JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Content of Chalconaringenin and Chlorogenic Acid in Cherry Tomatoes Is Strongly Reduced during Postharvest Ripening

RUNE SLIMESTAD*,† AND MICHÈL J. VERHEUL‡

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

The contents of chalconaringenin, chlorogenic acid, rutin, ascorbic acid, lycopene, and β-carotene were analyzed during postharvest and vine ripening of cherry tomatoes (*Lycopersicon esculentum* Mill.) (cv. Jennita) produced in a greenhouse. A remarkable decrease in the content of chalconaringenin took place during postharvest ripening. The tomatoes were found to contain 15.26 mg 100 g^{-1} fresh weight (FW) at harvest but held only 0.41 mg after 3 weeks at 20 °C in darkness. Chalconaringenin did not convert into naringenin. The content of chlorogenic acid fell from 0.51 to 0.06 mg 100 g^{-1} FW at the same conditions. The content of rutin and that of total phenolics remained stable during postharvest ripening. The amounts of lycopene as well as β-carotene and ascorbic acid increased during postharvest ripening. No significant change in the amount of methanol soluble antioxidants or total soluble solids was found during postharvest ripening of the tomato fruits. During vine ripening, the total amount of phenolics and that of soluble solids (% Brix) increased. The content of phenolics correlated well with the content of methanol soluble antioxidants ($p < 0.001$). The amount of ascorbic acid increased from 9.7 mg in green-yellow tomatoes to 17.1 mg 100 g^{-1} FW in red tomatoes. The amount of chalconaringenin decreased to 8.16 mg 100 g^{-1} FW, whereas no significant change was observed for chlorogenic acid or rutin. Possible causes for the decrease in chalconaringenin are discussed.

KEYWORDS: Ascorbic acid; β-carotene; chalconaringenin; cherry tomatoes; chlorogenic acid; FRAP; HPLC; lycopene; *Lycopersicon esculentum*; rutin; total phenolics

INTRODUCTION

Tomatoes constitute the predominant source of lycopene in most diets, and this compound has been associated with a range of health benefits (*1*, *2*). Tomatoes also contain lower amounts of other carotenoids such as β-carotene, which is known for its provitamin A activity (*3*). Consumption of tomatoes and tomato products has thus been considered as a nutritional indicator of good dietary habits and healthy life styles. The carotenoids in tomatoes are also key precursors for nor-isoprenoid aroma compounds such as β-ionone and geranylacetone (*4*).

In addition to the particular interest in lycopene, an awareness of other more or less well-known tomato constituents has emerged in recent years. Ascorbic acid is important in the protection of the tomato itself against oxidative damage that might increase with ripening due to enhanced respiration. This maintains firmness and improves shelf life of the fruit. During the last two decades, several papers have reported on the presence of flavonoids in tomatoes (*2*). This group of polyphenols, which comprises a variety of chemical structures with subtle biological properties, is in general important in conferring antioxidative benefits (*5*). Several flavonoids have been identified from different tomato varieties. Most of these structures belong to the flavonols, and the most predominant compound is quercetin 3-rutinoside (rutin) (**Figure 1**). The average amount of rutin and other quercetin compounds has been estimated to be 8 mg kg^{-1} FW relative to quercetin aglycone (*6*). Some few papers report on chalconaringenin (**Figure 1**) as the main flavonoid compound in fresh tomatoes (*7*, *8*). Chalconaringenin has recently been found to be the predominant phenolic compound in cherry tomato (cv. Jennita) (*9*). This compound is unstable and is easily converted to naringenin. It is therefore interesting that several papers report on naringenin and in some cases prunin (naringenin 7-glucoside) as main phenolic constituents in fresh tomatoes (*10*, *11*). Chalconaringenin from tomato extracts has been found to inhibit histamine release in an in vitro assay. This may indicate that such extracts can reduce allergic reactions (*12*).

Some phenolics (chlorogenic acid and rutin) have been suggested as regulants of auxin (like indole 3-acetic acid) metabolism (*13*). The production of photoprotective compounds such as flavonols in the skins of tomato fruits may afford protection against UV-B-induced oxidative damage (*14*).

Cherry tomatoes are small, tasty tomatoes that on average contain a higher amount of lycopene than traditional tomatoes. The taste advantage is primarily connected to their high content

* To whom correspondence should be addressed. Tel: +47-5178-9831. Fax: +47-5178-9801. E-mail: rune@plantchem.com.
† PlantChem.
‡ Planteforsk.

10.1021/jf050737d CCC: $30.25
Published on Web 08/10/2005

Table 1. Content of Plant Constituents and Physical Properties of Cherry Tomatoes during Postharvest and Vine Ripening[a]

treatment	weight (g)	size (mm)	°Brix	phenolics	FRAP	ascorbic acid	chlorogenic acid	rutin	chalconaringenin	*trans*-lycopene	*cis*-lycopene	β-carotene
none	17.3 ± 2.6	31.7 ± 1.9	7.6 ± 0.5	21.5 ± 7.5	583 ± 134	13.0 ± 3.7	0.51 ± 0.37	0.56 ± 0.12	15.26 ± 8.33	2.89 ± 1.57	0.05 ± 0.23	0.44 ± 0.08
4 °C, 1w	15.7 ± 3.6	30.7 ± 2.0	7.3 ± 0.9	25.2 ± 8.5	813 ± 151	12.6 ± 5.1	0.56 ± 0.22	0.48 ± 0.10	16.50 ± 5.25	2.27 ± 1.82	0.00 ± 0.02	0.44 ± 0.09
4 °C, 2w	17.1 ± 2.2	31.9 ± 2.0	7.7 ± 0.6	22.0 ± 2.9	654 ± 92	12.0 ± 4.1	0.51 ± 0.15	0.46 ± 0.09	11.44 ± 2.97	2.83 ± 0.79	0.00 ± 0.10	0.48 ± 0.05
4 °C, 3w	15.8 ± 3.2	31.7 ± 2.7	7.7 ± 0.5	22.9 ± 2.8	603 ± 201	20.4 ± 4.0	0.36 ± 0.06	0.51 ± 0.17	9.81 ± 3.02	2.85 ± 0.92	0.02 ± 0.17	0.41 ± 0.06
12 °C, 1w	16.4 ± 3.4	31.8 ± 2.4	7.6 ± 0.5	19.8 ± 2.5	640 ± 99	10.7 ± 4.3	0.43 ± 0.09	0.45 ± 0.07	9.03 ± 1.59	3.18 ± 1.38	0.30 ± 0.32	0.57 ± 0.12
12 °C, 2w	16.4 ± 3.0	32.1 ± 2.2	7.7 ± 1.0	19.4 ± 4.3	617 ± 118	13.4 ± 3.7	0.23 ± 0.10	0.43 ± 0.12	4.61 ± 0.93	8.01 ± 3.10	1.34 ± 0.39	0.75 ± 0.15
12 °C, 3w	15.5 ± 2.6	30.6 ± 1.8	7.7 ± 0.8	20.2 ± 1.6	610 ± 41	19.1 ± 4.5	0.11 ± 0.08	0.48 ± 0.17	3.66 ± 0.82	11.66 ± 3.57	2.01 ± 0.66	0.91 ± 0.26
20 °C, 1w	16.0 ± 2.7	30.1 ± 2.3	7.6 ± 0.4	18.1 ± 3.0	681 ± 255	13.3 ± 6.1	0.32 ± 0.09	0.42 ± 0.14	2.26 ± 0.59	9.08 ± 4.50	1.84 ± 1.02	0.57 ± 0.17
20 °C, 2w	15.9 ± 3.8	30.6 ± 1.8	7.7 ± 0.8	19.6 ± 4.2	558 ± 227	14.3 ± 3.8	0.10 ± 0.05	0.36 ± 0.10	0.53 ± 0.19	14.52 ± 6.02	2.51 ± 0.58	0.55 ± 0.11
20 °C, 3w	16.1 ± 3.4	30.8 ± 2.0	7.1 ± 0.9	20.5 ± 1.1	614 ± 67	18.6 ± 8.8	0.06 ± 0.01	0.36 ± 0.15	0.41 ± 0.22	17.62 ± 3.81	3.09 ± 1.58	0.56 ± 0.12
T	NS	NS	NS	**	NS	NS	***	**	***	***	***	***
S	NS	NS	NS	NS	**	***	***	**	***	***	***	***
T × S	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	**	***
II	14.8 ± 2.5	30.0 ± 2.2	6.1 ± 0.9	18.3 ± 4.9	513 ± 105	9.7 ± 2.5	0.53 ± 0.18	0.63 ± 0.16	11.25 ± 5.94	0.08 ± 0.31	0.00 ± 0.01	0.35 ± 0.16
IV	17.3 ± 2.6	31.7 ± 1.9	7.6 ± 0.5	21.5 ± 7.5	583 ± 134	13.0 ± 3.7	0.51 ± 0.37	0.56 ± 0.12	15.26 ± 8.33	2.89 ± 1.57	0.05 ± 0.23	0.44 ± 0.08
VI	14.7 ± 4.4	30.1 ± 3.0	9.3 ± 0.9	25.3 ± 5.9	670 ± 107	17.1 ± 3.7	0.63 ± 0.31	0.68 ± 0.18	8.16 ± 2.21	13.20 ± 2.89	1.06 ± 1.07	0.70 ± 0.10
RS	*	NS	***	*	*	***	NS	NS	*	***	***	***

[a] The contents of specific compounds are listed as total amount in mg/100 g FW ± SD, phenolics are given as mg GAE /100 g FW, and FRAP values are given as μmol Fe^{II}/100 g FW. For postharvest ripening of fruits harvested at stage IV (orange-yellow), the storage temperature (T) is given in °C, and the storage duration (S) is given in weeks; for vine ripening, ripening stage (RS) is given as stage II (green-yellow), IV, and VI (red) of seven stages of ripeness. Significance of differences between treatments is given: NS, not significant; *P < 0.05; **P < 0.01; and ***P < 0.001.

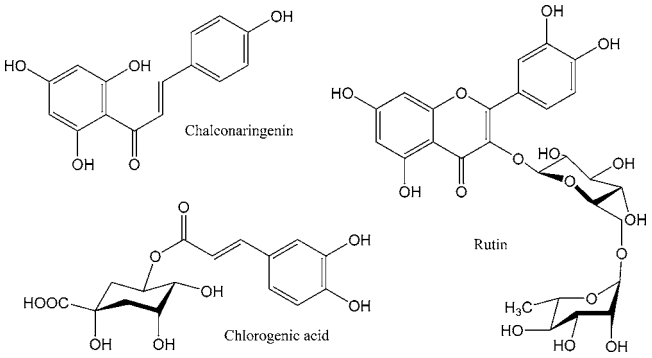

Figure 1. Molecule structures of chalconaringenin, rutin, and chlorogenic acid.

of soluble solids (sugars) and titrable acidity (*1*). Some varieties have also been found to contain high amounts of flavonols, primarily as quercetin (*15*). This observation has been used to develop genetically modified cherry tomatoes with an extraordinary high level of flavonols because of the potential health benefits of these compounds (*8*, *16*).

This work is part of a project where the overall aim is to develop a strategy for a non-GMO greenhouse grown tomato to achieve a documented minimal level of certain plant compounds (lycopene, β-carotene, ascorbic acid, specific flavonoids, and chlorogenic acid). Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes, harvested at an orange-yellow stage of ripening and analyzed immediately after harvest, were described earlier (*9*). The present work reports on the variations in content of specific plant compounds during postharvest and vine ripening. This information will be used to establish agronomic efforts and postharvest management in order to increase or maintain the content of specific compounds in the fruits above a certain minimal level.

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 January 15, 2004, on standard rockwool slabs (90 cm × 10 cm × 15 cm) in a commercial greenhouse in southwestern Norway (58° 47′N, 5° 41′E). The stand was trained as a layering system in single rows at a density of 3.5 plants m^{-2}. Air temperature, air humidity, and CO_2 concentration together with outdoor global radiation were recorded once per minute, whereas the electrical conductivity and pH in the growing medium were recorded three times per week (*9*). 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 used for vine ripening were harvested at stages II, IV, and VI 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) (*17*). The average daily mean air temperature in the greenhouse during vine ripening was 19.6 °C, while outdoor photosynthetic flux density varied from 12.7 to 51.5 mol m^{-2} day^{-1}. Fruits used for postharvest ripening were harvested at stage IV, which is a standard ripening stage at harvest in Norway, and stored in darkness at temperatures of 4, 12, or 20 °C (±0.5 °C) during 1, 2, or 3 weeks. The third tomato on the truss of four different plants was collected at 09:00 am on June 17, July 17, and September 17, giving a total of 12 samples for each treatment. Fruit weights and sizes are listed in **Table 1**. Tomatoes were analyzed separately, and mean values were calculated.

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 by elution with 50% methanol on a bed of Sephadex LH-20 (Amersham Biosciences, Denmark) in a 3 cm × 50 cm glass column. Chlorogenic acid (5′-caffeoylquinic acid), rutin (quercetin 3-rhamnosylglucoside), L-ascorbic acid, gallic acid, lycopene (ψ,ψ-carotene; all-*trans*-lycopene) and β-carotene were all purchased from Sigma-Aldrich. Methanol, trifluoroacetic acid (TFA), 2,4,6-*tri*-(2-pyridyl)-*S*-triazine, 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, whereas ethyl acetate was purchased from Tamro Med-lab (Norway).

Sample Preparation. Every fruit was frozen in N_2 (liquid) soon after collection and kept at −20 °C until analysis. The tomatoes were measured by weight and size and then transferred to a bath of N_2 (liquid) 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 weighted 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 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 NaH_2PO_4 in 24 mm × 150 mm sample tubes for 60 s and then kept in darkness at ambient temperature for another 15 min. The extract was finally separated from the residue by centrifugation (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 cm × 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 (*18*).

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

HPLC. A liquid chromatograph (Agilent 1100 system, Agilent Technologies) supplied with an autosampler and a photodiodearray 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 mm × 150 mm, 5 μm) 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 μL were injected on the column, and the column oven was set to 30 °C. Detection was measured 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 recondition 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 at 320 nm, whereas chalconaringenin and rutin were recorded at 370 nm. Ascorbic acid was analyzed on a Kromasil-NH_2 column (4.6 mm × 250 mm, 5 μm) (Supelco) by a single mobile phase consisting of 5 mM KH_2PO_4−acetonitrile (80:20) at 1.0 mL/min. Detection occurred at 260 nm. The method was slightly modified from that of Cano et al. (*18*). All HPLC samples were filtered through a 13 mm syringe filter (Nylon 0.45 μm, VWR International) prior to injection. Individual standard curves were made for each of the single compounds that were analyzed by HPLC. A lycopene derivative was eluted prior to all-*trans*-lycopene on the HPLC column. This compound exhibited three similar absorption bands as all-*trans*-lycopene, but an additional band appeared 140 nm in hypsochromic direction from that at the longest wavelength ("cis peak"). The absorption spectrum is in accordance with a *cis*-lycopene (*19*). No other peaks with similar absorption spectrum appeared in the chromatogram. The content of the cis isomer was measured by use of the same standard curve as that of all-*trans*-lycopene.

Total Phenolics. The method of Price and Butler (*20*) as modified by Graham (*21*) 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; 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).

FRAP. Antioxidants from the methanol extracts were measured by the FRAP assay (*22*). This method has been modified for the determination of antioxidants in plant extracts and has been renamed to ferric reducing antioxidant power assay (*23*). A 50 μL sample solution was diluted with 150 μL 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 UV−vis spectrophotometer (Agilent Technologies). Aqueous ferrous solutions in the concentration range of 100−1000 μM ($FeSO_4 \cdot 7H_2O$) were used for calibration, and the data were expressed as micromoles of ferrous equivalents per 100 g FW.

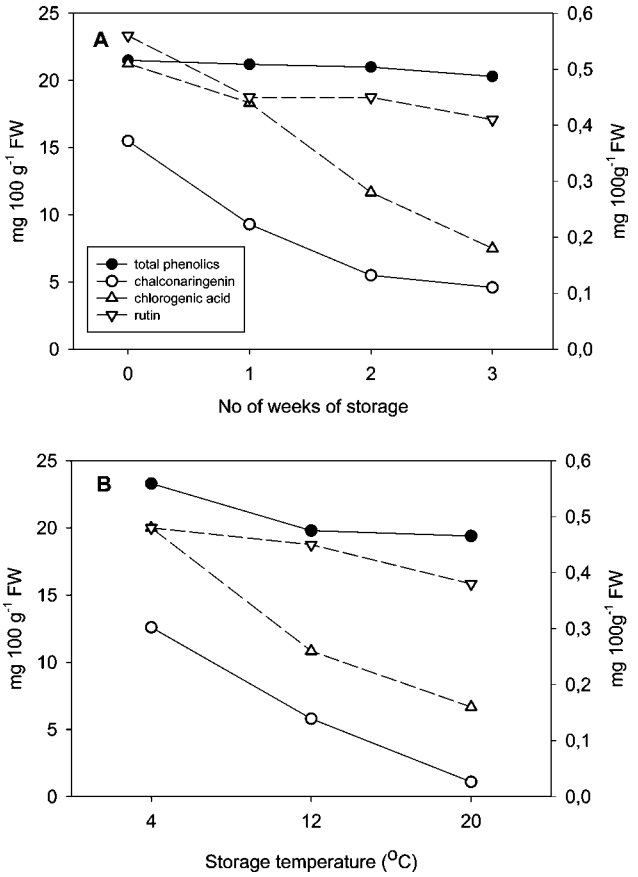

Figure 2. Content of total phenolics (expressed as equivalents of gallic acid) (left axis), chalconaringenin (left axis), chlorogenic acid (right axis), and rutin (right axis) during storage (**A**) (average of three storage temperatures, SE = 0.81, 0.70, 0.032, and 0.021, respectively) and (**B**) at different storage temperatures (average of storage duration, SE = 0.70, 0.61, 0.027, and 0.018, respectively).

Soluble Solids Content (SSC). SSC was determined by use of a digital PR-100α refractometer (Atago Co., Japan) of room-temperated tomato juice. Data were expressed as equivalent °Brix (or % SSC) (*24*).

Statistics. The results were subjected to analysis of variance using the GLM procedure from the SAS statistical computer program (version 8.02). The SNK test was used to determine significant differences between ripening stages or ripening conditions. Correlations between parameters of data sets from vine ripening and postharvest ripening were calculated using the CORR procedure in SAS. Only significant correlations are mentioned in the text.

RESULTS AND DISCUSSION

Chalconarengenin was found to be the main phenolic compound found in freshly picked cherry tomatoes (cv. Jennita). The average amount of chalconarengenin in fruits picked on three harvesting dates at standard ripening stage (IV) was found to be 15.26 mg 100 g^{-1} FW (**Table 1**). The amount varies during the growing season (*9*). The content of chalconaringenin decreased dramatically during postharvest ripening to a minimum of 0.41 mg 100 g^{-1} FW after 3 weeks of storage at 20 °C (**Table 1**). On average for all storage temperatures, the chalconaringenin content decreased with 39, 64, and 70% after, respectively, 1, 2, and 3 weeks of storage (**Figure 2a**). The loss of chalconaringenin was more pronounced at higher temperature. Storage temperatures of 4, 12, or 20 °C lowered the chalconarengenin content with 18, 62, and 93%, respectively (**Figure 2b**). No interaction was found between storage time, storage temperature, and harvesting date for chalconaringenin. To

maintain a high amount of chalconarengenin in the fruits, a storage temperature of 4 °C for less than 2 weeks should be used. In vine-ripened cherry tomatoes (stage VI), the content of chalconaringenin fell from 15.26 to 8.16 mg 100 g^{-1}. Because vine ripening from standard to red in a greenhouse at 20 °C normally endures 2−3 days, the loss in content is comparable to the decrease found in postharvest-ripened fruits at 20 °C.

Only a limited number of reports describe chalconaringenin as the main flavonoid in tomatoes. The compound has been found to accumulate in peel tissue. It was also found that the chalconaringenin production occurred simultaneously with color formation of the fruit (*7*, *8*). Our observations do not support this development-dependent approach (**Table 1**). A reasonable explanation of the disappearance of chalconaringenin during ripening would be its conversion into naringenin, which has been reported to be one of the main phenolic compounds present in tomatoes (*10*, *11*). However, no evidence was found for this conversion here. Naringenin was detected only as a minor compound in all of the samples, which was similar to the level detected during the whole growing season (*9*). The amount of chlorogenic acid was far below that of chalconaringenin (**Table 1**). The two compounds showed, however, a similar metabolic turnover during postharvest ripening with a decrease in level as a function of time and temperature (**Figure 2**). After 3 weeks of postharvest storage, the content of chlorogenic acid at overall temperature was found to be 35% as compared to that found in the freshly sampled cherry tomatoes. The loss was greatest at the highest temperature. During vine ripening, no significant change in the content of chlorogenic acid was observed. The metabolic turnover of chalconaringenin and chlorogenic acid during postharvest ripening has not been described previously. However, the highest content of chlorogenic acid has elsewhere been found at early stages of tomato development followed by a decrease during vine ripening (*25*).

As compared to the metabolic change that was detected for chalconaringenin and chlorogenic acid, the total amount of phenolics did not decrease during postharvest ripening of the tomatoes (**Figure 2**). This content was found to be stable with a mean value at 21.0 mg GAE 100 g^{-1}. During vine ripening, the amount rose from 18.3 mg 100 g^{-1} in green fruit to 21.5 and 25.3 mg 100 g^{-1} in standard ripened and red fruits, respectively.

As the amount of total phenolics was found to be relatively constant during ripening, the imbalance between the phenolic level and the decrease in content of chalconaringenin and chlorogenic acid during ripening has to be made up by other compounds. Rutin has been the far most highlighted flavonoid in tomatoes due to its antioxidant properties and bioavailability to humans. No development-dependent production of rutin in the cherry tomatoes seems to take place from the green to the red ripening stages. Rutin has been reported to be the most important flavonoid in tomatoes. Favorita, a red cherry tomato, contains 2.15 mg 100^{-1} g whereas other varieties are reported to contain from 0.13 to 2.22 mg 100^{-1} g of total flavonols (*26*). The chemical constituents in tomatoes are often reported with limited information about the growth conditions of the plant. However, most of the tomato production in the world takes place in open fields. It is therefore assumed that the tomatoes that are subjects of most inspections on chemical composition have been grown in open fields if no other information has been given. Direct exposure to sun, with higher levels of UV-B, might explain the high flavonol content (*27*). However, in the present investigation, the amount of rutin was found to be stable during ripening, and an overall amount was found to be 0.63 mg 100 g^{-1} in green through red cherry tomatoes during vine ripening. The amount of rutin was too low to explain the high amount of phenolics still present after the reduction in content of chalconaringenin and chlorogenic acid. A previous investigation has also reported that during tomato vine ripening chlorogenic acid declined whereas no changes were observed in rutin (*28*). The variation in the total content of phenolics has previously been evaluated during vine and postharvest ripening of tomato cv. Moneymaker grown in a greenhouse. The total phenolic content was found to be higher in postharvest-ripened samples (about 10−20 mg 100^{-1} g) than in vine-ripened fruit (about 7−11 mg 100^{-1} g) (*29*). The present data of the cherry tomatoes gave 20.0 and 25.3 mg GAE 100^{-1} g in postharvest- and vine-ripened fruits, respectively. Beside the higher amount of phenolics in this variety, the effect of ripening is opposite of that found in Moneymaker.

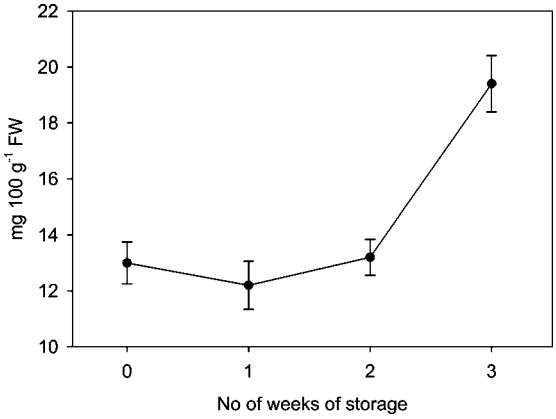

Figure 3. Content of ascorbic acid during storage (average of three storage temperatures ± SE).

Ascorbic acid increased in content during tomato ripening and reached maximum levels in red fruits (**Table 1**). The amounts detected in postharvest- and vine-ripened red tomatoes were on the same level. In one of the most popular cherry tomato cultivars, Favorita, the amount was found to be 18.3 mg 100^{-1} g (*30*). The content of ascorbic acid has elsewhere been found to decrease during ripening. This decrease has been linked to its antioxidant role when the ripening cells absorb higher amounts of oxygen, a result of an increase in respiration, which is characteristic of climacteric fruits (*31*). However, this pattern of ascorbic acid content is opposite to our results for postharvest ripening (**Figure 3**). One reason for this divergence is probably the reduced light exposure in our experiment. Another reason may perhaps be found in the level of other compounds within the cell vacuoles due to interaction between ascorbic acid and for example flavonoids. It is known that flavonoids seem to have vitamin C sparing effects and that this phenomenon can be related to their antioxidant properties (*32*). This seems to fit with the present observations: a raise in the amount of ascorbic acid at the expense of chalconaringenin and chlorogenic acid. The stable level of the well-known antioxidant rutin may in this context be explained by a higher reduction potential than chalconaringenin and chlorogenic acid. In general, half-cell reduction potentials of flavonoids are relatively low ($0.23\ V < E < 0.75\ V$ at pH 7.0) and are therefore thermodynamically able to reduce highly oxidizing free radicals (*33*). Ascorbic acid has a half-cell reduction potential at 0.28 V, whereas that of rutin is 0.6 V. No data has been found for chalconaringenin nor chlorogenic acid. However, it seems that the difference in redox potential is just one part of complex pattern with respect to vitamin C sparing effects (*34*). It has been found that

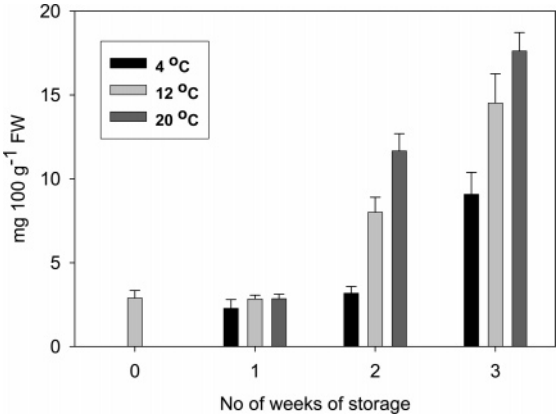

Figure 4. Content of all-*trans*-lycopene in cherry tomatoes as a function of storage temperature and storage duration (±SE).

chalconaringenin acts as a prooxidant by increasing the amount of thiobarbituric acid reactive substances during incubaction of LDL with Cu^{2+} (*35*). The present data show that there is a correlation between chalconaringenin and FRAP values during postharvest ripening ($p < 0.05$) and vine ripening ($p < 0.01$). Further inspections have to be made to interpret these results with respect to redox effects of this compound.

Lycopene was continuously produced in all experiments during tomato ripening, except at 4 °C, and maximum content of the all-trans isomer (17.62 mg 100^{-1} g FW) was detected in postharvest-ripened fruits after 3 weeks at 20 °C (**Figure 4**). Vine-ripened tomatoes were found to contain 13.20 mg 100^{-1} g all-*trans*-lycopene (**Table 1**). The total tomato lycopene content may range from 4.3 to 18.1 mg 100^{-1} g FW, with the most frequent values between 5.5 and 8.0 mg 100^{-1} g (*36*). The amount of the single *cis*-lycopene was strongly correlated with the amount of all-*trans*-lycopene ($p < 0.001$), and a maximum of 3.09 mg 100^{-1} g was detected after 3 weeks of postharvest ripening at 20 °C. The content of β-carotene followed a somewhat different pattern during fruit ripening as compared to that of lycopene. A maximum content of 0.91 mg 100^{-1} g was detected after 3 weeks of storage at 12 °C. Green-yellow tomatoes contained 0.35 mg 100^{-1} g, which made up half of what was found in vine-ripened fruits (0.70 mg 100^{-1} g) (**Table 1**). Among the carotenoids, lycopene has been found to be one of the strongest antioxidants (*37*). However, we did not measure antioxidant capacities for fat soluble compounds as part of this work.

In fruits stored at various temperatures, it has been observed that, at 20 °C, the biosynthesis and accumulation of phytoene and lycopene were fast, and those of β-carotene were slow (*38*). By contrast, at 30 °C, the biosynthesis and accumulation of lycopene and β-carotene were fast and phytoene accumulation was slow. In conclusion, the formation of lycopene depends on temperature range and seems to occur between 12 and 32 °C (*39*). When harvested at the pink-ripe stage and stored for ripening, it was found that tomato (cv. Sunny) had an optimum lycopene production in the temperature range 16−26 °C (*40*). The present results fit these previous reported data with respect to lycopene accumulation. It also supports the data found for β-carotene at 20 °C. It shows, however, that there are two temperature levels that might stimulate the accumulation of β-carotene, one close to 12 °C and one at 30 °C.

The content of soluble solids (% Brix) increased during vine ripening and reached a level at 9.3 in vine-ripened tomatoes harvested at stage VI. No increase in Brix value was detected during postharvest ripening (**Table 1**), and 7.7 was found to be the highest value. These results are in accordance with earlier observations (*41*), indicating a sweeter taste of vine-ripened tomatoes.

The sizes of the tomato fruits used in the experiment were not significantly different. A slightly higher weight was detected for tomatoes harvested at stage IV when compared to those harvested at stage II or VI (**Table 1**).

Tomatoes in Norwegian greenhouses are normally harvested at an early ripening stage (IV, orange-yellow) in order to extend shelf life. During transport and storage, the fruits become fully red (ripening stage VI). It is assumed that the tomatoes are consumed 1−3 weeks after harvest. Much of this time, the tomatoes are kept in dark or under reduced light conditions. Storage in the dark resulted in a significant decrease in contents of chalconaringenin and chlorogenic acid. The decrease correlated with increased temperature. The decrease is less pronounced for chalconaringenin during vine ripening, and no decrease in content was detected for chlorogenic acid upon vine ripening. The level of rutin has been found to be more or less constant during both postharvest and vine ripening. The importance of the changes in chalconaringenin and chlorogenic acid has to be further studied, as these constituents are present in cherry tomatoes at higher values than previously reported.

The time and temperature conditions during postharvest ripening clearly show that cherry tomatoes become ripe with respect to color and lycopene content within 1 week at 20 °C or within 2 weeks at 12 °C. Postharvest-ripened tomatoes contain the amount of β-carotene that is normally reported from ripe tomatoes. Ascorbic acid increased upon storage at all temperature levels and also during vine ripening. Postharvest-ripened tomatoes contain similar levels of carotenoids and ascorbic acid that are found in vine-ripened cherry tomatoes. The postharvest regimes gave lower sugar contents (measured as % Brix) as compared to vine-ripened cherry tomatoes. Vine ripening combined with a short storage period at lower temperatures might therefore be a strategy to increase sweetness and to maintain the content of plant constituents at a high level.

LITERATURE CITED

(1) George, B.; Kaur, C.; Khurdiya, D. S.; Kapoor, H. C. Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. *Food Chem.* **2004**, *84*, 45−51.

(2) 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 Cedex, France, 2001.

(3) Olson, J. A. Provitamin A functions of carotenoids: The conversion of beta-carotene into vitamin A. *J. Nutr*. **1989**, *119*, 105−108.

(4) Stevens, M. A. Relationship between polyene-carotene content and volatile compound composition in tomatoes. *J. Am. Soc. Hortic. Sci.* **1970**, *95*, 461−464.

(5) Halliwell, B. Antioxidant activity and other biological effects of flavonoids. In *Wake up to Flavonoids*; Rice-Evans, C., Ed.; Royal Society of Medicine Press Ltd.: London, 2000; pp 13−23.

(6) Beecher, G. R. Nutrient content of tomatoes and tomato products. *Proc. Soc. Exp. Biol. Med.* **1998**, *218*, 98−100.

(7) 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.

(8) 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.

(9) Slimestad, R.; Verheul, M. J. Seasonal varations in the level of plant constituents in greenhouse production of cherry tomatoes. *J. Agric. Food Chem.* **2005**, *53*, 3114−3119.
(10) Hunt, G. M.; Baker, E. A. Phenolic constituents of tomato fruit cuticles. *Phytochemistry* **1980**, *19*, 1415−1419.
(11) 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.
(12) Yamamoto, T.; Yoshimura, M.; Yamaguchi, F.; Kouchi, T.; Tsuji, R.; Saito, M.; Obata, A.; Kikuchi, M. Anti-allergic activity of naringenin chalcone from a tomato skin extract. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1706−1711.
(13) Bhuta, J. G.; Spaulding, D. W. Endogenous levels of phenolics in tomatoes during growth and maturation. *J. Plant Growth Regul.* **1997**, *16*, 43−46.
(14) Guidi, L.; Lorefice, G.; Pardossi, A.; Malorgio, F.; Tognoni, F.; Sodatini, G. F. Growth and photosynthesis of *Lycopersicon esculentum* (L.) plants as affected by nitrogen deficiency. *Biol. Plant.* **1998**, *40*, 235−244.
(15) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.
(16) Le Gall, G.; DuPont, M. S.; Mellon, F. A.; Davis, A. L.; Collins, G. J.; Verhoeyen, M. E.; Colquhoum, I. J. Characterization and content of flavonoid glycosides in genetically tomato (*Lycopersicon esculentum*) fruits. *J. Agric. Food Chem.* **2003**, *51*, 2438−2446.
(17) 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.
(18) 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.
(19) Rao, A. V.; Agarwal, S. Role of antioxidant lycopene in cancer and heart disease. *J. Am. Coll. Nutr.* **2000**, *19* (5), 563−569.
(20) 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.
(21) Graham, H. D. Stabilization of the Prussian blue color in the determination of polyphenols. *J. Agric. Food Chem.* **1992**, *40*, 801−805.
(22) 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.
(23) Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **2000**, *48*, 3396−3402.
(24) Mitcham, B.; Cantwell, M.; Kader, A. Methods for determining quality of fresh commodities. *Perishables Handl. Newsl.* **1996**, *85*, 1−5.
(25) Aziz, A. B. A. Seasonal changes in the physical and chemical composition of tomato fruits as affected by nitrogen level. *Meded. Landbouwhogeschool Wageningen* **1968**, *68*, 6.
(26) 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.
(27) Olsson, L. C.; Veit, M.; Weissenbock, G.; Bornman, J. F. Differential flavonoid response to enhanced UV-B radiation in Brassica napus. *Phytochemistry* **1998**, *49*, 1021−1028.
(28) Buta, J. G.; Spaulding, D. W. Endogenous levels of phenolics in tomato fruit during growth and maturation. *J. Plant Growth Regul.* **1997**, *16*, 42−46.
(29) Giovanelly, G.; Lavelli, V.; Peri, C.; Nobili, S. Variation in antioxidant components in tomato during vine and post-harvest ripening. *J. Sci. Food Agric.* **1999**, *79*, 1583−1588.
(30) 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.
(31) Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC: Boca Raton, 1990.
(32) Meltzer, H. M.; Malterud, K. E. Can dietary flavonoids influence the development of coronary heart disease? *Scand. J. Nutr.* **1997**, *41*, 50−57.
(33) Pietta, P.-G. Flavonoids as antioxidants. *J. Nat. Prod.* **2000**, *63*, 1035−1042.
(34) Bors, W.; Michel, C.; Schikora, S. Interaction of flavonoids with ascorbate and determination of their univalent redox potentials: A pulse radiolysis study. *Free Radical Biol. Med.* **1995**, *19*, 45−52.
(35) 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.
(36) 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.
(37) Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: New York, 1976; pp 38−165.
(38) Hamauzu, Y.; Chachin, K.; Ueda, Y. Effects of postharvest temperature on the conversion of 14C-mevalonic acid to carotenes in tomato fruit. *J. Jpn. Soc. Hortic. Sci.* **1998**, *67*, 549−555.
(39) Leoni, C. Industrial quality as influenced by crop management. *Acta Hortic.* **1992**, *301*, 177−184.
(40) Türk, R.; Seniz, V.; Ozdemir, N.; Suzen, M. A. Changes in the chlorophyll carotenoid and lycopene contents of tomatoes in relation to temperature. *Acta Hortic.* **1994**, *368*, 856−862.
(41) Verheul, M. J. Kvalitet i tomat som påvirket av noen dyrknings- og lagringsforhold. Planteforsk Grønn forskning 45, 2002; ISBN 82-479-032-3.

Received for review April 1, 2005. Revised manuscript received July 5, 2005. Accepted July 12, 2005.

JF050737D