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Seasonal Variations in the Level of Plant Constituents in Greenhouse Production of Cherry Tomatoes

RUNE SLIMESTAD^{*,†} AND MICHÈL J. VERHEUL[‡]

PlantChem and Planteforsk, Særheim Research Centre, N-4353 Klepp Station, Norway

The content of selected plant constituents was measured in cherry tomatoes (*Lycopersicon esculentum* Mill. cv. Jennita) during conventional Norwegian tomato production in a greenhouse from May until October 2004. Samples were collected according to standard production procedure with orange-yellow colored fruits at weight in the range of 12.4–19.3 g and size in the range of 28.9–33.0 mm (diameter). The content of selected compounds based on 100 g FW were found to vary in the following range during the season: 7.38–28.38 mg of chalconaringenin, 0.32–0.92 mg of rutin, 0.24–1.06 mg of chlorogenic acid, 5.60–20.02 mg of ascorbic acid, 1.60–5.54 mg of lycopene, and 0.37–0.55 mg β -carotene. Only minute amounts of naringenin together with kaempferol 3-rutinoside and caffeic acid, which previously have been reported from tomatoes, were detected. The content of chalconaringenin to rutin and that of lycopene to β -carotene showed a strong correlation during the season (p < 0.001). The content of total phenolics and methanol-soluble antioxidants also showed a correlation (p < 0.001), and were found in the range 14.6–32.6 mg of gallic acid equivalents (GAE)/100 g fresh weight (FW) and 445–737 μ mol of Fe^{II}/100 g FW, respectively. Seasonal variation in the level of plant constituents is shown to be related to photon flux density and fertilization level.

KEYWORDS: Cherry tomatoes; *Lycopersicon esculentum;* lycopene; β -carotene; ascorbic acid; chalconaringenin; rutin; chlorogenic acid; total phenolics; HPLC; antioxidants; photon flux density; fertilization level

INTRODUCTION

Tomato is one of the most important fresh vegetables in the industrialized world. It is also important for the food industries as raw material for the production of, for example, purees and ketchup. In Europe and the U.S., tomatoes (fresh and processing) are second only to potatoes in economic importance and consumption within the vegetable sector. According to the Economic Research Service (http://ers.usda.gov), the U.S. is one of the world's leading producers of tomatoes, second only to China. Annual per capita use of fresh-market tomatoes in the U.S. increased 15% between the early 1990s and the early 2000s to nearly 4.8 kg. The average consumption of fresh tomatoes in Norway is 6.4 kg capita/year (data from Opplysningskontoret for Frukt og Grønt; http://www.ofg.no). The consumption of fresh tomatoes in the Norwegian market has increased in conjunction with the introduction of new varieties (beefsteak, cherry, cocktail, plum, and yellow tomatoes).

Tomato is also one of the most used vegetables of the Mediterranean diet, a diet known to be beneficial for health, especially with regard to the development of chronic degenerative diseases. The large tomato consumption together with its importance in public health has attracted the attention of scientists toward the content of lycopene as well as β -carotene, ascorbic acid, and tocopherols. Moreover, there is a growing interest in other compounds present in tomatoes such as folates, flavonoids, and other phenolics, though not enough human studies are available to estimate the impact of particular compounds on human health (1).

Tomato is the most important dietary source of lycopene $(\psi,\psi$ -carotene), a red-colored antioxidant and a free radical quencher (2,3). Lycopene has in recent years been recognized as a target molecule that prevents incidence of prostate cancer (4). Lycopene content are reported to vary significantly among the different varieties, for example, from 1.6 mg/100 g fresh weight (FW) in Tigerella to 5.7 mg/100 g FW in Flavortop (5). Tomato is also a significant source of β -carotene (0.1–3.7 mg/ 100 g FW), a photoprotectant that act as a light-harvesting pigment in photosynthesis. It has also been recognized as provitamin A, and a range of other health benefits have been associated with this compound (I). The content of ascorbic acid is not high in tomatoes but has been highlighted especially due to its antioxidant properties. These properties seem to be associated with the presence of phenolic compounds (6). Among the classes of phenolics, that of flavonoids is the largest. The functions of flavonoids in plants are in no way fully understood. Several properties of a plant have, however, been associated with specific flavonoid structures within the plant. Some of these properties have been reviewed (7). However, it seems that each

^{*} Corresponding author: phone +47-5178-9831; fax +47-5178-9801; e-mail rune@plantchem.com.

[†] PlantChem.

[‡] Planteforsk.

plant is able to produce an arsenal of similar flavonoid structures that have different chemical properties (8). Several flavonoids have been reported to occur in fresh tomatoes: naringenin, chalconaringenin, naringenin 7-glucoside, rutin, quercetin 3-rhamnosyldiglucoside, kaempferol 3-rutinoside, and kaempferol 3-rhamnosyldiglucoside (9).

This work is the first report in a series where the overall aim is to develop a production strategy for a tomato with a documented level of certain plant compounds (lycopene, β -carotene, ascorbic acid, and specific flavonoids and chlorogenic acid). Environmental factors (light, temperature, air composition, mineral nutrition, growth medium) and cultural practices (variety, ripening stage at harvest, training system, irrigation system) are known to affect tomato composition (I, 9-18). In practical production, however, these factors are often variable and highly connected with each other. Most reports describe how production factors impact the composition of tomatoes in open field. Further documentation is recommended for tomato production in greenhouses, which also have a better possibility to control and adjust a range of growth variables.

This first part of the project reports on the variations in content of specific plant compounds during a growth season in conventional greenhouse production. The information from this mapping can indicate which agronomic efforts should be considered in order to keep the content of the plant compounds at a steady level throughout the production. A cherry tomato variety was chosen as subject for this study, as cherry tomatoes are generally known to contain high levels of carotenoids, flavonoids, and ascorbic acid and as this tomato is especially appreciated as a tasty tomato. All samples were harvested at an orange-yellow stage of ripening, according to conventional practices, and analyzed for specific compounds immediately after harvest. The report will therefore not give the content of lycopene as found in fruit at an edible stage. The reason for doing this has been to detect any metabolic correlation that may exist between ascorbic acid and specific phenolic compounds ahead of any possible metabolic turnover that can be expected for flavonoids or other phenolics during maturation of plant tissues (19). Thus, effects of storage are excluded. However, the next report from this project will give the amount of the individual compounds as found in tomatoes after postharvest ripening and vine ripening of the fruits.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Seedlings, 31 days old, of cherry tomato (*Lycopersicon esculentum* Mill. cv. Jennita) grown in 0.5 L rockwool cubes were planted on January 15, 2004, on standard rockwool slabs (90 \times 10 \times 15 cm) in a commercial nursery in southwestern Norway (58° 47' N, 5° 41' E). The stand was trained on a layering system in single rows at a density of 3.5 plants m⁻². Air temperature, air humidity, and CO₂ concentration together with outdoor global radiation was recorded once per minute, whereas the electrical conductivity (EC) and pH in the growing medium were recorded three times per week. All values were reported as mean values for a period of 2 weeks before sampling (**Table 1, Figure 1**). Photosynthetic photon flux density (PPFD) has been estimated to comprise 45% of the global radiation (*20*). Plants were fertilized with a standard complete nutrient solution by use of a drip irrigation system. Top shoots were removed according to standard practice on September 14.

Fruits were harvested at a standard ripening stage, comparable to stage IV (orange-yellow) of seven stages of ripeness: I (mature green), II (green-yellow), III (yellow-orange), IV (orange-yellow), V (orange-red), VI (red), and VII (deep red) (10). The third tomato on the truss of four different plants was harvested at 9:00 am on dates listed in **Table 2**. Tomatoes were analyzed separately, and mean values together with standard deviation were calculated.

Table 1. Abiotic Factors during the Growing Season^a

	outdoor PPFD, mol m ⁻² day ⁻¹	temp, °C	vapor pressure deficit, g m ⁻³	CO ₂ , ppm	acidity, pH	electrical conductivity, mS cm ⁻¹
April 15	29.3	18.8	3.2	560	5.0	4.6
April 29	39.9	18.5	3.5	400	4.6	5.2
May 13	43.5	18.9	3.3	503	5.1	5.2
May 27	53.7	19.6	4.1	556	5.4	5.0
Jun 10	48.8	19.4	3.9	462	5.9	5.2
Jun 17	51.5	19.3	4.2	525	5.7	4.3
Jun 23	44.0	19.4	4.4	444	5.6	4.7
Jul 7	46.2	19.8	4.5	537	5.4	5.6
Jul 21	47.3	20.2	4.6	424	5.7	5.0
Jul 27	57.2	22.3	5.6	442	6.0	4.8
Aug 6	41.1	23.7	5.3	375	5.9	5.2
Aug 18	29.4	19.6	3.3	311	5.6	6.2
Sep 3	28.3	19.6	3.6	432	5.5	5.9
Sep 17	12.7	b	b	b	b	6.8
Oct 1	17.5	b	b	b	b	8.5
Oct 15	14.9	b	b	b	b	b

 $^a\,\mathrm{All}$ values represent the mean value for the preceding period of 2 weeks. $^b\,\mathrm{No}$ data.



Figure 1. Outdoor PPFD and electrical conductivity (EC) in growth medium during the season of 2004 (average values for the preceding period of 2 weeks).

Chemicals and Standards. Naringenin was obtained from Carl Roth GmbH, Germany. Chalconaringenin was prepared by dissolving naringenin in 0.2 M NaOH. The solution was kept at 90 °C for 1 h and neutralized by addition of 0.2 M HCl. Chalconaringenin was isolated on a 3×50 cm glas column packed with Sephadex LH-20 (Amersham Biosciences, Denmark) by elution with 50% methanol. Kaempferol 3-rutinoside was isolated from needles of Norway spruce (Picea abies) (21). Chlorogenic acid (5'-caffeoylquinic acid), caffeic acid, rutin (quercetin 3-rhamnosylglucoside), L-ascorbic acid, gallic acid, lycopene $(\psi, \psi$ -carotene; *all-trans*-lycopene), and β -carotene were all purchased from Sigma-Aldrich, Norway. Methanol, trifluoroacetic acid (TFA), 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), sodium acetate, acetic acid, ferric chloride hexahydrate, and ferrous sulfate heptahydrate were bought from Sigma-Aldrich. Acetonitrile, gum arabic, orthophosphoric acid, and potassium ferricyanide were purchased from VWR International, Norway, whereas ethyl acetate was purchased from Tamro Med-lab, Norway.

Sample Preparation. Each sample, consisting of four fruits, was frozen in $N_2(liq)$ soon after collection and kept at -10 °C until analysis. The tomatoes within each sample were measured by weight and size and then transferred to a bath of $N_2(liq)$ for 1 min in order to make the tomatoes brittle. Each tomato was homogenized in a coffee grinder (Bosch, Germany) for 15 s, giving a white, farinaceous powder. Part of that powder was split in three: About 1 g was exactly weighed and transferred to a sample tube for analysis of ascorbic acid; another 1 g was mixed with 10 mL of acidic methanol (0.05% TFA, v/v); whereas

Table 2. Content of Plant Constituents and Physical Properties of Cherry Tomatoes

sampling	weight, g	size, mm	°Brix	phenol ics	FRAP	ascorbic acid	chlorogenic acid	rutin	chalconaringenin	lycopene	β -carotene
May 13	14.44 ± 2.18	29.65 ± 2.21	6.9 ± 0.6	17.5 ± 2.0	527 ± 37	5.65 ± 0.62	0.56 ± 0.12	0.32 ± 0.05	12.90 ± 3.03	2.65 ± 0.79	0.38 ± 0.03
May 27	12.42 ± 0.22	28.93 ± 0.91	6.7 ± 0.5	26.1 ± 3.3	606 ± 63	6.64 ± 0.53	0.43 ± 0.06	0.92 ± 0.30	24.23 ± 4.26	3.01 ± 0.44	0.44 ± 0.01
Jun 10	17.24 ± 2.56	30.97 ± 1.75	7.8 ± 0.5	32.6 ± 3.3	724 ± 41	14.81 ± 2.31	0.50 ± 0.08	0.45 ± 0.06	13.56 ± 3.74	2.11 ± 0.51	0.43 ± 0.06
Jun 17	17.77 ± 1.77	31.89 ± 1.34	8.2 ± 0.5	19.8 ± 2.3	598 ± 59	16.47 ± 2.98	0.84 ± 0.06	0.71 ± 0.14	17.25 ± 2.86	4.20 ± 0.87	0.55 ± 0.12
Jun 23	17.51 ± 2.23	31.38 ± 1.21	7.9 ± 1.2	29.3 ± 1.6	618 ± 135	15.66 ± 3.35	0.73 ± 0.09	0.61 ± 0.09	28.38 ± 8.94	5.54 ± 0.92	0.51 ± 0.05
Jul 7	17.67 ± 2.52	30.94 ± 2.32	7.7 ± 0.7	26.1 ± 5.3	708 ± 110	15.24 ± 1.71	0.72 ± 0.35	0.65 ± 0.12	23.74 ± 3.62	2.47 ± 0.52	0.44 ± 0.04
Jul 21	19.33 ± 3.32	32.79 ± 1.14	7.6 ± 0.4	28.8 ± 5.4	652 ± 72	9.97 ± 2.24	0.92 ± 0.04	0.76 ± 0.12	18.00 ± 2.57	2.90 ± 0.32	0.40 ± 0.03
Jul 27	19.28 ± 1.69	32.76 ± 1.11	7.7 ± 0.6	30.7 ± 7.8	701 ± 161	12.21 ± 2.09	0.83 ± 0.27	0.91 ± 0.15	22.63 ± 5.37	2.42 ± 0.94	0.43 ± 0.06
Aug 6	18.94 ± 1.87	32.68 ± 0.50	6.7 ± 0.7	25.8 ± 5.7	604 ± 23	9.38 ± 0.71	0.68 ± 0.07	0.48 ± 0.03	11.73 ± 0.63	2.41 ± 0.54	0.46 ± 0.03
Aug 18	16.21 ± 1.13	30.80 ± 0.77	7.4 ± 0.4	21.7 ± 3.6	737 ± 126	5.60 ± 0.72	0.84 ± 0.23	0.65 ± 0.11	12.54 ± 1.88	2.16 ± 0.54	0.49 ± 0.07
Sep 3	15.88 ± 1.99	30.26 ± 0.63	7.5 ± 0.3	21.0 ± 3.2	629 ± 61	14.26 ± 4.05	0.87 ± 0.28	0.49 ± 0.07	12.32 ± 1.39	2.35 ± 0.37	0.40 ± 0.03
Sep 17	17.21 ± 3.42	33.00 ± 2.83	7.4 ± 0.2	14.6 ± 1.2	445 ± 28	13.99 ± 2.17	0.24 ± 0.13	0.56 ± 0.07	7.38 ± 2.71	1.60 ± 0.06	0.40 ± 0.02
Oct 1	12.49 ± 1.91	29.93 ± 1.88	7.4 ± 0.4	19.4 ± 1.6	663 ± 21	13.08 ± 0.63	1.06 ± 0.09	0.36 ± 0.04	12.54 ± 1.57	1.98 ± 0.53	0.37 ± 0.02
Oct 15	14.08 ± 2.04	29.19 ± 1.82	7.5 ± 0.7	21.2 ± 4.3	652 ± 72	20.02 ± 0.64	0.51 ± 0.10	0.39 ± 0.08	10.97 ± 3.84	2.35 ± 0.55	0.47 ± 0.03
significance	***	ns	*	***	***	***	**	***	***	***	***

^a Samples were harvested according to conventional procedure, that is, the color of the fruits was orange-yellow (stage IV of seven stages of ripeness). The content of specific compounds is listed as total amount in milligrams per 100 grams FW \pm SD (n = 4); phenolics are given as milligrams of GAE per 100 grams FW \pm SD; and FRAP values are given as micromoles of Fe^{II} per 100 grams FW \pm SD. Significance of differences between sampling dates is given: ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

about 3 g was transferred to a 100 mL baker for thawing followed by the analysis of soluble solids.

Extraction. Ascorbic acid was extracted by vortexing (Vortex Genie2, Scientific Industries, New York) the samples with 10 mL of 50 mM NaH₂PO₄ in 24×150 mm sample tubes for 60 s and then keeping the tubes in the dark at ambient temperature for another 15 min. The extract was finally separated from the residue by use of a centrifuge (Jouan BB3V, Labo and Co., France) at 2500 rpm for 3 min. The residue left after extraction of ascorbic acid was re-extracted four times with 1 mL of ethyl acetate in a 17 \times 120 mm centrifuge tube. The sample was vortexed for 60 s followed by centrifugation at 2500 rpm for 3 min each time. The four extracts were combined and the solution was immediately analyzed for carotenoids (*11*).

The acidic methanol extracts were vortexed for 10 s, then the 24×150 mm sample tubes were sealed and the extracts were kept in darkness at ambient temperature for 24 h. Samples were prepared for HPLC analysis, analysis of total phenolics, and analysis of antioxidants by the ferric reducing ability plasma (FRAP) assay.

HPLC. A liquid chromatograph (Agilent 1100 system, Agilent Technologies) supplied with an autosampler and a photodiode-array detector was used for the analysis of carotenoids, ascorbic acid, and individual phenolics. Carotenoids and phenolics were separated on an Eclipse XDB-C8 (4.6 \times 150 mm, 5 μ m) column (Agilent Technologies). The carotenoids were eluted isocratically by a mixture of acetonitrile and methanol (70:30 v/v) at a flow rate of 0.6 mL/min. Aliquots of 20 μ L were injected on the column and the column oven was set to 30 °C. Peaks were detected at 450 nm. The flavonoids and aromatic acids were separated by use of a binary solvent system consisting of (A) 0.05% TFA in water and (B) 0.05% TFA in acetonitrile. The gradient (percent B in A) was linear from 5% to 10% in 5 min, from 10% to 25% for the next 5 min, from 25% to 85% in 6 min, from 85% to 5% in 2 min, and finally reconditioning of the column by 5% in 2 min. The flow rate was 0.8 mL/min, 10 µL samples were injected on the column, and separation took place at 30 °C. Naringenin was recorded at 280 nm and chlorogenic acid and caffeic acid at 320 nm, whereas chalconaringenin, rutin, and kaempferol 3-rutinoside were recorded at 370 nm. Ascorbic acid was analyzed on a Kromasil-NH₂ column (4.6 \times 250 mm, 5 μ m) (Supelco) by a single mobile phase consisting of 5 mM KH₂PO₄-acetonitrile (80:20) at 1.0 mL/min. Detection occurred at 260 nm. The method was slightly modified from that of Cano et al. (11). All HPLC samples were filtered through a 13 mm syringe filter (Nylon 0.45 μ m, VWR International) prior to injection. Individual standard curves were made for each of the single compounds that were analyzed by HPLC.

Total Phenolics. The method of Price and Butler (22) as modified by Graham (23) to enhance color stability was used in the determination of total phenolics in the samples. According to Graham's modifications, the method should be less sensitive for color change after addition of the stabilizer. However, some changes were observed and hence all measurements were done within 1 min after addition of the stabilizer. Absorbance was read at 700 nm, and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g fresh weight (FW). Gallic acid has become a commonly accepted standard as equivalent for total phenolics (24). As the conceptual basis of this Prussian blue method is to quantify phenolic hydroxyl groups calibrated against gallic acid, there will be an overestimation of total phenolics if the sample contains phenolics in major amounts that have more than three phenolic hydroxyl groups each and have similar molecular weight as the standard. However, chalconaringenin, which presumably dominates among the phenolics in this work, has substantially fewer hydroxyl groups to its molecular weight than gallic acid. This will correspondingly underestimate the phenolic content as this is reported as milligrams gallic acid equivalents.

FRAP. Antioxidants from the methanol extracts were measured by the ferric reducing ability plasma (FRAP) assay (25). A 50 μ L sample solution was diluted with 150 μ L of distilled water and vortexed with 1.5 mL of freshly prepared FRAP solution in a sample tube. The reaction mixture was kept at 37 °C for exactly 10 min. Absorbance was measured at 596.5 nm on an Agilent 8453 system (Agilent Technologies). Aqueous ferrous solutions in the concentration range 100–1000 μ M (FeSO₄·7H₂O) were used for calibration, and the data were expressed as micromoles of ferrous equivalents per 100 g fresh weight.

Soluble Solids Content. SSC was determined by use of a digital PR-100 α refractometer (Atago Co., Japan) of room-temperated tomato juice. Data were expressed as equivalent °Brix (or % SSC) (26).

Statistics. The results were subjected to analysis of variance by the GLM procedure from the SAS statistical computer program (version 8.02). The SNK test was used to determine significant differences among harvest dates. Correlations between average values of parameters were calculated by the REG procedure in SAS.

RESULTS AND DISCUSSION

Chalconaringenin (2',4',6',4-tetrahydroxychalcone) was detected as the main phenolic compound that occurred in the cherry tomatoes throughout the production season (**Figures 2–4**). The amount varied significantly from 7.38 to 28.38 mg/ 100 g FW (**Table 2**). This level was in several cases close to that of total phenolics (**Figure 4**), and the correlation of these parameters throughout the season was significant (p < 0.005). However, a slight underestimation of total phenolics has possibly occurred as chalconaringenin contain one more hydroxyl group than gallic acid (see Materials and Methods). It has been shown that the amount of phenolics increases with increasing light



Figure 2. Molecular structures of the most abundant flavonoids, chlorogenic acid, and carotenoids in cherry tomatoes at time of harvest.

intensity (12). This is confirmed in the present experiment, where a significant correlation between PPFD and total phenol was found (p < 0.05).

Some previous investigations on tomato content have found chalconaringenin and rutin to be the main flavonoid compounds with a total amount of 0.5-1.0 mg/100 g FW (27, 28). Most papers report, however, on naringenin as the main flavonoid (0.8-4.2 mg/100 g FW) in tomatoes (13, 14, 29, 30). In addition

some have pointed out naringenin 7-glucoside (Prunin) as the main flavonoid compound (31). In all cases the flavonoids are reported to occur in the peel (by 95–98%) and not in the pulp (27). Though naringenin and chalconaringenin have similar chromatographic properties by our HPLC method, they were easily distinguished due to their different UV spectra that were obtained by the photodiode-array detector (DAD). For analytical purposes the two compounds can also be easily interconverted (32). To the best of our knowledge, a physiological role of chalconaringenin has not been suggested in any plants. However, a positive correlation was observed between the content of chalconaringenin and the FRAP values (p < 0.05).

Rutin (quercetin 3-rutinoside) was found to be the only flavonol that was detected regularly in the sample series. The content was far below but correlated well with that of chalconaringenin (p < 0.001) and ranged from 0.32 to 0.92 mg/100 g FW. This is in accordance with previous reports on tomatoes. The content of rutin through the season also correlated with that of total phenolics (p < 0.005) and with the FRAP values (p < 0.05). Chlorogenic acid was detected in all samples at a mean value of 0.71 mg/100 g FW and correlated with the methanol-soluble antioxidants (p < 0.001). The content of this compound is close to that of rutin at a mean value of 0.60 mg/ 100 g FW, or twice that of rutin on a molar scale, 2.00 to 0.99 mol (MW 610 and 354 g/mol for rutin and chlorogenic acid, respectively) (Table 2). Chlorogenic acid has been detected as the main phenol in some cherry tomato varieties (33). This was not the case in the present work as the amount of chalconaringenin far exceeded that of chlorogenic acid. Another study found that the total flavonol content in 20 different ripened tomato varieties differed from 0.13 to 2.22 mg/100 g FW (34). Among these, cherry tomatoes contained the highest amount of flavonols, with rutin as the dominating flavonol compound. Another flavonol, kaempferol 3-rutinoside, was also detected in this work, but only in a few samples and at a level that was close to the limit of detection.

The content of ascorbic acid varied from 5.60 to a maximum of 20.02 g/100 g FW (**Figure 4**) and correlated with the amount of total phenolics (p < 0.05). High light intensity is closely



Figure 3. Chromatogram of phenolics found in cherry tomatoes. Detection was at 320 nm (upper trace) and at 370 nm (lower trace).



Figure 4. (Upper panel) Content of total phenolics (expressed as equivalents of gallic acid) and chalconaringenin during the season. (Middle panel) Variation in the content of ascorbic acid. (Lower panel) Methanol-soluble antioxidants as measured by the FRAP method.

related to the content of ascorbic acid (1, 15). The low amount of ascorbic acid detected in the samples of May 13 and 27 and August 19 may be explained by the low PPFD ahead of these sampling times. Also, increased salinity $(3-9 \text{ mS cm}^{-1})$ in rockwool slabs is shown to increase the content of ascorbic acid in greenhouse-grown tomato fruit (16). The high EC level, despite a low PPFD, might explain the high amount of ascorbic acid detected in the samples at the end of the growing season. Overall, tomatoes provide 10-20 mg of vitamin C/100 g of edible product (17, 35). Ascorbic acid is widely being used by the industry as a food ingredient or additive because of its reducing and antioxidant properties, and it is believed that these are the main functions of ascorbic acid in plants as well. The antioxidant role of ascorbic acid is multifunctional. It effectively inhibits enzymatic browning by reducing o-quinone products and protects certain oxidizable compounds (e.g., folates) by reductive effects, free radical scavenging, oxygen scavenging, and reduction of metal ions (36). It was therefore expected that the content of ascorbic acid in the tomatoes would follow, at least to a certain extent, the variation in the amount of methanolsoluble antioxidants. The mean level of these antioxidants, as measured by the FRAP assay, was found to be 633 μ mol of Fe^{II}/100 g FW (Figure 4). A drop in this content was observed at September 17 (445 μ mol), but no drop in the content of ascorbic acid was detected that day. It seemed rather to be connected to the low amount of phenolics in general and especially that of chalconaringenin and chlorogenic acid. It has been reported that chalconaringenin acts as a prooxidant (32). The present data do not support that suggestion.

Two carotenoids were measured during the season: lycopene and β -carotene (Figure 2). The content of lycopene was found to vary from 1.60 to 5.54 mg/100 g FW. As the tomatoes in this study were harvested at an early maturity stage, the level of lycopene was lower compared to that expected in fully ripened fruit. In addition, the carotenoid content might also be lower under glass when compared to the open field (1). Lycopene has been reported to range from 4.3 to 18.1 mg/100 g FW in red tomato fruit produced in open field, with a typical range between 5.5 and 8.0 mg/100 g (13). The level of lycopene did not change much during the period. A peak level was, however, detected in the middle of June. This was first believed to correlate with the increased PPFD. However, no such evidence was found as the level in PPFD reached a maximum in the middle of May and July (Figure 1). No cis-isomer of lycopene was detected in the samples. β -Carotene content was positively correlated to PPFD (p < 0.05). β -Carotene was found to have levels similar to those of lycopene during the period, and a signicant correlation between the two compounds was found (p < 0.001). It has been suggested that lycopene is attached to the insoluble and fibrous parts of the fruit and that skin contain 5 times more lycopene than the tomato pulp (18). Both lycopene and β -carotene are important in photoprotection of plant tissues because of their ability to quench and inactivate reactive oxygen species formed by exposure to light and air.

Cherry tomatoes showed a high level of soluble solids content (**Table 2**). Increasing light intensity and EC in the growing medium often increase the amount of soluble solid contents (*I3*). Results show that the amount of soluble solids tended to increase with increasing PPFD during spring and summer and to decrease in early autumn with decreasing PPFD. In late autumn, however, the amount of soluble solids did not decrease. This might have been due to a strong increase in the EC level in the growing medium (**Figure 1**) and/or to the removal of the top shoot. A positive correlation was detected between the level of soluble solids and that of methanol-soluble antioxidants (p < 0.05) and between soluble solids and the content of ascorbic acid (p < 0.001).

At September 17 the samples were found to contain minimum amounts of chalconaringenin, chlorogenic acid, lycopene, total phenolics, and methanol-soluble antioxidants. This might be related to a combination of high temperature and low PPFD in the weeks before September 17. Also, removal of the plant heads on September 14, which changes the plant/fruit balance, may have played a role. In addition to the increase in EC level in the growing medium after September 17, removal of plant heads might explain the increase in most of the compounds during the last month of production.

Among the samples, fruit diameter varied from 28.9 to 32.8 mm and fruit weight from 12.4 to 19.3 g. For the sampled fruits, no significant differences in fruit diameter were observed throughout the growing season. Fruit weight was lower in the harvests on May 27 (12.4 g) and October 1 (12.5 g) when compared to the harvests on July 21 (19.3 g) and August 6 (18.9 g). Even though it is known that most of the compounds in tomato can be influenced by environmental and nutritional factors, it seems to be possible to keep a relatively constant level throughout the season from May to October due to cultural practices in commercial greenhouse production in Norway.

LITERATURE CITED

- Bilton, R., Gerber, M., Grolier, P., Leoni, C., Eds. *The white* book on antioxidants in tomatoes and tomato products and their health benefits; Final report of the Concerted Action Fair CT97-3233; CMITI Sarl: Avignon, France, 2001.
- (2) Djuric, Z.; Powell, L. C. Antioxidant capacity of lycopenecontaining foods. *Int. J. Food Sci. Nutr.* 2001, 52, 143–149.
- (3) Di Mascio, P.; Kaiser, S.; Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* 1989, 274, 532–538.
- (4) Giovannucci, E.; Ascherio, A.; Rimm, E. B.; Meir, J.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.* **1995**, 87, 1767–1776.
- (5) Hart, D. J.; Scott, K. J. Development and evaluation of an HPLC method for the analysis of Carotenoids in foods, and the measurement of the Carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem.* **1995**, *54*, 101– 111.
- (6) Meltzer, H. M.; Malterud, K. E. Can dietary flavonoids influence the development of coronary heart disease? *Scand. J. Nutr.* 1997, 41, 50–57.
- (7) Harborne, J. B. Introduction to Ecological Biochemistry; Academic Press: London, 1993.
- (8) Slimestad, R.; Francis, G. W.; Andersen, Ø. M. Directed search for plant constituents: a case study concerning flavonoids in Norway spruce. *Euphytica* **1999**, *105*, 119–123.
- (9) Woldedecke, M.; Herrmann, K. Flavanole und flavone der Gemusearten III. Flavanole und flavone der tomaten und des gemusepaprikas. Z. Lebensm. Unters. Forsch. 1974, 155, 216– 219.
- (10) Lopez-Andreu, F. J.; Lamela, A.; Esteban, R. M.; Collado, J. G. Evolution of quality parameters in the maturation stage of tomato fruit. *Acta Hortic.* **1986**, *191*, 387–394.
- (11) Cano, A.; Acosta, M.; Arnao, M. B. Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). *Postharvest Biol. Technol.* 2003, 28, 59–65.
- (12) Wilkins, R. T.; Spoerke, J. M.; Stamp, N. E. Differential responses of growth and to soluble phenolics of tomato to resource availability. *Ecology* **1996**, *77*, 247–258.
- (13) Davies, J. N.; Hobson, G. E. The constituent of tomato fruit— The influence of environment, nutrition and genotype. *Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 205–280.
- (14) Minoggio, M.; Bramati, L.; Simonetti, P.; Gardana, C.; Iemoli, 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.
- (15) Venter, F. Solar radiation and vitamin C content of tomato fruits. *Acta Hortic.* **1977**, *58*, 121–127.
- (16) Petersen, K. K.; Willumsen, J.; Kaack, K. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. J. Hortic. Sci. Biotechnol. **1998**, 73 (2), 205–215.
- (17) Wold, A. B.; Rosenfeld, H. J.; Holte, K.; Baugerød, H.; Blomhoff, R.; Haffner, K. Colour 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.
- (18) Dumas, Y.; Dadomo, M.; DiLucca, G.; Grolier, P. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J. Sci. Food Agric. 2003, 83, 369–382.
- (19) Strack, D.; Heilemann, J.; Wray, V.; Dirks, H. Structures and accumulation patterns of soluble and insoluble phenolics from Norway spruce needles. *Phytochemistry* **1989**, 28, 2071–2078.

- (20) Blackburn, W. L.; Proctor, J. T. A. Estimating photosynthetically active radiation from measured solar irradiance. *Sol. Energy* **1983**, *2*, 233–234.
- (21) Slimestad, R.; Andersen, Ø. M.; Francis, G. W.; Marston, A.; Hostettmann, K. Syringetin 3-O-(6"-acetyl)-β-glucopyranoside and other flavonols from needles of Norway spruce, *Picea abies*. *Phytochemistry* **1995**, 40, 1537–1542.
- (22) Price, M. L.; Butler, L. G. Rapid visual estimation and spectrophotometric determination of tannin content in sorghum grain. *J. Agric. Food Chem.* **1977**, *25*, 1268–1273.
- (23) Graham, H. D. Stabilization of the Prussian blue color in the determination of polyphenols. J. Agric. Food Chem. 1992, 40, 801–805.
- (24) Jakubas, W. J.; Gullion, G. W.; Clausen, T. P. Ruffed grouse feeding behaviour and its relationship to secondary metabolites of quaking aspen flowers buds. *J. Chem. Ecol.* **1989**, *15*, 1899– 1918.
- (25) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" in plasma: the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.
- (26) Mitcham, B.; Cantwell, M.; Kader, A. Methods for determining quality of fresh commodities. *Perishables Handl. Newsl.* **1996**, *85*, 1–5.
- (27) Krause, M.; Galensa, R. Determination of naringenin and naringenin-chalcone in tomato-skins by reversed phase HPLC after solid-phase extraction. Z. Lebensm. Unters. Forsch. 1992, 194, 29–32.
- (28) Muir, S. R.; Collins, G. J.; Robinson, S.; Hughes, S.; Bovy, A.; De Vos, C. H. R.; van Tunen, A. J.; Verhoeyen, M. E. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nat. Biotechnol.* 2001, 19, 470–474.
- (29) Bugianesi, R.; Catasta, G.; Spigno, P.; D'Uva, A.; Maiani, G. Naringenin from cooked tomato paste is bioavailable in men. J. Nutr. 2002, 132, 3349–3352.
- (30) Paganga, G.; Miller, N.; Rice-Evans, C. The polyphenolic content of fruit and vegetables and their antioxidant activities: what does a serving constitute? *Free Radical Res.* **1999**, *30*, 153–162.
- (31) Caris-Veyrat, C.; Amiot, M.-J.; Tyssandier, V.; Grasselly, D.; Buret, M.; Mikolajczak, M.; Guilland, J.-C.; Bouteloup-Demange, C.; Borel, P. Influence of Organic versus Conventional Agricultural Practice on the Antioxidant Microconstituent Content of Tomatoes and Derived Purees; Consequences on Antioxidant Plasma Status in Humans. J. Agric. Food Chem. 2004, 52, 6503-6509.
- (32) Miranda, C. L.; Stevens, J. F.; Ivanov, V.; McCall, M.; Frei, B.; Deinzer, M. L.; Buhler, D. R. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. J. Agric. Food Chem. 2000, 48, 3876–3884.
- (33) Fleuriet, A.; Macheix, J. J. Quinyl esters and glucose derivatives of hydroxycinnamic acids during growth and ripening of tomato fruit. *Phytochemistry* **1981**, *20*, 667–671.
- (34) Stewart, A. J.; Bozonnet, S.; Mullen, W.; Jenkins, G. I.; Lean, M. E. J.; Crozier, A. Occurrence of flavonols in tomatoes and tomato-based products. J. Agric. Food Chem. 2000, 48, 2663– 2669.
- (35) Willcox, J. K.; Catignani, G. L.; Lazarus, S. Tomatoes and cardiovascular health. *Crit. Rev. Food Sci. Nutr.* 2003, 43, 1–18.
- (36) Gregory, J. F. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 566–567.

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