

## The Influence of Different Electrical Conductivity Values in a Simplified Recirculating Soilless System on Inner and Outer Fruit Quality Characteristics of Tomato

SANDRA KRAUSS,\* WILFRIED H. SCHNITZLER, JOHANNA GRASSMANN, AND  
 MARKUS WOITKE

Institute of Vegetable Science, Life Science Center Weihenstephan, TUM, Dürnast 2,  
 85350 Freising, Germany

Irrigation with saline water affects tomato fruit quality. While total fruit yield decreases with salinity, inner quality characterized by taste and health-promoting compounds can be improved. For a detailed description of this relationship, the influence of three different salt levels [electrical conductivity (EC) 3, 6.5, and 10] in hydroponically grown tomatoes was investigated. Rising salinity levels in the nutrient solution significantly increased vitamin C, lycopene, and  $\beta$ -carotene in fresh fruits up to 35%. The phenol concentration was tendentially enhanced, and the antioxidative capacity of phenols and carotenoids increased on a fresh weight basis. Additionally, the higher EC values caused an increase of total soluble solids and organic acids, parameters determining the taste of tomatoes. Total fruit yield, single fruit weight, and firmness significantly decreased with rising EC levels. Regression analyses revealed significant correlations between the EC level and the dependent variables single fruit weight, total soluble solids, titrable acids, lycopene, and antioxidative capacities of carotenoids and phenols, whereas vitamin C and phenols correlated best with truss number, and  $\beta$ -carotene correlated best with temperature. Only pressure firmness showed no correlation with any of the measured parameters. As all desirable characteristics in the freshly produced tomato increased when exposed to salinity, salinity itself constitutes an alternative method of quality improvement. Moreover, it can compensate for the loss of yield by the higher inner quality due to changing demands by the market and the consumer. This investigation is to our knowledge the first comprehensive overview regarding parameters of outer quality (yield and firmness), taste (total soluble solids and acids), nutritional value (vitamin C, carotenoids, and phenolics), as well as antioxidative capacity in tomatoes grown under saline conditions.

**KEYWORDS:** *Lycopersicon esculentum* Mill; cv. Durinta; salinity; NaCl; Brix°; phenols; carotenoid; vitamin C; antioxidative capacity

### INTRODUCTION

Over the past years, consumer awareness increased regarding foodstuffs as a source of health-supporting functions, which help to prevent many chronic diseases and dysfunctions. Although a wealth of functional foodstuffs are created to fulfill these requirements, one should keep in mind that “conventional food” like fruits and vegetables can serve this purpose probably even better (1). A customary vegetable like tomato, which is the most important vegetable worldwide, can fully fit the requirements for a balanced diet. It contains a series of beneficial health compounds and can be easily integrated in daily nutrition: Besides their fresh uptake, consumers use tomatoes in soups, sauces, pizza, pasta, and many other dishes.

The tomato’s importance as a nutraceutical, phytochemical, and chemopreventive vegetable (1) is based on its different

health-promoting ingredients: First, they contain several carotenoids (CARs) and are the main source of lycopene (LYC) in our diet (2). Antioxidative abilities of LYC and  $\beta$ -carotene ( $\beta$ -CAR) are a likely mechanism by which tomatoes prevent diet-related diseases, as the involvement of reactive oxygen species (ROS) in those is probable (3). In addition, LYC probably reduces the risk of prostate cancer (4). Besides CARs, polyphenolic compounds also contribute to the nutritional value of tomato (5). Depending on the cultivar, they differ in amounts and patterns with several antioxidative effects (6). Vitamins C and E (Vit C and E) are further naturally occurring antioxidants found in tomatoes (5). There is convincing evidence that a diet rich in naturally occurring phytochemicals is more effective than the consumption of single substances due to synergistic interactions (7). Thus, the consumption of “naturally designed” tomatoes is more advantageous for health than consuming LYC,  $\beta$ -CAR, phenols (PHEs), or Vit C alone.

\* To whom correspondence should be addressed. Tel: +49-8161-715593. Fax: +49-8161-714491. E-mail: krauss@wzw.tum.de.

For that reason, food-producing and -processing industries are highly interested in the improvement and enrichment of so-called health compounds in fruits and vegetables by either genetic engineering, choice of better cultivars, or well-directed cultivation methods. Besides manipulations of the isoprenoid pathway, genetic modifications of the flavonoid biosynthesis in tomatoes are also under investigation (8). The idea of increasing health benefits in daily consumed vegetables is promising and alluring, but most consumers do not accept genetic engineering as the method of choice. As many of the health compounds belong to the plant defense system, the well-directed application of stress to plants can probably increase the concentration of desirable components.

As several investigations implicated, salt stress enhances inner quality, i.e., compounds contributing to taste and nutritional value, of tomatoes (9, 10). Increased electrical conductivity (EC), reached by adding NaCl to a nutritional solution, leads to higher contents in LYC,  $\beta$ -CAR, and Vit C in controlled atmospheres. The antioxidative capacity (AC) is enhanced (11, 12). Additionally, the taste of the tomatoes is improved due to higher sugar and acid contents under salinity (11, 12). Thus, the introduction of controlled and defined salinity levels can be an effective method to design higher quality tomatoes. Admittedly, the implementation of stress toward the plants will lead to more or less pronounced losses in total and marketable yields (kg/plant or t/ha), which needs compensation by raising the price for a tastier and healthier produce of interest to the producer. Another aspect when using higher salinity levels in a simplified recirculating soilless growing system is the increase of water use efficiency and the application of poor quality irrigation water, which is of major concern in many regions worldwide facing water shortages. We like to show that a tradeoff between an environmentally sound production (water reuse, use of poor quality water), which is accompanied by a decrease in yield, can be balanced at least partly by the increase in fruit quality. To what extent and if economically feasible will depend on water costs and on consumer acceptance.

To get a complete impression of the pros and cons of cultivating tomatoes under saline conditions, we conducted a comprehensive investigation on this topic, regarding parameters of outer quality (yield and firmness), taste [total soluble solids (TSS) and sugars], and nutritional value (Vit C, CARs, and phenolics) with a special emphasis on secondary plant metabolites and their ACs.

## MATERIALS AND METHODS

**Fruit Sampling.** The experiment was conducted in 2004 at the Research Station Dürnast, Chair of Vegetable Science, Life Science Center Weihenstephan, Technische Universität München, in Freising (southern Germany). The investigation was carried out under controlled greenhouse conditions. The air temperature was set at 22 (day) and 16 °C (night). The relative humidity (RH) was approximately  $73.8 \pm 16.2\%$  with min and max values of 98.3 and 23.3%.

The longlife truss tomato cultivar Durinta (F1 hybrid, Fa. Western Seed) was planted. Ten week old tomato plants were transplanted into the greenhouse on the 10th of March. Plants were grown in 10 L pots (two plants per pot) filled with uncomposted wood fiber (Toresa spezial, Fa. Intertoresa). For a good plant establishment, all seedlings were first fertigated with a nonsaline full nutrient solution adjusted to pH 5.5–6.5 and with an EC value of approximately  $3 \text{ dS m}^{-1}$  in closed recirculating nutrient solution with macronutrients  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{SO}_4^{2-}$  and micronutrients Fe, Mn, Zn, B, Cu, and Mo at 10.0, 1.25, 6.5, 3, 1.25, 1.0, and 1.0  $\text{mmol L}^{-1}$  and 15, 10, 4, 20, 0.75, and 0.50  $\mu\text{mol L}^{-1}$ , respectively. The EC level and pH were measured daily with a handheld pH–EC meter in the supply solution and adjusted accordingly. Three weeks later, a solution of 17

and 34  $\text{mmol L}^{-1}$  NaCl was added to the nutrient solution of the saline treatments, resulting in a final EC value of 6.5 and 10  $\text{dS m}^{-1}$ . The NaCl concentration in the nutrient solution was measured biweekly and adjusted to requested levels (Test kits, Fa. Merck, Germany). The drain solution was analyzed fortnightly for element constitution. The drainage rate was  $\geq 70\%$ . Plants were watered at fixed intervals [every 20 min for 5 min (8 L/h)] using a drip irrigation system. The plants were drained in the high wire system, all axillary shoots were removed weekly, pruning of the lowest leaves was carried out to commercial practice, and deployment of bumble bees was used for pollination. Cultivation stopped on August 2, 2004. Each treatment [control (EC 3), EC 6.5, and EC 10] consisted of three replicates (14 experimental plants per replicate) arranged as a fully randomized block design.

**Evaluation Parameters/Methods.** The first three fruits of clusters 4, 6, and 10 were harvested at the same ripening stage (stage “9–10” at the color scale, Ctfl, Bergere, France) for measuring parameters of the fresh tomatoes: single fruit weight (SFW), pressure firmness (PF), TSS (refractometric index Brix° at 20 °C, measured with a DBX-55A refractometer from Atago, Japan), and dry weight (DW). Firmness is given as PF using the proposed formula in Brückner and Shewfelt,  $2000 \{P = F \times 1000 / [(h/2)^2 - (h/2 - d/2)^2] \times 2 \times \pi\}$  compensating for fruit size ( $P = \text{PF}$  in kPa,  $F = \text{deformation force}$  in N,  $h = \text{fruit height}$  in mm,  $d = \text{deformation}$  in mm, and  $\pi = 3.14$ ) (13). Deformation was examined with a texture analyzer (Instron TA-XT2). For detection of SFW, 30 fruits per replicate were measured ( $n = 90$ ), and PF and TSS were attained from 15 fruits per replicate ( $n = 45$ ). One mixed sample of three fruits from each replicate was taken to analyze DW ( $n = 3$ ).

The plant height (PH) was measured from all plants per replicate ( $n = 42$ ). The total fruit yield (TFY) per plant was calculated as an average using the measured total yield per replicate over the period from May 24, 2004, to May 8, 2004, divided by the number of plants per replicate ( $n = 3$ ).

**Preparation/Extraction.** To evaluate the inner quality of the experiment, 15 representative fresh tomatoes of each replicate were used for the analysis mentioned above; that is, for each treatment, a mixed sample containing 45 fruits of the selected tomatoes was freeze-dried and subsequently ground to powder. The following analyses were attained from freeze-dried material.

Organic acids were extracted from 1 g of tomato powder with 50 mL of water. Samples were put in a water bath (Typ 1092 Gesellschaft für Labortechnik MbH) at 25 °C. An aliquot of 10 mL from the filtered extract was titrated with 0.1 M NaOH by adding phenolphthalein as an indicator to the extract. The content of organic acids is given as citric acid (14). For each treatment—EC 3, 6, and 10—four titrations were made ( $n = 4$ ).

A 0.4 g amount of tomato powder was extracted with 5 mL of 1.5% *meta*-phosphoric acid to analyze Vit C. The extract was homogenized in the ultrasonic bath for 10 min, centrifuged for 3 min (10000 U/min), and filtered prior to HPLC injection.

CARs were extracted according to Hart and Scott (19): A 0.1 g amount of powder and 0.01 g of potassium carbonate were mixed with 0.9 mL of methanol (MeOH)/THF 1:1 (v/v), thoroughly vortexed, and treated for 3 min in the ultrasonic bath at 6 °C. In case of extraction for HPLC analysis, 0.18 mL of  $\beta$ -apo-carotenol (500  $\mu\text{M}$ ) was added as an internal standard and butylated hydroxytoluene (BHT) as an antioxidant. Samples for AC tests contained neither an internal standard nor BHT.

The resulting suspension was mixed with 0.5 mL of hexane and 0.5 mL of 10% NaCl, again vortexed, and centrifuged for 3 min (4000 U/min). The hexane phase, which contained the CARs, was withdrawn. Again, 0.6 mL of hexane was added followed by the centrifugation step and the hexane phase was withdrawn. This step was repeated another four times (altogether, six extraction steps with hexane). The collected hexane phases were evaporated to dryness in a vacuum concentrator and stored at  $-20$  °C until use.

The extraction of PHEs was modified after a method of Tura and Robards (15): A 0.4 g amount of powder was mixed with 4 mL of 80% MeOH, thoroughly vortexed, and treated ultrasonically for 30 min at 6 °C. The MeOH phase was withdrawn, filtered, evaporated to dryness in a vacuum concentrator, and stored at  $-20$  °C until use.

**Table 1.** Gradient of HPLC Analysis for CARs

min	solvents (%)		
	MeOH + 0.005% TEA	acetone	H <sub>2</sub> O + 0.05M NH <sub>4</sub> Ac
0	86	10	4
10	56	40	4
30	10	90	0
35	0	100	0
40	0	100	0
45	86	10	4
60	86	10	4

**Analysis. HPLC.** The Vit C content was detected by high-performance liquid chromatography (HPLC) (Dionex UVD 340S PDA-Detektor; Dionex P580 pump; Dionex Gina 50 Autosampler; Phenomenex Synergi Hydro RP; 250 mm × 4.60 mm, 4 μm column) at 245 nm with a flow rate of 1 mL/min. Twenty microliters was injected. The solvent was water containing 0.4% *ortho*-phosphoric acid (8.5%) and 1% 10 mM ethylenediaminetetraacetic acid disodium salt (EDTA) in H<sub>2</sub>O for 12 min modified after Davies (16). The column oven was set at 20 °C. Two extracts were measured two times ( $n = 4$ ).

CARs were resolved in 500 μL of CH<sub>2</sub>Cl and 1500 μL of ethanol (EtOH) and analyzed by HPLC according to Sander et al. (17) using a Merck Hitachi L 6200 A intelligent pump with a Phenomenex Develosil RP aqueous C30 column (250 mm × 46 mm, 5 μm) and an UV-vis Detector (Thermo Separation Products). Peaks were quantified with OMEGA software from Perkin-Elmer. The gradient consisted of MeOH + 0.05% triethylamin, acetone, and H<sub>2</sub>O + 0.05% ammonium-acetate (NH<sub>4</sub>Ac) as solvents (Table 1). The injection volume was 20 μL, the column oven was set at 30 °C, the detection wavelength was 450 nm, and the flow rate was 1 mL/min. For each treatment, two independent extracts were analyzed each two times ( $n = 4$ ).

**Folin Test.** The PHE extract was resolved in 1 mL of 80% MeOH, and reducing agents were quantified by means of Folin-Ciocalteu (18). Results are given as gallic acid equivalents (GAEs). For each treatment, two independent extracts were analyzed three times each ( $n = 6$ ).

**AC.** For detection of AC, a 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) decoloration assay was used (19). The assay contained in a total volume of 2 mL 1 mM ABTS, 6 μM peroxidase (POD), 0.7% acidified EtOH (0.35 mL of *ortho*-phosphoric acid in 50 mL of EtOH), water, and EtOH. All reagents were solved in EtOH, with the exception of POD, which was solved in H<sub>2</sub>O. The ABTS radical cation was generated by adding 35 μM hydroxyl peroxide (H<sub>2</sub>O<sub>2</sub>). CAR samples were solved in 500 μL of CH<sub>2</sub>Cl and 500 μL of EtOH, and PHEs were solved in 1 mL of 80% MeOH. The absorption of the radical was determined at 734 nm (UV-vis Photometer Pharmacia LKB Biochrom 4060). After 2 min, 100 μL of sample was added, and after 6 min, absorption was determined again. The absorption difference is a measure for AC. AC is given as Trolox equivalents (TE). For each treatment, two independent extracts were analyzed three times each ( $n = 6$ ).

**Instruments.** The instruments used were as follows: Texture Analyzer Instron TA-XT2 [Stable Micro Systems (United Kingdom) apparatus], Refractrometer DBX-55A (Atago, Japan), UV-vis Photometer Pharmacia LKB Biochrom 4060, Ultrasonic bath Sonorex Super 10 P from Bandelin, and Water bath Type 1092 Gesellschaft für Labortechnik MbH.

**Chemicals.** ABTS was obtained from Merck; POD was from Roche; gallic acid, citric acid, sodium carbonate, and Trolox were from ACROS; *meta*- and *ortho*-phosphoric acid, Folin-Ciocalteu reagent, tetrahydrofuran (THF), EDTA, hexane, and EtOH were from Merck; BHT was from Sigma; MeOH and acetone were from Fisher Scientific; LYC was from Wako; β-CAR, ascorbic acid, phenolphthalein, and β-apo-8'-carotenal (trans) were from Fluka; dichlormethane was from J. T. Baker; and potassium carbonate was from the pharmacy.

**Statistical Evaluation of Data.** For statistical analysis, SPSS version 12.0 for windows was applied. EC level main effect was analyzed by one-factorial analysis of variance (ANOVA). Means were compared using Tukey's test. The main effect and pairwise comparison of means were estimated significant at a  $p$  level < 0.05. Different letters in tables

**Table 2.** Effects of Salinity Levels on TFY Per Plant, PH, and DW<sup>a</sup>

	TFY (kg/plant)	PH (cm)	DW (%)
control	2.2 ± 0.2 a	456 ± 21 a	4.74 ± 0.03 c
EC 6.5	2.1 ± 0.1 a	413 ± 25 b	5.43 ± 0.14 b
EC 10	1.7 ± 0.3 a	369 ± 20 c	6.70 ± 0.34 a
$p$ ( $n$ )	0.055 (3)	0.000 (42)	0.000 (3)

<sup>a</sup> In this and the following tables, numbers indicate means ± SD, sample size ( $n$ ), and significance level ( $p$ ) of ANOVA. Different letters behind numbers indicate statistical significance of means estimated using Tukey's test,  $p > 0.005$ .

and graphs indicate significant differences between treatments. Stepwise regression analysis was carried out to determine correlation and between measured dependent (SFW, TSS, TA, Vit C, LYC, β-CAR, PF, AC CAR, AC PHE, and PHE) and independent variables [EC level, truss number, irradiation (W/m<sup>-2</sup>), temperature (°C), and RH (%)]. Values integrated in the analysis for irradiation, temperature, and RH were found to be relevant during the period of 30 days before fruit harvest, i.e., because fruit growth can be divided into three phases: slow growth in the first 2–3 weeks after anthesis (mainly cell division), rapid growth (cell expansion) when most weight is accumulated, and slow growth again for about 2 weeks before ripening (20). Best fits were indicated by Pearson's correlation coefficient and estimated significant at  $p \leq 0.05$ . The probability of  $F$  value for elimination or retention of variables in the model was set at  $\geq 0.1$  and  $\leq 0.05$ , respectively.

## RESULTS

Results are depicted and graphically shown only for cluster 6, as trends according to fruit quality parameters were basically comparable between the clusters. Means and significant influences of the evaluated parameters are additionally shown for clusters 4 and 10 in Table 3 on the basis of 1 g DW and in Figures 2–4 calculated on 100 g fresh weight (FW).

Stepwise regression analysis (Table 4) revealed the EC level as best fitting and significantly correlating with all quality parameters with the exception of Vit C (RH), β-CAR (temperature), and PHEs (truss number).

**Total Yield, Fruit Height, and DW.** To achieve an overview of the influence of salinity to vegetative growth parameters, TFY per plant and PH are shown in Table 1. TFY decreased from 2.2 kg/plant in the control to 2.1 kg/plant in EC 6.5 and 1.7 kg/plant in EC 10. PH was 456 cm in the control, 413 cm in EC 6.5, and 369 cm in EC 10. DW increased highly and significantly with rising salt stress from 4.74 (control) to 5.43 (EC 6.5) and 6.7% (EC 10) (Table 2).

**Parameters of the Fresh Tomatoes.** SFW decreased significantly from 70.2 g in the control to 57 g in EC 6.5 and to 42.5 g in EC 10 (Figure 1A). This relation correlated highly and significantly ( $r = -0.939$ ,  $p \leq 0.001$ ). Fruit firmness also decreased with increasing salt concentrations from 44.5 kPa PF to 43.3 kPa (EC 6.5) and 39.0 kPa at EC 10 (Figure 1C). Fruit firmness was the only quality parameter; no correlation could be attributed according to regression model settings. Even the effect of EC level on TSS was highly significant causing an increase from 4.58 (control) to 5.31 (EC 6.5) and 6.63 (EC 10) (Figure 1B) correlating highly and significantly ( $r = 0.884$ ,  $p \leq 0.001$ ). The same was observed for organic acids; concentrations on a FW basis rose from 329 (control) to 396 (EC 6.5) and 454 mg (EC 10) (Figure 2). Calculated on a DW basis, titrable acids (TAs) of control and EC 10 treatments comprised 67 mg and, with 73 mg, only slightly more at EC 6.5 (Table 3). Correlation was still significant ( $r = 0.673$ ,  $p \leq 0.05$ ) but less pronounced as compared to other parameters of fresh tomato.

**Table 3.** Effects of Salinity on TAs in Citric Acid, Vit C, LYC,  $\beta$ -CAR, AC of CARs and PHEs in TEs and PHEs in GAEs Per 1 g DW

TA (mg)			
cluster	4	6	10
control	77.1 ± 0.80 a	67.5 ± 1.6 b	65.8 ± 0.4 a
EC 6.5	72.1 ± 1.58 b	73.3 ± 1.0 a	60.2 ± 0.7 b
EC 10	68.4 ± 0.56 c	66.9 ± 1.3 b	58.4 ± 0.7 c
<i>p</i> ( <i>n</i> )	0.000 (4)	0.000 (4)	0.000 (4)
Vit C (mg)			
cluster	4	6	10
control	1.82 ± 0.05 a	1.73 ± 0.13 a	1.93 ± 0.03 a
EC 6.5	1.64 ± 0.02 b	1.38 ± 0.08 b	1.54 ± 0.04 b
EC 10	1.42 ± 0.04 c	1.35 ± 0.13 b	1.43 ± 0.04 c
<i>p</i> ( <i>n</i> )	0.000 (4)	0.000 (4)	0.000 (4)
LYC (mg)			
cluster	4	6	10
control	1.01 ± 0.17 a	0.91 ± 0.03 a	0.80 ± 0.11 a
EC 6.5	0.96 ± 0.11 a	0.99 ± 0.19 a	0.75 ± 0.16 a
EC 10	0.95 ± 0.20 a	0.87 ± 0.03 a	0.82 ± 0.08 a
<i>p</i> ( <i>n</i> )	0.833 (4)	0.343 (4)	0.729 (4)
$\beta$ -CAR (mg)			
cluster	4	6	10
control	0.08 ± 0.00 2	0.09 ± 0.00 a	0.06 ± 0.00 a
EC 6.5	0.09 ± 0.01 a	0.08 ± 0.00 b	0.05 ± 0.01 a
EC 10	0.06 ± 0.00 b	0.08 ± 0.01 b	0.06 ± 0.02 a
<i>p</i> ( <i>n</i> )	0.000 (4)	0.022 (4)	0.589 (4)
PHE (mg)			
cluster	4	6	10
control	4.16 ± 0.16 a	4.34 ± 0.03 a	4.74 ± 0.08 a
EC 6.5	3.68 ± 0.22 b	4.27 ± 0.15 a	4.84 ± 0.09 a
EC 10	3.81 ± 0.22 b	4.33 ± 0.24 a	4.26 ± 0.12 b
<i>p</i> ( <i>n</i> )	0.003 (6)	0.817 (6)	0.001 (6)
AC PHE (mg)			
cluster	4	6	10
control	4.86 ± 0.21 a	4.66 ± 0.23 a	4.57 ± 0.18 a
EC 6.5	4.72 ± 0.34 a	4.31 ± 0.13 a	4.04 ± 0.31 c
EC 10	4.26 ± 0.25 b	4.59 ± 0.52 a	4.35 ± 0.18 b
<i>p</i> ( <i>n</i> )	0.004 (6)	0.378 (6)	0.000 (6)
AC CAR (mg)			
cluster	4	6	10
control	0.94 ± 0.18 a	0.84 ± 0.12 a	1.00 ± 0.31 a
EC 6.5	0.98 ± 0.14 a	0.76 ± 0.09 a	1.08 ± 0.43 a
EC 10	0.96 ± 0.08 a	0.84 ± 0.10 a	1.08 ± 0.30 a
<i>p</i> ( <i>n</i> )	0.923 (6)	0.535 (90)	0.338 (6)

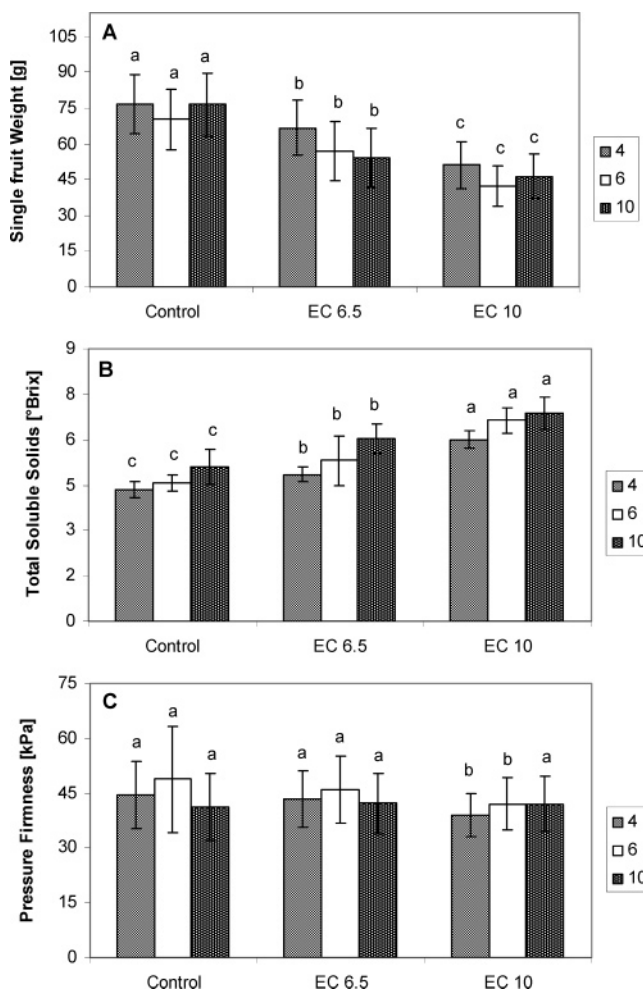
**Content of Antioxidants.** In general, both hydrophilic and lipophilic antioxidants rose with increasing EC levels on a FW basis. In contrast, salt concentrations had a weak influence when component concentrations were referred to as DW.

Vit C was influenced significantly by salinity, although not linearly. Regression analysis revealed RH (%) best fitting with Vit C concentration ( $r = 0.703$ ,  $p \leq 0.05$ ). Its content was 9.41 mg/100 g FW in the control treatment, only 8.61 mg in EC 6.5 and 10.33 mg/100 g FW at EC 10. Referred to as DW, 1.73 mg was found in the control treatment, 1.38 mg/1 g DW at EC 6.5, and 1.35 mg/1 g DW at EC level 10 (**Figure 3A** and **Table 3**). LYC increased from 4.31 mg/100 g FW (control) and 5.36 mg/100 g FW (EC 6.5) up to 5.80 mg/100 g FW (EC 10) (**Figure 3B**) and was shown to be positively correlated highly

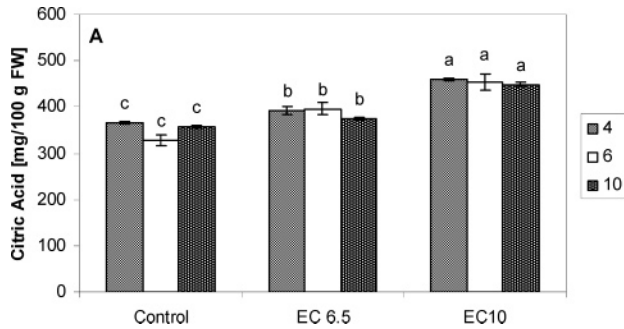
**Table 4.** Results of Stepwise Regression Analysis between Measured Dependent (SFW, TSS, TA, Vit C, LYC,  $\beta$ -CAR, PF, AC CAR, AC PHE, and PHE) and Independent Variables (EC Level, Truss Number, Irradiation, Temperature, and RH) Indicated by Pearson's Correlation Coefficient (*P*) and Significance Levels (*p*)<sup>a</sup>

dependent	variable		Pearson's correlation coefficient	<i>p</i>
	independent			
SFW	EC (mS/cm)		-0.939	0.000
TSS	EC (mS/cm)		0.884	0.000
TA	EC (mS/cm)		0.673	0.018
Vit C	RH (%)		0.703	0.035
LYC	EC (mS/cm)		0.816	0.000
$\beta$ -CAR	temp (°C)		-0.759	0.018
AC CAR	EC (mS/cm)		0.816	0.000
AC PHE	EC (mS/cm)		0.918	0.000
PHE	Truss no. ( <i>n</i> )		0.769	0.001

<sup>a</sup> *p* values  $\leq 0.05$  were estimated as significant ( $n = 9$ ).

**Figure 1.** Effects of salinity levels on single fruit FW (A), TSS (B), and PF (C). In this and the following figures, columns indicate means and error bars  $\pm$  SD. Different letters above columns indicate statistical significance of means estimated using Tukey's test,  $p > 0.005$ .

and significantly ( $r = 0.816$ ,  $p \leq 0.001$ ). On a DW basis (mg/1 g DW), 0.91 LYC was found in the control, slightly increasing to 0.99 mg/1 g DW at EC 6.5 with the lowest concentrations at EC 10 (0.87 mg/1 g DW) (**Table 3**). The salinity effect on  $\beta$ -CAR concentrations was comparable to LYC with no remarkable difference between 0.43 mg/100 g FW (control) and 0.44



**Figure 2.** Effects of salinity levels on organic acid concentrations in citric acid (TA) per 100 g FW.

mg/100 g FW (EC 6.5) but more pronounced up to 0.53 mg/100 g FW (**Figure 3C**). A slight decrease could be observed when calculated on a DW basis. The control concentration was 0.09 mg and 0.081 (EC 6.5) and 0.08 mg/1 g DW (EC 10; **Table 3**). There was no correlation observed between EC level and  $\beta$ -CAR, instead with temperature ( $r = 0.759$ ,  $p \leq 0.05$ ). Another class of substances, the PHEs, rose slightly from 20.5 mg GAE/100 g FW in the control to 21.7 mg GAE at EC 6.5 and was even more pronounced with 24.9 mg GAE at EC 10 (**Figure 3D**). GAE (PHEs) fit best in the regression analysis when plotted against truss number ( $r = 0.769$ ,  $p \leq 0.01$ ). Referred to as DW concentrations, there were no differences among the treatments (**Table 3**).

**AC.** Higher concentrations of secondary plant metabolites calculated on a FW basis in tomato fruit (**Figure 3B–D**) correlated with higher ACs as shown in **Figure 4**. In accordance, the AC of CARs (**Figure 4A**) increased with salinity from 4.0 mg TE (control) and 4.1 mg TE (EC 6.5) to 5.6 mg TE (EC 10). On the other hand, DW concentrations ranged from 0.84 mg TE (control) to 0.76 mg TE (EC 6.5) and back to 0.84 mg TE (EC 10) (**Table 3**). The AC of the phenolic extracts was comparable to the CARs with a small increase from control EC to 6.5 (22.1 and 23.4 mg) and again a steep increase at EC 10 (30.4 mg) (**Figure 4B**). From the same samples, DW concentrations of 4.7 mg TE (control), 4.3 mg TE (EC 6.5),

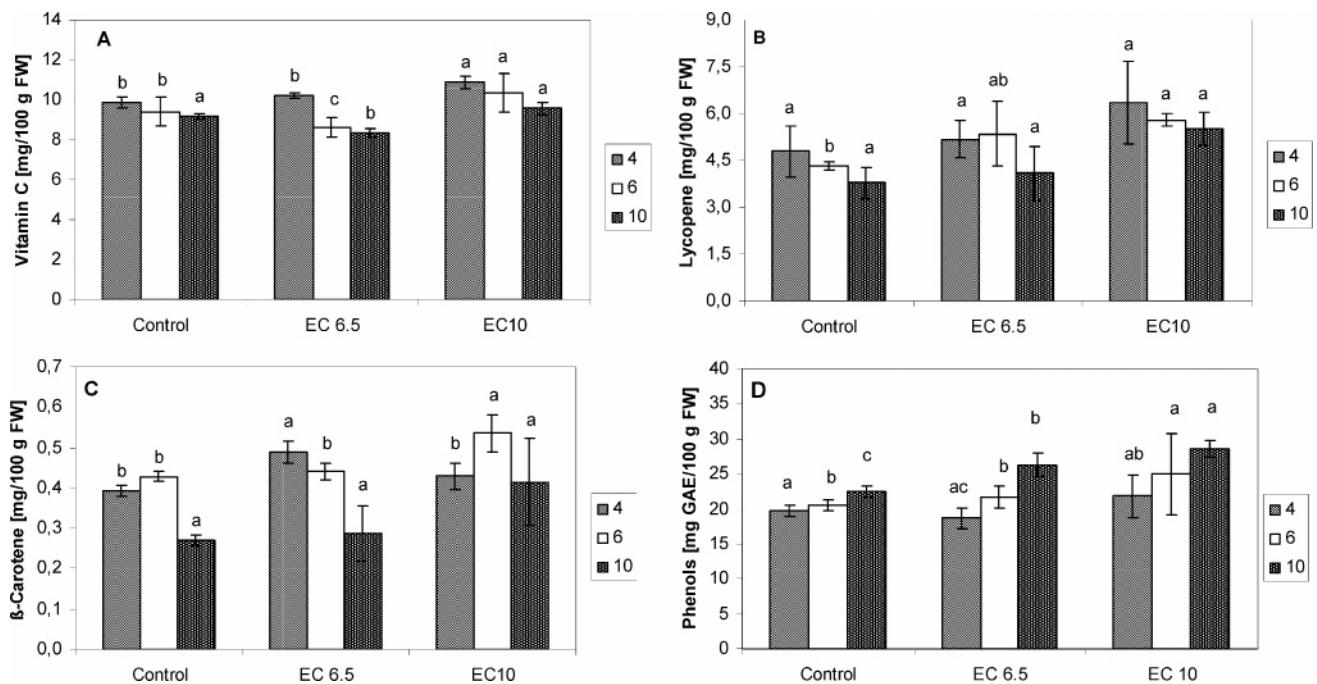
and 4.6 mg TE (EC 10) were found (**Table 3**). Both parameters correlated best when plotted against EC level as independent variables (AC CAR,  $r = 0.816$ ,  $p \leq 0.001$ ; AC PHE,  $r = 0.918$ ,  $p \leq 0.001$ ).

## DISCUSSION

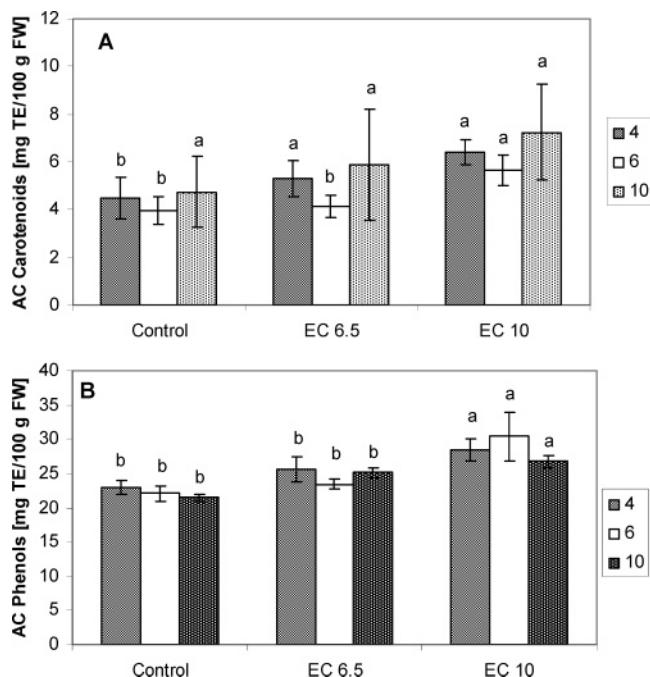
The results of our experiment show that for most tomato quality-related parameters a significant correlation between the EC level of the nutrient solution as revealed by stepwise regression analysis with the exception of some parameters (firmness,  $\beta$ -CAR, and Vit C) indicating that other parameters such as water/climate conditions during growth additionally serve to modulate their development gradually (21, 22).

**Influence on Outer Fruit Quality (PH, Yield, and Firmness) and Taste Parameters (TSS and Organic Acids).** Increasing salinity resulted in a markedly reduced PH as well as in a reduction in TFY. The loss in TFY is due to a reduction of SFW, which decreased within our experiment about 19% (EC 6.5) and 40% (EC 10), respectively. This is in accordance with the literature where a 10% reduction in fruit weight is proposed for EC 5–6, 30% for EC 8, and about 40% for EC values above 8 (9). De Pascale and co-workers (12) found a reduction in fruit yield of about 50% at EC 15.7 and 20% for EC 8, and Maggio et al. (23) reported a loss in fruit weight of about 59% for EC 15.7 as compared to a nonsalinized control. The loss in fruit yield can be ascribed to passive as well as active responses of the plant. First, plants under salinity accumulate less water and have a reduced water uptake (10). However, this reduced water uptake is counteracted by a reduced transpiration rate (via reduced stomata density and conductance as well as a reduced leaf surface) but a less reduced root growth as compared to the shoot (24). To maintain water uptake, additionally, an osmotic adjustment takes place to deal with water or salt stress (23, 25), which in turn leads to the observed higher levels of TSS and TA (see below). The reduced water uptake as a passive mechanism therefore cannot serve as sole explanation for the reduction in fruit yield.

Furthermore, the plant reacts actively with changes in hormone levels. Abscisic acid (ABA) synthesis in the roots



**Figure 3.** Effects of salinity levels on Vit C, LYC,  $\beta$ -CAR, and PHEs in GAEs per 100 g FW.



**Figure 4.** Effects of salinity levels on AC of CARs and PHEs in TEs per 100 g FW.

increases under salinity. Stress-induced ABA is an important signaling factor in the root to shoot communication causing appropriate reactions in the vegetative and generative part of the plant. Stomata closure and reduced leaf growth are active responses to higher salt concentrations in the root area (25). Moreover, the exponential phase of fruit growth has been reported to be particularly sensitive to ionic and osmotic changes (26). Additionally, ABA can be the reason for the increase of osmotically effective substances, measurable as TSS and organic acids in our experiment. Also, other researchers found an increase of osmotically effective metabolites with rising EC levels (11, 12). Additionally, enhancement of TSS and acidity is an active adaptation of plants to deal with salinity (27) as it guarantees further water uptake. Accumulation of these substances as an osmotic adjustment also makes up part of the increase in DW under salinity (28).

Fruit PF decreased in our experiment, although fruit firmness is reported repeatedly to increase with salinity (11); however, a decrease or unchanged firmness is also reported (14). The cultivar may play a major role in this concern (14). A strong requirement for a valid comparison of fruit firmness among different treatments is to have fruits of the same ripening stage; already, minor deviations can lead to different results. Sampling practice solely along a color chart might not be sufficiently exact for that purpose and should be better supported by additional measurements confirming color status of the fruit.

**Influence on Health-Promoting Compounds (Vit C, CARs, and Phenolics) and AC.** Salinity enhanced the content of Vit C, LYC,  $\beta$ -CAR, and PHEs related on a FW basis. The Vit C content was 9.7 mg/100 g FW for EC 3 and 10.1 mg/100 g FW for EC 10. These values are comparatively low since in the literature mean values between 15 and 23 are reported (29). However, the used cultivar Durinta has low Vit C values as compared to other cultivars (30). Moreover, light and temperature variations may lead to large variations in Vit C content (29) and possibly RH, as we see from its significance in our experiment.

LYC (4.3 mg/100 g FW for EC 3 and 5.8 mg/100 g FW for EC 10) and  $\beta$ -CAR (0.43 and 0.53 mg/100 g FW, respectively)

contents are in accordance with literature data, where LYC values between 3 and 5 mg/100 g FW and  $\beta$ -CAR values between 0.1 and 0.6 mg/100 g FW are found (29, 31). The increase with rising salinity was only observed when calculating on a FW basis, so it seems reasonable to suppose that this increase can be ascribed to the concentration effect discussed above (10, 23) and not as an active accumulation of ingredients. However, carotene biosynthesis is strongly stress sensitive and reacts on environmental factors such as light and temperature as well as on water stress (29). Our findings of a significant correlation of  $\beta$ -CAR content with temperature confirm this. De Pascale et al. (12) found  $\beta$ -CAR values of 0.31 mg/100 g FW for EC 4.4 and 0.44 mg/100 g FW for EC 15.7; LYC even increased from 5.9 to 10.22 mg/100 g FW. These authors could not detect differences in DW, which implies another mechanism than only concentration effects. A possible explanation would be an increased synthesis of CARs under salt stress, since several genes encoding for enzymes involved in LYC synthesis are upregulated under stress conditions. Because plants under salt stress show reduced leaf areas, the fruits are more exposed to sunlight; therefore, CARs biosynthesis additionally may be upregulated (29).

Phenolics increased by about 20% for EC 10 as compared to the control when calculated on a FW basis; that is, this increase is less pronounced than that for LYC and  $\beta$ -CAR, which increased by 33%, in sum. The content of phenolics expressed as GAE rose from 20.5 mg/100 g FW for EC 3 to 24.9 mg/100 g FW for EC 10. However, the content of polyphenolics in tomato can vary between 10 and 57 mg GAE/100 g FW, mainly depending on cultivar and growing conditions (32). The increased synthesis under saline conditions may reflect some kind of defense against the stress conditions, i.e., against oxidative burden since water stress was found to be accompanied by an increased production of reactive oxygen species (33). Besides increased generation of more effective antioxidants and thus a higher antioxidative activity at EC 10, radical scavenging abilities could have been increased as a consequence of different fruit size: Most of the flavonoids (34) accumulate in the peel of tomatoes. The pericarp marks a barrier in fruits to protect against several threats. Concerning salt and water stress, it minimizes transpiration and thereby uncontrolled water loss of the fruit. The functional importance of this boundary layer is obvious with regard to high concentrations of antioxidants in it. As the surface of tomatoes decreases with the square of its diameter but their volume with its cube, different sizes can have a multiplying effect on concentrations when the whole fruit is used as a scale basis. Furthermore, the pericarp of tomatoes grown under salinity contains smaller cells with thicker cell walls (35). Thus, percentage of peel in samples from the EC 10 treatment comprises both, and it is higher as a consequence of the described surface/volume ratio but also of cell density. Analysis and exact quantification of tomatoes' peel and pulp ingredients is underway for a better understanding.

To determine to what extent the increase in secondary plant metabolites also results in a higher AC, we tested CAR and phenolic extracts in the ABTS decolorization assay. When calculated on dry matter, a slight decrease in AC was observed regarding the phenolics; their calculation on a FW basis revealed an increase by 37% (control as compared to EC 10). The obtained values were about 19 mM TE/100 g DM and thus distinctly higher than those found in comparable studies where values between 2 and 3 mM TE/100 g DM were found (32).

Therefore, the higher content of phenolic compounds found also resulted in a higher AC. De Pascale et al. (12) tested a "hydrophilic fraction" of tomatoes on AC and found a nearly linear increase with rising salinity. However, the results are given in ascorbic acid equivalents and it is not clear whether they refer to FW or dry matter. Moreover, the aqueous extraction technique used by them will extract ascorbic acid effectively but will extract phenolic compounds only to a very small extent. For this reason, in this investigation, the pattern of ascorbic acid accumulation reflected the increased AC. We, however, used 80% MeOH as the extraction solvent and therefore received mainly phenolic compounds such as chlorogenic, caffeic, or ferulic acid in our extract but no ascorbic acid.

Regarding the CAR extracts, the increase in AC was about 40% and thereby slightly higher than the increase in  $\beta$ -CAR plus LYC. Besides LYC and  $\beta$ -CAR, other substances also occur in the lipophilic CAR extract (not detected by the used HPLC analysis) and can influence the results for the CAR extract. These substances can be other CARs such as  $\gamma$ -carotene, phytoene, or neurosporene or further lipophilic antioxidants such as  $\alpha$ -tocopherol, also found in tomatoes (5). Preliminary examinations indicate increasing contents of Vit E, especially in tomato peel (results not yet published). Also, de Pascale et al. (12) found an increasing AC until EC 8.5; however, at EC 15, no further increase was observed.

Increasing or improving the contents of flavoring and health-promoting compounds is becoming a new task for growers as the increased interest in genetic modification of foodstuffs denotes. Concerning tomatoes and their CARs and flavonoids, genetic engineering is in full progress (36, 37). These experiments led to an increase in  $\beta$ -CAR by 35% and LYC by 65%, respectively. Because the increases in CARs in our investigation were only slightly lower and genetically modified foodstuffs are not accepted by large consumer groups, irrigating with saline water is an alternative to enrich desirable compounds in tomatoes.

The benefits of producing tomatoes under salinity are obvious: salt treatment leads to enhanced sugar and acid contents accounting for a good taste of tomatoes (32). Higher contents of TSS also constitute a sales-promoting aspect for the canning industry. Increased contents of Vit C, LYC,  $\beta$ -CAR, and PHEs meet expectations of health-conscious consumers. The outcome comprises both tastiness and a highly valued food.

A marked decrease in fruit yield, 40% in our case for EC 10, reveals the distinctive negative aspects of salinity, at least for the producer in terms of income loss. This loss actually cannot be balanced out neither by the water savings occurring under saline conditions nor by higher prices for healthier tomatoes. Water consumption is until now only a small part of the whole cost in tomato production; therefore, savings in water do not actually lead to a great reduction in production costs. On the other hand, water already is a limiting factor in many regions of the world as well as poor quality water is reality in vegetable production. Therefore, these factors will deserve more attention in future production and their consequences for produce quality.

#### ABBREVIATIONS USED

EC, electrical conductivity; AC, antioxidative capacity; DW, dry weight; FW, fresh weight; TEs, Trolox equivalents; GAEs, gallic acid equivalents; BHT, butylated hydroxytoluene; ABTS, 2,2'-azinobis(3-ethyl-benzothiazoline)-6-sulfonic acid; EDTA, ethylenediaminetetraacetic acid disodium salt; POD, peroxidase; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; SFW, single fruit weight; PF, pressure

firmness; TSSs, total soluble solids; TAs, titrable acids; LYC, lycopene;  $\beta$ -CAR,  $\beta$ -carotene; PHEs, phenols; CARs, carotenoids; ABA, abscisic acid; RH, relative humidity.

#### LITERATURE CITED

- (1) Ferrari, C. K. B. Functional foods, herbs and nutraceuticals: Towards biochemical mechanisms of healthy aging. *Biogerontology* **2004**, *5*, 275–289.
- (2) Johnson, E. J. The role of carotenoids in human health. *Nutr. Clin. Care* **2002**, *5* (2), 56–65.
- (3) Stanner, S. A.; Hughes, J.; Kelly, C. N. M.; Buttriss, J. A review of the epidemiological evidence for the "antioxidant hypothesis". *Public Health Nutr.* **2004**, *7*, 407–422.
- (4) Giovannucci, E. A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp. Biol. Med.* **2002**, *227*, 852–859.
- (5) Beecher, G. R. Nutrient content of tomatoes and tomato products. *S. Exp. Biol. Med.* **1998**, *218*, 98–100.
- (6) Minoggio, M.; Bramati, L.; Simonetti, P.; Gardana, C.; Lemoli, L.; Santangelo, E.; Mauri, P. L.; Spigno, P.; Soressi, G. P.; Pietta, P. G. Polyphenol pattern and antioxidant activity of different tomato lines and cultivars. *Ann. Nutr. Metab.* **2003**, *47*, 64–69.
- (7) Liu, R. H. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* **2003**, *78*, 517S–520S.
- (8) Verhoeyen, M. E.; Bovy, A.; Collins, G.; Muir, S.; Robinson, S.; Vos de, C. H. R.; Colliver, S. Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway. *J. Exp. Bot.* **2002**, 2099–2106.
- (9) Cuartero, J.; Fernandez-Munoz, R. Tomato and salinity. *Sci. Hortic.* **1999**, *78*, 83–125.
- (10) Ebert, D. L.; Ho, L. The effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. *J. Hortic. Sci.* **1986**, *61* (3), 361–367.
- (11) Petersen, K.; Willumsen, J.; Kaak, K. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. *J. Hortic. Sci. Biotechnol.* **1998**, *73* (2), 205–215.
- (12) de Pascale, S.; Maggio, A.; Fogliano, V.; Ambrosino, P.; Ritieni, A. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *J. Hortic. Sci. Biotechnol.* **2001**, *76* (4), 447–453.
- (13) Brückner, B.; Auerswald, H. Instrumental data-consumer acceptance. In *Fruit & Vegetable Quality*; Shewfelt, R. L., Brückner, B., Eds.; Technomic Publishing Company, Inc.: Lancaster, Pennsylvania, 2000; pp 178–198.
- (14) Auerswald, H.; Schwarz, D.; Kornelson, C.; Krumbein, A.; Brückner, B. Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution. *Sci. Hortic.* **1999**, *82*, 227–242.
- (15) Tura, D.; Robards, K. Sample handling strategies for the determination of biophenols in food and plants. *J. Chromatogr. A* **2002**, *975*, 71–93.
- (16) Davey, M. W.; Dekempeneer, E.; Keulemans, J. Rocket-powered high-performance liquid chromatographic analysis of plant ascorbate and glutathione. *Anal. Biochem.* **2003**, *316*, 74–81.
- (17) Sander, L. C.; Sharpless, K. E.; Craft, N. E.; Wise, S. A. Development of engineered stationary phases for the separation of carotenoid isomers. *Anal. Chem.* **1994**, *66*, 1667–1674.
- (18) Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1998**, *299*, 152–178.
- (19) Cano, A.; Acosta, M.; Arnao, M. B. A method to measure antioxidant activity in organic media: Application to lipophilic vitamins. *Redox Rep.* **2000**, *5*, 356–370.
- (20) Ho, L. C.; Hewitt, J. D. Fruit development. In *The Tomato Crop: A Scientific Basis for Improvement*; Thereon, J. G., Rudich, J., Eds.; Chapman and Hall: London, 1986; pp 201–239.

- (21) Stanghellini, C. V. M.; W. Th. M.; Corver, F.; Van Dulleman, E.; Simonse, L. Combined effect of climate and concentration of the nutrient solution on a greenhouse tomato crop. *Acta Hort.* **1998**, *458*, 231–237.
- (22) Li, Y. L. Analysis of greenhouse tomato production in relation to salinity and shoot environment. Ph.D. dissertation, Agricultural University, Wageningen, 2000; 97 pp.
- (23) Maggio, A.; Pascale de, S.; Angelino, G.; Ruggiero, C.; Barbieri, G. Physiological response of tomato to saline irrigation in long-term salinized soils. *Eur. J. Agric.* **2004**, *21*, 149–159.
- (24) Dalton, F. N.; Poss, J. A. Water transport and salt loading: A unified concept of plant response to salinity. *Acta Hort.* **1990**, *278*, 187–194.
- (25) Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* **2002**, *25*, 239–250.
- (26) Adams, P.; Ho, L. C. Effects of constant and fluctuating salinity on the yield, quality and calcium status of tomatoes. *J. Hort. Sci.* **1989**, *64*, 725–732.
- (27) Hasegawa, P. M.; Bressan, R. A.; Zhu, J.-K.; Bohnert, H. J. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 463–499.
- (28) Plaut, Z.; Grava, A.; Yehezkel, C.; Matan, E. How do salinity and water stress affect transport of water, assimilates and ions to tomato fruits? *Physiol. Plant* **2004**, *122*, 429.
- (29) 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.
- (30) Wold, A.-B.; Rosenfeld, H. J.; Holte, K.; Baugerod, H.; R. B.; Haffner, K. Color of post-harvest ripened and vine ripened tomatoes (*Lycopersicon esculentum* Mill.) as related to total antioxidant capacity and chemical composition. *Int. J. Food Sci. Technol.* **2004**, *39*, 295–302.
- (31) Shi, J.; Le Maguer, M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 1–42.
- (32) Kaur, R.; Lister, C. E.; Savage, G. P. Antioxidant components of tomato: Effect of cultivar and size. In *Mineral Nutrition of Higher Plants*; Marschner, H., Ed.; Academic Press Jovanovich, Harcourt Brace: London, Orlando, San Diego, New York, Boston, Sydney, Tokyo, Toronto, 1986; pp 1–29.
- (33) Aktas, H.; Krani, L.; Aloni, B.; Bar-Tal, A. Physiological and biochemical mechanisms leading to blossom-end rot in greenhouse-grown peppers irrigated with saline solution. *Acta Hort.* **2003**, *609*, 81–88.
- (34) Muir, S. R.; Collins, G. J.; Robinson, S.; Hughes, S.; Bovy, A.; de Vos, C. H. R.; Tunen van, A. J.; Verhoeven, M. E. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nat. Biotechnol.* **2001**, *19*, 470–474.
- (35) Taiz, L.; Zaiger, E. Stress physiology: water deficit and drought resistance. In *Plant Physiology*, 2nd ed.; Taiz, L., Zaiger, E., Eds.; Sinauer Associates, Inc. Publishers: Sunderland, Massachusetts, 1998; pp 725–734.
- (36) Rosati, C.; Aquilani, R.; Dharmapuri, S.; Pallara, P.; Marusic, C.; Tavazza, R. Metabolic engineering of beta-carotene and lycopene content in tomato fruit. *Plant J.* **2000**, *24* (3), 413–419.
- (37) Bramley, P. M. Regulation of carotenoid formation during tomato fruit ripening and development. *J. Exp. Bot.* **2002**, *53* (377), 2107–2113.

---

Received for review August 8, 2005. Revised manuscript received November 14, 2005. Accepted November 20, 2005. This work was supported by the European Union within the fifth Framework of the INCO-MED 2 RTD Cost Projects (Ecoponics Project 2003-2006/Project ICA3-CT-2002-10020).

JF051930A