Research Article

Antioxidant nutritional quality of tomato

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Regular consumption of tomatoes has been associated with decreased risk of chronic degenerative diseases. Epidemiological findings confirm the observed health effects are due to the presence of different antioxidant molecules such as carotenoids, particularly lycopene, ascorbic acid, vitamin E and phenol compounds, particularly flavonoids. In this work, eight components contributing to the healthy quality of tomato (*i. e.* lycopene, β-carotene, other carotenoids, flavonoids, phenolic acids, vitamins C and E, dry residue) were studied in the framework of breeding programs aiming to develop nutritional superior genotypes. Twelve tomato advanced breeding lines and six open pollinated cultivars were grown in strictly controlled conditions and analysed for their content of antioxidants. Among the 18 genotypes analysed, 10 showed a high level of total carotenoids, 6 high level of β-carotene, 9 high lycopene levels, 15 high flavonoids and 2 relevant concentration of vitamin E. Based on such data and on a literature survey on tomato composition, an index, called index of antioxidant nutritional quality (I_{QUAN}), was proposed as a tool to address the breeding programs in selecting tomato genotypes with antioxidant nutritional qualities.

Keywords: Antioxidants / Carotenoids / Flavonoids / Genotypes / Tomato

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1 Introduction

Tomato is one of the most popular and extensively consumed vegetable crop worldwide. This vegetable is not only an important component of traditional Mediterranean diet, but also of other diets. There is evidence that regular tomato consumption decreases the incidence of chronic degenerative diseases such as certain types of cancer [1] and cardiovascular diseases [2]. The beneficial effects of tomato consumption are generally attributed to carotenoids, which are able to reduce the risk of certain types of cancer, arteriosclerosis and cataract formation [3, 4]. Two main carotenoids are present in tomato: lycopene, which is the major carotenoid compound (~80–90%) giving the red colour to the fruit [5], and β-carotene, which is 7–10% of the total carotenoid content [6]. Lycopene has been shown to have a strong antioxidant activity and to exhibit the highest physical quenching rate constant with singlet oxygen [7]. On the other hand, β-carotene is of special interest due to its provitamin A activity [8]. Tomatoes represent by far the main source of lycopene, whereas many other dietary sources contribute to the daily intake of β-carotene. However, the tomato fruit is a reservoir of other potentially healthy molecules, such as ascorbic acid, vitamin E and phenolic compounds, particularly flavonoids [9, 10]. Among antioxidant vitamins, the relevance of tomatoes for the daily intake of vitamins C is variable according to the dietary habits. However, in many countries tomatoes are together with oranges the main source of vitamin C [11]. On the other hand, the contribution of tomato to the whole vitamin E intake is marginal. Moreover, tomatoes contain also phenolic compounds, which also exhibit a strong antioxidant activity [12]. The antioxidant and free radical-scavenging properties of polyphenol compounds in several plant extracts have been recently reported, suggesting possible protective roles of polyphenol compounds in reducing risk of cardiovascular diseases in humans [13]. Kahkonen *et al*. [14] reported that the total phenolic content of tomatoes is up to 200 mg of gallic acid equivalent *per* 100 g (as dried weight). Tomato polyphenols, mainly phenolic acids, are present in free soluble form and in insoluble form when they are bound to the fibre. Moreover, tomato contains flavonoids, in particular rutin and naringenin. Some papers pointed out

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Abbreviation: I_{QUAN}, index of antioxidant nutritional quality

that tomato flavonoids, due to their high antioxidant power and to the significant biological activities, can have a substantial role in the health benefits attributed to the tomato consumption [15, 16].

Finally, tomatoes represent a relevant dietary source of soluble and insoluble dietary fibres, constituted by pectins, hemicelluloses and cellulose [17], with a concentration ranging from 0.8 to 1.3 g/100 g of fresh weight (USDA National Nutrient Database for Standard Reference, Release 15, http://www.nal.usda.gov/fnic/foodcomp; accessed June 30 2004) and they contains 1 – 3 g/100 g of organic acids, mainly citric acid.

Based on the epidemiological evidence of tomato consumption, in the last years the antioxidant composition as well as total antioxidant capacity has been extensively studied also with the aim to produce cultivars having a high antioxidants content. At present, the development of such tomato cultivars is a significant trend in plant research. However, for the antioxidants feature, it is difficult to carry out genetic analyses due to the complex inherence of traits involved and to the not complete understanding of the processes. Besides that, environmental effects, ripening stage of fruit and post-harvest storage conditions can also influence the traits detection. Considering the time- and money-consuming efforts necessary to obtain a new tomato cultivar, reliable tools to identify genotypes of "potential interest" to be used on the breeding program are very much needed. The identification of genotypes with high nutritive value, which more likely contains a high proportion of genes affecting the traits, represent a useful approach to select tomato cultivars with better health-promoting properties.

The aim of this work was to study the genetic variation of components contributing to the antioxidant composition (lycopene, β-carotene, other carotenoids, flavonoids and phenolic acids, vitamins C and E, dry residue) to identify superior genotypes useful to develop fresh market cultivars with good nutrition value. Moreover, based on the antioxidant composition of tomato genotype studied, an approach to obtain a global index of the antioxidant nutritional quality of the tomato genotypes denominated index of antioxidant nutritional quality (I_{QUAN}) is proposed.

2 Materials and methods

2.1 Materials and experimental design

Twelve tomato advanced breeding lines (143, POLY 20, POLY 56, 977, 988, 1512, 1438, 1513, 981, poly 27, 1447 and selection 6) and six open pollinated cultivars (Motelle, Momor, Cayambe, Ontario, Stevens and Heline) were grown in a field located in the S. Marzano area during the summer of 2003. Twenty plants for each genotype were transplanted in a randomised complete block design with two replicas. Planting distance were 30 cm on the row and 100 cm between rows. A random sampling of about 20 full ripe fruits *per* plot was performed. Fruits were homogenised, divided in aliquots and stored at −80°C for the determination of chemical and biochemical parameters. For the analysis of flavonoids and phenolic compounds, samples were cut and freeze-dried, after which they were powered and stored at −20°C.

All solvents used were from Merck (Darmstadt, Germany) and the reagents were purchased from Fluka (Switzerland) when not specified. Lycopene, β-carotene, phytoene, phytofluene, gallic acid, quercetin, rutin, naringenin, morin, kaempferol, chlorogenic, caffeic, *p*-coumaric and ferulic acids and 2,6-di-*tert*-butyl-*p*-cresol were from Sigma (St. Louis, MO, USA).

2.2 Chemical determinations

The following parameters were determined in all samples: pH at 20°C (HI 9017 Microprocessor pH meter, Hanna Instruments), refractive index at 20°C (BX) and total solids. The Brix degrees were determined in duplicate on the homogenate by a refractometer (ATAGO). Total solids were obtained drying 5 g of the product in an oven (Ehret) set at 70°C until constant weight was reached. Results were expressed in percentage.

2.3 Carotenoids

Carotenoids were determined by HPLC analysis as previously described [18]. Briefly, 5 g of homogenised tomato was extracted with 25 mL of dichloromethane and centrifuged at $3000 \times g$ for 5 min at 4°C. The supernatant was collected and the procedure was repeated with another 25 mL of solvent; the supernatants were combined. After appropriate dilution, an aliquot of the extract was used for lycopene, β-carotene, phytoene and phytofluene quantification using an HPLC (Shimadzu LC10, Japan) with DAD and a Prodigy column (5 μm, 250 × 4.6 mm; Phenomenex). The carotenoids were eluted with a flow of 0.8 mL/min following this linear gradient: starting condition 82% A and 18% B; at 20 min 76% A and 24% B; at 30 min 58% A and 42% B; at 40 min 40% A and 60% B; 45 min 82% A and 18% B. The phase A was a mixture of ACN, *n*-hexane, methanol and dichloromethane (2:1:1:1 v/v), while the phase B was ACN. Lycopene, β-carotene, phytoene and phytofluene were identified and quantified by a calibration curves built with pure standard compounds. Data were expressed as milligram of each carotenoid *per* 100 g of fresh weight.

2.4 Flavonoids and phenolic acids

The procedure described by Crozier *et al.* [19] with few modifications was followed. Aliquots of 1 g of lyophilised tomato were extracted with 20 mL of 60% aqueous metha-

nol solution containing 0.25 mg of morin as an internal standard. About 1.5 mL of this solution was kept down; the remaining part was added with 20 mM sodium diethyldithiocarbamate and with 5 mL of 6 M HCl, then it was refluxed at 90°C for 2 h. Extract aliquots, taken both before and after hydrolysis, were analysed. HPLC was carried out at a flow rate of 1 mL/min using an HPLC (Shimadzu LC 10), with DAD and a Prodigy (5 μm, 250 × 4.6 mm^2; Phenomenex) column. Elution of flavonoids and phenolic acids was achieved using the following linear gradient: starting condition, 95% A, 5% B; 5 min, 80% A, 20% B, 7 min, 70% A, 30% B; 10 min, 50% A, 50% B; 18 min, 40% A, 60% B; 23 min, 20% A, 80% B; 28 min, 10% A, 90% B; 33 min, 70% A, 30% B, 40 min 95% A, 5% B. Phase A: deionised water/formic acid 95:5 v/v, phase B methanol. Flavonoids were reported as sum of quercetin, naringerin and kaempferol, whereas phenolic acids as sum of chlorogenic, caffeic, *p*-coumaric and ferulic acids. All samples were analysed in duplicate.

2.5 Vitamins E and C

The vitamin E content was determined by HPLC according to AOAC procedure [20]. Ten g of tomato homogenate was saponified by refluxing with 40 mL of 95% ethanol and 10 mL of 50% KOH for 30 min at 100°C. After cooling the flask, the extract was put in a separator funnel and 100 mL of CH_3CHCl_2, 50 mL of CH_3CH_2OH and 150 mL of 1 N KOH were added. The CH_3CHCl_2 phase was collected, first it was washed with 40 mL of 0.5 N KOH and then with 40 mL of distilled water for three times and finally dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum, the residues were redissolved with CH_3OH, filtered through a nylon filter (0.45 μm) and dried by nitrogen flow. The sample was resuspended in CH_3OH and analysed by means of HPLC (Shimadzu LC10) with DAD and a Prodigy column (5 μm; 250 × 4.6 mm; Phenomenex). The vitamin E was eluted with MeOH/H_2O (98:2 v/v) at 1.0 mL/min flow under isocratic conditions and UV detection at 290 nm. The data obtained were expressed as milligram of vitamin E *per* 100 g of fresh weight.

The vitamin C content of tomato samples was determined by the titrimetic method using 2,6-dichloroindophenol (Fluka) [20].

2.6 Total dietary fibre

The amount of total dietary fibre was determined according to a gravimetric enzymatic method as previously described by Prosky *et al.* [21] (AOAC method 991.43). The enzymatic kit for fibre determination was from Megazyme (Bray, Co. Wicklow, Ireland) and was used according to the manufacturer's instructions. The analytical protocol was described in detail elsewhere [22].

2.7 Statistical analysis

Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) package version 13.0. In particular, the effect of the lines on lycopene, phytoene and phytofluene, total phenol, vitamins C and E content, I_{QUAN} (see after) and dry matter were estimated with a significance of $p < 0.01$ and $0.01 < p < 0.05$ by MANOVA procedure. Both multiple comparisons and homogeneous groups were estimated according to Sheffe's test. The *H* test of Kruskal–Wallis was performed for β-carotene, pH and Brix.

3 Results

3.1 Compositional data

The 18 genotypes of tomatoes analysed were not different in terms of surface colour (data not shown) since they were all harvested at the commercial ripening stage and their pH value ranged from 4.13 (Motelle) to 4.60 (1512; $F_{17,36} = 731.12$, $p < 0.001$; Table 1). The majority of genotypes showed a pH value below 4.5 that is considered an important quality commercial requirement for fresh tomato fruit [23].

In Table 1, data of dry matter, total dietary fibre, vitamins C and E, flavonoids and phenolic acids are shown. Dry matter value was considered in this study as in tomatoes it is mainly determined by dietary fibre and organic acid content, which have both a role in the determination of antioxidant capacity. The average dry matter content is 5.5%. The highest value content was observed for POLY 20 and Stevens genotypes (9.2 and 6.8%, respectively), while 988 showed the lowest value (4%) ($F_{16,34} = 11.34$, $p < 0.001$). The total dietary fibre of tomato samples ranged between 21 and 27% of the dry matter in agreement with data reported by Saura-Calixto *et al*. [24]. Thus, we consider that the value of dry matter represents a good indication of the concentration of dietary fibre in tomatoes.

Vitamin C content showed significant variation among lines ($F_{17,36} = 55.70$, $p < 0.001$). The highest values of vitamin C were obtained for Heline (16.3 mg/100 g), Motelle (15.6 mg/100 g), 981 (14.6 mg/100 g), Momor (13.8 mg/100 g) and Poly 56 (11.4 mg/100 g) lines.

As far as vitamin E, the lowest value was found in Motelle (0.17 mg/100 g), while the highest values were obtained in Poly 56 (0.62 mg/100 g) and Momor lines (0.60 mg/100 g; $F_{17,36} = 137.51$, $p < 0.001$).

Flavonoids showed a significant variation among all lines ($F_{17,36} = 40.95$, $p < 0.001$). The value of flavonoids ranged from 2.13 (Heline) to 5.18 mg/100 g (Ontario).

The content of phenolic acids was also significantly different among the lines, ranging from 2.04 (Cayambe) to 3.75 mg/100 g (Motelle; $F_{17,36} = 97.06$, $p < 0.001$).

Table 1. Proximal data, vitamins and total phenolic compounds in different tomato lines[a)]

Line	pH	Dry matter	Vitamin C	Vitamin E	Total fibre[b)]	Flavonoids	Phenolic acids
			mg *per* 100 g of FW[c)]				
143	4.47 ± 0.00	4.4 ± 0.1	9.03 ± 0.14	0.23 ± 0.01	22.3	3.41 ± 0.15	2.31 ± 0.02
Stevens	4.20 ± 0.00	6.8 ± 0.2	12.3 ± 0.43	0.25 ± 0.02	27.0	3.39 ± 0.08	2.26 ± 0.03
Poly20	4.42 ± 0.01	9.2 ± 0.0	9.24 ± 0.03	0.46 ± 0.01	21.0	3.56 ± 0.05	2.47 ± 0.02
Ontario	4.55 ± 0.00	5.5 ± 0.4	11.5 ± 0.46	0.49 ± 0.02	21.5	5.18 ± 0.27	2.95 ± 0.05
Sel6	4.24 ± 0.01	5.1 ± 0.6	11.8 ± 0.70	0.39 ± 0.03	23.7	3.40 ± 0.07	2.56 ± 0.06
Poly56	4.37 ± 0.01	5.4 ± 0.4	11.4 ± 0.24	0.62 ± 0.00	25.8	3.32 ± 0.13	2.61 ± 0.08
1447	4.57 ± 0.03	5.7 ± 0.5	11.4 ± 0.05	0.44 ± 0.00	24.1	3.57 ± 0.09	3.22 ± 0.02
977	4.14 ± 0.01	4.3 ± 0.0	11.7 ± 0.18	0.22 ± 0.02	27.0	3.48 ± 0.11	2.49 ± 0.06
1513	4.50 ± 0.00	5.7 ± 0.5	13.3 ± 0.08	0.23 ± 0.00	25.3	3.26 ± 0.08	2.63 ± 0.03
988	4.53 ± 0.00	4.0 ± 0.0	12.8 ± 0.28	0.34 ± 0.01	25.8	4.09 ± 0.08	2.34 ± 0.05
Cayambe	4.23 ± 0.00	4.5 ± 0.0	8.00 ± 0.00	0.22 ± 0.00	25.4	3.04 ± 0.07	2.04 ± 0.06
Heline	4.43 ± 0.00	6.2 ± 0.2	16.3 ± 0.26	0.28 ± 0.01	23.2	2.13 ± 0.17	2.45 ± 0.03
1512	4.60 ± 0.00	5.7 ± 0.1	13.6 ± 0.27	0.34 ± 0.00	22.1	3.00 ± 0.14	2.80 ± 0.11
1438	4.26 ± 0.00	4.5 ± 0.0	11.7 ± 0.08	0.28 ± 0.00	21.5	3.85 ± 0.18	2.85 ± 0.05
Motelle	4.13 ± 0.01	5.4 ± 0.2	15.6 ± 0.50	0.17 ± 0.01	23.5	2.92 ± 0.05	3.75 ± 0.10
Momor	4.49 ± 0.01	6.4 ± 0.9	13.8 ± 0.06	0.60 ± 0.00	26.2	2.84 ± 0.05	3.07 ± 0.05
981	4.35 ± 0.01	4.9 ± 0.1	14.6 ± 0.27	0.30 ± 0.02	22.4	3.55 ± 0.21	3.02 ± 0.08
Poly27	4.42 ± 0.08	6.5 ± 0.3	10.3 ± 0.04	0.22 ± 0.00	21.4	3.34 ± 0.15	2.14 ± 0.08

a) Values are means ± SD.
b) Values are given as percentage of the dry matter.
c) Fresh weight.

Carotenoids obtained on the 18 tomato lines by HPLC analysis are reported in Table 2. In particular, concentrations of lycopene, β-carotene, phytoene and phytofluene were measured. Hereinafter, phytoene and phytofluene were considered as their sum and they are considered as other carotenoids. Among all characters, carotenoids content accounts for the major variability explained by the genotype. In fact, lycopene content showed significant variation among all analysed genotypes ($F_{17,36} = 330.11$, $p < 0.001$), as well as both β-carotene and other carotenoids contents (β-carotene $F_{17,36} = 337.63$, $p < 0.001$; phytoene $F_{12,26} = 61.22$, $p < 0.001$; phytofluene $F_{12,26} = 14.61$, $p < 0.001$). The lines Motelle and Poly 20 showed the highest average lycopene content (16.9 and 16.0 mg/100 g, respectively) followed by Momor, Cayambe, 988 and Poly 56 group. Conversely, line 981 showed the lowest average value (2.33 mg/100 g). The β-carotene content of each line ranged from 0.28 (1447) to 1.00 mg/100 g (Poly 20). The last line showed also a very high content of lycopene as reported above. It is interesting to point out the complete lack of phytofluene in Sel 6, Heline and 1512 lines and a very low content in 981 (0.39 mg/100 g). These samples exhibited also a low content of total carotenoids (10.60, 10.71, 3.98 and 3.87 mg/100 g, respectively) compared to the values of about 18 mg/100 g measured in Motelle and Poly 20. Additionally, the same lines showed also the lower contents of phytoene (0.50, 0.51, 0.43 and 0.44 mg/100 g, respectively), which does not compensate the lower content of phytofluene. Among those lines, both 1512 and 981 showed also lower content of lycopene (3.25 and 2.33 mg/100 g, respectively), while both 1447 and 1512 showed the lower β-carotene average content (0.28 and 0.30 mg/100 g, respectively).

3.2 I_{QUAN}

The definition of a tomato nutritional quality index based on the antioxidant content could be of interest for breeders aiming to obtain tomato lines with high antioxidants content. In this frame, the final goal is to summarise the various parameters which contribute to the antioxidant quality of this fruit in one number. The proposed approach is illustrated in Table 3 and it relies on the concentration of each component contributing to the antioxidant quality and a relative weight which was attributed to them. For the definition of such components and their relative weight, a literature survey was carried out (see first column of Table 3). Based on this, eight main components and their ranges were identified: in particular, lycopene, β-carotene, other carotenoids, flavonoids, phenolic acids, vitamins C and E, dry residue (dry residue can be further split in dietary fibre and organic acid when data are available). In the case of outliers concentrations reported occasionally in some studies, data were discarded.

The first step for the index construction was the definition of a so-called "optimal concentration" for all parameters as the average literature value increased by 50% and calculated as follows:

$$C_{opt} = C_a + 0.5\, C_a \quad (1)$$

where C_{opt} is the optimal concentration and C_a is the average concentration for each parameter.

Table 2. Carotenoid content in tomato lines analysed[a)]

Line	Lycopene	β-Carotene	Phytoene	Phytofluene	Total carotenoids
		mg *per* 100 g of FW[b)]			
143	9.47 ± 0.01	0.41 ± 0.00	0.51 ± 0.00	0.47 ± 0.00	10.9 ± 0.00
Stevens	10.2 ± 0.20	0.43 ± 0.00	0.55 ± 0.01	0.47 ± 0.01	11.7 ± 0.22
Poly20	16.0 ± 0.18	1.00 ± 0.01	0.66 ± 0.00	0.50 ± 0.00	18.2 ± 0.20
Ontario	6.54 ± 0.22	0.41 ± 0.00	0.51 ± 0.00	0.44 ± 0.00	7.42 ± 0.90
Sel6	9.73 ± 0.08	0.37 ± 0.01	0.50 ± 0.01	0.00 ± 0.00	10.6 ± 0.09
Poly56	14.2 ± 0.72	0.51 ± 0.01	0.56 ± 0.01	0.47 ± 0.00	15.8 ± 0.75
1447	5.61 ± 0.39	0.28 ± 0.00	0.53 ± 0.01	0.44 ± 0.00	6.87 ± 0.37
977	10.0 ± 0.35	0.45 ± 0.03	0.51 ± 0.00	0.54 ± 0.00	11.0 ± 0.36
1513	9.21 ± 0.02	0.73 ± 0.00	0.60 ± 0.00	0.46 ± 0.02	11.0 ± 0.00
988	14.1 ± 0.08	0.68 ± 0.00	0.57 ± 0.02	0.42 ± 0.01	15.7 ± 0.09
Cayambe	13.5 ± 0.01	0.32 ± 0.00	0.60 ± 0.00	0.47 ± 0.01	14.9 ± 0.00
Heline	9.46 ± 0.02	0.74 ± 0.04	0.51 ± 0.02	0.00 ± 0.00	10.7 ± 0.04
1512	3.25 ± 0.03	0.30 ± 0.00	0.43 ± 0.01	0.00 ± 0.00	3.98 ± 0.04
1438	6.35 ± 0.12	0.31 ± 0.00	0.62 ± 0.01	0.51 ± 0.00	7.90 ± 0.01
Motelle	16.9 ± 0.68	0.47 ± 0.01	0.66 ± 0.00	0.46 ± 0.01	18.5 ± 0.67
Momor	13.3 ± 0.16	0.96 ± 0.01	0.57 ± 0.01	0.44 ± 0.01	15.3 ± 0.18
981	2.33 ± 0.02	0.71 ± 0.04	0.44 ± 0.00	0.39 ± 0.00	3.87 ± 0.02
Poly27	11.0 ± 0.65	0.39 ± 0.00	0.65 ± 0.02	0.46 ± 0.01	12.5 ± 0.67

a) Values are means ± SD.
b) Fresh weight.

Table 3. Literature concentration range and optimal concentration of different antioxidant components present in tomato

Reference	Line	Literature concentration range	Optimal concentration	Coefficient (*K*)
		mg *per* 100 g of FW[a)]		
[10, 28, 32–36, 54, 55]	**Lycopene**	**1.86–14.62**	**10.63**	**20**
[10, 32, 35, 47]	**β-Carotene**	**0.11–1.07**	**0.57**	**10**
	Other carotenoids			**10**
[10, 18, 36]	Phytoene	0.47–1.34	1.19	(3)
[10, 18, 36]	Phytofluene	0.23–1.16	0.90	(2)
[19, 32, 56]	Lutein	0.08–0.34	0.22	(5)
[10, 32, 35, 37, 57]	**Vitamin E**	**0.11–1.84**	**0.70**	**5**
[10, 34, 58]	**Phenolic acids**	**2.75–4.68**	**5.36**	**5**
[10, 19, 34, 36, 58, 59]	**Flavonoids**	**1.15–8.16**	**5.02**	**15**
[10, 32–39, 47, 60]	**Vitamin C**	**2.20–21**	**13.7**	**15**
[10, 18, 38, 47, 61]	**Dry matter**	**5.09–9.49**	**9.16**	**20**
[27]	Fibre	0.8–1.3	1.04	(15)
[10, 61]	Organic acids	1–3	2.4	(5)

Main components are shown in bold.
a) Fresh weight.

The second step was the assignment of a relative weight to each component contributing to the antioxidant action. This coefficient was indicated by the letter *K*. The *K* values for each parameter are reported in the last column of Table 3 and fixed based on three main considerations:

(i) the contribution that tomato gives for the intake of that particular nutrient in the diet (*i.e.* very high for lycopene, intermediate for β-carotene and vitamin C, low for vitamin E);

(ii) the scientific evidence demonstrating a specific health benefit associated to the intake of that specific compound (*i.e.* high for lycopene and lutein and flavonoids, relatively low for β-carotene) [1, 2, 4, 16, 25–28];

(iii) the bioavailability of compounds (high for carotenoids and vitamins C and E, lower for phenolic compounds and flavonoids) [29–31].

The value of *K* can be eventually adapted for different populations in consideration of the role of tomato in the overall diet or as consequence of new discoveries about the healthy properties of tomato antioxidant components.

Summarising, in our model, the nutritional quality of the tomato fruits is given by the following equation:

Table 4. Total value of I_{QUAN} and its component based on the compositional data of tomato analysed

Tomato lines	Lycopene	β-Carotene	Other carotenoids	Vitamin E	Flavonoids	Phenolic acids	Vitamin C	Dry matter	I_{QUAN}
143	18.83	7.25	7.51	1.67	10.19	2.15	9.78	9.61	66
Stevens	20.36	7.58	7.77	1.82	10.13	2.11	13.14	14.83	77
Poly20	31.89	17.40	8.88	3.26	10.64	2.30	10.09	20.04	103
Ontario	13.00	7.19	7.25	3.51	15.46	2.75	12.26	11.90	73
Sel6	19.34	6.58	7.61	2.80	10.16	2.39	12.45	11.18	68
1447	11.17	4.95	7.43	3.15	9.91	2.43	12.56	12.34	64
977	19.90	7.95	8.09	1.56	10.67	3.00	12.72	9.28	72
1513	18.31	12.89	8.01	1.66	10.40	2.32	14.54	12.34	80
988	27.99	11.96	7.55	2.46	9.74	2.45	15.69	8.73	84
Cayambe	26.84	5.54	8.17	1.56	12.22	2.18	8.76	9.74	74
Heline	18.81	12.95	3.86	1.99	9.07	1.90	17.52	13.43	79
1512	6.46	5.18	3.31	2.44	6.36	2.29	14.65	12.34	53
1438	12.62	5.44	8.63	2.03	8.96	2.61	12.90	9.83	63
Motelle	33.60	8.32	8.50	1.24	11.49	2.65	16.80	11.70	93
Momor	26.50	16.88	7.63	4.30	8.71	3.49	11.35	13.97	96
981	4.63	12.51	6.31	2.14	8.47	2.86	11.77	10.70	64
Poly27	21.99	6.88	8.44	1.55	10.61	2.81	11.20	14.19	77
Poly56	28.31	8.89	7.91	4.46	9.98	1.99	15.56	11.75	91

$$I_{\mathrm{QUAN}} = \sum \frac{C_x K_x}{C_{\mathrm{opt}}} \qquad (2)$$

where I_{QUAN} is the index of nutritional quality, C_x the concentration of the component (x) in the sample, C_{opt} the optimal concentration of the component and K_x is the coefficient of relative weight of the component (x).

Applying the formula (2) to the compositional data of the tomatoes analysed in this work, the values shown in Table 4 have been obtained. The value of I_{QUAN} highlights significant differences among the lines ($F_{17,36} = 383.8$, $p < 0.001$). Tomatoes analysed in this work can be divided into three categories according to their I_{QUAN} values: high antioxidant quality ($I_{QUAN} > 90$), intermediate antioxidant quality ($70 < I_{QUAN} < 90$) and low antioxidant quality ($I_{QUAN} < 70$). Comprehensively, the multivariate ANOVA showed a statistically significant difference among all breading lines ($p < 0.001$) on the basis of the overall normal distributed parameters that have been studied. This feature suggests that chemical composition can be used to select tomato lines with healthy promoting proprieties.

Finally, applying the proposed approach to three different sets of literature data [10, 32, 33], the I_{QUAN} ranges from 53 to 103.

4 Discussion

The tomato lines analysed in this study are an excellent source of biomolecules with different antioxidant properties. Comparing the data of this study with those reported in the literature, of 18 genotypes analysed, 10 showed a high level of total carotenoids, 6 high level of β-carotene, 9 high lycopene levels, 15 high flavonoids levels and 2 relevant concentration of vitamin E, whereas phenolic acids showed lower amount comparing literature [10, 18, 32–40]. Among the phytochemicals present in the tomato, carotenoids and particularly lycopene content are emerging as a factor that can be used to increase the commercial value of tomato production thanks to the nutritional benefit correlated to their consumption. Data of the tomato sampling considered for this study confirmed that the concentration of carotenoids can be very different (from 4 to 19 mg/100 g of fresh weight) also when tomatoes having a similar intensity of red colour are considered. These data suggest that the simple colour measure, also if it is carried out by instrumental colorimeters, cannot be used to estimate carotenoid amount and, particularly when the full red stage is reached, the direct HPLC measurement of lycopene should be performed. Lycopene value of our sampling (average value 10.1 mg/100 g of fresh weight) was quite high with respect to the literature (average value 7.1 mg/100 g of fresh weight). This is not surprisingly considering that the lines where selected among those supposed to have high carotenoids content.

Antioxidant compounds play a major role in determining tomato fruits nutritional quality. In the last few years, total antioxidant capacity, an index taking into account the antioxidant capacity of single compounds present in food as well as their potential synergistic and redox interactions [41–46], of tomatoes measured by different assays [47, 48] has been largely applied to drive the selection of healthy tomato lines. The measure of antioxidant capacity on an organic solvent extract (acetone or dichloromethane) is usually well correlated to carotenoid and lycopene concentration, as carotenoids are included into the lipophilic extract [18]. However, even though the carotenoid component is of great importance to determine the nutritional

quality of tomatoes, several other more polar compounds should be considered. In fact, it has been demonstrated that also the water extract contributes to the total antioxidant capacity [10, 33, 46, 49, 50]. Such extract contains polyphenols and vitamin C. Moreover, besides these well-known antioxidants, there are other phenolic compounds bound to the dietary fibre that should be considered [51, 52].

Despite the nutritional importance of measuring the total antioxidant capacity of tomatoes, the bottleneck of this tool is that scientists involved in the breeding programs aiming to improve the nutritional characteristics of tomatoes require to define precisely the concentration of which nutrients must be increased. In fact, plant breeders would like to consider each individual component that contribute to yield and quality and emphasise selection for the attributes important for specific commercial use. The index of antioxidant nutritional quality (*i. e.* I_{QUAN}) here proposed is a comprehensive tool that could be used to evaluate the nutritional quality of tomato lines. In the formulation of I_{QUAN}, the K coefficients represent an equilibrium point among many different considerations related to the specificity of tomato antioxidants, the bioavailability, the contribution of tomato to the overall intake of each component. Based on the proposed index, the importance of polyphenols and particularly flavonoids in selecting tomato genotypes should be increased in the near future. Similarly, the dry matter, which is a parameters considered mainly for technological aspects up to now, will also be evaluated for the content and the typology of dietary fibre and associated phenolic substances. In the light of the nutritional relevance of these components [27, 53], we have attributed in the I_{QUAN} coefficient a relevant weight (15) both to dietary fibre and flavonoids. On the other hand, considering the actual scientific evidence the carotenoid intake (particularly lycopene) from tomatoes remains the most relevant nutritional component of this fruit and therefore the highest weight in the I_{QUAN} (40).

Applying the proposed I_{QUAN}, the values demonstrate that it is possible to join the analysed tomato elite lines into three categories with statistically significant differences. This finding is very interesting and strongly indicate that it would be advisable that future breeding programs, aiming to improve tomato nutritional quality, will consider the individual measurement of all I_{QUAN} components.

In conclusion, the proposed index, which considers all the components related to the nutritional characteristics of tomatoes, would be a reliable tool to compare the nutritional qualities of different tomatoes. It is advisable that future studies in this field will schedule the determination of all analytical parameters considered in the I_{QUAN} to compare the potential health-promoting properties of the selected tomato cultivars.

5 References

[1] Giovannucci, E., Tomatoes, tomato-based products, lycopene and cancer: Review of epidemiologic literature, *J. Natl. Cancer Inst.* 1999, *91*, 317–331.

[2] Pandey, D. K., Shekelle, R., Selwyn, B. J., Tangney, C., Stamler, J., Dietary vitamin C and beta carotene and risk of death in middle-aged men. The Western Electric Study, *Am. J. Epidemiol.* 1995, *142*, 1269–1278.

[3] Sandstrom, B., Astrup, A. V., Dyerberg, J., Holmer, G., *et al.*, The effect on health of dietary antioxidants and antioxidant supplements, *Ugeskr. Laeger* 1994, *156*, 7675–7679.

[4] Weisburger, J. H., Evaluation of the evidence on the role of tomato products in disease prevention, *Proc. Soc. Exp. Biol. Med.* 1998, 140–143.

[5] Nguyen, M. L., Schwartz, S. J., Lycopene: Chemical and biological properties, *Food Technol.* 1999, *53*, 38–45.

[6] Gould, W., Color and color measurement, in: *Tomato Production, Processing and Quality Evaluation*, Avi Publishing, Westport 1974, pp. 228–244.

[7] Di Mascio, P., Kaiser, S., Sies, H., Lycopene as the most efficient biological carotenoid singlet oxygen quencher, *Arch. Biochem. Biophys.* 1989, *274*, 532–538.

[8] Sies, H., *Oxidative stress: Oxidants and Antioxidants*, Academic Press, London 1991.

[9] Beecher, G. R., Nutrient content of tomatoes and tomato products, *Proc. Soc. Exp. Biol. Med.* 1998, *218*, 98–100.

[10] Raffo, A., Leonardi, C., Fogliano, V., Ambrosino, P., *et al.*, Nutritional value of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1) harvested at different ripening stages, *J. Agric. Food Chem.* 2002, *50*, 6550–6556.

[11] Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., *et al.*, The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition, *Free Radic. Res.* 2002, *36*, 217–233.

[12] Shahidi, F., Wanasundara, P. K., Phenolic antioxidants, *Crit. Rev. Food Sci. Nutr.* 1992, *32*, 67–103.

[13] Velioglu, Y. S., Mazza, X., Gao, L., Oomah, B. D., Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, *J. Agric. Food Chem.* 1998, *46*, 4113–4117.

[14] Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., *et al.*, Antioxidant activity of plant extracts containing phenolic compounds, *J. Agric. Food Chem*. 1999, *47*, 3954–3962.

[15] Bourne, L. C., Rice-Evans, C., Bioavailability of ferulic acid, *Biochem. Biophys. Res. Commun.* 1998, *253*, 222–227.

[16] Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., Kromhout, D., Dietary antioxidant flavonoids and the risk of coronary heart disease; the Zutphen elderly study, *Lancet* 1993, *342*, 1007–1011.

[17] Claye, S. S., Idouraineb, A., Webera, C. W., Extraction and fractionation of insoluble fiber from five fiber sources, *Food Chem.* 1996, *57*, 305–310.

[18] Leonardi, C., Ambrosino, P., Esposito, F., Fogliano, V., Antioxidant activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes, *J. Agric. Food Chem*. 2000, *48*, 4723–4727.

[19] Crozier, A., Lean, M. E. J., McDonald, M. S., Black, C., Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery, *J. Agric. Food Chem.* 1997, *45*, 590–595.

[20] AOAC, *Official method of analysis-vitamins and other nutrients*, 1990, pp. 1058 – 1060.

[21] Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., Furda, I., Determination of unsoluble, soluble, and total dietary bran foods and food products. Interlaboratory study, *JAOAC* 1998, *71*, 1017 – 1023.

[22] Esposito, F., Arlotti, G., Bonifati, A. M., Napolitano, A., *et al.*, Antioxidant activity and dietary fibre in durum wheat bran by-products, *Food Res. Int*. 2005, *38*, 1167 – 1173.

[23] Nisen, A., Graffiadellis, M., Jiménez, R., LaMalfa, C. T., *et al.*, *Protected cultivation in Mediterranean climate. Plant production and protection paper*, FAO, Rome 1990.

[24] Saura-Calixto, F., Garcia-Alonso, A., Goni, I., Bravo, L., In vitro determination of the indigestible fraction in foods: an alternative to dietary fiber analysis, *J. Agric. Food Chem.* 2000, *48,* 3342 – 3347.

[25] Khachik, F., Carvalho, L., Bernstein, P. S., Muir, G. J., *et al.*, Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health, *Exp. Biol. Med.* 2002, *227*, 845 – 851.

[26] Stahl, W., Sies, H., Antioxidant activity of carotenoids, *Mol. Aspects Med.* 2003, *24*, 345 – 351.

[27] Lupton, J. R., Turner, N. D., Dietary fiber and coronary disease: Does the evidence support an association? *Curr. Atheroscler. Rep.* 2003, *5*, 500 – 505.

[28] Rao, A. V., Waseem, Z., Agarwal, S., Lycopene content of tomatoes and tomato products and their contribution to dietary licopene, *Food Res. Int.* 1998, *31*, 737 – 741.

[29] Williamson, G., Manach, C., Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies, *Am. J. Clin. Nutr.* 2005, *81*, 243S – 255S.

[30] Rodriguez-Bernaldo de Quiros, A., Costa, H. S., Analysis of carotenoids in vegetable and plasma samples: A review, *J. Food Compos. Anal.* 2006, *19*, 97 – 111.

[31] van den Berg, H., Faulks, R., Granado, H. F., Hirschberg, J., *et al.*, The potential for the improvement of carotenoid levels in foods and the likely systemic effects, *J. Sci. Food Agric.* 2000, *80*, 880 – 912.

[32] Abushita, A. A., Daood, H. G., Biacs, P. A., Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors, *J. Agric. Food Chem.* 2000, *48*, 2075 – 2081.

[33] Binoy, G., Charanjit, K., Khurdiyaa, D. S., Kapoorb, H. C., Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype, *Food Chem.* 2004, *84*, 45 – 51.

[34] Martìnez-Valverde, I., Periago, M. J., Provan, G., Chesson, A., Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato, *J. Sci. Food Agric.* 2002, *82*, 323 – 330.

[35] Abushita, A. A., Hebshi, E. A., Daood, H. G., Péter, A. B., Determination of antioxidant vitamins in tomatoes, *Food Chem.* 1998, *60*, 207 – 212.

[36] Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P., Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes, *J. Sci. Food Agric.* 2003, *83*, 369 – 382.

[37] Podsedek, A., Sosnowska, D., Anders, B., Antioxidative capacity of tomato product, *Eur. Food Res. Technol.* 2003, *217*, 296 – 300.

[38] Sahlin, E., Savage, G. P., Lister, C. E., Investigation of the antioxidant properties of tomatoes after processing, *J. Food Compos. Anal.* 2004, *17*, 635 – 647.

[39] Toor, R. K., Savage, G. P., Antioxidant activity in different fractions of tomatoes, *Food Res. Int.* 2005, *38*, 487 – 494.

[40] Takeoka, G. R., Dao, L., Flessa, S., Gillespie, D. M., *et al.*, Processing effects on lycopene content and antioxidant activity of tomatoes, *J. Agric. Food Chem.* 2001, *49*, 3713 – 3717.

[41] Miller, J. N., Sampson, J., Candeias, L. P., Bramley, P. M., Rice-Evans, C. A., Antioxidant activities of carotenes and xanthophylls, *FEBS Lett.* 1996, *384*, 240 – 242.

[42] Fogliano, V., Verde, V., Randazzo, G., Ritieni, A., Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines, *J. Agric. Food Chem.* 1999, *47*, 1035 – 1040.

[43] Benzie, I. F., Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, *Anal. Biochem.* 1996, *239*, 70 – 76.

[44] Rice-Evans, C. A., Miller, J. N., Paganga, G., Antioxidant properties of phenolic compound, *Trends Plant Sci.* 1997, *2*, 152 – 159.

[45] Miller, J. N., Rice-Evans, C. A., Factors influencing the antioxidant activity determined by the ABTS radical cation assay, *Free Radic. Res.* 1997, *26*, 195 – 199.

[46] Pellegrini, N., Re, R., Yang, M., Rice-Evans, C. A., Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying the 2,2′-azobis(3-ethylenebenzothiazoline-6-sulfonic) acid radical cation decolorization assay, *Methods Enzymol.* 1999, *299*, 379 – 389.

[47] Scalzo, J., Politi A., Pellegrini, N., Mezzetti, B., Battino, M., Plant genotype affects total antioxidant capacity and phenolic contents in fruit, *Nutrition* 2005, *21*, 207 – 213.

[48] Arnao, M. B., Cano, A., Acosta, M., Methods to measure the antioxidant activity in plant material: A comparative discussion, *Free Radic. Res.* 1999, *31*, 89 – 96.

[49] Scalfi, L., Fogliano, F., Pentangelo, A., Graziani, G., *et al.*, Antioxidant activity and general fruit characteristics in different ecotypes of *Corbarini* small tomatoes, *J. Agric. Food Chem.* 2000, *48*, 1363 – 1366.

[50] Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., *et al.*, Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays, *J. Nutr.* 2003, *133*, 2812 – 2819.

[51] Borrelli, R. C., Esposito, F., Napolitano, A., Ritieni, A., Fogliano, V., Characterization of a new potential functional ingredient: Coffee silverskin, *J. Agric. Food Chem.* 2004, *52*, 1338 – 1343.

[52] Pérez-Jiménez, J., Saura-Calixto, F., Literature data may underestimate the actual antioxidant capacity of cereals, *J. Agric. Food Chem.* 2005, *53*, 5036 – 5040.

[53] Depeint, F., Gee, J. M., Williamson, G., Johnson, I. T., Evidence for consistent patterns between flavonoid structures and cellular activities, *Proc. Nutr. Soc.* 2002, *61*, 97 – 103.

[54] Markovic, K., Hruškar, M., Vahcic, N., Lycopene content of tomato products and their contribution to the lycopene intake of Croatians, *Nutr. Res.* 2006, *26*, 556 – 560.

[55] Giordano, I., Graziani, G., Pentangelo, A., Gennaro, L., Fogliano, V., Yield level and qualitative characteristics of small fruited tomato in relation to plant protection with plastic screens, *Acta Hort.* 2003, *614*, 735 – 740.

[56] Khachik, F., Goli, M. B., Beecher, G. R., Holden, J., *et al.*, Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables, *J. Agric. Food Chem.* 1992, *40*, 390 – 398.

[57] Piironen, V., Syvaoja, E. L., Varo, P., Salminen, K., Koivistoinen, P., Tocopherols and tocotrienols in Finnish foods: vegetables, fruits, and berries, *J. Agric. Food Chem.* 1986, *34*, 742 – 746.

[58] Raffo, A., La Malfa, G., Fogliano, V., Maiania, G., Quaglia, G., Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1), *J. Food Compos. Anal.* 2006, *19*, 11 – 19.

[59] Dewanto, V., Wu, X., Adom, K. K., Liu, R. H., Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, *J. Agric. Food Chem.* 2002, *50*, 3010 – 3014.

[60] Gahler, S., Otto, K., Bohm, V., Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products, *J. Agric. Food Chem.* 2003, *51*, 7962 – 7968.

[61] Loiudice, R., Impembo, M., Laratta, B., Villari, G., *et al.*, Composition of San Marzano tomato varietes, *Food Chem.* 1995, *53*, 81 – 89.

