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Fractionate analysis of the phytochemical composition and antioxidant activities in advanced breeding lines of high-lycopene tomatoes

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This study investigates the antioxidant components [lycopene, total phenolics, total flavonoids, ascorbic acid (AsA) and dehydroascorbic acid (DHA)] as well as antioxidant activities of the hydrophilic and lipophilic fractions (AAHF and AALF) of peel, pulp and seed fractions isolated from red-ripe berries of the ordinary tomato cultivar Rio Grande and the two high-lycopene tomato breeding lines HLT-F61 and HLT-F62 simultaneously grown in an open-field of Northern Tunisia. Significant differences ($p < 0.05$) were found among cultivars for each trait studied. All fractions isolated from the red-ripe berries of HLT lines showed higher lycopene, total phenolics and total flavonoid contents, as well as higher AAHF and AALF, than those isolated from Rio Grande. Regardless of the fraction, HLT-F61 had the highest lycopene content (893.0 mg per kg fw, 280.0 mg per kg fw, and 47.5 mg per kg fw in peel, pulp and seed fractions, respectively) and total phenolics at least 2-fold and 3-fold higher than HLT-F62 and Rio Grande, respectively. Peel and seed fractions from HLT-F61 red-ripe tomato berries had the highest AsA content (345 mg per kg fw and 115 mg per kg fw, respectively), while no significant difference was found in the seed fraction between HLT-F62 and Rio Grande. The HLT-F62 pulp fraction showed the highest content of AsA (186 mg per kg fw) and DHA (151 mg per kg fw) among all the assayed cultivars. Except for the peel fraction, where HLT-F61 had similar AAHF values to HLT-F62, the high-lycopene line HLT-F61 showed higher AAHF values than HLT-F62 and Rio Grande. Regardless of the fraction, the highest AALF values were recorded in HLT-F61 berries. Thus, both HLT tomato lines are promising for the introduction, as advanced hybrids, in either fresh market or processing industry.

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1. Introduction

Tomato (*Solanum lycopersicum* L.) berries, commonly consumed in the Mediterranean diet, offer a diverse mixture of nutrients that are essential for human nutrition and contribute to the promotion of good health and wellbeing. Increased consumption of fresh or processed tomato products (canned tomatoes, sauce, juice, ketchup, soup, *etc*.) is directly associated with a reduced risk of contracting several widespread human pathologies, including cardiovascular diseases, prostate, lung and stomach cancers, osteoporosis and UV radiation associated skin disorders.[1–5] Flavonoids, phenols, ascorbic acid (vitamin C), tocochromanols (vitamin E) and carotenoids, mainly lycopene, are important bioactive molecules of ripe tomato fruits.[6–11] These compounds synergize to exert positive effects on human health through oxidative and still not fully understood non-oxidative mechanisms.[1,3–5,12] Consequently tomato fruits are increasingly considered as "functional food".[9–11,13] Besides pulp, tomato peels and seeds are also characterized by high contents of lycopene and phenolic compounds.[14] Together peel and seeds constitute the major agro-industrial by-product (pomace) obtained from tomato fruit processing for juice, paste and ketchup, represent a cheap and abundant (4% by weight of processed tomatoes) source for the extraction of bioactive molecules, not only provide natural antioxidants for nutraceutical, cosmetic and pharmaceutical usage, but also offer important economic advantages and help in resolving the environmental issue of tons of agro-industrial waste.[15–17] Recently, consumers concern about the safety of different synthetic antioxidant food additives has increased shifting the interest toward natural antioxidant molecules.[18]

Tomato seeds account for approximately 10% of the fresh tomato fruit and 60% of the pomace weight. They are a good source of protein (35%) and fat (25%).[19] Al-Wandawi *et al.*[20]

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reported that tomato peel contains a significantly higher amount of lycopene compared to other fruit fractions; they also reported higher levels of amino acids in tomato peel compared to wheat and a considerably high level of minerals in both seed and peel fractions compared to rice, wheat and barley. However, they did not focus on other antioxidant classes. Toor and Savage[14] focused on the bioactive compounds and antioxidant activities in different fractions of three tomato cultivars namely 'Excell', 'Tradiro' and 'Flavourine' grown using a hydroponic fertigation system under greenhouse conditions. They found that peels, followed, in most cases by seed, were not only characterized by the highest content of total phenolics, flavonoids and lycopene but also by the highest AAHF and AALF. Similarly, Chandra and Ramalingam[21] and Chandra *et al.*[22] confirmed that peel and seed fractions of different tomato cultivars grown in India under polyhouse conditions accumulated high levels of lycopene, AsA and phenolics. They also found the antioxidant activities of seed and peel fractions of all studied cultivars much higher compared to pulp, using either the Ferric Reducing Antioxidant Power (FRAP) or the DPPH radical scavenging activity assays.

Recently, Vínha *et al.*[23] appraised the effect of peel and seed removal on the nutritional value and antioxidant activities of four typical Portuguese cultivars (Cereja, Chucha, Rama and Redondo). The authors found that peeling was in general detrimental, attaining on average a 71% decrease in lycopene, 50% in β-carotene, 32% in total phenolics and 14% in ascorbic acid contents, as well as an 8–10% decrease in antioxidant activities. Besides, although seed removal increased both color and sweetness of the processed product, valuable bioactive compounds (11% of carotenoids and 24% of phenolics) as well as antioxidant activities (5%) were lost. Siddiqui *et al.*[13] recently assessed different bioactive compounds in the peel and pulp of sixteen newly developed tomato hybrids containing *dg*, *ogc* and *rin* genes. The authors found that tomato peel is a source of valuable phytochemicals for nutraceutical and functional food applications. However, the peel and pulp lycopene content in different tomato crosses was rather low when compared to those generally accumulated by genotypes harboring genes leading to an increased carotenoid content (*dg* and *ogc* genes).

In vitro studies revealed that lycopene is 2-fold and 10-fold more effective in quenching reactive oxygen species than β-carotene and α-tocopherol, respectively and has the highest Trolox Equivalent Antioxidant Capacity (TEAC) value among all carotenoids.[24] This stressed the need for increasing lycopene levels in tomato fruits,[25] leading to a large number of new tomato lines with increased levels of lycopene (high-lycopene tomatoes) being recently developed by conventional plant breeding techniques to satisfy the increasing demand of growers, processors and consumers for high nutritive quality foods.[7] Several studies focused on the antioxidant compounds and antioxidant activities in different high-lycopene tomato cultivars.[6,7–11,26–31] It has been established that high-lycopene tomato hybrids are characterized by a considerable higher level of carotenoids, particularly lycopene, in comparison with the ordinary tomato cultivars. However, in all these studies, antioxidants have been measured in whole fresh tomatoes or processed tomato products without separating the different fruit portions. Although many authors reported that most of the antioxidants in ordinary tomato cultivars are associated with the peel and seed fractions,[8,14,32,33] still there is a lack of information on the level of various antioxidants in the peel and seed fractions of high-lycopene tomato cultivars grown under open-field conditions.

In this study, the main phytochemical contents (lycopene, total phenolics, total flavonoids, AsA and DHA) as well as the AAHF and AALF were assessed in the peel, pulp and seed fractions of two high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) and the ordinary (Rio Grande) tomato cultivar grown simultaneously under open-field conditions.

2. Results and discussion

2.1. Lycopene content

The lycopene content in peel, pulp and seed fractions of the ordinary (Rio Grande) and high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) grown in open-fields in Tunisia are reported in Table 1. The lycopene content in the peel, pulp and seed fractions was significantly different among the studied tomato cultivars ($p < 0.05$). In all fractions, the highest and the lowest lycopene contents were recorded for

Table 1 Lycopene, total phenolics, total flavonoids, ascorbic acid and dehydroascorbic acid contents in the ordinary (Rio Grande) and high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) grown in an open-field. Values represent mean ± S.E. of three replicates. For each trait, values within the column followed by the same superscript letter are not significantly different (LSD test, $p < 0.05$)

Fractions	Peel	Pulp	Seeds
Lycopene		(mg per kg of fw)	
HLT-F61	893.0 ± 8.8^a	280.0 ± 10.0^a	47.5 ± 1.6^a
HLT-F62	508.2 ± 7.8^b	167.2 ± 9.6^b	28.5 ± 0.9^b
Rio Grande	423.7 ± 9.1^c	100.9 ± 6.1^c	18.4 ± 0.6^c
Total phenolics		(mg GAE per kg of fw)	
HLT-F61	930.3 ± 5.8^a	256.2 ± 18.2^a	941.8 ± 7.3^a
HLT-F62	430.3 ± 11.5^b	216.1 ± 10.0^a	436.8 ± 14.6^b
Rio Grande	331.7 ± 12.7^c	166.0 ± 2.9^b	319.2 ± 7.0^c
Total flavonoids		(mg RE per kg of fw)	
HLT-F61	783.5 ± 14.6^a	552.1 ± 25.2^a	650.0 ± 7.7^a
HLT-F62	512.6 ± 6.3^b	222.0 ± 10.2^b	318.7 ± 6.3^b
Rio Grande	303.4 ± 3.5^c	144.3 ± 2.6^c	215.2 ± 4.7^c
Ascorbic acid		(mg per kg of fw)	
HLT-F61	344.6 ± 8.0^a	125.3 ± 10.2^b	115.3 ± 3.0^a
HLT-F62	261.5 ± 5.7^b	186.0 ± 12.7^a	82.3 ± 2.6^b
Rio Grande	170.5 ± 6.9^c	118.8 ± 5.8^b	74.2 ± 2.2^b
Dehydroascorbic acid		(mg per kg of fw)	
HLT-F61	153.2 ± 5.0^a	86.9 ± 8.8^b	75.2 ± 2.8^a
HLT-F62	98.1 ± 4.1^b	150.6 ± 5.8^a	74.9 ± 5.4^a
Rio Grande	134.3 ± 3.0^a	64.6 ± 6.0^b	64.4 ± 2.4^a

Table 2 Lycopene, total phenolics, total flavonoids, ascorbic acid, dehydroascorbic acid as well as the antioxidant activities of the hydrophilic and lipophilic fractions in peel, pulp and seeds (means ± standard error of mean of the three tomato cultivars). Values within the column followed by the same superscript letter are not significantly different (LSD Test, $p < 0.05$)

Fractions	Lycopene (mg per kg of fw)	Total phenolics (mg GAE per kg of fw)	Total flavonoids (mg RE per kg of fw)	Ascorbic acid (mg per kg of fw)	Dehydroascorbic acid (mg per kg of fw)	Antioxidant activity of the hydrophilic fraction (μM Trolox per 100 g of fw)	Antioxidant activity of the lipophilic fraction (μM Trolox per 100 g of fw)
Peel	608.3 ± 144.6[a]	564.1 ± 184.8[a]	534.8 ± 140.6[a]	258.8 ± 50.3[a]	128.5 ± 16.2[a]	414.1 ± 80.0[a]	546.8 ± 46.5[a]
Pulp	182.7 ± 52.3[b]	212.8 ± 20.1[a]	306.1 ± 125.2[c]	143.4 ± 21.4[b]	101.0 ± 25.8[a]	188.5 ± 43.9[b]	239.2 ± 58.0[b]
Seeds	31.5 ± 8.5[b]	566.1 ± 190.8[a]	394.7 ± 131.3[b]	90.6 ± 12.6[b]	71.5 ± 3.6[a]	251.0 ± 34.0[b]	323.8 ± 83.0[c]

HLT-F61 and Rio Grande, respectively. The lycopene content ranged from 423.7 to 893.0 mg per kg fw in the peel, from 100.9 to 280.0 mg per kg fw in the pulp and from 18.4 to 47.5 mg per kg fw in the seed fractions. Compared to Rio Grande, variations ranging from 19% to 110% in the peel, 66% to 177% in the pulp, and 55% to 158% in the seeds of HLT-F61 and HLT-F62 were detected. In this study, significantly ($p < 0.05$) higher levels of lycopene were detected in the peel of tomatoes compared to pulp and seed fractions (Table 2). The peel was found to contain 3 to 4 times the lycopene content found in the pulp, consistently with previous results on ordinary grown tomato cvs grown under greenhouse conditions. George *et al.*[34] reported that tomato peels had 2.5-fold the lycopene content found in pulp. Al-Wandawi *et al.*,[20] Ilahy and Hdider[33] and Ilahy *et al.*[8] reported a 3 to 5 times higher peel lycopene content compared to pulp. The obtained lycopene values are in line with those reported by many authors ranging from 50 to 1000 mg per kg fw for peel of tomato cultivars from different geographical areas.[8,20,33–35] George *et al.*[34] studied the variation in contents of various bioactive compounds in tomato pulp and peel of twelve different genotypes. The lycopene content ranged from 48.3 to 141.0 mg per kg fw in peels and from 20.4 to 115.0 mg per kg fw in the pulp. Toor and Savage[14] reported that the peel lycopene content of three New Zealand greenhouse-grown tomato cultivars ranged from 65 to 102 mg per kg fw. Chandra and Ramalingam[21] and Chandra *et al.*[22] measured the lycopene content in peel, pulp and seed fractions of different Indian tomato cultivars grown under greenhouse conditions. The authors detected variations ranging from 53.2 to 240.8 mg per kg fw in the peel, from 25.5 to 169.7 mg per kg fw in the pulp and from 8.1 to 43.9 mg per kg fw in seeds. Also, Vínha *et al.*[23] conducted a study with four Portuguese tomato cultivars reporting that peel removal caused a significant loss (65–80%) of lycopene in fruits of every cultivar, while seed elimination decreased mainly the amount of total phenolics. Recently Siddiqui *et al.*[13] assessed the bioactive attributes of tomatoes possessing *dg*, *ogc* and *rin* genes. Although the authors reported that hybrids developed from parental lines harboring the *dg* genes were superior to those developed from parental lines carrying the *ogc* and *rin* genes, the lycopene content was lower than those obtained in this experiment and ranged from 80.6 to 246.0 mg per kg fw in peel and from 21.9 to 42.5 mg per kg fw in the pulp.

2.2. Total phenolics content

The total phenolics content in peel, pulp and seed fractions of the investigated tomato genotypes is reported in Table 1. Significant differences ($p < 0.05$) were found between fractions of the same cultivar and among cultivars for each fraction. Peel and seed fractions were characterized by the highest total phenolics content compared to pulp, in all investigated cultivars, which ranged from 331.7 to 930.3 mg GAE per kg fw in the peel, 166.1 to 256.2 mg GAE per kg fw in the pulp and from 319.2 to 941.8 mg GAE per kg fw in the seeds. Compared to Rio Grande, variations ranging from 30% to 180% in the peel, 30% to 54% in the pulp, and 37% to 195% in the seeds of HLT-F61 and HLT-F62, respectively were detected. Tomato peels showed 2 to 3.6 times higher total phenolics content compared to pulp. Although quantitatively higher in peel and seeds, the mean total phenolics contents in the peel, pulp and seeds of the three cultivars were statistically similar (Table 2). Similarly to lycopene, in peel pulp and seed fractions, the highest total phenolics content was recorded for HLT-F61 and the lowest was recorded for Rio Grande. HLT-F61 and HLT-F62 showed similar pulp total phenolics contents. However, HLT-F61 had a very high peel and seed total phenolics content compared to HLT-F62 and Rio Grande. Phenolic compounds tend to accumulate in tomato peel in higher levels compared to the other tomato fractions because of their role in protection against ultraviolet radiation and as defense chemicals against pathogen and predators.[36] The obtained values were in accordance with those reported by Ilahy *et al.*[8–10] and Hdider *et al.*[31] ranging from 105.6 to 877.0 mg of GAE per kg fw. Ilahy *et al.*[8] reported that the total phenolics content of different Tunisian field-grown tomato cultivars ranged from 436.6 to 915.2 mg GAE per kg fw in the peel, and from 166.6 to 247.7 mg GAE per kg fw in the pulp. Recently Ilahy *et al.*[9,10] and Hdider *et al.*[31] reported that the total phenolics content ranged from 105 to 877 mg GAE per kg fw in different high-lycopene tomato cultivars depending on the ripening stage and from 105.8 to 394.5 mg GAE per kg fw at the red-ripe stage depending on the cultivar. Even higher values (ranging from 1200 to 1330 mg GAE per kg fw) were reported by Lenucci *et al.*[6] for whole red-ripe berries of high-pigment cultivars grown in Southern Italy. Lower values were generally reported for greenhouse grown tomatoes compared to those grown in open-fields. Toor and Savage[14] reported hydrophilic phenolic

values ranging from 269.0 to 303.3 mg GAE per kg fw in peels, 87 to 152 mg GAE per kg fw in pulp and 158 to 288 mg GAE per kg fw in seeds of three tomato cultivars grown in New Zealand under greenhouse conditions. Chandra and Ramalingam[21] and Chandra *et al.*[22] reported that total phenolics contents values ranged from 236.7 to 399.6 mg GAE per kg fw in peels, 90.3 to 177.5 mg GAE per kg in the pulp, and 107.6 to 218.8 mg GAE/ per kg in seeds of different Indian tomato cultivars. The high solar radiation and temperature typical of Tunisian climate, particularly during spring and summer, could be the reason for the enhancement of the phenolics and flavonoid content in field-grown tomatoes. This observation has also identified the seed fraction as an important supplying source of phenolic compounds. Recently, Siddiqui *et al.*[13] reported that total phenolics content values ranged from 623.2 to 834.8 mg catechol equivalent per kg fw in peels, 179.8 to 301.5 mg catechol equivalent per kg in the pulp of different hybrids carrying *dg*, *ogc* and *rin* genes.

2.3. Total flavonoid content

The total flavonoid content in peel, pulp and seed fractions of the investigated tomato genotypes are reported in Table 1. Flavonoid contents in the peel, pulp and seed fractions were significantly different between cultivars ($p < 0.05$). The flavonoid content ranged from 303.4 to 783.5 mg RE per kg fw in the peel, 144.3 to 552.1 mg RE per kg fw in the pulp and 215.2 to 650.0 mg RE per kg fw in the seeds. Compared to Rio Grande, variations ranging from 69% to 158% in the peel, 54% to 283% in the pulp, and 48% to 200% in the seeds of HLT-F61 and HLT-F62 were noticed. Tomato peel showed 1.4 to 2.3 times higher flavonoid content than pulp. The mean total flavonoid content in the peel of the three cultivars was significantly ($p < 0.05$) higher than the mean flavonoid contents of their pulp and seeds (Table 2). Our values are in accordance with those of Lenucci *et al.*[6] who reported that flavonoids are the major components of the total phenolics content of tomatoes. They reported values ranging from 186 to 622 mg of RE per kg fw in different high-pigment and cherry tomato cultivars grown in Italy. Recently Ilahy *et al.*[9,10] and Hdider *et al.*[31] reported flavonoid content values ranging from 105.6 to 590.6 mg RE per kg fw in different high-lycopene tomato cultivars depending on the ripening stage and from 105.6 to 394.5 mg RE per kg fw at the red-ripe stage depending on the cultivar. Similarly to lycopene and total phenolics contents, in all investigated tomato fractions, the highest flavonoid values were detected for HLT-F61 and the lowest were detected for Rio Grande. In addition, higher flavonoid contents were obtained in peel and seed fractions compared to pulp. The reported values for the flavonoid content in green-house grown tomato cultivars were lower than those obtained in the present study, ranging from 82 mg RE per kg fw to 204 mg RE per kg fw in the peel. Variations can be ascribed to the high-lycopene trait. In fact, it has been reported that in red-ripe tomato fruits, naturally occurring mutations that increase the carotenoid content, such as Beta (B) and old-gold (og, og^c) colour mutations or high pigment (hp-1, $hp\text{-}1^w$, hp-2, $hp\text{-}2^j$, $hp\text{-}2^{dg}$) photomorphogenic mutations, were also characterized by a dramatic increase in plastid biogenesis and in the production of other compounds such as flavonoids and vitamin C.[37–39] In this context, Siddiqui *et al.*[13] assessed bioactive compound levels in the peel and pulp fractions of different hybrids carrying *dg*, *ogc* and *rin* genes. The authors reported total phenolics content values ranging from 623.2 to 834.8 mg catechol equivalent per kg fw in peels and from 179.8 to 301.5 mg catechol equivalent per kg in the pulp.

2.4. Ascorbic acid and dehydroascorbic acid contents

AsA and DHA contents in peel, pulp and seed fractions of the ordinary (Rio Grande) and high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) grown in open-fields in Tunisia are reported in Table 1. AsA contents in the peel, pulp and seed fractions were significantly different between the studied tomato cultivars ($p < 0.01$). The AsA content ranged from 170.5 to 344.6 mg per kg fw in the peel, 118.8 to 186.0 mg per kg fw in the pulp and 74.2 to 115.3 mg per kg fw in the seeds of the studied tomato fruits. HLT-F61 showed the highest AsA content in the peel fraction, while HLT-F62 ranked first for the AsA content in the pulp fraction. Rio Grande showed, statistically, similar pulp AsA content to HLT-F61 and similar seed AsA content to HLT-F62. Compared to Rio Grande, variations ranging from 53% to 102% in the peel, 5% to 56% in the pulp, and 10% to 55% in the seeds of HLT-F61 and HLT-F62 were detected. Tomato peels showed 1.4 to 2.7 times higher AsA content compared to pulp. The mean AsA content in the peel of the three tomato cultivars was significantly ($p < 0.05$) higher than the mean AsA content of their pulp and seeds (Table 2).

In this study, the DHA content ranged from 98.1 to 153.2 mg per kg fw in the peel, 64.6 to 150.6 mg per kg fw in the pulp and 64.4 to 75.2 mg per kg fw in the seeds of the studied tomato fruits. In all the investigated tomato fractions, HLT-F61 and Rio Grande showed statistically similar DHA contents. However in seed fraction, all the investigated tomato cultivars showed statistically similar DHA contents. Compared to Rio Grande, both HLT lines showed significant variations in the DHA content of the pulp (from 34% to 133%) and seed (from 16% to 17%) fractions. The mean DHA contents in the peel, pulp and seeds of the three tomato cultivars were not statistically different (Table 2). These results provide evidence that, besides the high storage levels of lycopene, the two selected HLT lines are also characterized by an over production of several other phytonutrients such as vitamin C. A similar increase in vitamin C contents was reported for the photomorphogenic tomato mutants (hp-1 and hp-2) by Mochizuki and Kamimura[37] and Mustilli *et al.*[38] Although AsA and DHA contribute to the total vitamin C content, few studies quantified DHA in tomato fruits. Nevertheless, the amounts of AsA and DHA were similar to those reported by Lenucci *et al.*,[6] Ilahy *et al.*[9,10] and Hdider *et al.*[31] ranging from 33 to 218 mg per kg fw for AsA and from 0 to 213 mg per kg fw for DHA. Generally, it is widely recognized that field-grown tomatoes

have higher AsA levels (up to 258 mg per kg fw) when compared to those produced under shade (155 mg per kg fw).

2.5. Antioxidant activities of the hydrophilic and lipophilic fractions

Antioxidant activities of the hydrophilic and lipophilic fractions (AAHF and AALF, respectively) determined by the TEAC assay in peel, pulp and seed fractions of the investigated tomato genotypes are reported in Table 3. The AAHF values in all the fractions were significantly different from the studied tomato cultivars ($p < 0.01$). In all the investigated tomato cultivars, the AAHF in peel and seed fractions were higher compared to the pulp. AAHF values ranged from 255.2 to 508.8 μM Trolox per 100 g fw in the peel, 114.4 to 266.0 μM Trolox per 100 g fw in the pulp and 198.7 to 314.8 μM Trolox per 100 g fw in the seeds. In the peel fraction, the highest AAHF values were recorded for both HLT lines and the lowest was recorded for Rio Grande. However, in the pulp and seed fractions, the highest AAHF values were found for HLT-F61 and the lowest for Rio Grande. Compared to Rio Grande, variations ranging from 87% to 99% in the peel, 62% to 132% in the pulp, and 20% to 58% in the seeds of HLT-F61 and HLT-F62 were recorded. Tomato peel showed 1.9 to 2.6 times higher AAHF compared to pulp. The contribution of AAHF to the total antioxidant activity ranged from 35% to 47% in the peel, 44% to 45% in the pulp and 40% to 53% in the seed. The mean value of the AAHF in the peel of the three tomato cultivars was significantly ($p < 0.05$) higher as compared to the AAHF mean values of their pulp and seeds (Table 2). To our knowledge, this is the first time that the antioxidant activities in the peel and seed of high-lycopene tomatoes have been reported. Nevertheless, our results are in line with those of Ilahy *et al.*[9] and Hdider *et al.*[31] ranging from 166 to 488.6 μM Trolox per 100 g fw for different high-lycopene tomato cultivars harvested at different ripening stages. Ilahy *et al.*[10] reported values ranging from 498.4 to 572.1 μM Trolox per 100 g fw for different high-lycopene tomato cultivars harvested at the red-ripe stage. Our results confirmed those reported by Lenucci *et al.*,[6] Ilahy *et al.*[9,10] and Hdider *et al.*,[31] who, using both FRAP and TEAC assays, found high-lycopene tomatoes being characterized by higher AAHF values compared to ordinary tomato cultivars. Lower AAHF values were reported by Toor and Savage[14] ranging from 197 to 242 μM Trolox per 100 g fw in the peel, from 63 to 94 μM Trolox per 100 g fw in the pulp and from 80 to 150 μM Trolox per 100 g fw in the seed. It is widely recognized that field-grown tomato berries accumulate higher amounts of antioxidants compared to those produced under shade. The high peel AAHF compared to pulp can be explained by the particular phenolic compounds presents in this fraction. In fact, it has been reported that some phenols occurring in large amounts in the cuticular layer of ripe tomato fruits, such as the flavonoid, chalcone chalconaringenin and the flavanone, naringenin, may express a pro-oxidative effect,[40,41] some other phenols, such as epicatechin, often surpass the antioxidant effects of well-known vitamins C and E.[42]

The AALF values ranged from 472.7 to 632.4 μM Trolox per 100 g fw in the peels, 139.9 to 340.4 μM Trolox per 100 g fw in the pulp and 175.7 to 462.1 μM Trolox per 100 g fw in the seeds of the studied tomato fruits. A similar trend to the AAHF was observed for the AALF. In all the investigated tomato cultivars, the AALF in the peel and seed fractions were higher than that of the pulp. In all the investigated fractions, the highest AALF was recorded for HLT-F61 and the lowest for Rio Grande. Compared to Rio Grande, variations ranging from 13% to 34% in the peel, 70% to 143% in the pulp, and 90% to 163% in the seeds of HLT-F61 and HLT-F62 were detected. Tomato peel showed 1.9 to 3.4 times higher AALF compared to pulp. This is expected, due to the high amount of detected lipophilic antioxidants in high-lycopene tomato lines. The contribution of the AALF to the total antioxidant activity ranged from 53% to 65% in the peel, 55% to 65% in the pulp and 47% to 59% in the seed fractions. The peel fraction showed the highest AALF mean value (546.8 μM Trolox per 100 g of fw) followed in the order by seed fraction (323.8 μM Trolox per 100 g of fw) and pulp (239.2 μM Trolox per 100 g of fw) (Table 2). Our results are in line with those of Ilahy *et al.*[9] and Hdider *et al.*[31] ranging from 139 to 488.6 μM Trolox per 100 g fw for different high-lycopene tomato cultivars harvested at different ripening stages. Ilahy *et al.*[10] reported values ranging from 348.8 to 540.1 μM Trolox per 100 g fw for different high-lycopene tomato cultivars harvested at the red-ripe stage. Our results confirmed those reported recently by Ilahy *et al.*[9,10] and Hdider *et al.*,[31] who, using the FRAP and TEAC assays, found that high-lycopene tomatoes are characterized by higher AALF values compared to ordinary tomato cultivars. Lenucci *et al.*[6] using the FRAP assay method, found an excessively low lipophilic antioxidant activity in some high-pigment tomato cultivars in comparison with the high amount of detected lipophilic antioxidants. This is probably due to the inability of carotenoids to reduce ferric chloride in the FRAP assay.[6,9,34] Lower AALF mean values, were reported by Toor and Savage,[14] of 20 μM Trolox per 100 g fw in the peel, 7 μM Trolox per 100 g fw in the pulp and 10.9 μM Trolox per 100 g fw in the seed.

The determination of the antioxidant activities in peel and pulp fractions of newly developed tomato hybrids carrying *dg*,

Table 3 Antioxidant activities of the hydrophilic and lipophilic fractions (AAHF and AALF) in the ordinary (Rio Grande) and high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) grown in an open-field. Values represent mean ± S.E. of the three replicates. For each trait, values within the column followed by the same superscript letter are not significantly different (LSD test, $p < 0.05$)

Fractions	Peel	Pulp	Seeds
AAHF	(μM Trolox per 100 g of fw)		
HLT-F61	508.8 ± 4.5[a]	266.0 ± 8.6[a]	314.8 ± 7.8[a]
HLT-F62	478.5 ± 14.5[a]	185.0 ± 4.3[b]	239.4 ± 2.3[b]
Rio Grande	255.2 ± 3.2[b]	114.4 ± 5.5[c]	198.7 ± 1.4[c]
AALF	(μM Trolox per 100 g of fw)		
HLT-F61	632.4 ± 19.1[a]	340.4 ± 10.2[a]	462.1 ± 7.7[a]
HLT-F62	535.4 ± 13.2[b]	237.3 ± 7.4[b]	333.7 ± 4.8[b]
Rio Grande	472.7 ± 6.4[c]	139.9 ± 4.6[c]	175.7 ± 3.3[c]

ogc and *rin* genes and grown under open field conditions in India revealed higher inhibition in the peel fraction. Values ranged from 45 to 78% in the peel and from 21 to 50% in the pulp when the DPPH assay was used. However, on using the metal chelating activity, the values ranged from 23 to 42 and from 15 to 26% in the peel and the pulp, respectively.[13]

The results obtained in this study also emphasize the valuable usage of high-lycopene tomato pomace for the higher yield extraction process of different antioxidant compounds compared to ordinary tomato cultivars as suggested by Siddiqui *et al.*[13]

The hopeful use of high-lycopene tomato lines for the development of new tomato-based products and the enrichment of such products with appropriately pre-treated peels and seeds will contribute to improve the nutritional value of tomato pastes and to significantly increase the concentration of all the major antioxidants in the final product and, as a consequence, their dietary intake as suggested by Reboul *et al.*[43] However, special care should be given to the sensory quality attributes of the final products. Peels and seeds can also be used to improve the qualitative traits of other food products. In fact, the enrichment of vegetal edible oil with tomato peels induced better thermal stability and ensured the release of highly valuable compounds (lycopene, rutin, and flavonoids) in the oil which can be regarded as an innovative, customer tailored, functional food.[44] Since bakery products are considered to be low in nutritional value, the enrichment of wheat flour with tomato peels and seed flour could lead to a positive outcome on the functional and nutritional properties of bakery products.[45] Likewise, the enrichment of meat farce or meat products using dried tomato peels could lead to a final product with better color and increased health benefits.[46,47]

2.6. Correlation

Many authors studied the correlation between bioactive compounds and antioxidant activities in numerous fruits and vegetables, particularly tomatoes.[9,10] However, little information is known about these types of correlation in different fractions of high-lycopene tomato cultivars. Considering our data, disregarding the fractions, no significant correlation between the antioxidant activity of the hydrophilic fraction values and DHA content was found (Table 4). This may be due to the fact that the hydrophilic extract contains other compounds that influence the antioxidant activities in all the fractions. Actually, significant correlation between the AAHF values and both total phenolics and total flavonoids contents were obtained, which may account for most of the antioxidant activity of the hydrophilic fraction values. Ilahy *et al.*[9,10] reported that the antioxidant capacity might not always correlate with the amount of total phenolics. Moreover, it seems that correlation depends on the stage of ripening. In fact, studying the nutritional value of ripening high-lycopene tomato fruits, Ilahy *et al.*[9] found that the antioxidant activity of the hydrophilic fraction was neither correlated to the ascorbic acid nor to the dehydroascorbic acid or total vitamin C contents. However, analysing the phytochemical content of red-ripe high-lycopene tomato fruits grown in Southern Italy, Ilahy *et al.*[10] found highly significant correlation between the antioxidant activity of the hydrophilic fraction and the contents of both dehydroascorbic acid and total vitamin C. Nevertheless, the antioxidant activity of the hydrophilic fraction often correlates with specific classes of hydrophilic antioxidants; it should be noted that it depends mainly on their synergistic effects and/or interactions with other constituents of the fraction.[6]

Considering the data from all tomato cultivars and fractions, significant correlation between the AALF values and lycopene content were obtained (Table 4). This is in agreement with the well-recognized idea that the antioxidant activity of the lipophilic fraction of tomato fruits was mainly attributed to the presence of carotenoids, particularly lycopene.[6,9,10,14]

3. Experimental

3.1. Plant culture

The open-field experiments were carried out in an experimental plot at the National Agricultural Research Institute of Tunisia in Northern Tunisia during the 2013 growing season (March–July). Three tomato cultivars were used: two high-lycopene tomato advanced breeding lines with the assigned names HLT-F61 and 'HLT-F62' (F6 generation), selected by the National Agricultural Research Institute of Tunisia, and the open-pollinated cultivar Rio Grande (Petoseed, Saticoy, CA, USA) commonly grown in Tunisia. The high-lycopene tomato cultivars, HLT-F61 and HLT-F62, have been developed through conventional plant-breeding techniques taking into account the careful selection of the high-lycopene trait.[7] This important commercial trait is commonly due to the presence of light-responsive high-pigment (hp) mutations such as hp-1, hp-1^{w}, hp-2, hp-2^{j}, hp-2^{dg}, and hp-3, which lead to an increase of carotenoid and flavonoid biosynthesis.[48,49] Sowing was carried out on 13 February 2013 in plug-seedling trays. One month-old tomato seedlings were transplanted in an open-field with a spacing of approximately 0.4 m within the row and 1.5 m between rows, matching a density of about 16 667 plants per ha and grown to maturity. The experimental design was a randomized complete block with three blocks (replicates). Irrigation was applied using a drip method with 4 L h^{-1} drippers placed at 0.4 m intervals along the irrigation line. Standard agronomical techniques were used for drip irrigation, plant nutrition and pathogen prevention as described by Ilahy *et al.*[9] All cultivars under analysis were grown simultaneously in the same field and subjected to identical treatments and, obviously, environmental conditions in order to minimize the influence of pre- and post-harvest factors, agronomic and cultural practices, ripening stage at harvest and storage conditions on genotype-related variability of field-grown tomatoes.[34,50,51]

3.2. Fruit sampling

Tomato fruits were hand harvested randomly from the rows and from the middle of the plant of each block at the red-ripe

Table 4 Pearson correlation coefficients of lycopene, total phenolics, total flavonoid, ascorbic acid, dehydroascorbic acid contents as well as the antioxidant activities of the hydrophilic and lipophilic fractions determined in peel, pulp and seeds

Trait[a]		LYC			TPC			FLAV			AsA			DHA			AAHF			AALF		
		Peel	Pulp	Seed	Peel	Pulp	Seed	Peel	Pulp	Seed	Peel	Pulp	Seed	Peel	Pulp	Seed	Peel	Pulp	Seed	Peel	Pulp	Seed
LYC	Peel	*																				
	Pulp	0.94**	*																			
	Seed	0.97**	0.96**	*																		
TPC	Peel	0.99**	0.94**	0.97**	*																	
	Pulp	0.81**	0.90**	−0.01^{ns}	0.82**	*																
	Seed	0.99**	0.92**	0.99**	0.99**	0.81**	*															
FLAV	Peel	0.99**	0.97**	0.95**	0.95**	0.87**	0.96**	*														
	Pulp	0.98**	0.95**	0.98**	0.98**	0.84**	0.99**	0.95**	*													
	Seed	0.99**	0.95**	0.99**	0.99**	0.85**	0.99**	0.97**	0.98**	*												
AsA	Peel	0.74*	0.55^{ns}	0.75*	0.75*	0.38^{ns}	0.74*	0.53^{ns}	0.74*	0.69*	*											
	Pulp	0.90**	0.78*	0.92**	0.92**	0.67*	0.89**	0.86**	0.85**	0.90**	0.68*	*										
	Seed	0.97**	0.93**	0.97**	0.97**	0.78*	0.98**	0.94**	0.98**	0.97**	0.74*	0.84**	*									
DHA	Peel	0.65^{ns}	0.41^{ns}	0.63^{ns}	0.63^{ns}	0.32^{ns}	0.61^{ns}	0.38^{ns}	0.62^{ns}	0.57^{ns}	0.96**	0.60^{ns}	0.60^{ns}	*								
	Pulp	−0.09^{ns}	0.16^{ns}	−0.11^{ns}	−0.11^{ns}	0.23^{ns}	−0.09^{ns}	0.17^{ns}	−0.07^{ns}	−0.04^{ns}	−0.70*	−0.17^{ns}	−0.08^{ns}	−0.81**	*							
	Seed	0.21^{ns}	−0.03^{ns}	0.26^{ns}	0.26^{ns}	0.00^{ns}	0.19^{ns}	0.05^{ns}	0.20^{ns}	0.18^{ns}	0.58^{ns}	0.46^{ns}	0.20^{ns}	0.67*	−0.65^{ns}	*						
AAHF	Peel	0.68*	0.79*	0.68*	0.68*	0.86**	0.68*	0.84**	0.68*	0.73*	0.06^{ns}	0.63^{ns}	0.65^{ns}	−0.05^{ns}	0.57^{ns}	−0.12^{ns}	*					
	Pulp	0.94**	0.97**	0.93**	0.93**	0.84**	0.94**	0.99**	0.93**	0.95**	0.49^{ns}	0.82^{ns}	0.93**	0.33^{ns}	0.22^{ns}	−0.00^{ns}	0.83**	*				
	Seed	0.96**	0.97**	0.96**	0.96**	0.90**	0.97**	0.97**	0.96**	0.98**	0.60^{ns}	0.87**	0.93**	0.48^{ns}	0.05^{ns}	0.08^{ns}	0.79*	0.95**	*			
AALF	Peel	0.96**	0.93**	0.92**	0.92**	0.74*	0.94**	0.96**	0.92**	0.93**	0.55^{ns}	0.83**	0.94**	0.37^{ns}	0.14^{ns}	0.03^{ns}	0.75*	0.98**	0.92**	*		
	Pulp	0.93**	0.98**	0.92**	0.92**	0.90**	0.93**	0.98**	0.92**	0.95**	0.47^{ns}	0.81**	0.89**	0.33^{ns}	0.21^{ns}	−0.05^{ns}	0.85*	0.97**	0.98**	0.92**	*	
	Seed	0.91**	0.97**	0.90**	0.92**	0.88**	0.91**	0.98**	0.90**	0.93**	0.42^{ns}	0.80**	0.89**	0.26^{ns}	0.29^{ns}	−0.04^{ns}	0.89**	0.98**	0.95**	0.95**	0.98**	*

[a] LYC = lycopene, TPC = total phenolics, FLAV = total flavonoid, AsA = ascorbic acid, DHA = dehydroascorbic acid, AAHF = antioxidant activity of the hydrophilic fraction, and AALF = antioxidant activity of the lipophilic fraction. ns = nonsignificant and *, ** = significant at $P < 0.05$ or 0.01 respectively.

stage and delivered quickly to the laboratory. Healthy tomato berries, homogeneous for intense red-color and size, without wounding or breakage, were visually selected (at least 2 kg for each cultivar and for each block). The selected tomato fruits were immediately separated into three different fractions: peel (pericarp), pulp (mesocarp) and seeds. Tomato peel was carefully separated as described by Ilahy and Hdider.[33] Generally, 15–20 fruits yielded 23–40.6 g of peels. Seeds were separated along with locular jelly parenchyma tissue. Tomato pulp was cut into small pieces and homogenized in a mixer (Waring Laboratory & Science, Torrington, CT, USA). Peels and seeds were homogenated with liquid nitrogen using a mortar and pestle. The obtained fractions were frozen at −20 °C and used to determine lycopene, total phenolics, total flavonoids, AsA and DHA contents as well as the antioxidant activities of hydrophilic and lipophilic fractions (AAHF and AALF, respectively) within less than one week, in order to minimize the depletion of nutrients that inevitably occurs even during frozen homogenate storage.[52]

3.3. Analytical procedures

3.3.1. Determination of lycopene content. Lycopene extraction and determination was conducted as described by Fish *et al.*[53] on triplicate independent aliquots (0.3 g) of each fraction. The method uses a mixture of hexane/ethanol/acetone (2 : 1 : 1 by vol.) containing 0.05% butylated hydroxytoluene (BHT). During the extraction process, some precautions were taken, like working in a reduced luminosity room and wrapping glass materials in aluminium foil to avoid lycopene loss by photo-oxidation. For lycopene quantification, the absorbance of the hexane extract was read at 503 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). Lycopene molar extinction $\varepsilon = 17.2 \times 10^4\ M^{-1}\ cm^{-1}$ in *n*-hexane was used for lycopene content determination and the results were expressed as mg per kg fresh weight (fw).

3.3.2. Determination of total phenolics content. Total phenolics were extracted as described by Martínez-Valverde *et al.*[42] on triplicate independent aliquots (0.3 g) of each fraction. 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 phenolics assay was performed by using the Folin–Ciocalteu reagent as described by Spanos and Wrolstad[54] on triplicate 50 μL aliquots of the supernatant. The absorbance was read at 750 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). The linear reading of the standard curve was from 0 to 300 μg gallic acid equivalents per mL. The results are expressed in mg of gallic acid equivalents (GAE) per kg fw.

3.3.3. Determination of total flavonoid content. The total flavonoid content was determined as described by Zhishen *et al.*[55] on triplicate independent aliquots (0.3 g) of each fraction. The resulting methanolic extract (50 μl aliquots) was used for the determination of total flavonoids. 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 Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). The linear reading of the standard curve was from 0 to 250 μg rutin per mL and the total flavonoid content was expressed as mg of rutin equivalents (RE) per kg fw.

3.3.4. Determination of ascorbic acid and dehydroascorbic acid contents. Ascorbic acid (AsA) and dehydroascorbic acid (DHA) contents were determined as reported by Kampfenkel *et al.*[56] on triplicate independent aliquots (0.1 g) of each fraction. AsA and DHA were extracted by using 6% metaphosphoric acid and detected at 525 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). The assay used for the determination of AsA and DHA is based on the reduction of Fe^{3+} to Fe^{2+} by AsA and spectrophotometric detection of Fe^{2+} complexed with 2,2′-dipyridyl. DHA is reduced to AsA by pre-incubation of the sample with dithiothreitol (DTT). Subsequently the excess DTT is removed with *N*-ethylmaleimide (NEM) and the total AsA is determined by using the 2,2′-dipyridyl method. The concentration of DHA is then calculated from the difference of the total AsA and AsA (without pretreatment with DTT). The vitamin C content is the sum of both (AsA + DHA) contents. The linear reading of the standard curve was from 0 to 700 μmol AsA.

3.3.5. Antioxidant activities of the hydrophilic and lipophilic fraction assays. The measurement of the antioxidant activities of the hydrophilic and lipophilic fractions (AAHF and AALF, respectively) was performed using the TEAC assay. The antioxidant activities were measured using the ABTS decoloration method.[57] The TEAC assay is standardly used for antioxidant activity assessment of fruits and vegetables, its numerous advantages consist in reproducibility, simplicity, and a good estimate of the antioxidant activities of pure compounds and complex matrices.[57,58] Hydrophilic and lipophilic antioxidants were extracted from 0.3 g of each fruit fraction (three independent 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. Supernatants were recovered and used for antioxidant activity measurements. The antioxidant activities were measured at 734 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). Two different calibration curves were constructed using freshly prepared Trolox solutions for AAHF and AALF determination. The linear reading of the standard curves was from 0 to 16 μM Trolox for both AAHF and AALF. Values are expressed as μM of Trolox per 100 g of fw.

3.3.6. Statistical analysis. The experimental design was a randomized complete block with three factors (cvs) and three blocks (replicates). The variations in the nutritional properties of the different fractions obtained from the red-ripe berries of the ordinary Rio Grande tomato cultivar and the two high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) were assessed by analysis of variance (ANOVA). When a significant difference was detected, means were compared using the

least significant difference (LSD) test ($p < 0.05$). Correlation was performed using Pearson's correlation coefficient (r). All statistical comparisons were performed using SAS Version 6.1 software (SAS Institute, Cary, NC, USA).

4. Conclusions

In this study, the antioxidant attributes of peel, pulp and seed fractions of two high-lycopene tomato advanced breeding lines (HLT-F61 and HLT-F62) were examined and compared to the ordinary cultivar Rio Grande. The high-lycopene tomato breeding lines had a considerably higher level of lycopene in peel, pulp and seed fractions, in comparison with the ordinary cultivar. On the other hand, in peel and seed fractions, total phenolics and flavonoid contents were very high compared to pulp in all the cultivars, although quantitatively higher in the high-lycopene lines. In all the studied tomato cultivars, peel AsA was very high compared to pulp and seed fractions. Except for HLT-F62, Rio Grande and HLT-F61 showed very high peel DHA compared to pulp and seed fractions. The study also highlights the importance of high-lycopene tomato lines as a promising material of choice for either fresh market or processing. Besides the importance of tomato pulp, this study highlights the importance of other tomato fractions (peels and seeds) as valuable nutrient suppliers. Tomato peels and seeds contain a great variety of biologically active substances. The enrichment in antioxidant compounds, primarily lycopene, is of particular importance in tomato subjected to industrial processing to compensate for the loss of antioxidant activities due to chemical, physical and biological factors. HLT-F62, with its high pulp AsA content, seems to be a useful tool for developing improved-ascorbic acid tomato lines.

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