Antioxidative Activity and Carotenoid and Tomatine Contents in Different Typologies of Fresh Consumption Tomatoes

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The phytonutrient intake associated with tomato consumption depends also on cultivar and fruit ripening stage. This work associates the antioxidative ability, the level of carotenoids, and the amount of glycoalkaloids to the main carpometric characteristics of four different typologies of tomatoes: "cherry", "cluster", "elongated," and "salad". These typologies have different weights and shapes, and they are usually consumed in the Mediterranean area at different ripening stages. Results showed that the considered tomato typologies also differ in their antioxidative ability and their carotenoid and glycoalkaloid contents. Growing conditions are also important in determining fruit characteristics: the analysis of the same cultivar of cherry tomato produced under the influence of moderate salt stress showed increases in the lipophilic antioxidative ability and the amount of carotenoid, whereas the level of glycoalkaloid decreased.

Keywords: *Tomato; quality; antioxidative activity; carotenoid; tomatine; fruit typology; growing conditions*

INTRODUCTION

Fruits and vegetables play a significant role in human nutrition (Goddard and Matthews, 1979). Among vegetables, tomato is the most important both for its large consumption and for its richness in health-related food components. Tomatoes represent a convenient way to supply several nutrients such as folate, vitamin C, and potassium, but the peculiar compounds of this vegetable are carotenoids, particularly lycopene (Beecher, 1997). It is well established that due to their antioxidant activity these compounds prevent cardiovascular disease and cancer (La Vecchia, 1997). Besides the compounds beneficial for human health, tomatoes could also contain tomatine and dehydrotomatine, glycoalkaloids having well-known toxic properties (Friedman and McDonald, 1997). The content of these glycoalkaloids decreases during ripening, whereas that of carotenoids increases (Kozukue et al., 1994; Rick et al., 1994). Therefore, it can be concluded that the consumption of well-ripened tomatoes should ensure maximum health benefit, with a high level of carotenoids coupled with the absence of glycoalkaloids. However, great efforts are in progress to elucidate the physiological process as well as the storage conditions that can control the phytonutrients content in foods (Goldman et al., 1999; Grusak et al., 1999).

Tomato is represented by several hundred cultivars and hybrids in response to the fresh consumption tomato market, which demands fruits having very different characteristics (Leonardi, 1994). Therefore, tomato cultivars for fresh consumption show great differences in fruit characteristics in terms of fruit size (from a few to some hundreds of grams), shape (from flattened to elongated), and color (from yellow to dark red). Moreover, according to consumer and market requirements, tomato fruits are harvested at different stages of ripening: from breaking to red color.

There are several works describing the variation of the qualitative characteristics of tomatoes in relation to cultivars [e.g., Davies and Winsor (1969), Gormley et al. (1983), and Stevens et al. (1977)] and growing conditions [e.g., Blanc (1986), La Malfa et al. (1995), and Mitchell et al. (1991)]. Most of these works have taken into consideration only some qualitative characteristics (e.g., dry matter and soluble solids), whereas the antioxidative ability, the carotenoid composition, and particularly the glycoalkaloid content have not been considered.

The objective of the present work was to establish the antioxidative ability and the level of carotenoids and glycoalkaloids of four typologies of tomatoes commonly used for fresh consumption and differing in their main carpometric characteristics (i.e., weight, shape, and stage of ripening). For one of the above typologies the effects of growing conditions were also analyzed.

MATERIALS AND METHODS

Sampled Materials. Greenhouse-grown tomatoes were sampled during May−June 1999 from southeastern Sicily (Ragusa province), a region of Italy widely exploited for tomato greenhouse cultivation. The following tomato typologies were taken into consideration (Figure 1): "cherry" (cv. Naomi F1), "cluster type" (cv. Felicia, F1), "elongated" (cv. Italdor, F1); and "salad" (cv. ES200, F1). For cherry typology, to verify if the salinity level of irrigation water could determine any effect on the considered parameters, a second sampling was taken in a close cultivation area (Siracusa province), where the electrical conductivity of irrigation water was at least 1 dS/cm higher; in the text and in the tables the two proveniences are indicated as "cherry Ragusa" and "cherry Siracusa".

Each typology was harvested at the ripening stage considered the most suitable for marketing: "full ripening" for the

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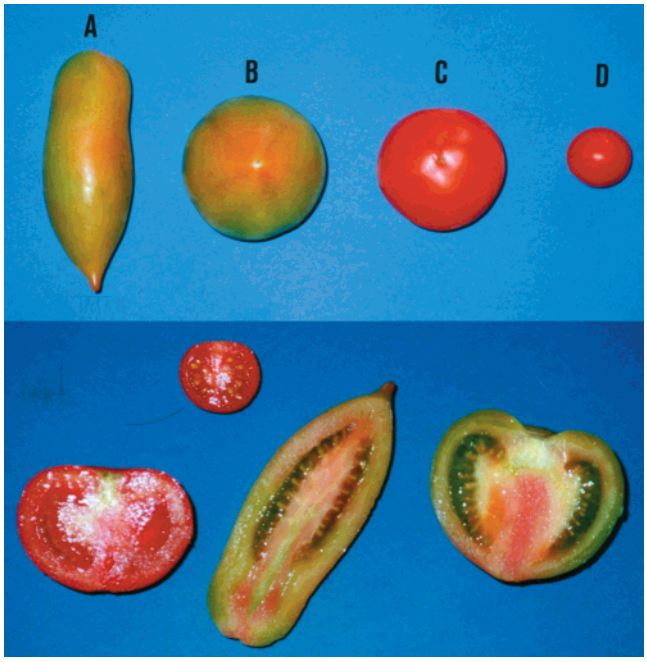

Figure 1. Tomato typologies analyzed: (A) elongated; (B) salad; (C) cluster; (D) cherry.

cherry and cluster types and "green-orange" for the elongated and salad types.

To mediate the effects of growing conditions, within each sample, fruits were harvested from five different farms, selected for their uniformity, and then pooled in one sample. After harvesting, tomatoes were kept for 2 days at ambient temperature, and then carpometric characteristics, antioxidant activity, and carotenoid and glycoalkaloid contents were determined separately on three groups of fruits, consisting of 30 fruits each chosen at random from each sample.

Carpometric Characteristics. The following determinations were performed on each sample: the unit fruit weight; the firmness, determined by measuring the force (g) to compress each fruit 2 mm between two steel plates using a Texture Analyzer model TA-XT2 Stable Micro Systems apparatus; the soluble solids, measured by a refractometer (Atago), results reported as °Brix at 20 °C; the dry matter (%), obtained by drying the fruits in a thermoventilated oven at 70 °C until constant weight was reached; the chromatic coordinates (L^*, a^*, and b^*), measured as described by McGuire (1992) by a tristimulus Minolta Chroma meter (model CR-200, Minolta Corp.). In the table color is described by lightness (L^*), hue angle ($h° = a^*/b^*$), and chroma (C^*).

Biochemical Analyses. *Carotenoid Content.* The procedure described by Tonucci et al. (1995) was followed with slight modification. Whole tomatoes were homogenized in a blender, extracted in THF in the presence of BHT, and resuspended in 5 mL of $CHCl_3$. A further 1:10 dilution of the extracted material in 40% CH_3CN, 20% methanol, 20% hexane, and 20% CH_2Cl_2 was performed before the chromatographic analysis. HPLC separation was carried out at a flow rate of 0.8 mL min^{-1} and a temperature of 30 °C using a Shimadzu HPLC with diode array detection and a Supelcosil C_{18} column (250 × 4.6 mm). Carotenoid elution was achieved using the following linear gradient: starting condition, 82% A, 18% B; 20 min, 76% A, 24% B; 30 min, 58% A, 42% B; 40 min, 39% A, 61% B. "A" was CH_3CN and "B" was methanol/hexane/CH_2Cl_2 1:1:1 v/v. Quantification of carotenoids was achieved by calibration curve obtained with authentic standard (β-carotene from Fluka) or HPLC-purified compound (lycopene). The concentration of the standards was calculated using the extinction coefficient.

Antioxidant Activity. One gram of tomato homogenate was washed twice with 5 mL of deionized water and centrifuged through a cheesecloth filter to separate the aqueous component from the insoluble fraction. The antioxidant activity was measured on a water-soluble fraction using the *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) method (Fogliano et al., 1999). Briefly, 20 μL of tomato aqueous extracts was added to 2 mL of a solution containing the DMPD radical cation in acetate buffer. The quenching of absorbance at 505 nm was compared with that obtained by a standard solution of ascorbic acid or Trolox.

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method performed as described by Pellegrini et al. (1999) was employed to assess the antioxidant activity of water-insoluble fractions. The assay was performed using different volumes (20−100 μL) of the material obtained from the carotenoid extraction procedure described above and used for HPLC analysis. The antioxidative activities of the lipophilic fraction were expressed in millimoles of Trolox present in 100 g of fresh tomato, whereas for the hydrophilic fraction ascorbic acid was used as reference compound.

Glycoalkaloids. One gram of freeze-dried tomato samples was extracted by 20 mL of 1% acetic acid for 2 h (Friedman and Levin, 1998). The extract was prepurified by a Sep-Pak column (Friedman and Levin, 1992). HPLC analysis with UV detection (200 nm) was performed using a C_{18} Phenomenex column (250 × 4.6 mm) and 100 mM $NH_4H_2PO_4$ in 32.5% CH_3CN, adjusted to pH 3.5 with phosphoric acid, as mobile phase using isocratic condition.

Statistical Evaluation of Data. Analysis of variance (ANOVA) was carried out with Sigmastat 2.0 (Jandel Scientific Software) to determine any significant difference. Data were analyzed considering fruit typology and growing conditions as experimental factors. When effects were significant ($P \leq 0.05$), we performed the Student−Newman−Keuls test; in the tables, different letters, within each parameter, indicate significant differences.

Table 1. Carpometric Characteristics of Considered Tomato Fruit Typologies[a]

typology	wt (g)	equat diam (cm)	shape (polar/equat diam)	locule (no.)	firmness (g/2 mm)
salad	155.6 a	7.0 a	0.76 d	3.3 a	1968 a
elongated	128.6 b	4.5 c	2.41 a	2.4 c	1482 b
cluster	106.6 c	6.1 b	0.81 c	3.0 b	1017 c
cherry Ragusa	15.9 d	3.1 d	0.97 b	2.0 d	674 d
cherry Siracusa	13.2 e	2.8 e	0.97 b	2.0 d	619 d

typology	color			soluble solids (°Brix)	dry matter (%)
	L^*	$h°$	C^*		
salad	45.5 b	0.16 b	24.5 b	5.08 c	5.61 c
elongated	49.4 a	0.05 b	29.7 a	4.64 c	5.09 c
cluster	38.0 c	1.11 a	29.9 a	4.78 c	5.37 c
cherry Ragusa	36.7 c	1.12 a	25.3 b	6.05 b	7.45 b
cherry Siracusa	37.4 c	1.10 a	24.8 b	7.87 a	9.49 a

[a] In this and the following tables different letters, within each parameter, indicate significant differences according to the Student−Newman−Keuls test.

RESULTS AND DISCUSSION

Carpometric Characteristics and Carotenoid Content. The tomato typologies considered for our investigation were harvested at the stage at which they are usually consumed in the Mediterranean area. It is worth noting that the studied tomatoes presented relevant differences in their appearance; thus, external fruit characteristics greatly varied (Table 1).

The stage of ripening at harvesting—which is one of the most important factors modifying fruit quality (Grierson and Kader, 1986)—can explain the relevant variations in terms of firmness and fruit color (a^*/b^*), observed on salad and elongated tomatoes (harvested at turning) compared to cluster and cherry tomatoes (harvested at full ripening). As already observed in other

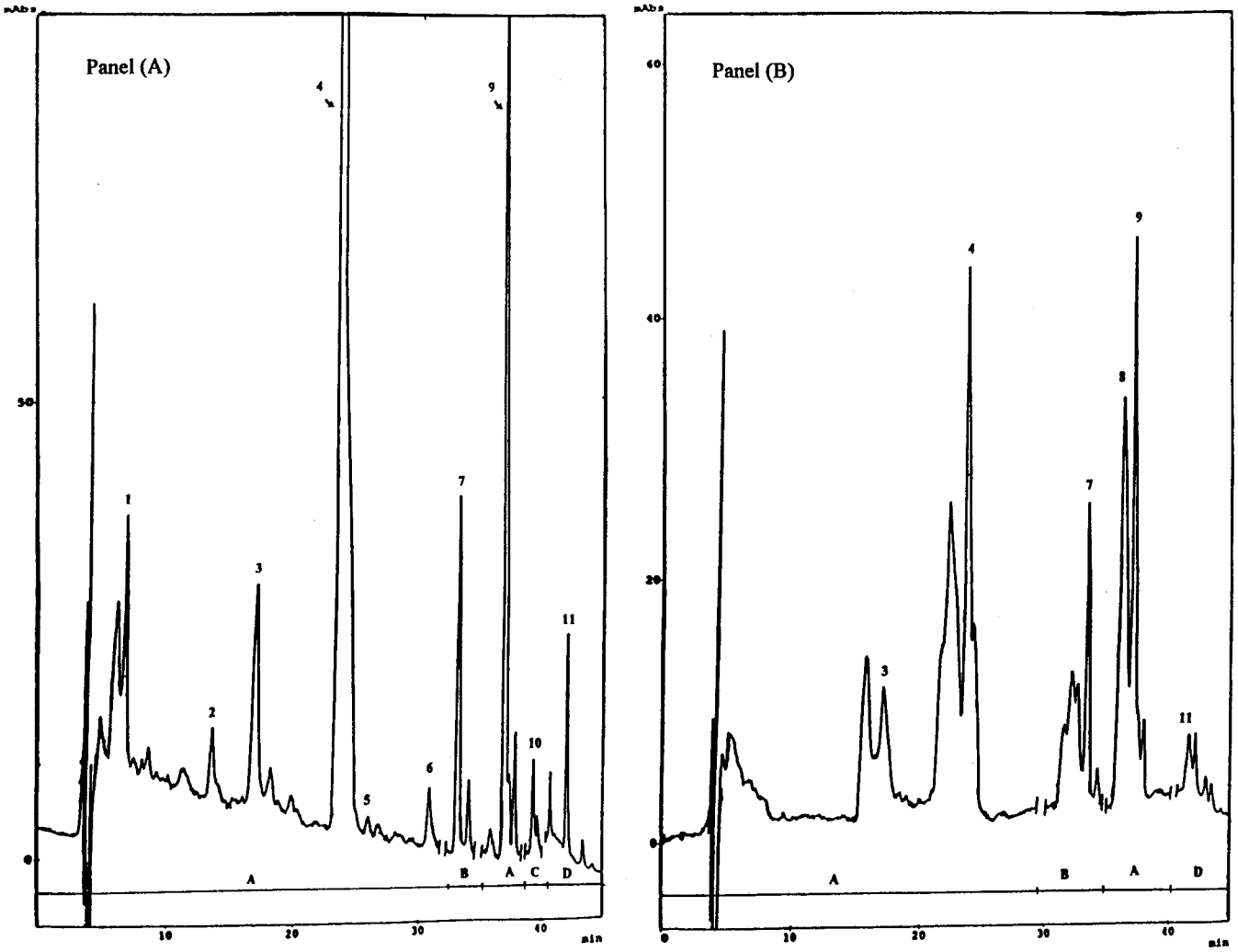

Figure 2. HPLC chromatograms of carotenoids extracted from elongated tomato (A) and salad tomato (B). Different wavelength detections are present: zone A, 450 nm; zone B, 400 nm; zone C, 350 nm; zone D, 290 nm. Peak identification: 1, lutein; 2, lycopene 5,6-diol; 3, lycopene 1,2-epoxide; 4, lycopene; 5, neurosporene; 6, γ-carotene; 7, ζ-carotene; 8, unknown; 9, β-carotene; 10, phytofluene; 11, phytoene.

Table 2. Carotenoid Content (Milligrams per 100 g of Fresh Weight) in Different Tomato Typologies

typology	lycopene	phytoene	phyto-fluen	lutein	ζ-carotene	β-carotene	lyc 5,6-diol	lyc 1,2-epoxide	neuro-sporene	γ-carotene	unident-ified	total carotenoids
salad	0.11 e	0.01 e	nd[a]	nd	0.05	0.08 e	nd	0.03 c	nd	nd	0.36	0.64 d
elongated	1.00 d	0.06 d	0.04 d	0.20a	0.90a	0.29 d	0.02 c	0.08 b	0.01 b	0.01 c	nd	2.60 c
cluster	7.90 b	0.47 c	0.23 c	0.01 b	0.01 c	0.49 c	0.06 b	nd	0.02 a	0.04 b	nd	9.24 b
cherry Ragusa	7.20 c	0.52 b	0.33 b	nd	nd	0.92 b	0.06 b	0.03 c	nd	0.03 b	nd	9.11 b
cherry Siracusa	10.80 a	0.61a	0.41a	nd	nd	1.05a	0.08a	0.17a	nd	0.07a	nd	13.19 a

[a] nd, not detected.

studies (Stevens et al., 1977; Giovannelli et al., 1999), soluble solids and dry matter contents were not clearly associated with ripening stage; in fact, only slight variations were observed among salad, elongated, and cluster types. In cherry tomatoes both parameters were significantly higher.

As expected, a great variation among the different samples is present in the carotenoid component in terms of both total amount and qualitative composition. In Figure 2 (panels A and B) the chromatograms of carotenoid extracts from two different cultivars are reported; the carotenoid pattern is quite different depending on tomato typology. The chromatogram of elongated tomato (panel A) is well resolved and contains a significant amount of all the identified carotenoids. On the other hand, in the chromatogram of salad tomato (panel B) the presence of several unknown compounds leads to peaks overlapping in different regions. The UV spectra of these unidentified compounds are typical of carotenoid compounds with a triplet of maximum of absorbance between 444 and 502 nm. Quantitative data of composition of the main carotenoids are presented in Table 2. Lycopene was always the most represented in all typologies, although in the salad tomato a relevant part (57%) of unidentified carotenoids was present. In cluster and cherry tomatoes lycopene represented 79 and 85% of total carotenoids, respectively.

The amount of carotenoids present in fully ripe fruits was for both cherry and cluster tomatoes in agreement with those reported in the literature (Tonucci et al., 1995). Considering the absolute content, tomato green-orange typologies do not represent an important way to supply carotenoids. In fact, the carotenoid content is very low, in both salad and elongated tomatoes (3 and

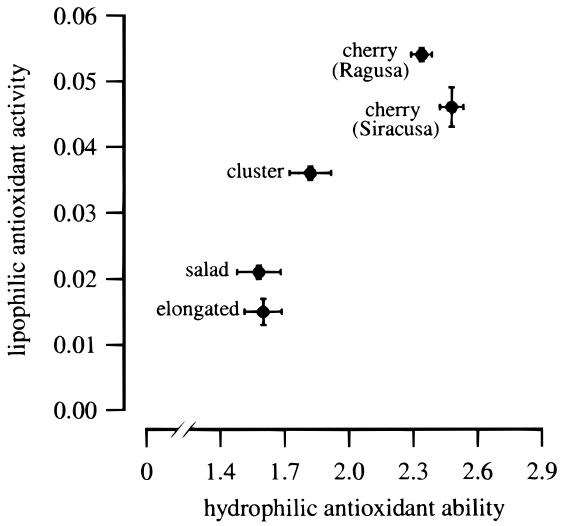

Figure 3. Antioxidant ability of different tomato typologies expressed in millimole equivalents of Trolox or ascorbic acid per 100 g of fresh weight (means ± SD).

20%, respectively, of the total carotenoids found in other tomato typologies).

Antioxidant Activity. A prerequisite to measure the antioxidant activity of tomato is the separation of aqueous and lipophilic fractions. Therefore, two procedures are necessary to evaluate the contribution of the different tomato components to the total antioxidative activity. Two radical cation assays were selected because these methodologies are cheap, not laborious, and, therefore, very useful for this kind of screening.

The hydrophilic activity is ~40% higher in the two cherry tomatoes, whereas the differences among the other varieties are negligible (Figure 3). It is reported in the literature (Giovannelli et al., 1999) that the concentration of hydrophilic antioxidant such as ascorbic and other organic acids is not clearly influenced by the ripening. Our data show that cherry tomato has a high hydrophilic antioxidative ability. Therefore, it can be argued that the cultivar mainly influences this parameter.

Results obtained for the lipophilic antioxidants are well related to the amount of carotenoid present in each sample. The antioxidant ability is significantly higher in cherry and cluster tomatoes and lower in salad and elongated tomatoes. Interestingly, the value of cherry tomato is higher than cluster tomato, although the two varieties have roughly the same amounts of total carotenoids. Also, the value of salad type is 40% higher than that of elongated tomato, notwithstanding it contains one-third of the total carotenoids (see Table 2). These discrepancies are likely related to the different compositions of the carotenoid extracts. For the salad type it is possible that the carotenoid-like unidentified compounds can account for the relatively high antioxidative ability.

The antioxidative ability expressed as millimoles of Trolox present in 100 g of fresh products is in good agreement with that reported by Pellegrini et al. (1999), considering that these authors reported the value of antioxidative ability for a kilogram of dry material. It this case it was preferred to refer the value to 100 g of fresh products to maintain the notation used for the food composition tables.

Content of Glycoalkaloids. It is well-known that the content of glycoalkaloids decreases during ripening, being negligible for fully red tomatoes (Kozukue et al., 1994). Glycoalkaloids are toxic in several in vitro assays (Friedman and McDonald, 1997); therefore, their consumption is potentially harmful. Several cases of glycoalkaloid poisoning have been described mainly due to ingestion of sprouted potatoes (McMillan and Thompson, 1979). Actually the effective in vivo toxicity of these compounds is still unclear. As a matter of fact, populations that normally eat tomato accessions having very high tomatine contents do not have any toxicity symptoms (Rick et al., 1994). Moreover, it was reported that a green tomato rich diet can contribute to cholesterol reduction due to the formation of a complex between tomatine and cholesterol (Friedman et al., 1997).

Table 3. Glycoalkaloid Contents of Different Tomato Typologies (Milligrams per Kilogram of Fresh Weight)

typology	tomatine	dehydrotomatine	total
salad	13.2 b	0.38 b	13.6 b
elongated	41.3 a	2.01 a	43.3 a
cluster	9.9 c	0.23 b	10.1 c
cherry Ragusa	8.0 c	0.22 b	8.2 c
cherry Siracusa	nd[a]	nd	nd

[a] nd, not detected.

In the tomato typologies we have studied, we observed outstanding variations in the total amount of glycoalkaloids, which varied between 8 and 43 mg/kg of fresh weight, with a ratio between tomatine (TOM) and dehydrotomatine (DHM) that is always between 1:20 and 1:10. These data are comparable with those reported in the literature, although the glycoalkaloid content of each typology is not strictly related to the ripening stage. In fact, the salad type, which was harvested at the green-orange stage, had a glycoalkaloid content comparable to that of the cluster type, which was taken at full ripening stage (Table 3). On the other hand, salad and elongated tomatoes (harvested at similar ripening stages) showed very different glycoalkaloid contents. It can be concluded that besides the ripening stage also the role of genotype is relevant in determining the glycoalkaloid content. The elongated typology, with a level of 40 mg/kg, has a relatively high content of these compounds considering that the current guideline for potato establishes a maximum allowed level of 200 mg/kg of glycoalkaloids. It should be noted that in vitro assays demonstrate that TOM is less toxic compared to the main potato glycoalkaloids (Friedman and McDonald, 1997). On the other hand, no data for DHT are available.

Antioxidative Activity and Carotenoid and Glycoalkaloid Contents According to Growing Conditions. The variation induced by water salinity on the nutritional parameters above studied was investigated. Two samples of the same cultivar of cherry tomato were examined. Cherry Ragusa was grown using irrigation water having an electrical conductivity of <~2 mS/cm, whereas in the growing area of cherry Siracusa the water used for irrigation has an electrical conductivity of ~3 mS/cm. The differences in the carpometric parameters fruit weight, dimension, and soluble solid and dry matter contents (see Table 1) could therefore be explained by the effect of salt stress widely described in previous works [e.g., Mitchell et al. (1991)]. Water stress induced by high salinity mainly restricts the amount of water supplied to the fruit by the phloem, whereas the concentration of the phloem sap is increased (Ho et al., 1987). The consequence is that cherry Siracusa has higher soluble solids and dry matter with

respect to cherry Ragusa; therefore, although the carotenoid patterns are similar, the amount of carotenoid per 100 g of fresh weight was ~50% higher in cherry Siracusa. It is worth noticing that this difference is not detectable in the measurment of the skin color (see Table 1). This evidence suggests that the color determination is not sufficient to quantify the carotenoid contents, particularly when ripening reaches the red stage. The lipophilic antioxidative ability was, according to the carotenoid content, higher in cherry Siracusa. On the other hand, the hydrophilic antioxidative abilities were very similar between the two cherry tomato samples and significantly higher respect to the other typologies.

The glycoalkaloid level is quite low in cherry Ragusa (8.2 mg/kg), whereas it is under the detection limit (i.e., <2 mg/kg) in cherry Siracusa. This finding could be of great interest, and it should be related to the regulation of the biosynthesis of secondary metabolites in plants grown under high salinity.

Conclusion. Tomato consumption is usually associated with the intake of lycopene and other antioxidants having healthy effects. The tomatoes analyzed in this work represent the typologies mainly used for fresh consumption in Mediterranean countries. They show outstanding differences in antioxidative ability and in the content of carotenoids and glycoalkaloids. Three factors seem to play a pivotal role in determining these differences: (i) cultivar; (ii) ripening stage; and (iii) growing conditions. A thorough investigation of the influence of each factor on the tomato composition is beyond the aim of this work; however, the data allow some speculation. Carotenoid content as well as lypophilic antioxidant activity was more affected by ripening stage than by cultivar, which determined slight even if significant effects. Glycoalkaloid content was dependent on both cultivar and ripening stage. Hydrophilic antioxidative activity depends on typology, and it is independent of the ripening stage. Cherry tomatoes have the highest lipophilic and hydrophilic antioxidative abilities; moreover, their high carotenoid level is combined with a low content of glycoalkaloids.

Future works will investigate the factors related to pre- and postharvest conditions that must be taken into account to better understand their influence in the synthesis and accumulation of components such as carotenoids and glycoalkaloids as well as the antioxidative ability. All of these factors contribute to the determination of tomato quality, particularly in terms of the health-related properties of this fruit.

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