Antioxidant Activity and General Fruit Characteristics in Different Ecotypes of *Corbarini* Small Tomatoes

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Sixteen ecotypes of *Corbarini* small tomatoes were studied. The antioxidant activity was evaluated with the DMPD (*N*,*N*-dimethyl-*p*-phenylenediamine) method in the water-soluble fraction (S-AA) and with the ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] method in the water-insoluble fraction (I-AA). S-AA and I-AA were strongly related to each other, varying to a large extent between the ecotypes considered. They were also significantly correlated with fruit weight, total solids, and refractive index. The combination of the latter variables (by multiple regression analysis) accounted for 36% and 44% of the total variability of S-AA and I-AA, respectively. Moreover, when the ecotypes were subdivided according to their shape (round, pear-shaped, oval, and long), S-AA and I-AA were both significantly higher in round tomatoes and lower in the long ones. In conclusion, the antioxidant activity varies to a considerable extent between different ecotypes of *Corbarini* small tomatoes. These differences are related to shape and some other fruit characteristics.

Keywords: *Antioxidant activity; tomatoes; ABTS; DMPD; Mediterranean diet*

INTRODUCTION

The information on food antioxidant content, especially with respect to phytonutrients, are still incomplete and sometimes wanting (Mangels et al., 1993; Hart and Scott, 1995; Vandenlangenberg et al., 1996). Rather than determining the concentration of each antioxidant molecule, in the past few years several authors have evaluated the "total" antioxidant activity of foods (Benzie, 1996) in terms of the capacity of substances extracted from food matrix to delay the oxidation process in a controlled system (Cao et al., 1996; Wang et al., 1996; Miller and Rice Evans, 1997; Fogliano et al., 1999, Pellegrini et al., 1999). In this regard, a particular interest may be focused on tomatoes and tomato products as they contain several antioxidants (Hertog et al., 1992; Khachik et al., 1992; Hart and Scott, 1995; Tonucci et al., 1995; Sharma and Le Maguer, 1996; Abushita et al., 1997; Crozier et al., 1997; Beecher, 1998) such as vitamin C and carotenoids (especially lycopene, Mangels et al., 1993; Beecher, 1998; Clinton, 1998) and also vitamin A and some flavonoids. In addition, previous works have shown a large difference in antioxidants among different tomato cultivars (Hertog et al., 1992; Mangels et al., 1993; Hart and Scott, 1995; Crozier et al., 1997) but did not specifically explore the potential sources of this variability.

Small tomatoes identified as "Corbarini" or "Pomodorini di Corbara" are widely and increasingly consumed (raw, cooked, or processed) with a constant rise in the surface area cultivated with them. The several ecotypes available (Giordano et al., 1999) differ with respect to size and shape (round, pear-shaped, oval, and long). Their antioxidant properties have never been studied.

The aim of the present study was therefore to evaluate the antioxidant activity of different ecotypes of *Corbarini* small tomatoes and to correlate its variability with shape and other general fruit characteristics.

MATERIALS AND METHODS

Chemicals. 4-Amino-*N*,*N*-dimethyl *p*-phenylendiamine dihydrochloride (DMPD) and gallic acid were from Fluka (Switzerland). L-Ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and butylated hydroxytoluene (BHT) were purchased from Aldrich (Germany). The solvents (HPLC grade) were all from Carlo Erba (Italy).

Spectrophotometric measures were recorded using a UV−vis Shimadzu 2100 instrument (Japan) equipped with a Peltier electronics temperature control and magnetic stirring.

Tomato Sampling. The experiment was carried out between spring and summer of 1998. Sixteen different ecotypes of *Corbarini* tomatoes were studied, belonging to the germoplasm collection of the Istituto Sperimentale per le Culture Industriali, Battipaglia, Italy.

The plants were transplanted in May, cultivated using tutors and berries, and harvested when the fruits on the first five to six branches were well ripe. For each ecotype three samples were collected, each of them consisting of 100 fruits from five plants placed in the same row. Sampling procedure excluded yellow as well as over-ripened and deteriorated fruits. Finally, 20 randomly selected whole fruits for each sample were then frozen at −20 °C and stored until analysis.

Sample Preparation and Basic Determinations. The analyses were carried out within 3 months after harvesting. The whole tomatoes were chopped and homogenized in a Warring blender for 2 min. For each sample, two independent procedures of extraction were then performed. Five g of

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homogenized material was centrifuged at 5000 rpm for 5 min, and the water-insoluble fraction was resuspended in 5 mL of distilled water and centrifuged according to the same procedure. The two above-mentioned supernatant fractions were pooled to constitute the waterly extract. On the other hand, the resulting water-insoluble fraction was extracted with 5 mL of petroleum ether and 5 mL of acetone and again centrifuged after each step. A theoretical volume of 10 mL was considered for both fractions to calculate the antioxidant activity.

Fruit weight was calculated as the mean value of the 60 fruits belonging to the same ecotype. The pH was measured on the homogenized material and refractive index (soluble solids) on the water-soluble fraction, while total solids (TS) were determined by the gravimetric method and expressed in absolute value or as percentage of fresh weight (FrWt).

Measurement of Antioxidant Activity. The antioxidant activity was measured on water-soluble and water-insoluble fractions using two different methods.

The *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) method, described in detail elsewhere (Fogliano et al., 1999), was used to determinate the water-soluble antioxidant activity (S-AA). Briefly: 20 μL of tomato watery 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.

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method (Miller et al., 1995; Miller and Rice-Evans, 1997a) was employed to assess the antioxidant activity of the water-insoluble fraction, with some modifications. The lipophilic extracts were pooled, dried under nitrogen, resuspended in CH_2Cl_2, and added to a solution containing the ABTS radical cation in ethanol.

According to standard reference regression lines, S-AA and I-AA were expressed, respectively, in mg ascorbic acid equivalent and in mg BHT equivalent, either per g FrWt or g TS.

Statistics. Data are expressed as mean ± standard deviation (SD).

The 16 ecotypes were considered as such or subdivided into four major groups according to their shape: round (2 ecotypes), pear-shaped (2), oval (5) and long (7). Simple correlation and multiple regression analysis were used to assess the relationships between variables. Differences between shape groups were evaluated by ANOVA or by analysis of covariance after adjustment for fruit weight, % TS, pH, and RI. The Tukey's test was chosen for pairwise comparisons. A cluster analysis base on S-AA and I-AA was also employed to arbitrary place ecotypes into three different groups. S-AA and I-AA (per FrWt or TS) were logarithmically transformed for simple correlation, regression, and cluster analyses because of their skewed distributions.

RESULTS

A normal distribution of values was observed for fruit weight, TS, pH, and IR, while data for S-AA and, even more, I-AA were positively skewed (skewness 1.05 and 2.04, respectively).

S-AA and I-AA values were very consistent for the three samples examined for the same ecotype, with a coefficient of variation (100 × SD/mean) always below 1.6%. Considering the 16 different ecotypes, S-AA ranged from 0.28 to 0.54 mg AscAc/g FrWt (median 0.36) and I-AA from 0.69 to 1.77 mg BHT/g FrWt (median 0.81), while the corresponding values per g TS were 4.38−8.08 mg AscAc (median 5.72) and 10.7−27.2 mg BHT (median 13.1).

I-AA was strongly correlated ($p < 0.001$) with S-AA when expressed either per FrWt ($r = 0.908$) or TS ($r = 0.885$). Moreover, as shown in Table 1, S-AA/FrWt and I-AA/FrWt were both related to fruit weight, % TS and RI, but not to pH, while S-AA/TS and I-AA/TS were associated (inversely) only with fruit weight. When the multiple regression analysis was used, the combination of RI and % TS accounted for 23% of the overall variability of S-AA/FrWt, but for only 15% of that of I-AA/FrWt. This figure increased to 36% for S-AA and to 44% for I-AA when fruit weight was also included as predictor.

Table 1. Simple Correlation Coefficient of Antioxidant Activity (per Fresh Weight or Total Solids) vs General Characteristics for *Corbarini* Small Tomatoes[a]

	fruit wt	TS	pH	IR
S-AA/FrWt	−0.467[b]	0.490[b]	−0.194	0.502[b]
S-AA/TS	−0.428[b]	0.075	−0.094	0.091
I-AA/FrWt	−0.622[b]	0.431[b]	−0.076	0.425[b]
I-AA/TS	−0.610[b]	0.132	0.007	0.162

[a] FrWt = fresh weight, TS = total solids. The data of AA were logarithmically transformed. [b] $p < 0.005$.

The four shape-groups differed significantly with respect to fruit weight, % TS, pH, and RI (Table 2): fruit weight was greater in the pear-shaped group, pH higher in the oval ones, and RI lower in the long fruits. As shown in Table 3, the ANOVA indicated that the antioxidant activity (per FrWt or TS) was also significantly different between groups for both fractions. On the average, S-AA was much higher in round tomatoes and lower in the long ones, with intermediate values for the pear-shaped and oval ecotypes. Similarly, I-AA was greater in round tomatoes than in the other three groups. These significant differences ($p < 0.001$) persisted even when fruit weight, % TS, and RI were taken as covariates.

Finally, by means of a cluster analysis based on S-AA and I-AA, the 16 ecotypes were classified into three groups (low, intermediate, and high antioxidant activity levels) as shown in Table 4: all the round tomatoes belonged to the higher level and all the pear-shaped and oval ones to the intermediate level, while six out of seven long samples fell within the low level.

DISCUSSION

Tomatoes and tomato products are known to contain significant amounts of different vitamins and other antioxidants (Hertog et al., 1992; Khachik et al., 1992; Hart and Scott, 1995; Tonucci et al., 1995; Sharma and Le Maguer, 1996; Abushita et al., 1997; Crozier et al., 1997; Beecher, 1998; Pellegrini et al., 1999). Previous studies have also indicated that the concentrations of these molecules widely vary among different tomato cultivars (Hertog et al., 1992; Mangels et al., 1993; Hart and Scott, 1995; Crozier et al., 1997). Indeed, most of these data have been obtained on tomatoes purchased directly at the market, without any control or information on growing conditions. In the present experiment, different ecotypes of *Corbarini* small tomatoes were cultivated according to well-controlled conditions in an experimental field and the plants harvested when the fruits of the first five to six branches were completely ripened.

The differences in antioxidant activity between tomato ecotypes were apparent and related to major fruit characteristics. To begin with, S-AA or I-AA were directly associated with TS. This finding can quite easily explained for I-AA, because carotenoids (and other lipophilic antioxidants) are localized in skin and other water-insoluble fractions (Sharma and Le Maguer, 1996). The corresponding increase in S-AA cannot be interpreted on the basis of the available results. Indeed, it should be noticed that S-AA and I-AA were very well

Table 2. General Characteristics of 16 Ecotypes of *Corbarini* Small Tomatoes Subdivided According to Their Shape[a]

	round	pear-shaped	oval	long	ANOVA $p <$
fruit wt (g)	$12.2 \pm 2.8^{a-c}$	19.2 ± 1.4^{a}	18.9 ± 1.7^{b}	18.1 ± 1.8^{c}	0.001
TS (%)	6.63 ± 0.17^{c}	6.23 ± 0.45	6.44 ± 0.38^{d}	$5.90 \pm 0.44^{c,d}$	0.001
pH (unit)	4.34 ± 0.07^{a}	4.37 ± 0.20^{b}	$4.45 \pm 0.08^{a-c}$	4.39 ± 0.06^{c}	0.005
RI (°Brix)	6.12 ± 0.24^{a}	5.67 ± 0.41	5.91 ± 0.38^{d}	$5.37 \pm 0.45^{a,d}$	0.001
					(mean ± SD)

[a] Significant pairwise differences are indicated by values in the same row with the same superscript letter.

Table 3. Antioxidant Activity of 16 Ecotypes of *Corbarini* Small Tomatoes Subdivided According to Their Shape[a,b]

	round	pear-shaped	oval	long	ANOVA $p <$
S-AA/FrWt (mg AscAc/g)	0.51 ± 0.02	0.41 ± 0.01	0.37 ± 0.01	0.30 ± 0.02	0.001
S-AA/TS (mg AscAc/g)	7.75 ± 0.26	6.62 ± 0.64	5.71 ± 0.37	5.14 ± 0.49	0.001
I-AA/FrWt (mg BHT/g)	1.55 ± 0.23	0.97 ± 0.02	0.88 ± 0.09	0.73 ± 0.03	0.001
I-AA/TS (mg BHT/g)	23.5 ± 3.9	15.6 ± 0.9	13.8 ± 1.9	12.4 ± 1.0	0.001
					(mean ± SD)

[a] FrWt = fresh weight; TS = total solids. [b] All the pairwise differences are significant with the exception of pear-shaped vs oval for I-AA/FrWt.

Table 4. Distribution of 16 Ecotypes of *Corbarini* Small Tomatoes in Different Levels with Increasing Antioxidant Activity (Cluster Analysis)

level of antioxidant activity	round	pear-shaped	oval	long
I: low	0	0	0	6
II: intermediate	0	2	5	1
III: high	2	0	0	0

related to each other, giving a similar antioxidant rank for the different ecotypes (as shown also by cluster analysis). Furthermore, a relationship between the concentrations of total carotenoids and vitamin C also occurred when data by Abushita et al. (1997) were reconsidered. Obviously, this point could be better investigated in the future by determining the concentration of different antioxidant molecules in water-soluble and water-insoluble fractions.

Among the other general fruit characteristics, S-AA and I-AA were well correlated with RI (an index of soluble solids and mainly of sugars) but not with pH. It is well-known that modifications in either pH or RI occur during the ripening process, when significant changes in the concentration of different antioxidant molecules are also observed (Abushita et al., 1997). However, it is unlikely that major differences in ripening stage can explain the variability observed in RI and antioxidant activity, as all the tomatoes were harvested by very experienced researchers when completely ripe and according to a number of standard—albeit subjective—criteria (color, tenderness, etc.). Harvesting accuracy is indirectly confirmed by the fact that fruit characteristics and antioxidant activity were very similar in the samples obtained for the same ecotype and also within the same shape group.

Furthermore, an inverse relationship, independent of % TS, was found between antioxidant activity and fruit weight. This is in agreement with a previous observation showing that the concentration of flavonoids is higher in small tomatoes compared to large tomatoes (Crozier et al., 1997). Again, it could be assumed that fruit weight affects the structure of tomatoes and—in particular—the proportions between skin and pulp or between different pulp fractions, which have been demonstrated to contain different amount of antioxidants (Sharma and Le Maguer, 1996). This is expected to be more relevant for lipophilic antioxidants; as a matter of fact, the overall percentage of antioxidant variability explained in the multiple regression analysis by fruit weight, percentage of TS and RI is quite high in both cases, but the relative contribution of fruit weight to the model seems to be greater for I-AA than for S-AA.

Last but not least, the ecotypes of *Corbarini* tomatoes can be clearly subdivided according to their shape into round, pear-shaped, oval, and long. With respect to antioxidant activity the coefficient of variation within groups was low (<5%) in each case for S-AA and in three out of four groups for I-AA. More interestingly, antioxidant activity significantly differed between shape groups with the lowest values in long tomatoes and the highest ones in round tomatoes (Table 3). This could be due to the fact that shape affect the ratio between surface (skin) and fruit total weight. Indeed, after adjusting for fruit weight, % TS, and RI, the differences in S-AA and I-AA due to shape still appear to be very significant ($p < 0.001$). Thus, it may be speculated that ecotypes with the same shape share genetic characteristics that affect directly or indirectly (for instance, through plant height, covering rate, etc.) the synthesis and concentration of different antioxidants.

In conclusion, the antioxidant activity of *Corbarini* tomatoes varies to a considerable extent for both the water-soluble fraction and the water-insoluble fraction. These differences are related to shape and some other fruit characteristics (weight, total solids, and refractive index).

ABBREVIATIONS USED

S-AA, antioxidant activity in the water-soluble fraction; I-AA, antioxidant activity in the water-insoluble fraction; RI, refractive index; FrWt, fresh weight; TS, total solids; AscAc, ascorbic acid.

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