



Determination of healthy natural antioxidants in selected muskmelon (*Cucumis melo*) cultivars

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ABSTRACT

Muskmelon (*Cucumis melo* L.) is a popular summer fruit consumed all over the world due to the combination of refreshingly sweet taste, pleasant flavour and nutritional value. Melon fruit is characterized by germplasm variability with superior quality. This study aimed to evaluate 5 promising local muskmelon cultivars (Maazoun, Galaoui, Stambouli, Trabelsi, Asli) for some physicochemical characteristics, the content of different bioactive compounds and the antioxidant activity. The experiments were conducted for two consecutive years 2018 and 2019 at the support station of the inter-professional vegetable group positioned in the North East of Tunisia. Significant differences were observed across the melon cultivars ($P < 0.05$) for soluble solids (9–12°Brix), pH (5.5–6.6), carotenoid (7.5–59.5 mg/kg FW), phenolic (400–1390 mg GAE/kg FW), flavonoid (251.3–390 mg RE/kg FW), vitamin C (138.2–193 mg/kg FW) contents, as well as hydrophilic (102.28–285.43 μ M Trolox/100 g FW) and lipophilic (30–180.22 μ M Trolox/100 g FW) antioxidant activities. Galaoui showed the highest levels of carotenoids, phenolics, flavonoids, vitamin C and antioxidant activities followed by Stambouli. Trabelsi cultivar also had a high level of flavonoids. The enhanced hydrophilic antioxidant activity in Galaoui was significantly correlated to phenolic content ($r = 0.91$). These findings proved that besides being refreshing summer fruit, muskmelon can be considered a promising healthy produce with a superior potential source of natural antioxidants.

Keywords: Antioxidants composition, Correlation, Hydrophilic antioxidant activity, Lipophilic antioxidant activity

Melon (*Cucumis melo* L.) commonly known as muskmelon, is a highly produced and consumed fruit in the world. According to Food and Agriculture Organization database, the total world's harvested area and the global annual production were 1068238 ha and 28.46 million tones, respectively in 2020 (FAOSTAT 2020). Muskmelon is also a favourite summer fruit in Tunisia. In 2020, a total of 9881 ha were dedicated to this crop and its production attained 104432 tonnes (FAOSTAT 2020). In addition to its superior consumer preference, it is an extremely healthful food choice as it provides wide dietary antioxidants with recognized biological relevance, such as carotenoids, phenols and vitamins, as well as a number of other human health compounds (Henane *et al.* 2016). These compounds provide its beneficial roles in the human diet, reducing the risk of certain diseases (Ayseli and Ayseli 2016). Indeed, muskmelon has been recognized as a good source of

carotenoids, especially β -carotene, the pigment responsible for the orange flesh colour of the ripe-fruit. Equally, muskmelons are known as being rich in polyphenols with important antioxidant activity potential (Rodriguez-Perez *et al.* 2013) and a range of other antioxidants including vitamin C (Lester and Hodges 2008).

Besides, antioxidant components and functional properties are also increasingly considered as quality traits in muskmelon (Henane *et al.* 2016). Measuring antioxidant activity in fractions better assesses potential of fruits and vegetables than analyzing single compounds. However, although their great health significance, only few studies has been done on the functional quality of muskmelon. The correlation between bioactive compounds and antioxidant activity is of primary importance in fruits and vegetables. Little information on these types of correlations in muskmelon was reported earlier (Şelale *et al.* 2012). The current study was designed to assess some physicochemical traits and bioactive compounds of 5 muskmelon cultivars. The antioxidant activities (hydrophilic and lipophilic) and their correlation with bioactive compound values were also determined to establish the relationship between these variables.

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MATERIALS AND METHODS

Plant materials: Five muskmelon cultivars, previously selected as part of the horticulture laboratory breeding programme based on their superior powdery mildew resistance at the National Agricultural Research Institute of Tunisia were utilized (Jebari *et al.* 2004). These cultivars were Galaoui (orange flesh colour), Stambouli (light orange flesh colour), Trabelsi (very light green flesh colour), Maazoun (light green flesh colour) and Asli (cream flesh colour) based on colour visual identification.

Plant culture: The experiments were conducted during 2018 and 2019 at the support station of the inter-professional vegetable group positioned in the North East of Tunisia (36°48'28"N, 10°6'4"E). Melon seeds were sown in plug-seedling trays at the beginning of March. The muskmelon plants were transplanted around mid-April in 2018–2019 with an in-row and between row spacing of 100–150 cm, respectively in a randomized complete block design. Three blocks (replicates) were used with 10 plants per cultivar. The cultivation methods recommended by the National Agricultural Research Institute of Tunisia comprised chemical fertilizers (85 kg/ha N, 70 kg/ha P₂O₅, 130 kg/ha KNO₃, 80 kg/ha MgSO₄), drip irrigation and hand-weeding control were used. Synthetic chemical pesticides: Imidacloprid (Promochimie, Tunis, Tunisia, 200 g/L), Acetamiprid (SEPCM, Tunis, Tunisia, 200 g/L) and Triforine (Saprol, BASF Agro SAS, 18 g/L) were used once in the cycle for pathogen treatments.

Fruit sampling and preparation: At the time of maturity, the melon fruits were picked at random and quickly taken to the laboratory for analysis. The fruit maturity was assessed in the field based on their ease of separation from the vine with a slight twist leaving a clean cavity. The melon fruits were cut longitudinally and the samples were taken from the central part of each sample fruit. The flesh of the muskmelons was divided, sliced into piece sections and homogenized using a mixer (Waring Laboratory and Science, Torrington, CT, US). Then covering the homogenates rapidly with aluminium to avoid the photo-oxidation and preserved kept under -80°C for additional fruit quality chemical analysis, which was performed in triplicates for each sample.

Physicochemical trait determination: The fruit, water soluble dry-matter content was estimated using a digital refractometer (Atago PR-100, NSG Precision Cells, Inc., Farmingdale, NY, USA) by applying the first juice drops on its surface and the results were expressed as Brix°. The fruit juice pH was determined using a pH meter (WTW, Microprocessor pH Meter, pH 539, Weilheim, Germany).

Carotenoids content determination: The fruit's carotenoid content was measured following the method reported by Henane *et al.* (2016). The extraction included a combination of hexane-ethanol-acetone (2–1–1) and 0.05% butylated hydroxytoluene. Carotenoid content was quantified by measuring the absorbance at 450 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) and the data were expressed as

milligrams (mg) of carotenoid equivalents per kg fresh weight (mg/kg FW).

Total phenolics content determination: The total phenol content of muskmelon was determined using the Folin-Ciocalteu formula. On aliquots (0.3 g) of each fraction, phenolics were extracted as defined by Martinez-Valverde *et al.* (2002). Each sample was given 5 ml of 80% aqueous methanol and 50 ml of 37% HCl. For 2 h, the mixture was held at 4°C with continuous stirring (300 rpm). The samples were centrifuged for 15 min at 10,000 g. Finally, the Folin–Ciocalteu agent was added to 50 µL samples of the supernatant. The absorbances were measured using a BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at a wavelength of 750 nm. Gallic acid was used to generate a linear calibration curve and the values were represented in milligrams of gallic acid equivalents per kilogram of fresh weight (mg GAE/ kg FW).

Flavonoid content determination: The flavonoid content in (0.3 g) aliquots of the homogeneous juice was analyzed following Zhishen *et al.* (1999) procedure. After 5 min, 60 µL of 10% AlCl₃ solution was applied, followed by 200 µL of 1 m NaOH after 6 min. The absorbances of the mixtures were measured at 510 nm using a Cecil BioQuest CE 2501 spectrophotometer. A standard Rutin calibration solution was used and data were expressed as milligrams of Rutin equivalent per kg fresh weight (mg RE/kg FW).

Vitamin C content determination: The method used to determine Vitamin C (ascorbic acid content) was as described by Kampfenkel *et al.* (1995) on a (0.1 g) sample of homogeneous juice. Vitamin C extraction was assayed using 6% metaphosphoric acid and determined using the absorbance at 525 nm by a Cecil BioQuest CE 2501 spectrophotometer. Data were expressed as milligrams of vitamin C per kg fresh weight (mg/kg FW).

Hydrophilic and lipophilic antioxidant activity determination: The ABTS radical scavenging capacities were measured according to the methods of Miller and Rice-Evans (1997). TEAC assay was applied and used for the measurement of the antioxidant activities of hydrophilic and lipophilic fractions (HAA and LAA, respectively). Sample centrifugation was done at 10,000 g for 7 min and the supernatant was collected prior to its utilization for antioxidant activity measurements. Absorbances of the samples were consequently measured at 734 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). A linear calibration curve was determined with Trolox solution (0–16 µM Trolox) and data were expressed as µM of Trolox per 100 g of fresh weight (µM Trolox/100 g FW).

Data analysis: SAS Version 9.1 program was used to statistically analyze all data using one-way analysis of variance (ANOVA) (SAS Institute, Cary, NC, USA). The least significant difference (LSD) procedure was used to compare the mean values (P<0.05). Correlations between parameters were evaluated using Pearson's correlation coefficient (r). There were no significant differences between the two studied years 2018–2019 (P>0.05) and therefore the

Table 1 Soluble solids, pH, carotenoids, total phenols, flavonoids and vitamin C contents in different muskmelon cultivars

Cultivar	Soluble solid (°Brix)	pH	Carotenoid (mg/kg FW)	Phenol (mg GAE/kg FW)	Flavonoid (mg RE/kg FW)	Vitamin C (mg/kg FW)
Maazoun	11.0 ± 0.1 ^b	5.6 ± 0.4 ^c	7.5 ± 2.1 ^d	450.0 ± 1.1 ^d	251.3 ± 1.2 ^c	158.7 ± 0.6 ^c
Galaoui	10.3 ± 0.1 ^c	6.1 ± 0.3 ^b	59.5 ± 3 ^a	1390.0 ± 2 ^a	390.0 ± 3.0 ^a	193.0 ± 0.5 ^a
Stambouli	9.0 ± 0.1 ^c	5.5 ± 0.3 ^c	41.3 ± 4.1 ^b	1080.0 ± 0.1 ^b	264.3 ± 1.5 ^b	186.5 ± 0.6 ^b
Trabelsi	9.5 ± 0.1 ^d	5.6 ± 0.4 ^c	8.5 ± 3.0 ^{cd}	400.0 ± 0.3 ^e	388.3 ± 3.1 ^a	151.2 ± 0.5 ^d
Asli	12.0 ± 0.1 ^a	6.6 ± 0.3 ^a	9.5 ± 2 ^c	885.8 ± 1.1 ^c	263.3 ± 1.5 ^b	138.2 ± 0.7 ^e
LSD	0.16	0.12	1.28	27.89	2.02	3.00

Values are mean of triplicate analyses ± standard error; Values with different letters are significantly different at P<0.05 by LSD test.

data presented were the mean value of the 2018–2019 years.

RESULTS AND DISCUSSION

Physicochemical characteristics: The soluble solids content and the pH are useful parameters for predicting the maturity and consumer preference for melon. The soluble solids content and the pH varied significantly between the studied muskmelon cultivars (P<0.05) (Table 1). The highest soluble solids content value was obtained for Asli (12°Brix) and the lowest value was obtained for Stambouli (9°Brix). The values obtained were in the line of those reported by Fundo *et al.* (2018) (10.35° Brix) in different melon cultivars. The pH, values ranged from 5.5 in Stambouli to 6.6 in Asli.

The pH data were close to the value (6.14) reported by Fundo *et al.* (2018) in cantaloupe melon. The different cultivars have interesting values for soluble solids and pH because muskmelon was generally appreciated by consumers for their sweetness and low level of acidity.

Carotenoid content: Values for carotenoid contents among cultivars varied significantly (P<0.05) and ranged from 7.5 mg/kg FW in the light green-flesh colour cultivar Maazoun to 59.5 mg/kg FW in the orange-flesh colour cultivar Galaoui. Galaoui had at least 9-fold greater than Maazoun. The cultivar Stambouli ranks second with 41.3 mg/kg FW. The reported values were in line with our previous results (Henane *et al.* 2016) and are comparable with Menon and Rao (2014) who reported that orange fleshed melon cultivars have higher levels of carotenoid than green-fleshed cultivars. Our results further proved that melon is a significant source of carotenoids (Maieitti *et al.* 2012).

Total phenol and flavonoid content: The total phenol and flavonoid contents varied significantly between the different studied melon cultivars (P<0.05) (Table 1). Total phenols ranged from 400 mg GAE/kg FW in Trabelsi to 1390 mg GAE/kg FW in Galaoui. The cultivar Stambouli ranked second with 1080 mg GAE/kg FW. These results are in agreement with those of Menon and Rao (2014) who noticed a substantial amount of phenolic compound in orange-fleshed compared to green-fleshed melon cultivars. Thus, these differences were largely attributed to genotypic variation affecting the melon total phenolic content. Although the genetic variation is the most important factor affecting the amount of phenols in fruits and vegetables,

environmental factors like light and temperature may also alter their levels (Ferreira *et al.* 2023). Muskmelon can therefore be considered a source of phenolic compounds due to its availability and high consumption, which can contribute considerably to increasing the daily dietary intake of these compounds.

Flavonoids are the most abundant and widely dispersed polyphenolic compounds found in plants. Flavonoid contents varied from 251.3 mg RE/kg FW to 390 mg RE/kg FW in Maazoun and Galaoui, respectively. Galaoui was found to have flavonoid content similar to Trabelsi, and then comes Stambouli. The outcomes were significantly higher than those published by Ibrahim and El-Masry (2016), who found that flavonoids content in cantaloupe varieties achieved 2.05 mg RE/kg FW. The increased flavonoid content of the cultivars studied is probably due to genotypic and environmental differences. High flavonoid contents ranging from 100.5–241.6 mg RE/Kg FW have also been reported in watermelon cultivars (Tlili *et al.* 2013). Based on our findings, melon cultivars can be considered an effective source of flavonoids.

Vitamin C content: Vitamin C varied significantly between the studied muskmelon cultivars (P<0.05) (Table 1). Vitamin C content ranged from 138.2–193 mg/kg FW in Asli and Galaoui, respectively, followed by Stambouli with 186.5 mg/kg FW. The obtained vitamin C values are in line with those reported for muskmelon varieties ranging from 100–300 mg/kg FW (Pandey *et al.* 2021). Higher values attaining 1075.9 mg/kg FW for the juice of cantaloupe melon were reported by Fundo *et al.* (2018). The data confirm the results of Hodges and Lester (2006) who reported that the synthesis of vitamin C was different in various cultivars grown in different conditions. These divergent results were probably due to the variety and/or climatic conditions (Bernal *et al.* 2014).

Hydrophilic and lipophilic antioxidant activities (TEAC assay): Hydrophilic and lipophilic antioxidant activity (HAA and LAA) varied significantly between the studied melon cultivars (P<0.05) (Fig 1). The HAA in Galaoui was at least 2.8-fold greater (285.43 µM Trolox/100 g FW) than that obtained in Trabelsi (102.28 µM Trolox/100 g FW) followed by cultivar Stambouli. Comparable values were reported by Henane *et al.* (2015) for different genotypes of melon.

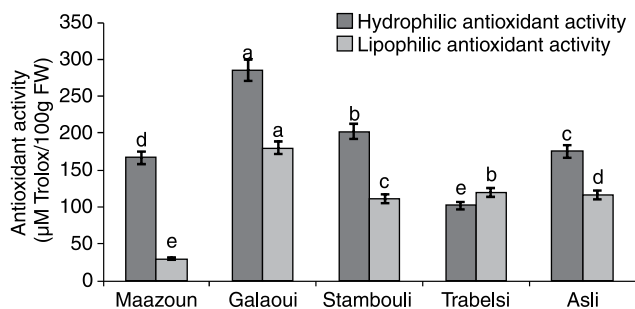


Fig 1 Hydrophilic and lipophilic antioxidant capacity of muskmelon cultivars determined by the Trolox equivalent antioxidant capacity assay.

Values are the mean of triplicate analyses \pm standard error; Values with different letters are significantly different at $P < 0.05$ by LSD test.

The lowest and the highest LAA values were recorded in Maazoun (30 μM Trolox/100 g FW) and Galaoui (180.22 μM Trolox/100 g FW) cultivars. The LAA in Galaoui was at least 6-fold greater than that obtained in Maazoun. Significant differences in lipophilic antioxidant activity were also obtained previously when comparing 4 melon cultivars (Henane *et al.* 2016). In other research, Hodges and Lester (2006) studied total hydrophilic and lipophilic antioxidant activities in 3 muskmelon cultivars during storage. They found no overall change in total hydrophilic and lipophilic antioxidant activity levels, expressed in Trolox equivalents per gram dry weight (Trolox/100 g FW), within cultivars. The highest hydrophilic and lipophilic antioxidant activities were detected in Galaoui with 283.63 μM Trolox/100 g FW and 160.92 μM Trolox/100 g FW, respectively. This emphasizes that orange-fleshed melon has a greater antioxidant potential than green-fleshed muskmelon.

The total antioxidant activity (HAA + LAA) varied from 197.23 μM Trolox/100 g FW in the green flesh colour cultivar Maazoun to 465.65 μM Trolox/100 g FW in the orange flesh cultivar Galaoui. The data reported that orange-fleshed muskmelon had higher antioxidant activities and higher levels of phenolic compounds. Nevertheless, the values were higher than those reported for different muskmelon cultivars ranging from 40.7–75 μM Trolox/100 g FW (Ionica *et al.* 2015). The differences were also probably due to genetic or agro-environmental factors.

Correlation analysis: The relationship between phytochemicals and antioxidant activities was examined in various fruits and vegetables. However, in muskmelon, these kinds of information are partial. In this study, significant correlation between HAA values and phenolic content was obtained (Table 2).

Therefore, it can be concluded that the HAA of muskmelon was mainly attributed to the phenolic compounds. Tristán *et al.* (2022) found a similar correlation in melon using total antioxidant activity and phenols. The results also showed that no significant correlation between LAA and carotenoids was detected. Hence, other lipophilic compounds may be responsible for most of the LAA values

Table 2 Pearson correlation coefficients (r) and related significance between antioxidant content and antioxidant activities

Trait	TEAC assay	
	r	p
Total phenolics	0.91	< 0.01
	0.75	ns
Vitamin C	0.11	ns
	0.29	ns
Flavonoids	0.29	ns
	0.29	ns
Carotenoids	0.29	ns
	0.29	ns

n (sample size) = 15; ns, no significant correlation.

in muskmelon fruits.

Based on the results, it can be concluded that some muskmelon cultivars are a significant source of carotenoids and phenolics in the Mediterranean diet and can be considered as a functional food. It also highlights the important role of genotypic differences in determining bioactive compounds and antioxidant activity of muskmelons. The orange and the light orange flesh coloured melon cultivars Galaoui and Stambouli, showed higher levels of carotenoid, phenolic, flavonoid, vitamin C contents as well as a higher antioxidant activity and were identified as superior healthy natural antioxidant genotypes. Phenolics showed a positive correlation with the hydrophilic antioxidant activity and are the major contributors of this activity in muskmelon. Future studies on the effect of agro-technical processes with antioxidant potential are required.

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