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Bioactive compounds, folates and antioxidant properties of tomatoes (*Lycopersicum esculentum*) during vine ripening

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Abstract

Bioactive compounds and their relationship with antioxidant activity were determined in three tomato cultivars (Ronaldo, Siena and Copo) during vine ripening. The lycopene, chlorophyll (total, a and b), total phenolic, flavonoid, vitamin C and folate contents, and the antioxidant activity, by the ferric reducing/antioxidant power assay and the β-carotene lineolate system, were determined in the samples. Tomato ripening involved the breakdown of chlorophylls, accompanied by a continuous increase in the lycopene content. Total phenolics, flavonoids and vitamin C increased significantly during ripening, whereas the folate content fell markedly as tomatoes turned from green to red. The lycopene and flavonoid content was highest in the Copo cultivar, vitamin C and folate highest in Ronaldo, and total phenolics highest in Siena. The antioxidant activity, as measured with the ferric reducing/antioxidant power assay, increased significantly during ripening in all extracts, and showed a positive correlation with the total phenolic and flavonoid contents. However, when measured with the β-carotene lineolate system, the antioxidant activity decreased significantly during ripening; perhaps due to the antioxidant activity of chlorophylls and the peroxidation activity of vitamin C.

Keywords: *Tomato, phenolic compounds, flavonoids, lycopene, folate, antioxidant activity, ripening*

Introduction

Tomato (*Lycopersicum esculentum*) is regarded as an important functional food, due to its beneficial effects on human health (Pennington 2002). A possible role has been suggested for tomatoes and derived products in the prevention of cardiovascular disease, some types of cancer (Weisburger 1998; Willcox et al. 2003) and ultraviolet light-induced erythema (Stahl et al. 2001). The disease-preventing potential of tomatoes is related to the antioxidant compounds (lycopene, β-carotene, vitamin C and phenolic compounds) they contain and to synergistic interactions between many of them. However, the individual levels of these components in tomatoes, as in other

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crops, vary widely, since they are influenced by genetic factors, cultivar and degree of ripeness, as well as by agronomic and environmental conditions during growth (Fleuriet and Macheix 1985; Grolier et al. 2001; Martínez-Valverde et al. 2002). Thus, the lycopene content reported for Hungarian, Indian and Spanish tomato varieties ranged from 1 to 11 mg/100 g (Abushita et al. 2000; Martínez-Valverde et al. 2002; George et al. 2004). A four-fold variation has been observed for the ascorbic acid content (8.40–32.40 mg/100 g; George et al. 2004) and total phenolics varied from 9 to 27 mg/100 g and from 25 to 50 mg/100 g in Indian and Spanish tomato varieties, respectively (Martínez-Valverde et al. 2002; George et al. 2004).

Tomato ripening involves the breakdown of chlorophylls and build-up of carotenoids, accompanied by a continuous increase in lycopene, the carotenoid responsible for the red colour of ripe tomatoes. Lycopene is a terpenoid with 13 double bonds, 11 of which are conjugated. Thus, it is a potent lipophilic antioxidant, exhibiting the highest physical quenching rate constant for singlet oxygen among the biological carotenoids (Di Mascio et al. 1989) and is also a potent inhibitor of lipid peroxidation and low-density lipoprotein oxidation *in vivo* (Agarwal and Rao 1998; Hadley et al. 2003). Tomato and tomato-derived products are considered the main source of lycopene in the human diet (Shi and Le Maguer 2000), and several epidemiological studies have reported that lycopene-rich diets have a beneficial effect for human health, helping to prevent cardiovascular disease and various epithelial cancers (Sharoni et al. 2000). Tomatoes also contain widely varying amounts of phenolic compounds, which enhance their antioxidant properties. A number of studies have shown that flavonoids and hydroxycinnamic acids are the major phenolics in tomatoes (Fleuriet and Macheix 1985; Crozier et al. 1997; Justesen et al. 1998; Martínez-Valverde et al. 2002). Moreover, tomatoes contain moderate amounts of ascorbic acid, so that their consumption contributes a good proportion of the recommended dietary allowance of vitamin C. Antioxidant activity (AA) in tomatoes is derived from the synergistic interaction of numerous antioxidant compounds, and so any study of AA must measure levels of all such compounds.

Folate is the generic term for folic acid (pteroylmonoglutamic acid) and related compounds exhibiting the biological activity of folic acid. Folates are present in vegetables in monoglutamate and polyglutamate forms; 5-methyltetrahydrofolate (5-MTHF) is one of the predominant natural forms, and is the molecule with the greatest biological activity. Folates have been recognized as a key nutrient for human health, with protective effects against cancer, cardiovascular disease and impaired foetal development (Ros et al. 2002). Although tomatoes are not generally considered a rich source of folates in the human diet, the fact that they are widely consumed in all diets may contribute to the overall beneficial effects of the tomato, particularly in preventing cardiovascular disease (Willcox et al. 2003).

The present study sought to identify the bioactive compounds present at three different stages of tomato ripening, and to ascertain the relationship with AA. This information is of interest to fresh-vegetable processors, food technologists, dieticians and nutritionists anxious to know at which stage the nutritional properties of tomatoes and their beneficial effects for human health are greatest.

Materials and methods

Raw materials

Three tomato cultivars (Ronaldo, Siena and Copo) were grown by the Production Department at Tropicana-Alvalle S.L. (Puente Tocinos, Murcia, Spain). The tomatoes were grown for local fresh consumption, using a hydroponic fertigation system in a commercial greenhouse located in Torre Pacheco (Murcia, Spain). Ten kilograms of each cultivar were harvested at three ripening stages according to the colour of the fruit and following the criteria of the Californian Tomato Commission (2002): green (stage 1), pink (stage 4) and red (stage 6). After washing, tomatoes of the same cultivar and ripeness stage were homogenized with an Omni-mixer (Giralt, International, Waterbury, CT, USA). Samples were stored at −80°C in plastic bottles until analysis. All analyses were carried out in triplicate.

High-performance liquid chromatography determination of lycopene

For lycopene extraction the method described by Sharma and Le Maguer (1996) was used, weighing 1 g tomato into a 125-ml flask wrapped with aluminium foil to exclude light. Fifty millilitres of a mixture of hexane–acetone–ethanol (2:1:1) were added to the flask to solubilize the carotenoids. Samples were shaken for 30 min and then 10 ml distilled water was added. The solution was left to separate into a distinct polar layer and a non-polar layer containing lycopene. The hexane solution containing lycopene was filtered through 0.22-μm filter paper before being directly injected into a high-performance liquid chromatography (HPLC) 2690 Waters system (Waters, Milford, MA, USA). System conditions were: injection volume, 20 μl; detector wavelength, 472 nm; column, Lichospher 100 RP-18 of 12.5 × 0.4 cm; particle size, 5 μm (Merck, Darmstadt, Germany); and a mobile phase of methanol:tetrahydrofuran:water (67:27:6, v:v:v; flow rate, 1 ml/min) (Sharma and Le Maguer 1996). A pure lycopene (Sigma, St Louis, MO, USA) was used for the preparation of calibration curves.

Determination of chlorophylls

Chlorophylls (a, b and total) were calculated spectrophotometrically after extracting 5 g tomato with acetone. The mixture was filtered and the precipitate was washed with 85% acetone until the green colour of the filtrate disappeared. The volume was brought to 50 ml, and the absorbance was read at 663, 652 and 645 nm with a Hitachi U-2000 spectrophotometer (Hitachi Ltd, Tokyo, Japan). The respective chlorophyll contents expressed in mg/100 g were obtained according to the following equations (Fuster and Prestamo 1979):

$$\text{Chlorophyll a} = 20 \times (12.7 \times A_{663} - 2.7 \times A_{645})$$
$$\text{Chlorophyll b} = 20 \times (22.9 \times A_{645} - 2.7 \times A_{663})$$
$$\text{Total chlorophyll} = 20 \times (27.8 \times A_{652})$$

High-performance liquid chromatography determination of ascorbic acid

The ascorbic acid content was measured by reversed-phase HPLC, as described by Esteve et al. (1995). Ten grams of sample were diluted to 100 ml with 1% (w/v)

meta-phosphoric acid solution (Merck), and shaken for 10 min. The extracts were filtered first through Whatman No 1 paper, and then through a 0.45-μm Millipore filter before being analysed by HPLC using a 2690 Waters system equipped with a Waters 996 UV diode array detector. System conditions were: injection volume, 20 μl, detector wavelength, 245 nm flow rate, 1 ml/min; column, Lichospher 100 RP-18 of 12.5×0.4 cm; particle size, 5 μm (Merck). The mobile phase was phosphate buffer 0.1 M (pH 3.5) and vitamin C was quantified as L-(+)-ascorbic acid (Merck).

Determination of total phenolics and total flavonoids

The total phenolics and total flavonoids were analysed spectrophotometrically using a Hitachi U2000 spectrophotometer (Hitachi Ltd). Phenolic compounds were extracted from homogenized tomato samples (2 g) with 80% (v/v) aqueous methanol containing 1% HCl, after shaking for 2 h at room temperature. Extracts were centrifuged at 3,500 rpm for 15 min, and 1 ml supernatant was taken to develop colorimetric reactions. Total phenolics were determined photometrically using Folin–Ciocalteau reagent. Tomato extracts were diluted with distilled water to obtain readings within the standard curve range. Afterwards, 200 μl sample solution was mixed with 800 μl Na_2CO_3 solution and 1 ml Folin–Ciocalteau reagent. The samples were allowed to stand for 120 min at room temperature before the absorbance at 750 nm was measured with a Hitachi U-2000 spectrophotometer. Gallic acid monohydrate (Sigma) was used as the standard using the following calibration solutions: 20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml and 100 μg/ml. The total phenolic content was expressed as milligrams of gallic acid equivalents per kilogram of tomato (Gahler et al. 2004). For total flavonoids, 200 μl tomato extract was mixed with 1.25 ml distilled water followed by the addition of 75 μl of 5% $NaNO_2$. After 6 min, 150 μl of a 10% $AlCl_36H_2O$ solution was added and allowed to stand for another 5 min before 500 μl of 1 M NaOH was added. The mixture was brought to 2.5 ml with distilled water and was mixed well. The absorbance was measured immediately against the blank at 510 nm using a Hitachi U-2000 spectrophotometer. (+)-Catechin (Sigma) was used as standard using the following calibration solutions: 4 μg/ml, 8 μg/ml, 12 μg/ml, 16 μg/ml and 20 μg/ml. Total flavonoid content was expressed as milligrams of catechin equivalents per kilogram of tomato (Dewanto et al. 2002).

High-performance liquid chromatography determination of folate

Folates were extracted with Ches–Hepes buffer, pH 7.85, and 2 mM mercaptoethanol, and were then incubated at 37°C for 3 h with a solution of hog kidney conjugase. Enzyme was inactivated by heating at 100°C for 10 min. A solid-phase extraction tube (DS-Sax; Supelco, Bellefonte, PA, USA) was used to purify and concentrate the sample extracts with a Baker SPE-21 vacuum manifold (Krackeler, Scientific Inc., Albany, NY, USA). The folate content was measured by HPLC with fluorescence and an ultraviolet detector, using a gradient elution of phosphate buffer 2.2 and acetonitrile, following the method described by Vatheristo et al. (1996). Peaks were compared with different standards to quantify compounds. Folic acid was provided by Sigma, and tetrahydrofolate (calcium salt), 5-formyl-tethrahydrofolate (sodium salt), 5-methyl-tethrahydrofolate (sodium salt) and pteroyltri-γ-L-glutamic acid were obtained from Dr Schirck's Laboratories (Jona, Switzerland).

Determination of antioxidant activity

The AA in tomatoes was determined using two methods: the ferric reducing/antioxidant power (FRAP) assay, and the β-carotene lineolate system.

Sample extraction. The AA was evaluated in tomato extracts and in hydrophilic fractions. For tomato extraction, homogenized tomatoes were centrifuged at 5,000 rpm for 20 min; the supernatant obtained was then filtered prior to analysis. To obtain hydrophilic fractions, two extractants were assayed—0.1 M phosphate buffer (pH 7.4) and methanol—following the procedure described in the scientific literature (Azuma et al. 1999; Lavelli et al. 2000; Takeoka et al. 2001; Cano et al. 2003). Four grams of homogenized tomato were mixed with 4 ml of 0.1 M phosphate buffer (pH 7.4) or 4 ml methanol and shaken for 30 min at room temperature. Samples were then centrifuged at 5,000 rpm for 20 min, and the filtered supernatant was placed in a water bath at 100°C for 2 min to inactivate the natural antioxidant enzymes. Extracts were again centrifuged, and were filtered to obtain the clear supernatant to be assayed.

Ferric reducing/antioxidant power assay. The AA was determined following the method described by Benzie and Strain (1996). At low pH, when a ferric–tripyridyltriazine complex is reduced to the ferrous form, an intense blue colour develops with an absorption maximum at 593 nm. FRAP reagent was freshly prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10 mM 2,4,6-tripyridyl-*s*-triazine in a 40 mM HCl solution and 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$. The results obtained with this technique were expressed as micromolar equivalents of Trolox using different standard solutions ranging between 0.1 and 1.55 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid (Sigma-Aldrich, St Louis, MO, USA).

β-*Carotene lineolate system.* The AA was measured following the method described by Al-Saikhan et al. (1995), based on the *in vitro* oxidation of the β-carotene/linoleic acid system. Two milligrams of β-carotene (Fluka, Buchs, Switzerland) was dissolved in 20 ml chloroform solution (Merck). A 3 ml aliquot of β-carotene chloroform solution was added to a conical flask along with 40 mg linoleic acid and 400 mg Tween 40 (both Sigma). Chloroform was evaporated to dryness under reduced pressure at low temperature. One hundred millilitres of H_2O_2 (Merck) was added to the dried mixture, and then shaken. Aliquots of 120 µl tomato extract were added to 3 ml β-carotene/linoleic/H_2O_2 solution in the test tubes before being thoroughly mixed. The tubes were sealed and incubated at 50°C for 40 min. *In vitro* oxidation of β-carotene-linoleic acid was monitored spectrophotometrically with a Hitachi U-2000 spectrophotometer (Hitachi Ltd), reading the sample absorbance at 470 nm, at different times (0, 10, 20, 30 and 40 min). For control purposes, 120 µl deionized water was used instead of sample. The degradation rates for tomato extracts and controls were calculated using the following equation:

$$\text{Degradation rate of the sample } (R) = \text{In } (a/b) \times 1/t$$

where a is the absorbance at time 0, b is the absorbance at 10, 20, 30 and 40 min, and t is the time at which the absorbance is read.

The AA of the samples tested was expressed both as the percentage of inhibition of peroxidation of the β-carotene/linoleic acid system (%AA) and as an oxidation rate

(OX), both calculated using the degradation rate values for samples and controls after 40 min of assay, following the equations described by Al-Saikhan et al. (1995).

$$\%\ \mathrm{AA} = (R_{\mathrm{control}} - R_{\mathrm{sample}}/R_{\mathrm{control}}) \times 100$$
$$\mathrm{OX} = R_{\mathrm{sample}}/R_{\mathrm{control}}$$

Statistical analysis

Statistical analysis of the data was performed using SPSS modules. An analysis of variance was included in the data treatment to study variations in the concentration of individual bioactive compounds and in the total tomato antioxidant activity. A *post hoc* test was carried out for pairwise comparison and to determine significant differences at a level of 5%. A Pearson correlation and linear regression analysis were performed to ascertain the correlation coefficient (r) and the relationship between all studied parameters.

Results and discussion

Chlorophylls and lycopene contents

Table I presents the moisture, chlorophyll and lycopene contents in the three tomato cultivars at each ripeness stage. The chlorophyll content decreased significantly from green to red, due to the breakdown of chlorophylls and the build-up of carotenes, which leads to colour changes. The highest total chlorophyll content was detected in the Ronaldo cultivar, followed by Copo and Siena. The decrease in chlorophyll content as a function of ripeness was also greater in Ronaldo tomatoes (98.6%), followed by Copo (97.2%) and finally Siena (90.70%). Similar behaviour was observed in chlorophylls a and b: both decreased with ripening, and a complete breakdown of chlorophyll b was noted for red tomatoes.

Table I. Moisture, chlorophyll and lycopene contents (mg/100 g) for Ronaldo, Siena and Copo cultivars at three stages of ripening.

Sample	Moisture (%)	Chlorophyll a	Chlorophyll b	Total chlorophylls	Lycopene
Ronaldo					
Green	91.46	48.08 ± 0.18^{a}	20.02 ± 0.56^{a}	65.98 ± 0.32^{a}	ND
Pink	93.07	5.08 ± 0.28^{d}	2.20 ± 0.34^{d}	5.74 ± 0.32^{d}	1.97 ± 0.13^{d}
Red	94.79	1.63 ± 0.29^{f}	ND	0.92 ± 0.32^{e}	2.99 ± 0.18^{c}
Siena					
Green	92.58	18.63 ± 0.13^{c}	8.14 ± 0.24^{c}	25.94 ± 0.64^{c}	ND
Pink	94.21	5.63 ± 0.62^{d}	2.35 ± 0.38^{d}	5.63 ± 0.10^{d}	1.39 ± 0.27^{d}
Red	94.55	2.69 ± 0.38^{ef}	ND	2.41 ± 0.32^{e}	5.15 ± 0.18^{b}
Copo					
Green	93.67	24.51 ± 0.82^{b}	11.82 ± 1.57^{b}	32.77 ± 1.83^{b}	ND
Pink	94.27	3.46 ± 0.06^{e}	2.37 ± 0.53^{d}	5.27 ± 0.75^{d}	1.88 ± 0.22^{d}
Red	94.75	1.95 ± 0.52^{f}	ND	0.93 ± 0.32^{e}	6.81 ± 0.56^{a}

Mean ± standard deviation of three determinations. Data with different superscript letters in columns are significantly different ($P<0.05$). ND, not detected.

Lycopene was not detected in green tomatoes, a finding also reported by other authors (Cano et al. 2003). In pink tomatoes, there were no significant differences in the lycopene content between the three cultivars, although the lowest values were recorded in Siena (1.39 mg/100 g). From the pink to red stage the lycopene content increased significantly (1.5-fold, 3.7-fold and 3.6-fold in Ronaldo, Siena and Copo, respectively), and ranged from 2.99 to 6.81 mg/100 g for Ronaldo and Copo, respectively, in red tomatoes. Thus, the lycopene content in red tomatoes varied 2.3-fold between cultivars, which may be attributed to factors such as plant nutrition, environment and genotype, which, taken together, can markedly affect carotenoid biosynthesis. Similar variations have been reported by a number of authors for tomatoes grown in several countries (Rao et al. 1988; Abushita et al. 2000; Martínez-Valverde et al. 2002; George et al. 2004; Toor and Savage 2005).

Total phenolic and flavonoid content

Figure 1 shows total the phenol and flavonoid content in the three tomato cultivars at each ripeness stage. Values for the phenolic compounds ranged between 66.66 and

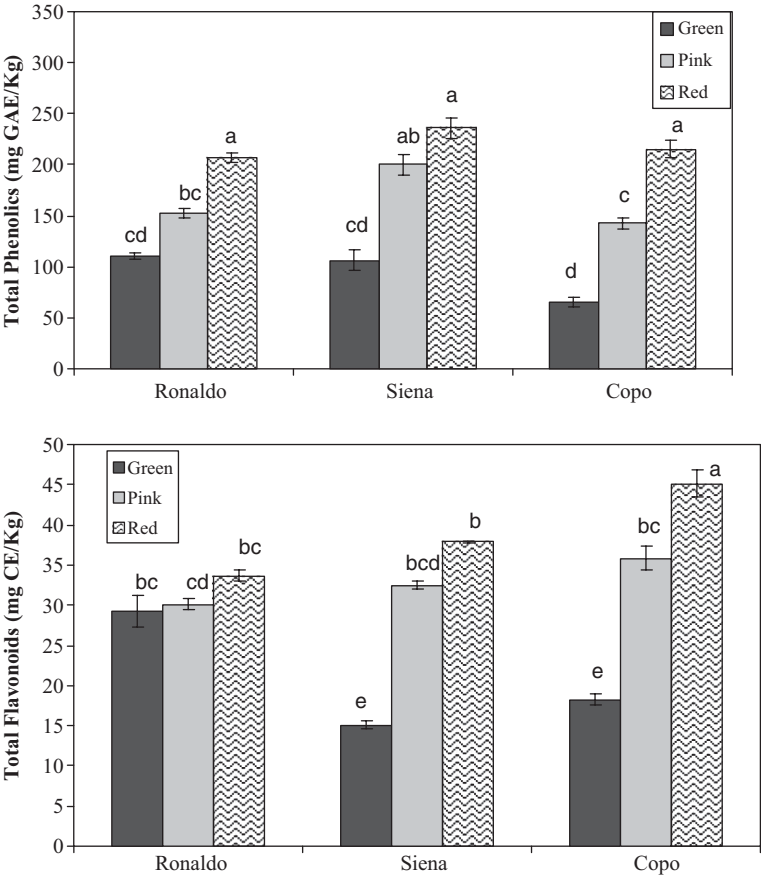

Figure 1. Total phenolic and flavonoid contents (mg/kg) for Ronaldo, Siena and Copo cultivars at three stages of ripening. GAE, gallic acid equivalents. Mean values with unlike superscript letters are significantly different ($p < 0.05$).

235 mg/kg, while the flavonoid content varied from 15.04 to 45.09 mg/kg. Levels of these compounds increased significantly from green to red tomatoes for Ronaldo, Siena and Copo cultivars. Siena displayed the highest total phenolic content, while Copo tomatoes had the highest flavonoid levels. As a general rule, the total phenolic content as determined by the Folin–Cicocalteau method is overestimated, since not only phenolic compounds but also other reducing compounds are determined simultaneously. Nevertheless, this method is still widely used to measure total phenolic levels in plant foods and to assess their antioxidant activity (Wang and Lin 2000; Vinson et al. 1998; Wu et al. 2004).

The total phenolic compound content is governed by a number of factors, including cultivar, ripeness, agronomic conditions and post-harvest handling, which would account for the considerable variability in reported data. The results obtained here agree with those of several authors. Martínez-Valverde et al. (2002), in a study of phenolic compounds in several commercial tomato cultivars grown in Spain, found values ranging from 200 to 500 mg/kg. Giovanelli et al. (1999) reported a range of 50–250 mg/kg depending on ripeness; post-harvest-ripened tomatoes displayed a higher content than vine-ripened fruits. Cano et al. (2003) found an increase in the aqueous phenol content as a function of ripeness, while lipophilic phenols remained unchanged. Several studies report a drop in the hydroxycinnamic acid content (mainly chlorogenic acid) in fully ripened tomatoes (Fleuriet and Macheix 1985; Buta and Spaulding 1997), whereas the flavonoid content increases during ripening and its synthesis might be favoured by light (Hunt and Baker 1980; Caputo et al. 2004).

Ascorbic acid content

The ascorbic acid contents for the three cultivars are shown in Figure 2. As can be seen, the ascorbic acid content was significantly higher in Ronaldo tomatoes than in Siena and Copo. The values ranged from 5.05 to 8.21 mg/100 g for green samples, from 5.99 to 8.26 mg/100 g for pink tomatoes and from 7.91 to 15.41 mg/100 g for

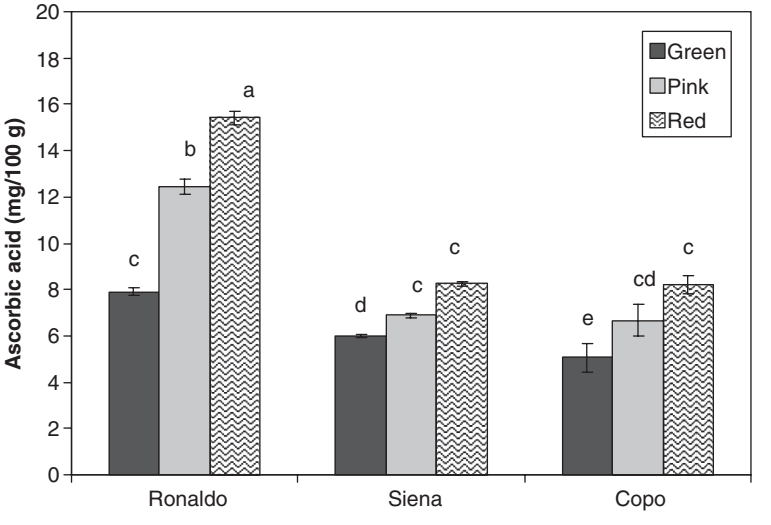

Figure 2. Ascorbic acid content (mg/100 g) for Ronaldo, Siena and Copo cultivars at three stages of ripening. Mean values with unlike superscript letters are significantly different ($p < 0.05$).

red tomatoes, thus displaying a significant increase during ripening. Comparison with the values reported in the literature showed that the vitamin C levels obtained here were in the range described by Bajaj et al. (1990) and Toor and Savage (2005), but lower than those reported by Abushita et al. (1997). The ascorbic acid content is known to depend on factors such as cultivar, ripeness, size, position on plant, light, soil type and indoor or outdoor cultivation. Although the effect of ripeness on total ascorbic acid content in food plants is considerable, it varies considerably (Davey et al. 2000). A number of authors (Jiménez et al. 2002; Cano et al. 2003) reported similar behaviour for the ascorbic acid content during tomato ripening, but their results did not agree with those reported previously by Abushita et al. (1997) and Giovanelli et al. (1999). In addition, variations have been reported as a function of the ripening process. Some authors (Audisio et al. 1993; Jiménez et al. 2002) described higher ascorbic acid contents in vine-ripened than in post-harvest-ripened tomatoes, whereas Giovanelli et al. (1999) found higher values in the last stage of post-harvest ripening than in vine-ripened tomatoes.

Folate content

Figure 3 shows the folate content in tomato samples, which was present only in the form of 5-MTHF, the predominant natural form. Values for the 5-MTHF content were significantly higher in Ronaldo than in the other cultivars, and the content decreased with increasing ripeness (Figure 3). Values ranged from 4 to 13.56 μg/100 g for green tomatoes, from 2.37 to 7 μg/100 g for pink tomatoes and from 1.93 to 6.44 μg/100 g for red tomatoes. In all three cultivars, the folate content decreased by more than 50% from green to red tomatoes, with losses of 52.21%, 51.76% and

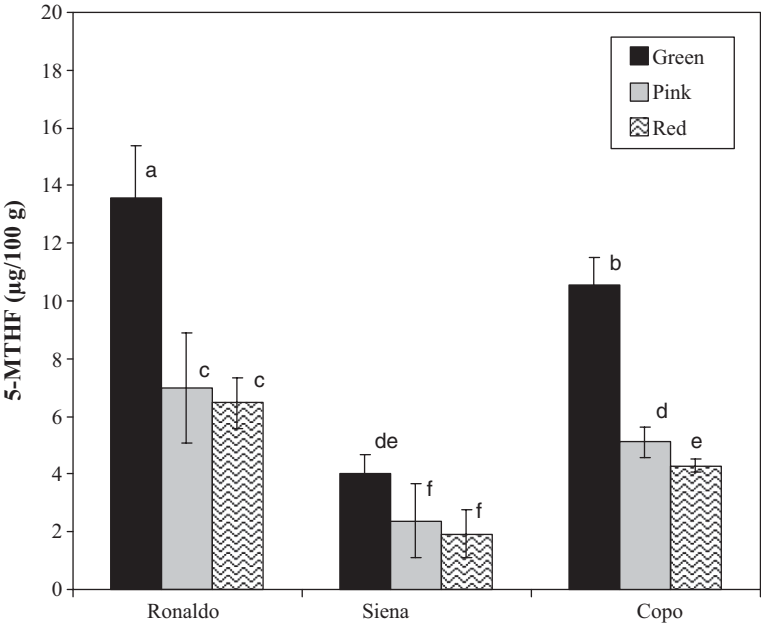

Figure 3. Folate content (5-MTHF μg/100 g) for Ronaldo, Siena and Copo cultivars at three stages of ripening. Mean values with unlike superscript letters are significantly different ($p < 0.05$).

59.47% for in Ronaldo, Siena and Copo, respectively. The folate content also varies with tomato cultivar; Olivares et al. (2004) recently reported values ranging between 6.93 and 16.43 μg/100 g for three tomato cultivars.

The folate content and distribution during higher plant development is currently under investigation, and a hypothesis has been formulated regarding the role of folate in leaves. The highest folate content is found in the plant cell cytosol, and this compound may be involved in the numerous methylation reactions taking place during the build-up of the photosynthetic apparatus (Gambonet et al. 2001). In fact, some chloroplastic enzymes are methylated (Black et al. 1987), and these reactions might require considerable amounts of *S*-adenosyl methionine to incorporate in the chloroplast. This compound is synthesized in the cytosol from 5-MTHF (Gambonet et al. 2001), and has been reported in the leaves of pea plants. The higher 5-MTHF content in green tomatoes than in red tomatoes may enable a methylation reaction of the photosynthetic apparatus, the folate level decreasing thereafter when the tomatoes turn red.

Antioxidant activity

Table II summarizes findings for the AA of tomato extracts measured by two different analytical methods. The ferric reducing ability (FRAP) of both tomato extracts and hydrophilic tomato extracts increased significantly from green to red tomatoes in all three cultivars, since ripe tomatoes had a higher antioxidant-compound content than unripe tomatoes. Although Takeoka et al. (2001) reported considerable AA in the methanolic fraction of tomatoes due to improved extraction of hydroxycinnamic acids (caffeic and chlorogenic acids), the results obtained here showed no significant difference in antioxidant activity as a function of the extraction method. This may be because methanolic and phosphate-buffer extracts solubilize similar amounts of total

Table II. AA of tomatoes (mmol Trolox equivalent/kg), and AA and oxidation ratio (OX) in a lipidic medium for Ronaldo, Siena and Copo cultivars at three stages of ripening.

		AA (mmol Trolox equivalent)		Aqueous extracts		Methanolic extracts	
Sample	Tomato extract	Aqueous extracts	Methanolic extracts	AA (%)	OX	AA (%)	OX
Ronaldo							
Green	0.20±0.02^{c}	0.27±0.02^{d}	0.28±0.01^{d}	58.84±2.78^{a}	0.48±0.026^{a}	62.41±6.78^{a}	0.37±0.01^{e}
Pink	1.19±0.13^{b}	1.21±0.09^{c}	1.26±0.02^{c}	50.88±1.03^{ab}	0.41±0.017^{a}	62.43±0.77^{a}	0.36±0.06^{e}
Red	1.28±0.01^{b}	1.26±0.01^{c}	1.36±0.01^{c}	30.00±2.40^{cd}	0.69±0.025	38.96±1.23^{c}	0.61±0.02^{c}
Siena							
Green	0.19±0.01^{c}	0.24±0.04^{d}	0.24±0.01^{d}	21.85±3.78^{de}	0.78±0.036^{ab}	37.73±1.09^{cd}	0.62±0.02^{ab}
Pink	1.30±0.08^{b}	1.30±0.02^{c}	1.45±0.26^{c}	20.84±1.08^{e}	0.79±0.01^{a}	33.31±2.39^{e}	0.66±0.03^{ab}
Red	1.77±0.11^{a}	1.59±0.03^{b}	1.65±0.10^{b}	19.15±0.96^{e}	0.81±0.01^{a}	32.93±1.61^{e}	0.67±0.02^{a}
Copo							
Green	0.18±0.02^{c}	0.17±0.01^{d}	0.17±0.01^{d}	44.51±2.38^{b}	0.55±0.03^{d}	50.98±2.16^{b}	0.48±0.02^{d}
Pink	1.18±0.04^{b}	1.26±0.01^{c}	1.39±0.06^{c}	33.48±2.39^{c}	0.65±0.01^{c}	40.46±1.58^{c}	0.59±0.01^{c}
Red	1.80±0.06^{a}	1.81±0.09^{a}	2.10±0.05^{a}	31.25±2.00^{c}	0.67±0.02^{c}	37.47±1.41^{cd}	0.62±0.01^{bc}

Mean ± standard deviation of three determinations. Data with different superscript letters in columns are significantly different ($P < 0.05$).

phenols from raw tomato, a finding reported by other authors (Lavelli et al. 2000). The antioxidant activity observed here was similar to that reported by Martínez-Valverde et al. (2002) and by George et al. (2004) for various commercial tomato cultivars and genotypes, by Cano et al. (2003) for four different stages of ripeness, and by Toor and Savage (2005) for different tomato fractions.

The AA determined by FRAP assay was higher in red tomatoes of Siena and Copo cultivars than in Ronaldo variety (Table II), which may be due to the differences in the antioxidant compound content and their synergistic effect in the AA measured. The relationship between AA and bioactive compound content was studied using a Pearson correlation and a linear regression analysis. The FRAP values of tomato displayed a significant positive correlation with total phenol and flavonoid contents ($r>0.87$, $P<0.000$ for both antioxidants), and a slight correlation with vitamin C content ($r=0.40$, $P<0.038$). AA can be estimated using the linear regression equation, taking into account the relative contribution of the different antioxidant compounds. The hydrophilic activity of tomato during development was determined by total phenol and flavonoid contents with the following equation, since these compounds account for 95% of total variance of FRAP values.

$$\text{mM Trolox equivalent/kg} = 5.30\ \text{total phenols} + 2.24\ \text{flavonoids} - 0.711\ (R^2 = 0.95)$$

With regard to the β-carotene lineolate system, which measures the inhibition of lipid oxidation, green tomatoes exhibited greater activity than red tomatoes (Table II). These results suggest an inverse relationship between the inhibition of lipid peroxidation and the antioxidant compound content. For this reason, a negative correlation with antioxidant levels and a weak positive correlation with total chlorophyll and chlorophyll a contents ($r=0.43$, $P<0.022$ in both cases) was found. Although chlorophylls are not considered a dietary antioxidant, these results are in agreement with those reported by Hunter and Flecher (2002), who described that chlorophyll from peas and spinach might contribute to the *in vitro* activity of these vegetables. Thus, in green tomatoes the content of chlorophylls contributed to the AA measured by the β-carotene lineolate system. However, the pink to red tomatoes showed lower AA values because vegetable extracts with a high ascorbic acid content exhibit prooxidant activities (Azuma et al. 1999; Böhm and Schlesier 2004), reducing the potential to inhibit lipid peroxidation. In this model, significant differences were observed between buffer phosphate and methanolic extracts, the latter showing the highest AA content, perhaps due to the greater extraction of antioxidant pigments, especially chlorophylls. It was not possible to obtain a predictive model to estimate the lipid peroxidation activity of tomato, due to the low contribution of the analysed bioactive compounds.

The extraction methods used in this study only provided information on AA because of the hydrophilic compounds present in tomatoes (mainly phenolics and ascorbic acid). Lipophilic compounds such as lycopene are responsible for lipophilic AA in tomatoes, but their contribution to total AA is considered low. In tomato, the hydrophilic fraction shows a higher AA content than the lipophilic fraction, although the extract difference depends on the analytical method used: hydrophilic activity accounted for around 92.5% of the total AA determined with the oxygen radical absorbance capacity (ORAC) method (Wu et al. 2004), approximately 87% as measured with the Xanthine Oxidase (XOD_/xanthine system (Lavelli et al. 2000) and practically 100% when evaluated with the Myeloperoxidose (MPO)/NaCl/H_2O_2 system (Lavelli et al. 2000).

The results obtained here show that hydrophilic antioxidant activity not only depended on the tomato cultivar, as reported previously (Martínez-Valverde et al. 2002; George et al. 2004), but that the stage of ripeness also had a significant effect. Since the extent of this biological activity differs with the evaluation method, it is highly advisable—as recommended by other authors (Böhm and Schlesier 2004)—to use more than one assay to determine the antioxidant potential of food extracts.

Contribution of tomato to dietary intake of bioactive compounds

When establishing the relative dietary contribution of a food, it is important to consider not only the nutrient levels but also the level of consumption of the food. Detailed consumption data for fruit and vegetables in 13 European countries show that potatoes are overwhelmingly the most highly consumed vegetable, with tomato coming second. The contribution of tomato to overall bioactive compound intake is therefore considerable (Table III), but it varies according to cultivar and stage of ripeness. Red tomato is known to be the main source of lycopene, and 30 mg lycopene per week may have beneficial effects for human health. The consumption of one red tomato per day (serving size 130 g) will cover the recommended level. Although tomato phenolic content is only moderate compared with that of some other vegetables, their high consumption in the diet makes them a good source of phenols. Vinson et al. (1998) list the tomato among the main dietary sources of phenolic compounds, but their contribution varies depending on the cultivar and maturity stage. For vitamin C, a serving size of 130 g red and pink tomatoes will provide around 15–32% of the recommended dietary allowances (60 mg vitamin C/day). However, the contribution of tomato to the recommended dietary allowances for folate is low, since the highest content is to be found in green tomatoes.

Table III. Contribution of tomato to total dietary intake of bioactive compounds.

Tomato	Lycopene (mg)	Total phenols (mg)	Flavonoids (mg)	Ascorbic acid (mg)	Folate (μg)
Ronaldo					
Green	–	14.4	3.8	10.3 (17%)	17.6 (9%)
Pink	2.6	19.8	3.9	16.2 (27%)	9.1 (5%)
Red	3.9	26.9	4.4	20.03 (33%)	8.4 (4%)
Siena					
Green	–	13.8	2.0	7.8 (13%)	7.8 (4%)
Pink	1.8	26.1	4.2	8.9 (15%)	3.1 (2%)
Red	6.7	30.6	4.9	10.7 (18%)	2.5 (1%)
Copo					
Green	–	8.4	2.4	6.6 (11%)	6.8 (3%)
Pink	2.4	18.5	4.7	8.7 (15%)	6.7 (3%)
Red	8.8	28	5.7	10.7 (18%)	5.6 (3%)

Data calculated using the mean content of bioactive compound, for a serving size of 130 g. Percentages in parentheses show the contribution of tomato to recommended dietary allowances for ascorbic acid and folate.

Conclusion

It is concluded that red tomatoes exhibit a better antioxidant composition, based on their higher lycopene, total phenolic, flavonoid and ascorbic acid contents. As a result of this antioxidant content, red tomatoes display greater ferric reducing capacity but a reduced lipid oxidation inhibition activity. The antioxidant activity of tomatoes is most probably due to hydrophilic antioxidants, especially total phenols and flavonoids. This information is of interest to fresh-vegetable processors, food technologists, dieticians and nutritionists since it illustrates the stages at which the nutritional properties of tomatoes and their beneficial effects for human health are greatest.

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