# AGRICULTURAL AND FOOD CHEMISTRY

## Irrigation with Diluted Seawater Improves the Nutritional Value of Cherry Tomatoes

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The aim of this study was to assess whether the nutritional value of cherry tomato can be improved by irrigating plants with diluted seawater (12%; EC = 10 mS/cm in comparison with a control at EC = 4 mS/cm). Berries of cherry tomato cv. Naomi were analyzed at the red-ripe stage for the contents of NADPH and NADP<sup>+</sup> as well as for the amounts of the main antioxidants, such as ascorbic acid, lipoic acid, tocopherols, and phenolic acids. As compared to the controls, the fruits of salt-treated plants showed a higher titratable acidity and a higher concentration of reducing sugars. The fruits picked from tomato plants irrigated with diluted seawater produced berries characterized by a higher nutritional value. Following salinity, berries showed higher amounts of vitamin C, vitamin E, dihydrolipoic acid, and chlorogenic acid. It was hypothesized that protocatechuic, vanillic, caffeic, and ferulic acids were utilized to counteract the damaging effects of salinity-induced oxidative stress, allowing tomato fruits to maintain a high reduced status even following salinization.

KEYWORDS: *Lycopersicon esculentum* L.; cherry tomato; salinity; ascorbic acid; lipoic acid; tocopherols; cell redox status; phenols

#### INTRODUCTION

Activated oxygen species (AOS) represent important threats to human health. It is known that about 2-3% of oxygen consumed by a cell is converted into free radicals that rise physiologically during cellular aerobic metabolism or, even more, following pathological events. Indeed, free radicals have been indicated as pathogenesis determinants of many degenerative and chronic diseases such as cancer, cardiovascular disease, cataracts, and immunity system dysfunctions that develop with age (1). Molecules with antioxidant properties, such as vitamin C (ascorbic acid), vitamin E (tocopherols), lipoic acid, and phenols, may counteract the damaging effects of free radicals. Tomato (Lycopersicon esculentum L.), commonly used in the Mediterranean diet, is a major source of antioxidants and contributes to the daily intake of a significant amount of these molecules (2). The consumption of fresh tomatoes and tomato products has been found to be inversely related to the incidence of some types of cancer (3) as well as to plasma lipid peroxidation (4). In addition to phenolic compounds (5), significant amounts of  $\alpha$ - and  $\gamma$ -tocopherol, ascorbic acid (AsA), and lipoic acid could be detected in tomatoes (6). In the physiology of plant cell, these substances are included in an antioxidative cycle, where AsA regenerates  $\alpha$ -tocopherol (the most important antioxidant in the lipid phase) and lipoic acid regenerates AsA. Both lipoic acid (LA) and dihydrolipoic acid (DHLA) have antioxidant power, and DHLA (the reduced form and the most effective one) is able to donate an electron to the oxidized form of AsA, thus strengthening the antioxidant network (7). LA and its reduced form are unique because they are soluble in both lipid and aqueous phases and can connect the activity of antioxidants in the cell membrane (tocopherols) to the antioxidants in the cytoplasm (AsA and glutathione). From a health point of view, LA has been used for the detoxification of heavy metal poisoning and the treatment of atherosclerosis and several side effects of diabetes and to prevent the formation of cataracts (7). However, a single compound or class of compounds cannot determine their positive effect on health as the fresh product does, because this effect is rather exerted by the whole pool of antioxidants with noticeable synergistic effects (8). For this reason, there is convincing evidence that a diet rich in naturally occurring phytochemicals is more effective than the consumption of a single substance (9).

As many of the health compounds belong to the plant defense system, the direct application of a stress to plants can increase the concentration of desirable components. The tolerance of plants to an environmental stress is sometimes associated with increases in the endogenous concentration of antioxidants, and tomato, a moderate-salinity-tolerant species, has been shown to respond positively to irrigation with diluted seawater, increasing the nutritional value of fruits (6). In addition, the

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 Table 1. Electric Conductivity (EC) and Ion Composition of the Nutrient

 Solutions Used for Rockwool Culture of Tomato Plants<sup>a</sup>

	mmol $L^{-1}$									
EC (mS/cm)	N (100% nitrate)	Ρ	S	CI	К	Ca	Mg	Na	Br	Fe
4.0 10.0	13 13	1 1	1.5 3.5	22 77	8.0 8	4.0 4.0	1.5 6.0	20 70	0.1	0.02 0.02

<sup>a</sup> The concentrations of micronutrients were according to Hoagland's nutrient solution.

taste of tomatoes is improved due to higher sugar and acid contents under salinity (6, 10). Thus, the introduction of a controlled irrigation with diluted seawater can be an effective method to produce higher quality tomatoes in addition to face the scarcity and salinization of water resources, which are of major concern in many regions worldwide such as Sicily. In this Mediterranean region, where protected horticulture is one of the most important economic sectors, irrigation with saline water has been put into practice to improve the quality of fresh tomato, in particular cherry types. For this reason, the commercial importance of cherry tomato is continuously increasing, being characterized by a higher content of dry matter and higher soluble solids levels than normal-sized fresh market cultivars (11). These differences are due to the higher contents of sugars (fructose and glucose) and organic acids (citric and malic), which, in turn, are the major factors in determining tomato sweetness and overall flavor intensity (11).

In a previous study (6), we investigated the combined effect of diluted seawater and ripening stage on the nutritional properties of different tomato cultivars and breeding lines, genetically modified for ripening, from an antioxidant point of view. It was found that both fruit development stage and salinity induced an oxidative stress and that the nutritional value of the fruits (as related to the presence of antioxidants) following salt treatment was genotype-dependent.

Little information is available about the compositional profile of cherry tomato with regard to antioxidant compounds (11). The present study focused on the changes in antioxidants and redox status of red-ripe cherry tomatoes (cv. Naomi) grown with diluted seawater under greenhouse conditions. In particular, the reduced and oxidized forms of ascorbic acid and lipoic acid, tocopherols, and phenolic acids, which play a significant role in determining the nutritional value of tomatoes, were analyzed to evaluate whether moderate salinity can improve the nutritional properties, especially the cherry ones.

#### MATERIALS AND METHODS

**Chemicals.** The standards LA, DHLA,  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol, and gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, and ferulic acids as well as glucose-6-phosphate, 2,6-dichlorophenol indophenol, phenazine methosulfate, NADPH, and NADP<sup>+</sup> were purchased from Sigma (Milan, Italy).

**Plant Material.** Cherry tomato (*Lycopersicon esculentum* L. cv. Naomi) plants were grown during the spring of 2003 in a glasshouse with a minimum night temperature and a daytime ventilation temperature of 16 and 28 °C, respectively; the mean value of daily radiation was 9.2 MJ/m<sup>2</sup> with a maximum photon flux density of 500–700  $\mu$ mol/m<sup>2</sup>s. A closed-loop rockwool culture was used. Artificial seawater was prepared according to the makeup of the Tyrrhenian Sea, and the concentrations of nutrients are reported in **Table 1**. Two salinity levels of the nutrient solutions were applied with EC of 4.0 (local irrigation water) or 10.0 mS/cm, which corresponded to 0 and 12% seawater concentration.

Salinization was initiated 3 weeks after planting with 1.5 mS/cm daily increments to avoid osmotic shock. The recycling nutrient

solutions were checked daily for EC and pH (5.5–6.0) and partially replaced every 2 weeks; the EC in each experimental treatment was adjusted every week by adding fresh irrigation water or seawater solution. Irrigation was controlled by a timer that opened the irrigation lines for 2 min up to 12 times per day, depending on growing stage and environmental conditions. A leaching fraction (i.e., the ratio between drainage and irrigation water) of 70–80% was adopted to avoid important differences in the salinity of the recycling solution and in the root zone.

The fruits were picked at red-ripe stage from the second and third truss of separate plants early in the morning. Fruits with >90% of the fruit surface red were considered to be at the red-ripe stage (12).

**Fruit Quality.** Several quality attributes were determined in tomato berries picked at the red-ripe stage. In addition to fresh and dry matter contents (determined by drying the whole fruits in a ventilated oven at 70 °C until constant weight was reached), pH, soluble solids (by a bench refractometer), titratable acidity, and reducing sugars (by colorimetric assay using dinitrosalicylic acid test) were measured on the solution obtained from the filtration of fruit puree.

Ascorbic Acid. Fresh tomato fruits were immediately homogenized at 4 °C in ice-cold 6% trichloroacetic acid (w/v) using first a Waring blender and then a cold mortar. Fresh weight/extraction solution ratio was 1:1. After centrifugation at 12000g for 30 min, total and reduced ascorbate were determined in the supernatants. Determinations were carried out according to the method of Sgherri and Navari-Izzo (13). Calibration curves for AsA and DHA in the range of 5–50 nmol were used.

Lipoic Acid. Both lipoic acid (LA) and dihydrolipoic acid (DHLA) were extracted from tomato berries by acidic hydrolysis according to the method of Vianey-Liaud et al. (14). After hydrolysis, samples were extracted with chloroform following the procedure of Sgherri et al. (15). The resultant organic phases were collected, evaporated to dryness under vacuum, and stored at 4 °C under nitrogen. LA and DHLA contents in the extracted solutions were determined by isocratic RP-HPLC using a Waters apparatus (model 515) with an electrochemical detector (Metrohom model 791) equipped with a glassy carbon electrode and a Millennium software (Waters) for integration of peaks. Detection was performed at +1.1 V at 25 °C with a Nova Pak C-18 4  $\mu$ m column (3.9 mm  $\times$  150 mm). Extracts were eluted at 25 °C at a flow rate of 1 mL/min using 227.5 g of acetonitrile, 31.5 g of 2-propanol, and 674.5 g of 0.05 M KH<sub>2</sub>PO<sub>4</sub> as mobile phase (pH 2.5). Chromatographic peaks were identified by comparing both retention times and spectra with those of the standards. Cochromatography of the standards with the samples was also used to identify peaks with close retention times. Mixtures of standards of LA and DHLA (Sigma) in the range of 4-100 ng were injected to calculate the calibration curve.

**NADPH and NADP<sup>+</sup>.** Fresh tomato fruits were immediately homogenized at 4 °C, using first a Waring blender and then a cold mortar with 0.1 N HCl (NADP<sup>+</sup> determination) or 0.1 N NaOH (NADPH determination). The assay was performed spectrophotometrically (Spectrophotometer UV–visible Varian Cary 1E) at 625 nm as previously described (15). The assay mixture contained extract (10  $\mu$ L), 50 mM Tris-HCl buffer (pH 7.8), 6 mM glucose-6-phosphate, 7.5 mM 2,6-dichlorophenol indophenol, 3.7 mM phenazine methosulfate, and 5 units of glucose-6-phosphate dehydrogenase in the final volume of 1 mL. The levels of NADPH and NADP<sup>+</sup> were calculated referring to standard curves in the range of 5–75 pmol for NADPH and 5–50 pmol for NADP<sup>+</sup>.

**Tocopherols.** Tocopherols were determined in lipid extracts from tomato berries. Extractions were performed in the dark according to the method of Quartacci et al. (16) and under continuous flux of nitrogen. The four tocopherol forms ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -) were determined by isocratic RP-HPLC using a Waters apparatus (model 515) with an electrochemical detector (Metrohom model 791) equipped with a glassy carbon electrode and a Millennium software (Waters) for the integration of peaks. Detection was performed according to the method of Galatro et al. (17) at +0.6 V at 25 °C with a Nova Pak C-18 4  $\mu$ m column (3.9 mm × 150 mm). The extracts were eluted with 95% methanol containing 20 mM LiClO<sub>4</sub> at a flow rate of 1 mL/min. For identification of peaks, the retention times and maximum spectra of tocopherols were

#### Seawater and Nutritional Value of Cherry Tomatoes

**Table 2.** Effect of Seawater Irrigation (12%, EC = 10 mS/cm) on Chemical and Physical Characteristics of Cherry Tomato, Cv. Naomi<sup>b</sup>

EC (mS/cm)	reducing sugars (mmol/L)	fruit wt (g)	titratable acidity (mequiv of NaOH/ 100 mL)	dry residue (% FW) <sup>b</sup>	total soluble solids (°Brix)
4 10	$\begin{array}{c} 287.1 \pm 7.8 \text{ a} \\ 392.3 \pm 9.3 \text{ b} \end{array}$	$\begin{array}{c} 32.6 \pm 1.1 \text{ b} \\ 18.4 \pm 0.8 \text{ a} \end{array}$	$\begin{array}{c} 8.7 \pm 0.4 \text{ a} \\ 12.7 \pm 0.7 \text{ b} \end{array}$	$\begin{array}{c} 7.43 \pm 0.5 \text{ a} \\ 9.85 \pm 0.7 \text{ b} \end{array}$	$5.55 \pm 0.3$ a 7.88 $\pm$ 0.8 b

<sup>*a*</sup> The means (n = 4) in a column followed by different letters are significantly different at  $P \le 0.01$ . <sup>*b*</sup> FW, fresh weight. Before statistical analysis an arc sine or angular transformation was applied.

compared with those of standards, which were also used for quantification. Standard mixtures of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol (Sigma) in the range of 25–75 ng were injected to calculate the calibration curve.

**Phenolics.** Phenolics were extracted from berries (1 g) with 50% methanol containing 1% HCl for 1 h under continuous stirring according to the procedure reported in Sgherri et al. (18). After centrifugation at 12100g for 15 min, the supernatant was collected and the extraction was repeated again twice on the pellet. The methanolic extracts were collected, vacuum-dried, and resuspended in 80% methanol. Before analysis, the samples were passed through a Sartorius (Goettingen, Germany) filter (Minisart 0.45  $\mu$ m) to remove any suspended material.

Qualitative and quantitative analysis was performed by a RP-HPLC (19). Twenty microliters was injected into a Waters model 515 HPLC system fitted with a 3.9 × 150 mm Nova-Pak C18 column (Waters, Milford, MA). Detection was at 280 nm using a Waters 2487 dual  $\lambda$  UV–visible detector. Mobile phase A contained 98% water and 2% acetic acid, and mobile phase B contained 68% water, 30% acetonitrile, and 2% acetic acid. A linear gradient of 0–30% mobile phase B was run for 30 min at 1 mL min<sup>-1</sup>. The identity of the phenolic acids was confirmed by cochromatography on HPLC with authentic standards, and quantification was performed using a standard curve in the range of 0.2–2  $\mu$ g of standard mixtures containing gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, and ferulic acids. Chromatogram analysis was performed by the software Millennium 32 (Waters).

**Statistical Analysis.** The results are the means from three replicates of four independent experiments (n = 4). All data are reported as mean value  $\pm$  SE. The significance of differences among mean values was determined by one-way ANOVA. Comparisons among means were performed using Duncan's multiple-range test. Reported means in table and figures accompanied by different letters are significantly different at  $P \leq 0.01$ . When necessary, an arc sin or angular transformation was applied before statistical analysis was performed.

#### RESULTS

All berries obtained from both treatments were marketable; they had a regular shape and did not show any defect or symptom of disorders such as blossom-end rot or cracking.

The higher salinity of the recyling water significantly reduced fruit weight and increased the dry residue and the content of total soluble solids by 33 and 42%, respectively (**Table 2**); the concentration of reducing sugars and titratable acidity increased as well in the salinized berries by 37 and 46%, respectively.

Berries of plants grown at 4 mS/cm (control) showed a lower presence of NADPH than NADP<sup>+</sup> (NADPH/NADP<sup>+</sup> ratio < 1), whereas in salinized plants the NADPH/NADP<sup>+</sup> ratio of berries approached 1 due to an increase in NADPH contents from 21.52 to 32.72 nmol/g of dry weight (DW) (**Figure 1**).

Higher contents of both AsA and AsA + DHA were found in salinized (10 mS/cm) berries compared to control (4 mS/ cm). Following salinity, the reduced form of vitamin C increased in comparison with the oxidized one, allowing an AsA/DHA ratio of 2 to be reached in the salinized sample (**Figure 2**).



**Figure 1.** Contents of NADPH (left axes) and NADPH/NADP<sup>+</sup> ratios (right axes) in the berries of cherry tomato plants grown in soilless culture with nutrient solutions of different salinities: EC = 4.0 and 10.0 mS/cm. The latter nutrient solution was prepared by simulating diluted seawater. The fruits were sampled at the red-ripe stage. Significant differences ( $P \le 0.01$ ) between treatments are accompanied by different letters.



Figure 2. Ascorbic acid (AsA, left axes) and total ascorbate (AsA + DHA, left axes) and AsA/DHA ratio (right axes) in cherry tomato berries at red-ripe stage of control and seawater-treated plants. Statistical analysis was as in Figure 1.



Figure 3. Dihydrolipoic (DHLA, left axes) and lipoic acid (LA, left axes) and DHLA/LA ratio (right axes) in cherry tomato berries at red-ripe stage of control and seawater treated plants. Statistical analysis was as in Figure 1.

Under saline conditions, DHLA increased by 31%, whereas a decrease by 54% in LA occurred, thus determining a change in the DHLA/LA ratio from 3.6 to 10.3 (**Figure 3**). The overall increment in the total content of lipoic acid following salt treatment was 13%.

Major tocopherols determined were  $\alpha$ - and  $\gamma$ -tocopherol (**Figure 4**);  $\delta$ -tocopherol was found only in trace amounts, whereas  $\beta$ -tocopherol was not detected. The presence of  $\alpha$ -tocopherol was 4-fold higher than that of  $\gamma$ -tocopherol in both the control and salinized berries; salt treatment increased significantly both forms by the same percentage (20%).

The phenolic acids identified in cherry tomato berries (**Figure 5**; **Table 3**) were protocatechuic, vanillic, chlorogenic, caffeic, *p*-coumaric, and ferulic acids. No gallic and syringic acids were detected, and *p*-hydroxybenzoic acid could be detected only at a concentration just above the detection limit. The major phenolic acids in the control berries were caffeic and protocat-



**Figure 4.**  $\alpha$ -Tocopherol and  $\gamma$ -tocopherol (left axes) and  $\alpha/\gamma$  ratio (right axes) in cherry tomato berries at red-ripe stage of control and seawater treated plants. Statistical analysis was as in **Figure 1**.

echuic acid, which showed values of 98.29 and 74.96  $\mu g/g$  of DW, respectively. Vanillic, chlorogenic, and ferulic acids were all present in the same amount, approaching 50  $\mu g/g$  of DW, whereas *p*-coumaric acid was the least representative. Upon salinization, protocatechuic, vanillic, caffeic, and ferulic acids all decreased by 22, 11, 30, and 14%, respectively; by contrast, chlorogenic acid increased by 14%, and the level of *p*-coumaric acid did not change.

#### DISCUSSION

The nutritional value of tomato fruits is dependent on the presence of antioxidant compounds such as vitamin C (ascorbic acid) and vitamin E (tocopherols), the consumption of which is related to oxidative processes that could be induced by natural physiological processes such as ripening or by environmental changes such as salinity and drought (6). Indeed, a high salt level in the root zone may stimulate the production of oxidizing agents in plant cells and may result in redox reactions leading to metabolic alterations and reduced fruit yield (Table 2). Nothwithstanding the reduction of fruit weight, cherry tomato irrigated with diluted seawater appeared to be tastier, with a higher increase in titratable acidity and concentration of reducing sugars in comparison with control berry (Table 2). Cherry tomatoes are generally characterized by higher dry matter and soluble solid levels (Table 2) than normal-sized fresh market cultivars (6). High dry matter and soluble solids are desirable characteristics for the canned tomatoes industry because they improve the quality of the processed product (20).

Compared to salad cultivars and lines genetically modified for ripening (6), cherry tomato showed, other than an increased tastiness, a higher nutritional value. Indeed, in cherry tomato the content of vitamins C and E (Figures 2 and 4) was higher than in normal-sized market cultivars [on average 13.0  $\mu$ mol/g of DW and 120.0  $\mu$ g/g of DW for AsA and  $\alpha$ -tocopherol, respectively (6)]. Cherry tomato showed in comparison with cv. Jama and Gimar WT (6) a great ability to maintain a high reduced status in stressful conditions such as high salinity (Figure 1). This is important to maintain, or even to increase, the reduced forms of antioxidants, which are more effective than their oxidized forms in counteracting the damaging effects of AOS (Figures 2 and 3), which increase following salinity (21). The presence or the increased synthesis of antioxidants could be seen as an adaptive mechanism to biotic and abiotic stresses. The salinity-induced increase in AsA could have been a result of a regeneration from DHLA (15), which indeed increased in salinized berries (Figures 2 and 3), as well as of de novo synthesis, because the total content of AsA and DHA was increased by salinity. Eventually, AsA could have been involved in the regeneration of  $\alpha$ -tocopherol, which also increased in the fruits of plants irrigated with diluted seawater (Figure 4). The presence in all berries and, in particular, in the salinized ones of higher amounts of DHLA compared with LA (**Figure 3**) is particularly meaningful. Indeed, the reduced form is more effective in performing antioxidant functions (22), because it is the only one able to regenerate endogenous antioxidants (15), thus maintaining the ascorbate redox status (**Figure 2**).

Our results are consistent with previous reports (20) on the potential benefits of irrigation with saline water on fruit quality of tomato berries. The antioxidant activity is important for assessing the nutritional value of fruits and vegetables (23), antioxidant compounds being recognized as beneficial for preventing widespread human diseases, including cancer and cardiovascular pathologies (24). In addition, an increase in fruit antioxidants may enhance the shelf life as well, thus improving the overall quality of marketeble fruits.

The increase in NADPH (**Figure 1**), which in turn induced the regeneration of AsA and DHLA (**Figures 2** and **3**), and the increase in vitamin E (**Figure 4**) in salinized cherry tomatoes indicate a high capacity of this tomato cultivar to tolerate the damaging effects of oxidative stress caused by high salinity. This capacity seems higher with respect to other genotypes previously studied (6), in which NADPH decreased following salinity. The higher tolerance of cherry tomato is also demonstrated by its capacity to give marketable fruits when irrigated with seawater diluted to 12% instead of 10%, which was employed for irrigation of cv. Jama and Gimar genotypes (6).

In agreement with this observation,  $\alpha$ -tocopherol concentrations showed significantly higher values in salt-tolerant plants than in more salt-sensitive ones when salt-treated (25, 26). Also, wild-type tobacco plants displayed a >6-fold increase in total tocopherol content from around 70 ng cm<sup>2</sup> in the controls to about 400 ng cm<sup>2</sup> in 400 mM NaCl treated plants (27). The total tocopherol content was closely correlated with the concentration of sodium chloride in the growing medium, with  $\alpha$ -tocopherol contributing to >90% of the tocopherol pool (27). Like photosynthetic tissues, tomato berries (6) accumulate predominantly  $\alpha$ -tocopherol (**Figure 4**), whereas tobacco seeds are rich in  $\gamma$ -tocopherol (27).

The maintenance with increasing salinity of the relative proportion between  $\alpha$ - and  $\gamma$ -tocopherol (Figure 4) did not affect the nutritional value of cherry tomato berries (6). Indeed, the two representative forms of vitamin E have different healthy properties that make  $\gamma$ -tocopherol a less powerful antioxidant than  $\alpha$ -tocopherol. On the other hand,  $\gamma$ -tocopherol represents the "other" vitamin E for human health (28) by trapping peroxynitrite formed in excess during inflammation (29). To date, little is known about the specific roles of  $\alpha$ - and  $\gamma$ -tocopherol in different plant tissues. According to Abbasi et al. (27),  $\gamma$ -tocopherol could be involved in the desiccation tolerance of seeds and leaves as well. This is presumably mediated by the higher in vivo lipid antioxidant activity of  $\gamma$ -tocopherol, which reduced the extent of lipid oxidative membrane damage and pigment loss following oxidative stress in transgenic tobacco plants silenced for  $\gamma$ -tocopherol methyltransferase ( $\gamma$ -TMT). Anyway,  $\gamma$ -tocopherol might not be able to influence cellular signaling like  $\alpha$ -tocopherol does and to substitute the latter to ensure a better plant survival in salt stress conditions (27). According to these authors,  $\alpha$ -tocopherol appears to provide a better protection of macromolecules from salt-induced denaturation than  $\gamma$ -tocopherol. Consequently, the tolerance of cherry tomato to increasing salinity could be in part explained by the increased amounts of both isoforms (Figure 4).



Figure 5. Chromatograms of phenolic acids in cherry tomato berries at red-ripe stage of control (red line) and seawater-treated plants (black line): (1) protocatechuic acid; (2) chlorogenic acid; (3) vanillic acid; (4) caffeic acid; (5) *p*-coumaric acid; (6) ferulic acid.

Table 3.	Effect of	Seawater	Irrigation	(12%, E	:C = 1	10 mS/cm	) on	Phenolic	Acids	of (	Cherry	Tomato,	Cv.	Naomi <sup>a</sup>
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		$\mu$ g/g of DW									
EC	protocatechuic acid	chlorogenic acid	vanillic acid	caffeic acid	p-coumaric acid	ferulic acid					
4	$74.96\pm4.30~\mathrm{b}$	56.47 ± 1.50 a	$49.55\pm2.30\mathrm{b}$	$98.29\pm2.6\mathrm{b}$	$17.91 \pm 0.8  \mathrm{a}$	$53.47\pm2.4$ b					
10	$58.15\pm3.50~\text{a}$	$64.65\pm1.65~\text{b}$	$44.13\pm2.50~\text{a}$	$68.53 \pm 3.1~\mathbf{a}$	$18.72\pm0.5a$	$45.88\pm1.9~\text{a}$					

<sup>a</sup> The means (n = 4) in a column followed by different letters are significantly different at  $P \le 0.01$ .

Even if the contents of protocatechuic and caffeic acid decreased upon salinity, their levels remained high in comparison with other phenolic acids, thus ensuring a reserve of these antioxidants during salt conditions (Table 3). Chlorogenic acid was the only phenolic acid, among those analyzed, that increased following irrigation with diluted seawater (Table 3). This observation supports the hypothesis that, due to its polyhydroxy nature, chlorogenic acid contributes significantly to the antioxidant activity of the tomato water-soluble fraction. In fact, the hydroxyl derivatives of cinnamic acid appear to be more powerful antioxidants than the hydroxyl derivatives of benzoic acid and efficient radical scavengers in vitro because they have the capacity to inhibit lipid peroxidation due to their H-donating abilities as well as their partition coefficients (23). Chlorogenic acid is one of the most abundant forms of phenolic acids, not only in tomato berries (30) but also in apple, pear, tomato, and peach extracts (31, 32). Moreover, it is the most representative phenolic acids in shoots of Raphanus sativus and Ramonda serbica (18, 33). Because the oxidation of chlorogenic acid by horseradish peroxidase is inhibited by AsA (34), the increase in the latter antioxidant with salinity could in part be accounted for by the absence of consumption of the former one (**Figure 2**; **Table 3**). The importance of chlorogenic acid in counteracting the damaging effects of AOS is confirmed by its unusually elevated contents in leaves of the resurrection plant *R. serbica* (*33*) and by its increase under copper treatment in *R. sativus* (*18*). In fact, phenolic acids are synthesized by plants in response to physical injury, infection, or other stresses (*35*), thus increasing the nutritional value of a plant product.

In conclusion, the use of controlled salinity levels can be an effective method to produce tomatoes of a superior organoleptic and nutritional quality and with a higher market price, which may compensate for the reduction in crop yield. This finding is a confirmation of what was previously found for other tomato genotypes (6), even if the present research demonstrates how well cherry tomato tolerates the damaging effects of oxidative stress caused by high salinity, thus improving its nutritional quality. In fact, this capacity appears to be higher with respect to that of the other genotypes previously studied (6), where NADPH decreased following salinity. Even the changes in

phenolic acid contents could be of some importance for what concerns the nutritional value of cherry tomato: even if the two major phenolic acids, caffeic and protocatechuic acid, decreased upon salinity, their content remained very high and chlorogenic acid was found to increase following salt treatment. In addition, considering that water is already a limiting factor in many regions worldwide such as Sicily, where cherry tomato represents one of the main fresh products, irrigation with diluted seawater deserves attention in future production. However, caution in the practice of over-irrigation with salty water should be held to avoid deleterious impact on the soil. Despite the enormous interest in phenolic compounds as potential protective agents against the development of human diseases, the real contributions of such compounds to health maintenance and the mechanisms through which they act are still unclear (36). Anyway, phenolic acids and, in particular, chlorogenic acid might have important biological effects for human health other than physiological roles in plants, and greater attention should be given to these compounds. Studies in this field are still in progress in our laboratory.

#### **ABBREVIATIONS USED**

AOS, activated oxygen species; AsA, ascorbic acid; DHA, dehydroascorbic acid; DHLA, dihydrolipoic acid; DW, dry weight; FW, fresh weight; LA, lipoic acid.

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Received for review November 12, 2007. Revised manuscript received February 13, 2008. Accepted February 15, 2008. This paper was supported by the Italian Ministry of University, Scientific and Technological Research (PRIN 2003) and by the University of Pisa (Fondi di Ateneo 2003).

JF0733012