

Effect of Growing Period on the Agronomic Characteristics and Phenolic Content of Different Watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) Cultivars Grown in Tunisia

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ABSTRACT

Besides some agronomic characteristics, total phenolics and flavonoid contents of different watermelon cultivars (*Citrullus lanatus* (Thunb.) Mansfeld), as influenced by two growing periods, were investigated. Fruits from plants grown under low plastic tunnel and open-field conditions were collected. Five watermelon cultivars (four commercial cultivars namely 'Crimson Sweet', 'Dumara', 'Giza', 'Aramis', and a new selection 'P503' produced by the National Agricultural Research Institute of Tunisia) were compared. The growing period significantly influenced yield, soluble solids, total phenolics and flavonoid contents of all investigated watermelon cultivars. The total phenolics in the watermelon cultivars ranged from 122.81 to 200.69 mg GAE kg⁻¹ FW in early and full seasons, respectively. The flavonoid content ranged from 150.60 to 226.44 mg RE/kg FW in early (January - May) and full (March - July) seasons, respectively. The mean total phenolics and flavonoid contents of the five cultivars was 62 and 66% higher, respectively, in full season than in early season. This study indicates that the total phenolics and flavonoid contents of watermelon can vary considerably with changes in environmental conditions.

Keywords: flavonoid, growing season, quality, temperature

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) is a popular horticultural crop in the Mediterranean area because of its economic importance and nutritional properties. Natural compounds in food are an important health-protecting factor. They have been recognized as being beneficial for prevention of the wide spread of human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts (Clinton 1998; Sies and Stahl 1998). Watermelon provides a wide variety of dietary antioxidants such as carotenoids (lycopene and β -carotene), phenols, vitamins (A, B, C and E) and specific amino acids (citrulline and arginine) (Perkins-Veazie 2002; Perkins-Veazie *et al.* 2007; Tlili *et al.* 2011), which are thought to exert a protective role in reducing the risk of certain types of cancers, cardiovascular diseases and age-related degenerative pathologies (Rice-Evans *et al.* 1996; Giovannucci 1999; Rao 2006).

In fact, watermelon contains moderate but significant quantities of phenolics. In a recent study, Tlili *et al.* (2011) reported that the highest average total phenolic content was detected in 'Giza' (147.3 mg GAE/kg fw). 'Aramis' (92.3 mg GAE/kg fw) and 'P503' (89.0 mg GAE/kg fw) cultivars showed the lowest average content of total phenolics. In addition, Brat *et al.* (2006) found a moderate amount (~116 mg GAE/kg fw) of phenolics in watermelon fruits sampled from French national markets. Much higher values ranging between 870 and 910 mg GAE/kg fw were obtained in red-fleshed watermelon by Perkins-Veazie *et al.* (2002). These key secondary metabolites exhibit an important peroxyl-radical scavenging activity and hence potential pharmacological effects (Larson 1988; Halliwell 1994; Manach *et al.* 1998). Among phenolics, flavonoids reduce low density lipoprotein (LDL) oxidation and quench reac-

tive oxygen radicals, decreasing thereby the risk of cardiovascular diseases and cancers (Pietta 2000; Karakaya *et al.* 2001). Although the biological effect of flavonoids is, in general, attributed to their antioxidant activity, recent investigations indicate that they might affect signalling pathways in animal cells (Williams *et al.* 2004).

It is known that the amount of each antioxidant in fruits and vegetables is strongly influenced by genotype differences and external factors such as agro-technical processes, environmental conditions, ripening degree at harvest and post harvest manipulation. This is particularly reported for various agricultural crops among them tomato (*Solanum lycopersicum* L.) (Abushita *et al.* 2000; Dumas *et al.* 2003) and pepper (*Capsicum annuum* L.) (Navarro *et al.* 2006).

In order to maximize the content of the important phytonutrients, the influences of genetics, agricultural practices and environment have been investigated. The effects of fertilisation (Owusu *et al.* 2000; Bar-Tal *et al.* 2001; Navarro *et al.* 2002), environmental conditions, and other cultural practices (Dorais *et al.* 2001) on yield and quality of many horticultural crops have been reported. However, most of these studies have focused on fruit appearance (colour, size, visual injuries, etc.) and marketable yield. In addition, the influence of growing conditions and seasonal fluctuations of bioactive compounds and antioxidant properties of many fruits and vegetables are well defined; nevertheless, studies on the effect of environmental factors on phytochemical and antioxidant properties of watermelon are very limited. The effects of growing conditions on the antioxidant properties, particularly on phenolic content of strawberry, were demonstrated (Wang and Zheng 2001). In this study, the effects of four (18/12, 25/12, 25/22, and 30/22°C) day/night growing temperature on phenolic acid, flavonol, and anthocyanin contents and antioxidant activities of two strawberry cultivars against peroxyl radicals

(ROO[•]), superoxide radicals (O₂^{•-}), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH[•]), and singlet oxygen (¹O₂) in fruit juice of 'Earliglow' and 'Kent' strawberry (*Fragaria × ananassa* Duch.) cultivars were evaluated. The authors reported that an increase in night temperature from 12 to 22°C, with the day temperature kept constant at 25°C, resulted in a significant increase in phenolic acid, flavonols, and anthocyanins. These conditions also resulted in a significant increase in antioxidant capacity.

Regarding phenolic compounds, although genetic control represents the main factor in determining their accumulation in vegetable foods, external factors may also have a significant effect on this (Macheix *et al.* 1990). In many plant species the flavonol content may be enhanced in response to elevated light levels, in particular to increased UV-B radiation (Brandt *et al.* 1995). As a matter of fact, it has been reported that cherry tomato plants grown in greenhouse under high light intensity (30% of shading) accumulated an approximately two-fold greater soluble phenols content (rutin and chlorogenic acid) than low-light intensity (73% of shading) (Wilkens *et al.* 1996).

Marked variations in quercetin contents have been observed in cherry tomatoes grown at different times of the year, but no definite seasonal trends have been evidenced (Crozier *et al.* 1997; Stewart *et al.* 2000).

On the basis of the above mentioned data for other horticultural crops, variations in climatic conditions between different seasons can be expected to significantly influence the compositional profiles of watermelon cultivars. To develop information on this subject, we compared some agronomic characteristics, total phenolics and flavonoid contents of five watermelon cultivars namely 'Crimson Sweet', 'Giza', 'Dumara', 'P503' and 'Aramis', grown in two different seasons.

MATERIALS AND METHODS

Plant culture and growth conditions

The field experiments were carried out in a field at the Research and Experimental Station of Teboulba, Monastir, Tunisia. Sowing was performed in plug-seedling trays on 19th January and 4th March 2008, respectively. Watermelons were transplanted to the soil on 29th February under a low plastic tunnel and on 16th April in open-field conditions for the early and full seasons, respectively. Transplantation was done with an in-row spacing of 125 cm and between-rows spacing of 150 cm. Plants were grown into a sandy soil, in two different growing periods (early season under low plastic tunnel and full season under field condition). In each growing season four blocks were used with 10 plants per cultivar. After transplanting, drip irrigation was applied with 4L⁻¹ drippers placed at 0.4 m intervals along the irrigation line. Drip irrigation ran for 1-3 h, at 1-2 day intervals, depending on potential evapotranspiration of the research station, climate data and crop coefficient. The production methods were in accordance with the procedures utilized by the research and experimental station of Teboulba, Monastir, Tunisia and recommended by The National Agricultural Research Institute of Tunisia (Jebbari *et al.* 2004). They included fertilization with synthetic chemical fertilizers (145 kg N ha⁻¹, 140 kg P₂O₅ ha⁻¹, 210 kg K₂O ha⁻¹). Chemical fertilizer solution was added to water irrigation by pump injection twice a week. The production methods also included a hand-weeding control and plant pathogen control with synthetic chemical pesticides. Imidacloprid (Bayer Crop Science, Nitokuno, Japan) (200 g L⁻¹) was used to reduce aphids, acetamipride (Bayer Crop Science, USA) (200 g L⁻¹) was applied to reduce thrips and abamectine (Syngenta Agro. SAS, USA) (18 g L⁻¹) was used to reduce mites. All these pesticides were applied once a cycle.

Watermelon harvest and sampling

Watermelon fruits were harvested from the rows at the appropriate ripening stage. Only watermelon fruits with an average soluble solid content ≥ 8%, were considered for analysis as recommended by Perkins-Veazie *et al.* (2006) to ensure that all watermelon

fruits reached the consumption stage. Three independent samples of at least 3 injury-free watermelon fruits for each cultivar were hand harvested randomly. Harvests were performed in both conditions 105 days after sowing (DAS) under low plastic tunnel production and 150 DAS under field condition. Watermelon fruits were quickly delivered to the laboratory and immediately cut longitudinally from the stem-end to the blossom-end through the ground spot. Flesh samples were taken from the heart area (between locular and the fruit centre) of each watermelon. Soluble solids content (°Brix) was measured immediately as described below. Approximately 1 kg of the obtained samples was homogenized in a mixer (Waring Laboratory & Science, Torrington, CT, USA) for 5 min. The homogenates were frozen at -80°C and used to determine the phenolic and flavonoid contents within less than one week to minimize the depletion of nutrients that inevitably occurs even during frozen homogenate storage (Phillips *et al.* 2010).

Soluble solid content determination

Soluble solid content was measured by cutting a wedge of flesh from the heart area and squeezing the juice into a digital refractometer (Atago PR-100, NSG Precision Cells, Inc, Farmingdale, NY, USA) calibrated with a 10% sucrose solution.

Total phenolic determination

Total phenols were extracted as described by Martínez-Valverde *et al.* (2002) on triplicate aliquots of homogenate juice (0.3 g). Briefly, 5 mL of 80% aqueous methanol and 50 µL of 37% HCl were added to each sample. The extraction was performed at 4°C, for 2 h, under constant shaking (300 rpm). Samples were centrifuged at 10,000 × g for 15 min. The total phenols assay was performed by using the Folin-Ciocalteu 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.

Flavonoid determination

The flavonoid content was determined as described by Zhishen *et al.* (1999) on triplicate aliquots of the homogenous juice (0.3 g). Aliquots (50 µL) 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 mL of 5% NaNO₂ was added. After 5 min, 60 mL of 10% AlCl₃ was added and finally 200 mL of 1 M NaOH was added after 6 min. The absorbance was read at 510 nm, using a Cecil BioQuest CE 2501 spectrophotometer, and flavonoid content was expressed as mg of rutin equivalents per kg of FW (mg RE/kg FW).

Statistical analysis

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

Climatic data

Climatic data in the area of production were recorded daily at the weather station in the Research and Experimental Station of Teboulba, Monastir, Tunisia. Mean daily maximum and minimum temperature and average global radiation detected during the whole cycle is presented in **Table 1**.

RESULTS AND DISCUSSION

Mean daily maximum and minimum temperatures during the whole cycle were: 29.2 and 14.1°C during the early growing season 39.5 and 17.5°C during the full season. Global radiation was, on average, 3245 and 3768 J cm⁻² during the first and second growing period, respectively. In

Table 1 Temperature (°C) and global radiation (joules/cm²) data recorded by the weather station of Teboulba research station, which was the closest to the experimental field where experiments were carried out. The reported values refer to 2008 and cover the entire watermelon plant growing season (January - July).

Months	Period of ten days	Extreme temperature (°C)		Global radiation (joules/cm ²)
		Minimum	Maximum	
January	1	4.7	12.8	2613
	2	3.2	10.7	2469
	3	4.7	13.2	2155
February	1	2.5	10.8	2499
	2	4.3	12.7	2679
	3	5.8	13.2	2399
March	1	8.5	20.8	2988
	2	9.2	18.7	3266
	3	10.4	22.2	3621
April	1	12.8	24.8	2698
	2	15.5	30.7	2866
	3	16.2	36.2	3127
May	1	18.5	33.0	3569
	2	17.7	35.8	3230
	3	18.1	40.5	3843
June	1	15.1	42.0	3599
	2	17.3	42.7	3762
	3	20.5	45.3	4900
July	1	19.1	46.0	4266
	2	16.3	42.7	4522
	3	17.5	47.3	4014

particular, during the month before the harvest, minimum and maximum temperatures were 18.1 and 36.4°C, 18.6 and 44°C, for the first and second growing period, respectively, whereas global radiation was 3547 and 4562 J cm⁻², respectively.

Crop yield

Marketable yields of the watermelon cultivars grown under the early (January - May) and full seasons (March - July) are presented in Fig. 1. The results showed that marketable yields were significantly different between cultivars ($P < 0.001$). 'Aramis' ranked among the best cultivar for yield in both trials.

When averaged across growing (period), marketable yield varied from 6.39 to 9.76 kg plant⁻¹ in 'P503' and 'Aramis', respectively. The results also showed that the watermelon marketable yields were significantly affected by the growing seasons independent of the cultivar ($P < 0.001$). Watermelon marketable yields were higher in full season in all cultivars. The average value of marketable yield was 6.12 and 9.78 kg plant⁻¹ in early and full seasons, respectively. The fruit yield depended mostly on the climatic conditions over the growing period.

Average fruit weight was significantly different between cultivars (Fig. 2) ($P < 0.001$). When averaged across growing seasons, the varieties produced fruit with average weight ranging between 3.66 and 7.36 kg. The fruit produced by 'Giza' and 'P503' were small sized fruit. The highest mean fruit fresh weight was recorded for 'Aramis'. The results also showed that mean fruit fresh weight was not affected by the growing seasons independent of the cultivar ($P > 0.05$).

Soluble solids

The soluble solids of the watermelon cultivars grown under low plastic tunnel and field conditions are presented in Fig. 3. The soluble solids were significantly different between cultivars ($P < 0.001$). 'Aramis' ranked among the best cultivar for soluble solids in both trials. When averaged across both growing periods, soluble solids varied from 9.13 to 11.26 in 'Giza' and 'Aramis', respectively. The results also

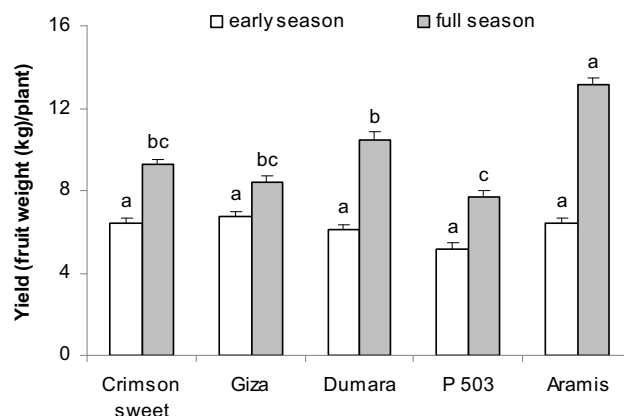


Fig. 1 Marketable yields of the watermelon cultivars grown under the early (January - May) and full seasons (March - July). Data are means of three replicates \pm standard error. Bars marked with the same letters are not significantly different (LSD test, $P < 0.05$).

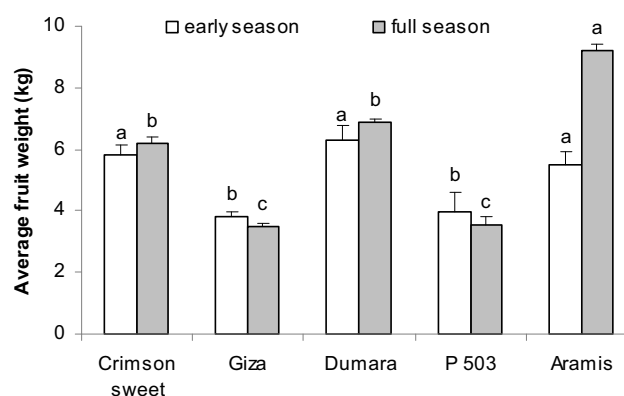


Fig. 2 Average fruit weight of the watermelon cultivars grown under the early (January - May) and full seasons (March - July). Data are means of three replicates \pm standard error. Bars marked with the same letters are not significantly different (LSD test, $P < 0.05$).

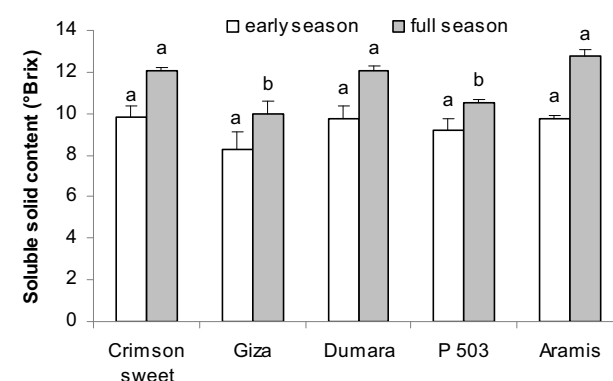


Fig. 3 The soluble solids of the watermelon cultivars grown under the early (January - May) and full seasons (March - July). Data are means of three replicates \pm standard error. Bars marked with the same letters are not significantly different (LSD test, $P < 0.05$).

showed that the watermelon soluble solids were significantly affected by the growing seasons independent of the cultivar ($P < 0.001$). Watermelon soluble solids were higher in full season in all cultivars. The average value of soluble solids was 9.36 and 11.48 in early and full seasons, respectively. In this respect, Winsor and Adams (1976) reported that an increase in the amount of solar radiation received by the plant leads to an increase in the rate of photosynthesis by the plants, which leads to an accumulation of carbohydrates (mainly in the form of sugars) in tomato (*Solanum lycopersicon* L.) fruit.

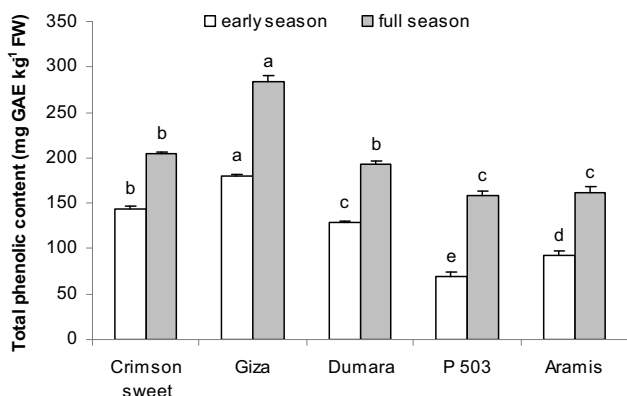


Fig. 4 The total phenolics of the watermelon cultivars grown under the early (January - May) and full seasons (March - July). Data are means of three replicates \pm standard error. Bars marked with the same letters are not significantly different (LSD test, $P < 0.05$)

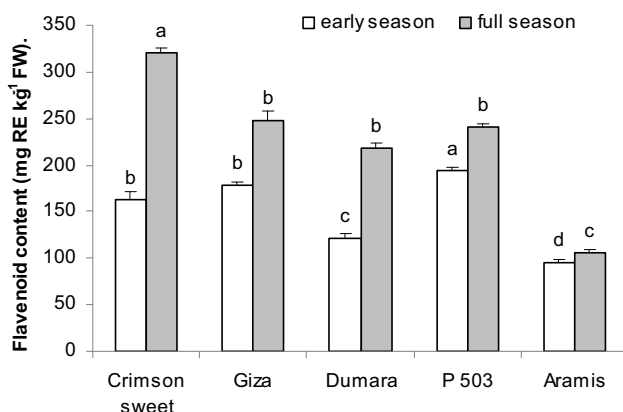


Fig. 5 Flavonoid content of the watermelon cultivars grown under the early (January - May) and full seasons (March - July). Data are means of three replicates \pm standard error. Bars marked with the same letters are not significantly different (LSD test, $P < 0.05$).

Total phenolics and flavonoids

The total phenolics and flavonoid contents of the watermelon cultivars grown under low plastic tunnel and field conditions are presented in Fig. 4 and 5, respectively. Concerning total phenolic contents, the results showed that values were significantly different between cultivars ($P < 0.001$). 'Giza' ranked among the best cultivar for total phenolics in both trials. When averaged across growing period, total phenolics varied from 113.93 to 232.15 mg GAE kg⁻¹ FW in 'P503' and 'Giza', respectively.

The total phenolics was also characterized by marked variations between growing period independent of the cultivar ($P < 0.001$), ranging from 122.81 to 200.69 mg GAE kg⁻¹ FW in early and full seasons, respectively. The mean total phenolics of the five cultivars was 62% higher in the samples collected under field conditions during summer (July) than collected under low plastic tunnel during spring (May).

Regarding flavonoids, the results showed that values were significantly different between cultivars ($P < 0.001$). 'Crimson sweet' ranked among the best cultivar for flavonoid contents in both trials. When averaged across growing period, flavonoid contents varied from 100.53 to 241.625 mg RE kg⁻¹ FW in 'Aramis' and 'Crimson sweet', respectively. The results also showed that the watermelon flavonoid contents were significantly affected by the growing seasons independent of the cultivar ($P < 0.001$). Watermelon flavonoid contents were higher in full season in all cultivars. The average value of flavonoid content was 150.60 and 226.44 mg RE kg⁻¹ FW in early and full seasons,

respectively. To our knowledge, these results are the first data on phenolic and flavonoid contents in watermelon cultivars as affected by growing seasons, even though this can be due to an increase in the amount of UV radiation associated with an increase in solar radiation received by the plants. The increased temperature and increase in the age of plants may have also caused an accumulation of phenolics in plants. Among studies on other crops, Howard *et al.* (2002) reported that the antioxidant capacity and phenolic content of spinach (*Spinacia oleracea* L.) was greatly affected by the growing season. They attributed the variation to the difference in temperature and light intensity. In addition, Toor *et al.* (2006) reported seasonal variation in the antioxidant components of tomato. They mentioned higher levels of phenolics (62% higher) in the tomato fruit collected during summer than the spring. Therefore, summer greenhouse tomato fruit which receives a higher amount of light and UV radiations have been reported to contain a higher amount of phenolics and flavonoids.

CONCLUSIONS

Our data highlight the important role played by environmental conditions in determining the phenolic and flavonoid contents of watermelon. A wide variation in phenolic content according to the season was observed. Therefore, our results confirmed that the hot temperatures and high solar radiation of mid-summer in the Mediterranean basin may produce a positive effect on this secondary metabolite accumulation.

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