only melons with mean soluble solids content ≥ 8% were sampled for analysis to ensure that all fruits were fully ripe.

#### *Lycopene content determination*

Frozen samples from every fruit were ground in a mortar with a pestle and again with a laboratory blender. Lycopene extraction and determination were conducted as described by Fish *et al*. (2002). The method uses a mixture of hexane-ethanol-acetone in (2-1-1) proportions containing 0.05% butylated hydroxytoluene (BHT). The absorbance of the hexane extract was measured at 503 nm with a spectrophotometer (CECIL CE 2501). Zeroing was done with hexane. During analysis, some precautions like working in reduced luminosity room and wrapping glass material with aluminium were adopted to minimise lycopene loss by photo-oxidation (Fish *et al*. 2002). Lycopene molar extinction ($\varepsilon = 17.2*10^4$/M/cm) was used for lycopene content determination (Beerh and Sidappa 1959). Results were expressed as mg $kg^{-1}$ fresh weight (FW).

### *Total phenolic content determination*

Total phenolics were determined according to the colorimetric method of Folin-Ciocalteu as modified by Eberhardt *et al*. (2000) and Singleton *et al*. (1999). Each sample (4 g) was extracted with 10 ml methanol for 24 h. 125 µl of the methanolic extract was mixed with 500 µl distilled water in a test tube followed by the addition of 125 µl of Folin-Ciocalteu reagent and allowed to stand for 3 min. Then, 1.250 ml 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 before measurement at 760 nm versus the blank on a spectrophotometer (CECIL CE 2501). The linear reading of standard curve was from 0 to 300 µg of gallic acid per ml. Results were expressed as milligrams gallic acid equivalent (GAE) per kilogram of fresh weight (mg GAE $kg^{-1}$ FW).

#### *Flavonoid content determination*

The flavonoid content was determined as described by Jia *et al*. (1999) on triplicate aliquots of the homogenous juice (0.3 g). 50 µL aliquots of the methanolic extract were used for flavonoid 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 using a Bechman DU 650 spectrophotometer and flavonoid content was expressed as milligrams of rutin equivalents (RE) per kilogram of fresh weight (mg RE $kg^{-1}$ FW).

### *Ascorbic acid (ASA) and dehydroascorbic acid (DHA) determination*

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 Beckman DU 650 spectrophotometer and expressed in mg $kg^{-1}$ FW.

#### *Antioxidant activity determination*

Antioxidant activity was measured using the ABTS decoloration method (Miller and Rice-Evans 1997). The method estimates the relative capacity of antioxidant substances to scavenge the radical cation of 2,2'-azinobis-(3ethylbenzothiazoline-6-sulfonate; $ABTS^{\cdot+}$) compared to the standard antioxidant Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid). For this purpose, $ABTS^{\cdot+}$ was produced with 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) after incubation overnight at 4°C under constant magnetic stirring. Hydro- and lipophilic antioxidants were extracted from 0.3 g of the homogenous juice (three replicates) with 50% methanol or 50% acetone, repectively at 4°C under constant magnetic shaking (300 rpm) for 12 h. Samples were centrifuged at 10000 × *g* for 7 min and the different supernatants were recovered and used for measurement of antioxidant activity. The decolourization caused by reduction of the cation by antioxidants from the samples present in different extracts were measured at 734 nm in a Beckman DU 650 spectrophotometer. Calibration curves were constructed with freshly prepared Trolox solutions for hyrophilic and lipophilic antioxidant determinations. Values were expressed as micromolar Trolox per 100 g of fresh weight (µM Trolox 100 $g^{-1}$ FW).

## Statistical analysis

The basic plots of the experiment were spread out in a randomised experimental design in three complete blocs. The analysis of variance was carried out according to the General Linear Models (GLM) procedure developed by the Statistical Analysis Systems Institute (SAS, V6.0, Cary, NC). Means and standard errors were calculated. The LSD test was also used for testing significant differences between means with a 95% confidence level.

## RESULTS AND DISCUSSION

## Lycopene content

The content of lycopene of the studied watermelon varieties is shown in **Fig. 1**. The lycopene content values varied significantly between studied watermelon varieties ($P<0.01$). Lycopene content reached very high levels (>90 mg $kg^{-1}$ FW) and varied from 47.40 mg $kg^{-1}$ FW in 'Dumara' to 112.00 mg $kg^{-1}$ FW in 'P503'. 'Giza' had a similar lycopene level to 'P503' (105.25 mg $kg^{-1}$ FW). Their values were more than 2-fold higher than those of 'Dumara' and 'P403'.

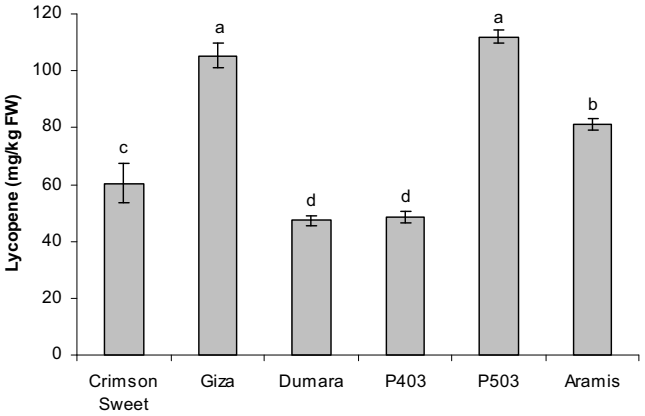


**Fig. 1 Lycopene content of the different studied watermelon varieties.** Data are means of three replicates ± standard error. Bars marked with the same letters are not significantly different (LSD test, P<0.05).

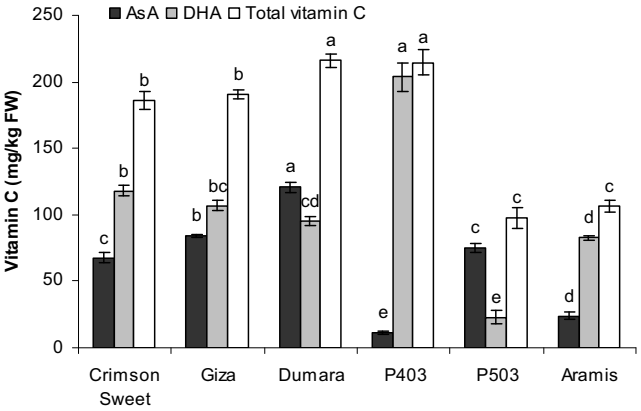


**Fig. 3 Ascorbic (AsA), dehydroacsorbic (DHA) and total vitamin C content of the different studied watermelon varieties.** Data are means of three replicates ± standard error. Bars marked with the same letters are not significantly different (LSD test, P<0.05).

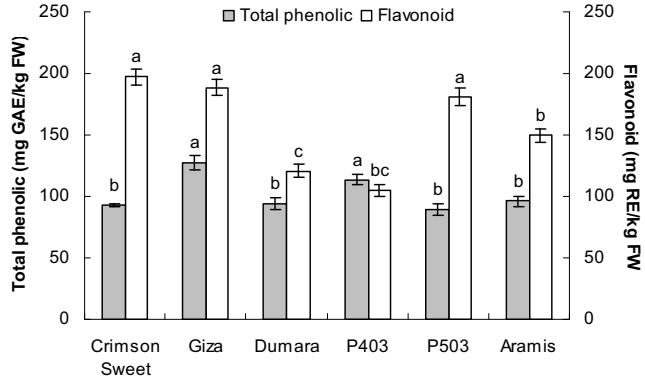


**Fig. 2 Total phenolic and flavonoid contents of the different studied watermelon varieties.** Data are means of three replicates ± standard error. Bars marked with the same letters are not significantly different (LSD test, P<0.05).

The results are in agreement with those reported by Perkins-Veazie *et al*. (2006) who found that lycopene content varies among watermelon cultivars and can reach very high values attaining 99.8 mg $kg^{-1}$ FW, while studying the carotenoid composition of 50 watermelon cultivars. As was reported for an American diet (Vinson *et al*. 1998), our data also proved that watermelon can constitute a predominant source of lycopene in the Tunisian diet because of its availability and high consumption. These results are also in agreement with our previous work on the same watermelon varieties (Tlili *et al*. 2009).

## Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the studied watermelon varieties are shown in **Fig. 2**. Both values varied significantly between studied watermelon varieties (P<0.01). For total phenolic content, values ranged from 89.76 mg GAE $kg^{-1}$ FW in 'P503' to 127.26 mg GAE $kg^{-1}$ FW in 'Giza'. 'P403' showed a similar phenolic content to 'Giza' (113.33 mg GAE $kg^{-1}$ FW) and 'Aramis' showed a similar value to 'P503' (95.93 mg GAE $kg^{-1}$ FW). The values obtained were in line with our previous results (Tlili *et al*. 2009) and those of Brat *et al*. (2006) who found a moderate amount (116 mg GAE/kg FW) of phenolics in watermelon fruits sampled from French national markets and confirm that watermelon is a good source of phenols, particularly in countries with high watermelon consumption (Vinson *et al*. 2001).

Regarding flavonoid content, values obtained ranged from 104.71 RE $kg^{-1}$ FW in 'P403' to 197.42 mg RE $kg^{-1}$ FW in 'Crimson Sweet'. 'Giza' and 'P503' showed similar flavonoid content to 'Crimson Sweet'. These results provide evidence that watermelon can constitute a significant source of flavonoids, as was reported for tomato. In fact, the obtained data are within the range of those reported for tomato varieties ranging from 134 to 622 mg RE $kg^{-1}$ FW (Lenucci *et al*. 2006).

## Vitamin C content

The content of ascorbic acid (AsA), dehydroascorbic acid (DHA) and total vitamin C of the studied watermelon varieties are shown in **Fig. 3**. Total vitamin C, AsA and DHA values varied significantly between the studied watermelon varieties (P<0.01). Total vitamin C ranged from 98.04 mg $kg^{-1}$ FW in 'P403' to 216.05 mg $kg^{-1}$ FW in 'Dumara'. The contribution of AsA and DHA to total vitamin C content was also dependent on variety. In fact, for 'Dumara', AsA and DHA accounted for 56 and 44%, respectively of the total vitamin C. However, they accounted for 5 and 95% in 'P403'. Lower values ranging from 38.2 to 69.8 mg $kg^{-1}$ FW were reported for different watermelon varieties grown under 100% evapotranspiration (ET) irrigation (Leskovar *et al*. 2004). However, higher values attaining 576.2 mg $kg^{-1}$ FW were reported by Mélo *et al*. (2006). The variations are probably ascribed to differences in genotypic and cultural practices as was reported by Leskovar *et al*. (2004). To our knowledge the presented data is among the first reports on vitamin C contents in watermelon varieties and confirms those of Leskovar *et al*. (2004) who reported that watermelon is considered to be a good source of vitamin C.

## Hydrophilic and lipophilic antioxidant activities (TEAC assay)

The hydrophilic and lipophilic antioxidant activities (HAA and LAA) of the studied watermelon varieties are shown in **Fig. 4**. Both values varied significantly between the studied watermelon varieties (P<0.01). HAA varied from 156.41 µM Trolox 100 $g^{-1}$ FW in 'Dumara' to 239.90 µM Trolox 100 $g^{-1}$ FW in 'Aramis'. 'P503' had a similar HAA to 'Dumara'. The HAA in 'Aramis' was at least 1.5-fold greater than 'Dumara' and 'P503'. This data is among the first reports on the hydrophilic and lipophilic antioxidant activities in watermelon varieties. Nevertheless, the obtained data were in the range of those found by Raffo *et al*. (2006) (170 to 420 µM Trolox 100 $g^{-1}$ FW) in greenhouse tomato and those reported for tomato varieties ranging from 81 to 218 µM Trolox 100 $g^{-1}$ FW (Cano *et al*. 2003; Toor and Savage 2004).

Regarding lipophilic antioxidant activity, values varied from 195.23 µM Trolox 100 $g^{-1}$ FW in 'P403' to 283.97 µM Trolox 100 $g^{-1}$ FW in 'Crimson Sweet'. 'Aramis' was placed in the lowest LAA value bracket with 'P403' (204.40 µM Trolox 100 $g^{-1}$ FW). The LAA in 'Crimson Sweet' was

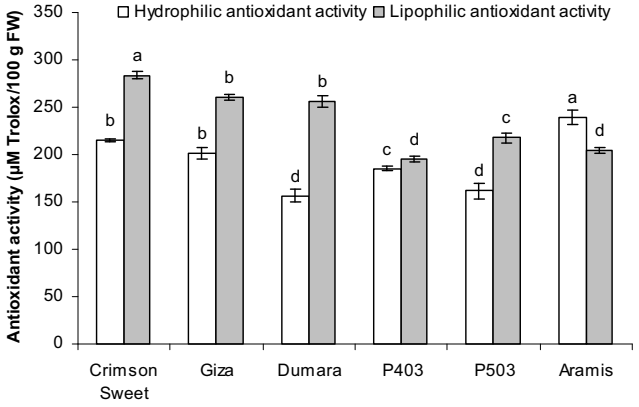


**Fig. 4 Hydrophilic and lipophilic antioxidant activities of the different studied watermelon varieties.** Data are means of three replicates ± standard error. Bars marked with the same letters are not significantly different (LSD test, P<0.05).

at least 1.5-fold higher than 'Aramis'. The obtained data were higher than those reported by Cano *et al*. (2003) and Toor and Savage (2004) ranging from 70 to 88 µM Trolox 100 $g^{-1}$ FW and those found by Raffo *et al*. (2006) ranging from 26 to 34 µM Trolox 100 $g^{-1}$ FW) in greenhouse tomato.

## CONCLUDING REMARKS

This study confirmed the important role played by genetic background in determining phytochemical compounds and the antioxidant activity of fresh watermelon. In the present study, 'Giza' showed the highest analyzed antioxidants levels. However, 'Aramis' and 'Crimson Sweet' showed the highest hydrophilic and lipophilic antioxidant activity, respectively. Therefore the amount of antioxidant compounds in a fruit does not necessarily correlate with its antioxidant activity. Due to the complexity of the composition of foods, their antioxidant power depends on the synergistic effects and redox interactions among the different nutrient and antinutrient molecules.

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