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Changes in Free Amino Acid, Phenolic, Chlorophyll, Carotenoid, and Glycoalkaloid Contents in Tomatoes during 11 Stages of Growth and Inhibition of Cervical and Lung Human Cancer Cells by Green Tomato Extracts

Suk-Hyun Choi,[†] Sang-Hwa Lee,[‡] Hyun-Jeong Kim,[#] In-Seon Lee,[#] Nobuyuki Kozukue,[§] Carol E. Levin,[⊥] and Mendel Friedman*,[⊥]

[†]Department of Food Service Industry, [‡]Department of Food Nutrition, and [§]Bio Organic Material and Food Center, Seowon University, Cheongju-city 361-742, Republic of Korea, [#]Center for Traditional Microorganism Resources, Keimyung University, Daegu 704-701, Republic of Korea, and [⊥]Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

Tomato (*Solanum lycopersicum*) plants synthesize nutrients, pigments, and secondary metabolites that benefit nutrition and human health. The concentrations of these compounds are strongly influenced by the maturity of the tomato fruit on the vine. Widely consumed Korean tomatoes of the variety Doturakworld were analyzed for changes in the content of free amino acids, phenolic compounds, chlorophylls, carotenoids, and glycoalkaloids at 11 stages (S1−S11) of ripeness. The results show that (a) the total content (in mg/100 g of FW) of the free amino acids and other nitrogen-containing compounds in the extracts ranged from about 41 to 85 in the green tomato extracts S1−S7 and then increased to 251 (S9) in the red extracts, followed by a decrease to 124 in S11 red extracts; (b) the total initial concentration and composition of up to 12 phenolic compounds of ∼2000 μg/100 g of FW varied throughout the ripening process, with the quantity decreasing and the number of individual compounds increasing in the red tomato; (c) chlorophyll *a* and *b* content of tomatoes harvested during S1 was 5.73 mg/100 g of fresh pericarp and then decreased continuously to 1.14 mg/100 g for S11; (d) the concentration (in mg/100 g of FW) of lycopene in the S8 red extract of 0.32 increased to 1.27 in S11; and (e) tomatoes harvested during S1 contained 48.2 mg of dehydrotomatine/100 g of FW, and this value continually decreased to 1.5 in S7, with no detectable levels in S8−S11. The corresponding α-tomatine content decreased from S1 (361) to S8 (13.8). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell assay IC_{50} values showed that Hel299 lung cells, A549 lung cancer cells, and HeLa cervical carcinoma cells were highly susceptible to inactivation by glycoalkaloid-rich green tomato extracts. Chang normal liver cells and U937 lymphoma cells were less susceptible. The possible significance of the results for plant physiology and the diet is discussed.

KEYWORDS: Tomato; free amino acids; phenolic compounds; chlorophylls; carotenoids; glycoalkaloids; cancer

INTRODUCTION

During growth on the vine, tomato fruits synthesize free amino acids, numerous phenolic compounds, the green pigments chlorophylls *a* and *b*, the carotenoids β-carotene and lycopene, and two glycoalkaloids (see **Figure 1** for structures). In a critical review, Dumas et al. (*1*) noted that, except for lycopene, few reliable studies have been done on changes in tomato composition of phenolic and other compounds during growth of tomatoes. In previous studies, we reported that the chlorophyll and tomatine contents of field grown green tomatoes decreased rapidly during fruit ripening on the vine and that β-carotene and lycopene levels were low in immature and high in mature tomatoes (*2*). We also found that extracts of green tomatoes inhibited human breast, colon, liver, and stomach cancer cells (*3*). The present study will follow the levels of these as well as the important components free amino acids and phenolic compounds during the ripening of tomatoes. Also, the effect of ripening stage on inhibition of cancer cells will be examined.

Free amino acids and secondary metabolites produced during tomato ripening play important roles both in the plant and in the diet. Plant foods such as potatoes and tomatoes contain high levels of free amino acids. Free amino acids represent a source of nitrogen and of nutritionally essential amino acids such as lysine, methionine, and threonine. On the other hand, during processing they can be converted to or be precursors for potentially deleterious compounds such as D-amino acids (*4*) or Maillard browning products. Free L-asparagine is the major precursor for potentially

*To whom correspondence should be addressed. E-mail: mendel.friedman@ars.usda.gov. Tel: 510-559-5615.

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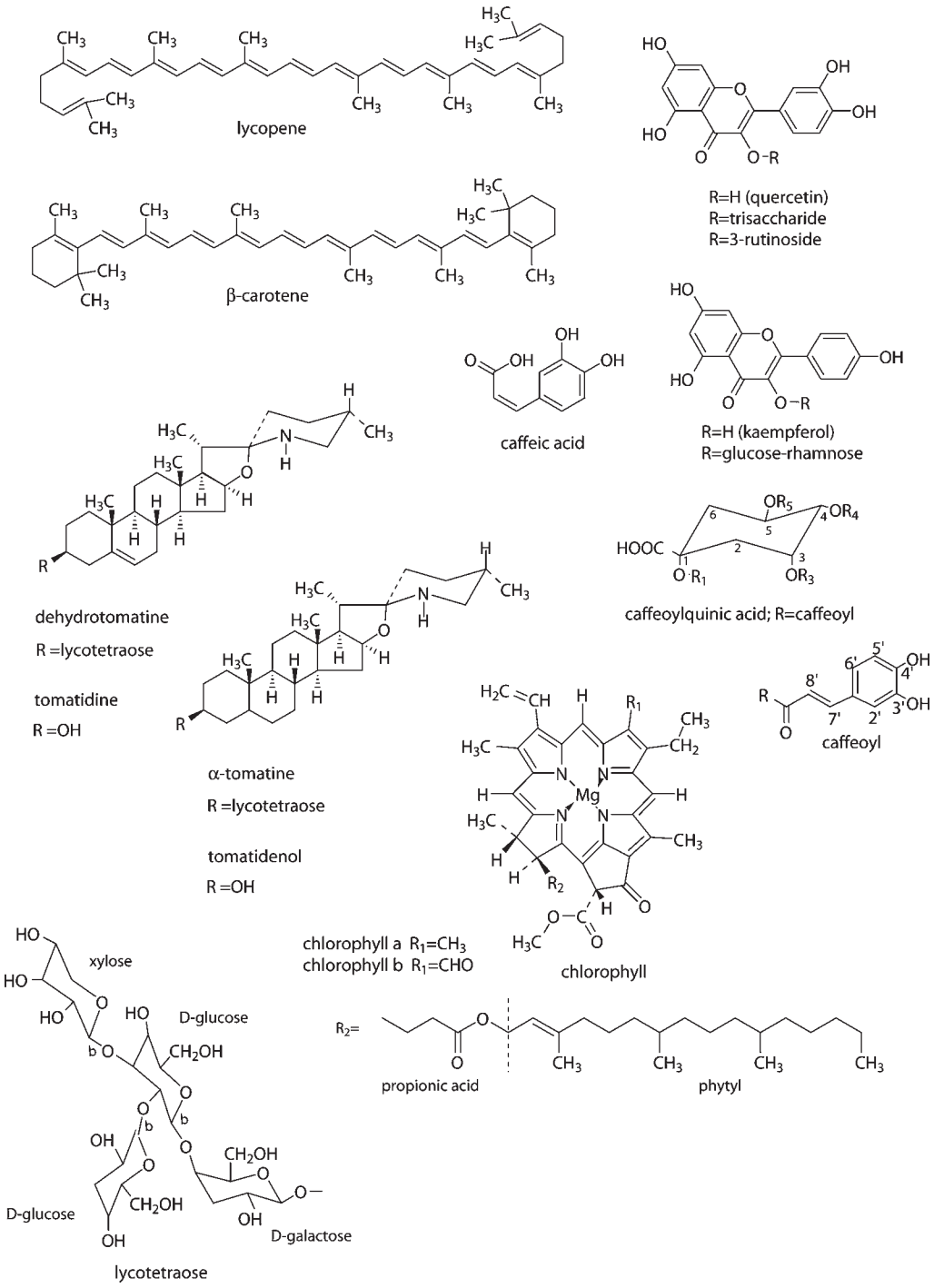

Figure 1. Structures of pigments, phenolic compounds, and glycoalkaloids in tomato extracts evaluated in the present study.

toxic acrylamide, found in processed foods (*5*). We and other investigators have suggested that one way to mitigate the formation of acrylamide during processing of plant foods is to reduce the biosynthesis of asparagine. These considerations suggest the need to determine changes in the free amino acid content of tomatoes during growth and maturation of the plant.

Phenolic compounds present in tomatoes contribute to the plant defense against phytopathogens (*6*). Tomato phenolics have been shown to inhibit Cox-2 expression (*7*), suggesting that they may have anticarcinogenic properties. Chlorophyll content of the tomato fruit decreases as the fruit ripens as chloroplasts change to pigment-producing chromoplasts (*8*). However, the extent of decrease is not apparent because the green chlorophyll in the unripe fruit may be masked by red pigments in the ripe fruit. Chlorophyll is reported to exhibit anticarcinogenic effects (*9*, *10*). The carotenoids lycopene and β-carotene are known to be present in ripe tomato fruit. Carotenoids are health-promoting antioxidants that have been shown to be effective against some cancer cells (*11*−*13*).

Previously we showed that tomato fruits synthesize the glycoalkaloids dehydrotomatine and α-tomatine and that commercial tomatine consisted of a ~10:1 mixture of α-tomatine and dehydrotomatine (*14*−*17*). We also found that tomatine was cytotoxic to frog embryos (*18*), inhibited human cancer cells in vitro (*19*), was not toxic and inhibited multiorgan carcinogenesis during long-term oral feeding to rainbow trout (*20*), and reduced plasma cholesterol and triglyceride levels in hamsters following oral consumption (*21*, *22*) and that tomatine-containing extracts of green tomatoes inhibited human breast, colon, liver, and stomach cancer

Table 1. Tomatoes Used in the Present Study

growth stage	days after flowering	color	length (mm)	width (mm)	weight (g)/fruit	ammonia precipitate (mg/100 g of fruit)	ammonia precipitate (mg/fruit)
S1	3	green	9.85 ± 1.02	8.75 ± 0.76	0.5 ± 0.05	481.6	2.41
S2	7	green	14.6 ± 3.07	10.53 ± 0.91	1.42 ± 0.32	531.59	7.55
S3	14	green	22.7 ± 1.79	20.9 ± 1.34	5.28 ± 1.01	334.32	17.65
S4	25	green	31 ± 0.92	28.03 ± 0.85	13.97 ± 0.52	95.18	13.3
S5	32	green	50.5 ± 0.1	40.5 ± 0.71	52.17 ± 4.13	71.04	37.06
S6	40	green	57.45 ± 2.05	47.7 ± 0.71	80.44 ± 2.72	41.67	33.52
S7	47	green	83.7 ± 0.71	51.55 ± 0.78	155.61 ± 6.24	26.53	41.28
S8	52	red (1/4)	85.31 ± 1.56	62 ± 0.99	223.76 ± 7.52	nd[a]	nd
S9	55	red (2/4)	75.8 ± 2.56	55.12 ± 2.35	179.38 ± 8.23	nd	nd
S10	57	red (3/4)	76.35 ± 2.23	58.31 ± 3.21	188.76 ± 4.32	nd	nd
S11	60	full red	65.32 ± 1.68	45.13 ± 2.52	139.36 ± 4.23	nd	nd

[a] nd, not detected.

cells (*3*). Other studies indicate that tomatine also inhibited the growth of bacteria, fungi, and viruses (reviewed in ref *23*).

The main objective of the present study was to extend the earlier observations by defining changes in the contents of free amino acids, phenolic compounds, chlorophylls *a* and *b*, carotenoids *β*-carotene and lycopene, and glycoalkaloids dehydrotomatine and α-tomatine in the fruit of one tomato variety as a function of 11 stages of maturation on the vine. We also determined the inhibition of human liver, lymphoma, and lung cancer cells by glycoalkaloid-rich tomato extracts. To our knowledge this is the first report on the dynamics of biosynthesis of all the mentioned tomato ingredients in a single study.

MATERIALS AND METHODS

Materials. Analytical grade potassium dihydrogen phosphate, ammonium hydroxide, acetone, methanol, ethanol, dichloromethane, *n*-hexane, butylhydroxytoluene, and magnesium carbonate were obtained from commercial sources. HPLC grade acetonitrile and formic acid were purchased from J. T. Baker (Phillipsburg, NJ) and Aldrich (Milwaukee, WI), respectively. Before use, solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA) and degassed in an ultrasonic bath. *β*-Carotene standard (GJ01 from carrots) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Lycopene standard (L9879 from tomatoes) and commercial tomatine were obtained from Sigma (St. Louis, MO). TRI-SIL-Z for the preparation of trimethylsilyl ester derivatives of sugars was purchased from GL Science Inc. (Tokyo, Japan). Pure dehydrotomatine and α-tomatine were isolated from Sigma tomatine by multiple collections of eluates from the HPLC column described in a previous paper (*16*).

Human cervical carcinoma (HeLa), histiocytic lymphoma (U937), lung cancer (A549), and normal human liver (Chang) and lung cell lines (Hel299) were obtained from American Type Culture Collection (ATCC, Rockville, MD) and from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were maintained in an MEM medium supplemented with 10% of fetal bovine serum, 50 units/mL of penicillin, and 50 mg/mL of streptomycin, at 37 °C in a 5% CO_2 incubator. Cell culture reagents were obtained from GibcoBRL (Life Technologies, Cergy-Pontoise, France). Each sample was dissolved in DMSO (2 mg/200 μL) and stored at −4 °C.

Sampling of Tomatoes. Tomato fruits of the variety Doturakworld have been widely cultivated in Korea and have resistance against tomato yellow leaf curl virus, leaf mold disease, and tobacco mosaic virus (resistant to TMV-Tm-1 type). Tomato seedlings were purchased from the cultivation section of the Agricultural Cooperative Department in Kyongju City, Korea, on January 10, 2009, and replanted in a greenhouse. In the greenhouse, temperatures were set in the range from 13 to 25 °C during the day and from 10 to 13 °C during the night. The date of each tomato bloom was written down on a label and attached to the flower stem. Specimens of three to four pieces of fruits of approximately the same weight were collected at 3, 7, 14, 25, 32, 40, 47, 52, 55, 57, and 60 days after blooming, representing 11 stages of ripeness. Fruits collected at their third to fourth trusses were used as the specimens. The collected fruits were weighed and measured for size. Fruits were grouped from stage 1 (very small green tomato) to stage 11 (fully ripe large red tomatoes) for size as shown in **Table 1**.

Extraction of Free Amino Acids and Phenolic Compounds from Tomato Fruits. Three fresh, uniform-sized tomato fruits from each stage were selected for analysis. The calyx was removed, after which the remainder of the fruits, including flesh, seeds, and gelatinous fluid, were cut with a knife into thin strips (2 × 2 mm). For S1, 1.50 g was macerated in a glass mortar to which was added 80% ethanol (10 mL). The suspension was then centrifuged at 12000*g* for 10 min at 10 °C. The residue was re-extracted three times with 80% ethanol (10 mL) and centrifuged. The combined supernatants were diluted to 50 mL with 80% ethanol. Stages S2 (3.15 g) to S11 (18.68 g) were extracted as described for S1 and brought up to a volume of 100 mL with 80% ethanol. An aliquot of each extract (10 mL) was concentrated in a rotary evaporator at 30 °C and the residue was then dissolved in 80% ethanol (1.0 mL). This extract was used for the analysis of both free amino acids and phenolic compounds.

In an exploratory study, we found that a ratio of solvent to fresh weight of 6:1 was sufficient for complete extraction. For an exhaustive extraction, we used a 10:1 ratio repeated four times with the same fresh sample.

Analysis of Free Amino Acids. The analysis was carried out by ion-exchange chromatography using methods adapted from the literature (*24*−*26*). Briefly, 10 μL of extract (obtained above) was injected directly into a Hitachi model L-8800 amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan) onto a column packed with Hitachi custom ion-exchange resin 2622 (4.6 i.d. × 60 mm, particle size = 5 μm), which was temperature controlled from 30 to 70 °C. Lithium citrate buffer and ninhydrin flow rate were 0.35 and 0.30 mL/min, respectively. The reaction coil temperature was set to 135 °C. Two separate analyses were carried out with each sample.

LC-MS Analysis of Phenolic Compounds. HPLC was carried out on an Agilent Technologies (Santa Clara, CA) 1200 series binary LC system with a photodiode array detector monitored at 340 nm. The system was coupled with a 3200 Q Trap LC-MS/MS system (Applied Biosystems Inc., Foster City, CA). An aliquot (10 μL) of the extract obtained above was directly injected into an Inertsil ODS-3v, 5 μm, 4.6 × 250 mm HPLC column (GL Science Inc., Tokyo, Japan). The mobile phase consisted of the following linear gradient: acetonitrile (A) and 0.5% formic acid (B), A = 5% (0−5 min), 18% (5.1−30 min), 70% (30.1−90 min), 90% (90.1−100 min), and 5% (100.1−120 min). The flow rate was 0.8 mL/min at 30 °C. The LC eluate was introduced into the mass spectrometer from 5 to 40 min. Mass (MS) and tandem mass spectrometry (MS/MS) were operated in the negative ion mode in the mass range of *m*/*z* 160−1200. Helium was used as the collision gas for the MS/MS spectrometric procedures, followed by the isolation of ions over a selected mass window of 2 Da. MS/MS represents multiple stages of precursor ion *m*/*z* selection followed by product ion detection for successive progeny ions. Mass selection of the analyte by *m*/*z* was followed by fragmentation and analysis of the fragments. For quantification, integrated chromatographic peak areas from stage 1 to stage 11 samples were compared to peak areas of known amounts of standard 5-caffeoylquinic acid. Three separate analyses were carried out with each sample.

Analysis of Chlorophylls *a* and *b*. Extraction of chlorophyll was performed in the dark at a room temperature of 10 °C. Tomato fruit sample, 0.2 g (stage 1) to 3.0 g (stage 11), crystalline sand, and $MgCO_3$ (0.1 g) were macerated in a glass mortar with 80% acetone and then centrifuged at 18000*g* for 10 min at 5 °C. The pellet was extracted three times with 80% acetone (10 mL) and centrifuged. The extracts were combined and adjusted to 50 mL with 80% acetone. This solution was then examined in a

Table 2. Concentrations (Milligrams per 100 g of Fresh Weight) of Free Amino Acids at Different Stages of Growth of Tomato Fruits[a]

	growth stage[b]										
amino acid[c]	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
p-Ser	0.32 ± 0.03	0.13 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	1.17 ± 0.18	1.77 ± 0.23	0.38 ± 0.06	0.51 ± 0.06
L-Asp	1.66 ± 0.17	1.28 ± 0.06	0.92 ± 0.06	1.00 ± 0.06	0.99 ± 0.08	1.18 ± 0.08	2.78 ± 0.20	6.58 ± 0.51	13.4 ± 1.6	1.36 ± 0.08	3.59 ± 0.17
L-Thr	1.16 ± 0.13	0.67 ± 0.06	1.01 ± 0.11	0.72 ± 0.11	0.67 ± 0.08	0.94 ± 0.13	1.35 ± 0.18	2.35 ± 0.28	2.84 ± 0.31	1.42 ± 0.13	1.72 ± 0.30
L-Ser	3.59 ± 0.28	2.24 ± 0.21	2.62 ± 0.06	1.43 ± 0.14	0.84 ± 0.07	1.33 ± 0.07	2.98 ± 0.16	10.62 ± 0.95	9.01 ± 0.38	0.21 ± 0.03	3.82 ± 0.31
L-Asn	9.13 ± 0.62	3.34 ± 0.20	4.34 ± 0.20	3.51 ± 0.14	2.84 ± 0.18	4.05 ± 0.14	6.54 ± 0.47	6.55 ± 0.04	8.83 ± 1.13	1.74 ± 0.11	3.03 ± 0.06
L-Glu	4.31 ± 0.03	2.72 ± 0.04	5.59 ± 0.06	3.19 ± 0.11	1.64 ± 0.10	1.62 ± 0.11	2.03 ± 0.11	29.7 ± 0.4	91.4 ± 1.6	37.4 ± 1.8	55.1 ± 0.3
L-Gln	22.3 ± 0.5	21.8 ± 1.5	19.9 ± 1.3	15.0 ± 0.2	12.4 ± 0.2	20.2 ± 0.2	21.1 ± 0.3	33.5 ± 0.7	36.3 ± 1.7	16.8 ± 1.0	18.6 ± 0.6
L-Pro	1.04 ± 0.08	0.21 ± 0.02	0.43 ± 0.04	0.20 ± 0.04	0.09 ± 0.01	0.08 ± 0.01	nd	1.03 ± 0.11	1.66 ± 0.27	0.80 ± 0.08	1.56 ± 0.21
Aad	0.11 ± 0.01	nd[d]	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gly	0.31 ± 0.04	0.18 ± 0.01	0.15 ± 0.03	0.12 ± 0.01	0.14 ± 0.01	0.19 ± 0.03	0.26 ± 0.04	0.90 ± 0.10	0.75 ± 0.08	0.24 ± 0.04	0.29 ± 0.04
L-Ala	2.01 ± 0.04	1.32 ± 0.10	1.42 ± 0.13	1.02 ± 0.06	1.02 ± 0.11	1.10 ± 0.13	0.94 ± 0.00	2.97 ± 0.14	2.59 ± 0.18	1.47 ± 0.21	5.31 ± 0.14
L-Cit	0.75 ± 0.06	0.87 ± 0.08	0.59 ± 0.06	0.41 ± 0.03	0.22 ± 0.03	0.67 ± 0.10	0.65 ± 0.07	0.41 ± 0.06	0.86 ± 0.07	0.14 ± 0.03	0.25 ± 0.04
L-Val	2.15 ± 0.10	0.84 ± 0.07	1.66 ± 0.16	1.01 ± 0.14	0.78 ± 0.06	1.21 ± 0.14	1.40 ± 0.11	1.83 ± 0.10	1.43 ± 0.13	1.34 ± 0.20	1.03 ± 0.11
L-Met	nd	nd	nd	nd	nd	nd	nd	0.49 ± 0.07	0.53 ± 0.03	0.41 ± 0.04	0.54 ± 0.04
L-Ile	1.41 ± 0.04	0.79 ± 0.04	0.96 ± 0.13	0.54 ± 0.07	0.42 ± 0.04	0.76 ± 0.07	0.98 ± 0.11	1.30 ± 0.06	1.25 ± 0.20	1.05 ± 0.01	1.10 ± 0.13
L-Leu	1.26 ± 0.04	0.50 ± 0.08	0.93 ± 0.14	0.45 ± 0.08	0.24 ± 0.04	0.34 ± 0.04	0.38 ± 0.06	1.06 ± 0.16	1.17 ± 0.23	0.90 ± 0.13	1.41 ± 0.08
L-Tyr	1.27 ± 0.04	0.29 ± 0.04	0.64 ± 0.06	0.30 ± 0.06	0.13 ± 0.03	0.18 ± 0.01	0.28 ± 0.03	0.74 ± 0.07	0.60 ± 0.07	0.40 ± 0.06	0.85 ± 0.08
L-Phe	1.54 ± 0.07	0.38 ± 0.04	0.83 ± 0.08	0.63 ± 0.07	0.44 ± 0.04	0.75 ± 0.07	1.12 ± 0.10	2.87 ± 0.37	2.59 ± 0.18	2.67 ± 0.17	3.17 ± 0.23
βAla	0.68 ± 0.08	0.19 ± 0.03	0.32 ± 0.07	0.23 ± 0.04	0.25 ± 0.04	0.19 ± 0.03	0.20 ± 0.04	0.92 ± 0.06	0.75 ± 0.06	0.33 ± 0.03	0.18 ± 0.03
4Abu	19.5 ± 0.5	6.8 ± 0.2	15.0 ± 0.8	15.2 ± 0.3	16.6 ± 0.6	16.9 ± 0.7	15.6 ± 0.8	50.8 ± 1.0	66.1 ± 1.2	46.8 ± 0.7	19.8 ± 0.5
$EtNH_2$[e]	0.40 ± 0.03	0.16 ± 0.03	0.15 ± 0.03	0.14 ± 0.03	0.13 ± 0.03	0.21 ± 0.03	0.07 ± 0.01	0.35 ± 0.06	0.20 ± 0.03	0.34 ± 0.08	0.39 ± 0.07
Hyl	0.73 ± 0.03	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.41 ± 0.06	0.41 ± 0.07	0.25 ± 0.06	0.24 ± 0.03
L-Lys	0.67 ± 0.10	0.35 ± 0.03	0.41 ± 0.06	0.20 ± 0.03	0.14 ± 0.03	0.17 ± 0.03	0.25 ± 0.03	0.70 ± 0.11	1.41 ± 0.14	0.05 ± 0.01	0.19 ± 0.03
MeHis	1.39 ± 0.17	0.28 ± 0.03	0.80 ± 0.07	0.32 ± 0.03	0.23 ± 0.04	0.13 ± 0.01	0.23 ± 0.01	0.30 ± 0.04	0.40 ± 0.07	0.04 ± 0.01	0.10 ± 0.01
L-His	0.35 ± 0.06	0.13 ± 0.03	0.22 ± 0.03	0.10 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.25 ± 0.03	0.52 ± 0.06	1.15 ± 0.14	nd	0.10 ± 0.03
L-Car[e]	5.69 ± 0.47	0.66 ± 0.11	0.69 ± 0.11	0.54 ± 0.07	0.76 ± 0.08	0.47 ± 0.07	0.60 ± 0.10	nd	2.15 ± 0.20	1.75 ± 0.11	1.27 ± 0.07
L-Arg	0.83 ± 0.08	0.44 ± 0.06	0.53 ± 0.08	0.31 ± 0.03	0.15 ± 0.03	0.39 ± 0.04	0.80 ± 0.07	0.47 ± 0.06	0.97 ± 0.11	0.08 ± 0.01	0.22 ± 0.03
total	84.6 ± 1.1	46.8 ± 1.5	60.3 ± 1.6	46.8 ± 0.5	41.4 ± 0.7	53.3 ± 0.8	61.0 ± 1.1	159 ± 2	251 ± 3	118 ± 2	124 ± 1

[a] Tomato variety: Doturakworld. [b] S1−S11 are expressed as the stages of tomatoes shown in **Table 1**. Values are average of duplicate determinations. [c] Amino acid abbreviations follow IUPAC standard. [d] nd, not detected. L-Cys and L-Trp were not determined in any sample. . [e] Amino acid derived N-containing compounds.

Shimadzu (Kyoto, Japan) UV mini model 1240 at 663 nm (chlorophyll *a*) and 642.5 nm (chlorophyll *b*). The concentrations of the two chlorophylls were calculated as described previously (*2*, *27*).

Isolation and Analysis of Lycopene and β-Carotene. Extraction of carotenoids was performed in the dark at a room temperature of 10 °C. The procedure described previously (*28*) for the extraction of carotenoids was modified as follows: Butylhydroxytoluene (0.05 g), $MgCO_3$ (0.1 g), a small amount of crystal sand, and acetone (50 mL) were added to the tomato samples, 0.5 g (stage 1) to 18.0 g (stage 11). The samples were then homogenized in a mortar. The mixture was filtered through a 3G3 glass filter, and the solid residue was washed three to four times with acetone (30 mL) until no more colored material was extracted. The extracts were combined and transferred to a decanting funnel containing diethyl ether. The ether solution was then partitioned with water. An equal volume of 20% KOH in methanol was added to the ether fraction, nitrogen gas was introduced into the headspace, and the saponification was completed overnight in a cold room at 5 °C. The unsaponifiable components were then washed out with water until the washings were at neutral pH, filtered through a bed of anhydrous Na_2SO_4, and evaporated to dryness in a rotary evaporator at 10 °C. The dried pigments were dissolved in dichloromethane (1 mL). The material was kept in the dark prior to analysis by HPLC.

HPLC was carried out on the above Agilent 1200 HPLC chromatograph, with the detector set at 470 nm, and an autosampler cooled to 4 °C (Agilent Technologies). A reversed-phase C18 column, particle size = 5 μM, packed with Eclipse XDB-C18, 250 × 4.6 mm i.d. (Agilent Technologies) and heated to 30 °C was used. Lycopene and β-carotene were eluted with acetonitrile/methanol/dichloromethane/*n*-hexane (50:40:5:5, v/v/v/v) at a flow rate of 1 mL/min. Peaks were compared with authentic β-carotene and lycopene. Peak areas were linear in the concentration range tested, from 30 to 500 ng.

Ammonia Precipitates. Potato (*29*) and tomato (*16*) glycoalkaloids are often isolated by treating a solution with ammonia and collecting the precipitate. Three uniform-sized whole fresh fruits from each stage were combined and chopped with a knife. After weighing, each sample, 1.0 g (stage 1) to 22.0 g (stage 11), was blended in a homogenizer with 2% acetic acid in methanol (100 mL). The resulting mixture was concentrated to 2−3 mL with the aid of a rotary evaporator. The concentrate was dissolved in 0.2 N HCl (40 mL) and centrifuged at 18000*g* for 5 min at 5 °C. The residue was rinsed twice with 0.2 N HCl (10 mL) and then centrifuged again. Concentrated NH_4OH (2 mL) was added to the combined supernatants to bring the solution to pH 10.5. This basic solution was placed in a 65 °C

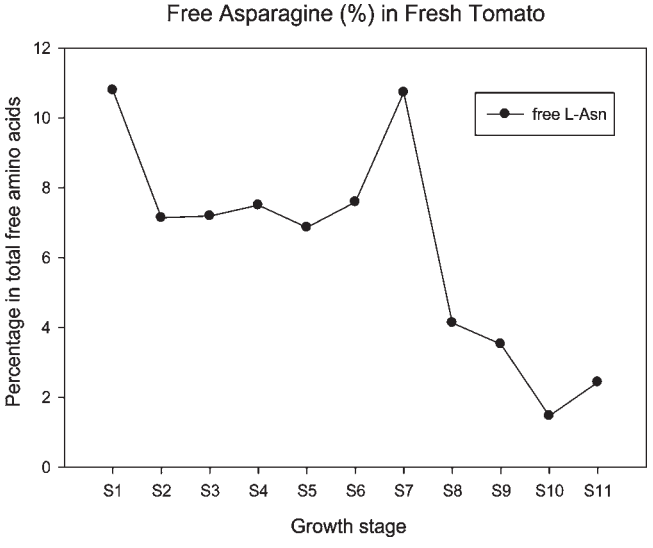

Figure 2. Percent of free L-asparagine relative to total free amino acids in tomato fruit as it grows.

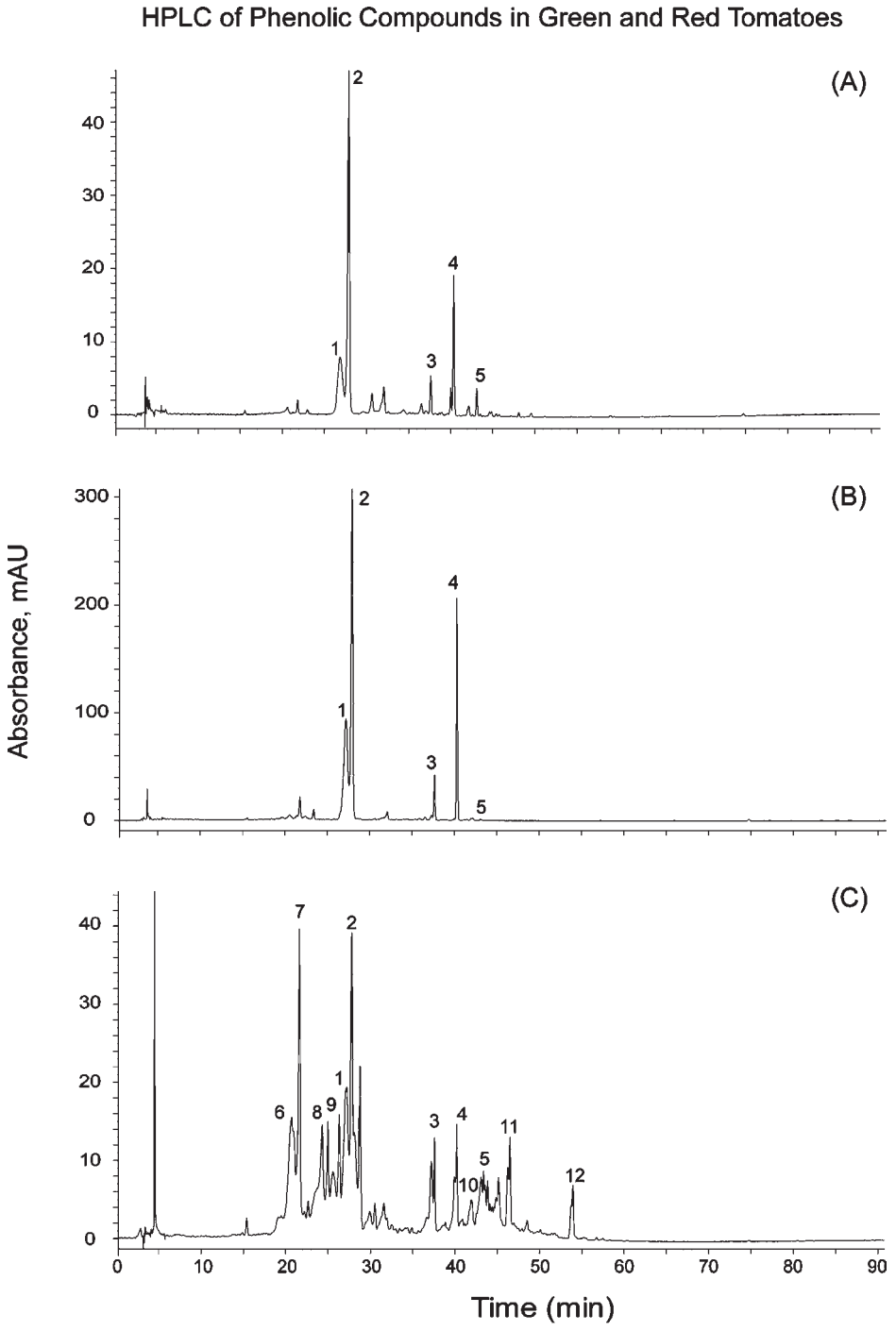

Figure 3. Separation of phenolic compounds (**Table 3**) in extracts of stage 1 (**A**), stage 5 (**B**), and stage 11 (**C**) of tomato fruit (**Table 1**) determined by LC-MS detected at 340 nm.

water bath for 50 min and then refrigerated overnight. The precipitate was collected after centrifugation at 18000*g* for 10 min at 5 °C and washed twice with 2% NH_4OH. The ammonia was dissipated, and the resulting pellet was dried at 30 °C under reduced pressure and then dissolved in 2% acetic acid in methanol (2 mL) and centrifuged at 18000*g* for 10 min at 5 °C. An aliquot of the supernatant (50 μL) was injected directly into the HPLC for α-tomatine/dehydrotomatine analysis. A second aliquot of the supernatant (1 mL) was dried and weighed. This dry fraction was used for MTT analysis described below. All extractions and precipitations were done in triplicate.

Analysis of Dehydrotomatine and α-Tomatine. HPLC was carried out on a Hitachi liquid chromatograph model 665-II equipped with a Shimadzu UV−vis detector (model SPD-10Avp) set to 208 nm. The column was an Inertsil ODS-3v, 5 μm, 4.0 × 50 mm HPLC column (GL Science Inc.), temperature controlled with a Shimadzu CTO-10Asvp thermometer. Chromatogram peak areas were integrated with a Hitachi D-2500 chromato-integrator. The mobile phase was acetonitrile and 20 mM KH_2PO_4 (24:76, v/v). The flow rate was 1.0 mL/min at a column temperature of 30 °C. Three separate analyses were carried out with each sample. Identifications of dehydrotomatine and α-tomatine are based on retention times on HPLC peaks of pure dehydrotomatine and α-tomatine compared to corresponding peaks from the tomato extracts. Quantification was accomplished with the aid of a Hitachi model D-2500 chromato-integrator by comparing the HPLC peak area from the sample to the peak area of known amounts of pure dehydrotomatine and α-tomatine isolated from tomato fruits. Plots of five concentrations of dehydrotomatine and of α-tomatine in the ranges from 2 to 20 μg and from 10 to 120 μg, respectively, against peak areas were linear.

Table 3. Phenolic Compounds Identified by LC-PDA, MS, and MS/MS in the Pulp Extracts from Immature Green to Full-Red Ripe Tomato Fruits

peak no. on HPLC	retention time[a] (min)	UV−vis (nm)	$[M-H]^-$ (*m*/*z*)	MS/MS fragments	identification
1	26.97 ± 0.10	326, 294, 248	353.3	191	3-caffeoylquinic acid (3-CQA)
2	27.92 ± 0.04	326, 294, 246	353.3	191.1	5-caffeoylquinic acid (5-CQA)
3	37.71 ± 0.12	354, 254.0	741.1	300.1	quercetin trisaccharide (QTS) (*39*)
4	40.39 ± 0.10	354, 314, 280	609.0	609.0, 301.1	quercetin-3-rutinoside (Q-3-R)
5	43.15 ± 0.10	346, 292, 266	593.5	593.5, 285.0	kaempferol−glucose−rhamnose (KGR)
6	20.64 ± 0.02	290,240	341.3	179.0, 135.0	caffeic acid−hexose isomer (I) (CHI (I))(*39*, *40*)
7	21.65 ± 0.03	310,290,240	341.3	179.0, 135.0	caffeic acid−hexose isomer (II) (CHI II)
8	24.32 ± 0.02	316, 294, 242	431.4	179.1, 161.1, 135.0	unidentified substance (UIS-1)
9	25.01 ± 0.02	316, 290, 242	431.4	179.1, 164.1, 135.0	unidentified substance (UIS-2)
10	42.04 ± 0.03	326, 294, 246	353.2	353.1,191.1,179.1, 173.1, 135.1	4-caffeoylquinic acid (4-CQA)
11	46.52 ± 0.01	326, 248	515.1	191.0, 179.1,173.1, 135.0	dicaffeoylquinic acid (di-CQA)
12	53.93 ± 0.02	326, 248	677.5	—	tricaffeoylquinic acid (tri-CQA)

[a] Average ± SD (*n* = 3).

MTT Assay for Growth Inhibition of Cells. The MTT assay that differentiates dead from living cells was adapted from the literature (*30*). The following reagents and instruments were used: MTT reagent, 5 mg/mL in phosphate-buffered saline, protected from light, and stored at 20 °C; MEM cell medium (containing 10% fetal bovine serum, 1% penicillin/streptomycin); microplate reader (Bio-Rad Co., Hercules, CA). Cell lines were seeded into a 96-well microplate (1−104 cells/well) and incubated for 24 h. Next, cells were treated with four concentrations (1, 10, 50, and 100 μg/mL ammonia precipitates) for 48 h. The MTT solution (0.1 mg/mL) was then added to each well. After 4 h of incubation at 37 °C, DMSO (200 μL) was added to each well. The absorbance (*A*) was then read at a wavelength of 540 nm. The decrease in absorbance in the assay measures the extent of decrease in the number of viable cells following exposure to the test substances calculated by using the following formula:

$$\%\ \text{inhibition of cells} = A_{\text{control}} - A_{\text{test substance}}/A_{\text{control}} \times 100$$

Statistical Analysis. Inhibitory concentrations at 50% (IC_{50}) values were calculated by constructing a four-parameter logistic curve using the values from the previous calculation, percent inhibition of cells, with the aid of SigmaPlot 11 (Systat Software, Inc., San Jose, CA). The resulting equation was solved for concentration at 50% cell inhibition. Statistical differences between samples were determined by ANOVA followed by Holm−Sidak tests using the SigmaPlot 11 software.

RESULTS AND DISCUSSION

Changes in the Composition of Tomatoes during Ripening. **Table 1** shows the changes in fruit size and weight during 11 stages (S1−S11) of growth and maturation of the tomato fruit. Stages S1−S7 refer to green and S8−S11 to red tomatoes harvested at the times indicated. Fruit size and weight is greatest at S8, just as the fruit is changing to red. The subsequent loss of weight may be related to the increasing transpiration in ripening fruits.

Free Amino Acids. In **Table 2**, we report the composition of free amino acids and two amino acid-derived nitrogen-containing compounds eluting from the column (ethylamine and L-carnitine) in tomato fruit during the stages of growth listed in **Table 1**. The total content of the combined free amino acids and other nitrogen-containing compounds (in mg/100 g of FW) for green tomatoes (S1−S7) ranged between 41.4 and 84.6. At the breaker stage (S8), the content increased to 159 mg/100 g of FW. It then peaked to 251 mg/100 g of FW at S9 before decreasing again in S10 and S11 to 118 and 124 mg/100 g of FW, respectively. Most of the individual amino acids also followed this pattern, peaking in content at S9. γ-Aminobutyric acid (4Abu) and L-Gln were the most abundant amino acids in green tomato. The content of L-Glu was low in green tomatoes and then increased rapidly from S7 to S11 to become the most abundant amino acid in the fully ripe tomato. This result agrees with a report that the increase of free amino acids, especially L-Glu, during ripening of tomatoes is the result of increased activities of peptidases, which catalyze the release of free amino acids from endogenous proteins (*31*). 4Abu is of interest because it has been shown to have an antihypertensive effect in humans (*32*). Tomatoes have previously been shown to contain significant amounts of this amino acid (*32*). In the present study, levels of this amino acid varied through the ripening process, with no apparent pattern.

L-Asn and L-Asp were also relatively abundant amino acids in the tomato samples. The L-Asn content is of interest because, as mentioned earlier, it is a precursor of potentially toxic acrylamide. However, except in the presence of other food, tomatoes alone do not produce significant amounts of acrylamide (*33*). Previous studies have shown that removal of free L-Asn can decrease the amount of acrylamide formed. The relative amount of L-Asn is also of interest because other free amino acids can compete with L-Asn for formation of browning products during food processing.

Figure 2 shows L-Asn content expressed as percent of total free amino acids. The illustrated trend shows that S1 tomato has relatively high L-Asn content. Levels then decrease in S2−S6 and rise again to a maximum at S7. Thereafter, the levels in S8−S11 decrease. This may be relevant to manufacturers of food products who may be processing tomatoes at the breaker stage S7−S8, which contains the highest levels of L-Asn.

There are few published studies on free amino acid content of tomatoes. Interest in free amino acid content of tomatoes is increasing because the amino acids affect tomato taste (*34*). Salts and conjugates of L-Glu are flavor enhancers. The glutamate content of tomato fruit was found to be unaffected by water stress, but increased 2-fold under salinity stress (*34*), improving their taste.

Phenolic Compounds. The HPLC, LC-MS, and UV−vis methods we previously used to determine the content of phenolic compounds in potatoes (*35a*) and sweet potatoes (*35b*) were adapted in the present study to the analysis of tomato phenolic compounds. **Figure 3** illustrates the HPLC separations of phenolic compounds in two green (S1 and S5) and one red tomato extract (S11). Structural identification of individual compounds in extracts was performed by associating the HPLC peak with the UV−vis spectrum and the corresponding parent ion and fragmentation patterns in mass spectra (**Table 3**) and by comparison of retention times for the same phenolic compounds reported by other investigators (*36*−*38*). Green tomato extracts at stages S1−S7 contained five phenolic compounds: 3-caffeoylquinic acid (3-CQA), 5-caffeoylquinic acid (5-CQA), quercetin trisaccharide (QTS), quercetin-3-rutinoside (Q-3-R), and kaempferol−glucose−rhamnose (KGR). The red tomato stages S8−S11 contained somewhat lower amounts of total phenolics, but a much larger variety of individual phenolic compounds, probably isomerized or derivatized 3- and 5-CQA.

On the basis of the studies by Moco et al. (*39*) and Mullen et al. (*40*), it is likely that the sugar in caffeic acid−hexoses I and II

Table 4. Concentration of Phenolic Compounds (See **Table 3**) in the Pulp Extracts from Immature Green to Full-Red Ripe Tomato Fruits[a]

	peak no. on HPLC[b]												
growth stage	1 3-CQA	2 5-CQA	3 QTS	4 Q-3-R	5 KGR	6 CHI (I)	7 CHI (II)	8 UIS-1	9 UIS-2	10 4-CQA	11 di-CQA	12 tri-CQA	total
S1	467.0 ± 17.8	1097.3 ± 55.3	81.3 ± 5.2^{1,2}	276.2 ± 17.3^{1}	49.6 ± 3.9	nd[c]	nd	nd	nd	nd	nd	nd	1971.4 ± 61.0
S2	202.2 ± 10.5^{1}	317.2 ± 15.5^{1}	40.7 ± 5.0^{3,4}	198.0 ± 15.4^{2,3}	13.2 ± 2.6^{1,2}	nd	nd	nd	nd	nd	nd	nd	771.3 ± 24.9^{1,2}
S3	226.7 ± 7.8^{1,2}	404.7 ± 14.0^{1}	37.3 ± 5.4^{3,4}	157.9 ± 8.3^{2}	7.9 ± 1.4^{1}	nd	nd	nd	nd	nd	nd	nd	834.4 ± 18.9^{1,3}
S4	250.9 ± 9.1^{2}	388.5 ± 11.5^{1}	26.1 ± 2.9^{3}	157.6 ± 12.9^{2}	2.6 ± 0.9^{1}	nd	nd	nd	nd	nd	nd	nd	825.7 ± 19.8^{1,3}
S5	364.8 ± 14.4^{3}	319.1 ± 14.0^{1}	22.1 ± 3.5^{3}	207.0 ± 11.7^{3}	tr[c]	nd	nd	nd	nd	nd	nd	nd	913.0 ± 23.5^{3}
S6	331.1 ± 8.9^{3}	399.6 ± 16.2^{1}	23.2 ± 1.9^{3}	300.9 ± 10.1^{1,4}	tr	nd	nd	nd	nd	nd	nd	nd	1054.7 ± 21.2
S7	368.6 ± 11.8^{3}	530.9 ± 8.4	84.0 ± 5.8^{1}	343.2 ± 12.2^{4}	nd	nd	nd	nd	nd	nd	nd	nd	1326.6 ± 19.8
S8	139.0 ± 14.2^{4}	148.9 ± 12.6^{2}	92.3 ± 6.8^{1}	36.1 ± 4.7^{5}	12.8 ± 3.4^{1,2}	106.4 ± 9.8	107.8 ± 12.4	74.4 ± 7.1	45.0 ± 5.7^{1}	113.8 ± 8.2	31.8 ± 2.9^{1}	19.5 ± 1.7^{1}	927.9 ± 29.2^{3}
S9	120.1 ± 7.6^{4}	147.1 ± 11.7^{2,3}	59.6 ± 5.4^{2,4}	33.0 ± 3.8^{5}	22.7 ± 3.7^{2}	72.7 ± 3.3^{1}	68.1 ± 2.5^{1}	40.2 ± 5.5^{1}	32.5 ± 6.6^{1,2}	22.7 ± 2.9^{1}	21.9 ± 4.6^{1}	21.9 ± 4.4^{1}	662.2 ± 19.7^{2}
S10	51.9 ± 2.3^{5}	59.3 ± 7.7^{2,3}	19.0 ± 2.1^{3}	13.2 ± 3.8^{5}	6.2 ± 1.8^{1}	68.4 ± 5.5^{1}	67.1 ± 3.2^{1}	45.1 ± 4.5^{1}	37.5 ± 3.6^{1,2}	18.4 ± 2.3^{1}	19.6 ± 2.0^{1}	12.5 ± 2.4^{1}	418.3 ± 13.2^{4}
S11	47.1 ± 3.8^{5}	53.9 ± 4.0^{3}	26.7 ± 5.4^{3}	21.3 ± 2.6^{5}	7.2 ± 1.9^{1}	58.4 ± 7.2^{1}	72.7 ± 5.3^{1}	35.6 ± 5.0^{1}	16.9 ± 3.2^{2}	12.5 ± 1.9^{1}	19.5 ± 4.3^{1}	13.4 ± 2.2^{1}	385.0 ± 14.5^{4}

[a] Tomato variety: Doturakworld. [b] Values (μg/100 g of FW) are expressed as chlorogenic acid equivalents. Values in the same column with the same superscript number are not statistically different for $p < 0.01$. [c] nd, not detected; tr, trace.

is glucose and that quercetin trisaccharide is quercetin−hexose−deoxyhexose−pentose (*39*). We were unable to assign structures to two isomeric compounds that were separated by HPLC (peaks 8 and 9) and that have identical molecular weights (431.4) and mass fragments. The fragments at ms/ms = 179.1 and 135.0 are specific for caffeic acids. No information about $m/z = 431.4$ was found in the tomato literature or in the mass spectra libraries.

Total phenolics in micrograms per 100 g of fresh weight (FW) (**Table 4**) were highest at stage 1 (1971), decreased by more than half in S2−S6, and then increased somewhat in S6−S7, the last fully green stage. As the tomato starts to turn to red (S8), total phenolic content decreases and the number of individual phenolics increases. There was a progressive decrease of total phenolics from S8 (928) to S11 (385). 5-CQA was found to be the most abundant phenolic compound in the tomato, followed by 3-CQA and Q-3-R. The gap between 5-CQA and the other phenolics narrowed as the tomato ripened, the 5-CQA content decreasing more rapidly than the other phenolics. Most of the changes in phenolics occur as the tomato begins to turn to red.

Chlorophylls a and b. **Table 5** shows that chlorophyll levels were high in S1 and S2, intermediate in S3−S7, and low in S8−S11. Most of the change in total chlorophyll during ripening was due to chlorophyll *a*. Changes in chlorophyll *b* throughout ripening were small. Levels in S1−S4 were equivalent ($P < 0.001$) as were levels in S5−S11 ($P < 0.001$). As mentioned earlier, because chlorophyll is reported to exhibit anticarcinogenic effects, it is likely that the green pigment contributes to the anticarcinogenic potential of green and to a lesser extent to red tomatoes. Relative anticarcinogenic effects of chlorophylls *a* and *b* merit study.

Lycopene and β-Carotene. Tomatoes harvested during stages S1−S7 of ripening did not contain detectable levels of β-carotene or lycopene (**Table 5**). The concentration of lycopene then increased 4-fold from S8 to S11. These large changes in lycopene content during maturation contrast with the observed minor changes for β-carotene. In contrast to lycopene, S8 contained the highest level of β-carotene, which was then found to decrease during ripening. These results agree with studies by Riggi et al. (*41*), who found that in contrast to lycopene levels, which increased progressively throughout the later ripening stages, the rate of accumulation of β-carotene was highest in the early stages of ripening, from mature green to yellow, and then leveled off in orange and red ripe tomatoes.

External factors may influence the biosynthesis of carotenoids during ripening of tomato fruit. Riggi et al. (*41*) found that water stress had a negative effect on lycopene accumulation during ripening, but no effect on β-carotene. The authors theorize that carotenoid levels could be influenced by involvement in the biosynthesis of water-stress phytohormones. Tomato variety was found to strongly influence the biosynthesis of carotenoids during ripening of tomatoes grown in the Czech Republic (*42*). For tomatoes grown in Tunisia, changes in lycopene content also varied among tomato varieties (*43*). The lycopene content of tomato skin decreased significantly in overripe tomatoes. For tomatoes grown in Germany, exposure to UV-B radiation before harvest resulted in increased lycopene and β-carotene contents (*44*).

Glycoalkaloid Content. **Table 6** shows the dehydrotomatine and α-tomatine contents of the tomatoes reported in both milligrams per 100 g of FW and in milligrams per unit fruit. As we previously reported (*14*), the range of tomatine narrows when examined on a per fruit basis. The levels per unit of fruit tend to increase with maturity until S8 is reached, when the tomato begins to ripen and levels fall to near zero. When measured on a weight basis, levels start out high (409 and 166 mg/100 g of FW for S1 and S2, respectively) and then decrease to near 20 mg/100 g for S3−S7 and to a nondetectable level for S8−S11.

Table 5. Concentrations of Lycopene, β-Carotene, and Chlorophylls *a* and *b* in Tomatoes during Different Stages of Growth[a]

growth stage[b]	lycopene	β-carotene	total carotenoids	ratio of lycopene/carotenoids	Chl *a*	Chl *b*	total Chl
S1	nd	nd	nd		4.40 ± 0.86^{1}	1.33 ± 0.37^{1}	5.73
S2	nd	nd	nd		4.74 ± 0.32^{1}	$1.23 \pm 0.07^{1,2}$	5.97
S3	nd	nd	nd		$2.76 \pm 0.12^{2,3}$	$0.90 \pm 0.14^{1,2,3,4,5}$	3.66
S4	nd	nd	nd		3.06 ± 0.01^{2}	$1.11 \pm 0.20^{1,2,3,4}$	4.17
S5	nd	nd	nd		1.94 ± 0.33^{4}	$0.54 \pm 0.06^{3,4,5}$	2.48
S6	nd	nd	nd		$2.02 \pm 0.26^{3,4}$	$0.74 \pm 0.44^{2,3,4,5}$	2.76
S7	nd	nd	nd		$2.20 \pm 0.07^{3,4}$	$0.42 \pm 0.08^{4,5}$	2.62
S8	0.32 ± 0.02^{1}	0.79 ± 0.05	1.11	0.40	1.0 ± 0.06^{5}	$0.67 \pm 0.09^{2,3,4,5}$	1.68
S9	0.43 ± 0.03^{1}	0.50 ± 0.03^{1}	0.93	0.87	0.82 ± 0.06^{5}	$0.65 \pm 0.051^{2,3,4,5}$	1.47
S10	0.90 ± 0.05	0.41 ± 0.04^{1}	1.31	2.18	0.41 ± 0.02^{5}	$0.52 \pm 0.05^{4,5}$	0.94
S11	1.27 ± 0.08	0.42 ± 0.03^{1}	1.69	3.06	0.43 ± 0.02^{5}	$0.71 \pm 0.07^{2,3,4,5}$	1.14

[a] Values are average mg/100 g ± SD ($n = 3$). Values with shared superscript numbers in columns are significantly the same, $p < 0.01$. [b] S1−S11 are the stages of harvested tomatoes shown in **Table 1** for the Doturakworld tomato variety.

Table 6. Dehydrotomatine and α-Tomatine Contents of Tomatoes Listed in **Table 1**[a]

	mg/fruit			mg/100 g of FW			% in ammonia precipitate	
growth stage	dehydrotomatine (A)	α-tomatine (B)	sum (B + A)[b]	dehydrotomatine (A)	α-tomatine (B)	sum (B + A)[b]	dehydrotomatine	α-tomatine
S1	0.3 ± 0.0^{1}	1.9 ± 0.1^{1}	2.1 ± 0.1^{1} (6.3)	48.2 ± 3.0	361 ± 25.5	409 ± 25.7 (7.5)	10.0	74.9
S2	0.2 ± 0.0^{1}	1.6 ± 0.1^{1}	1.7 ± 0.1^{1} (8.0)	11.1 ± 0.1	155 ± 13.4	166 ± 13.4 (14.0)	2.1	29.2
S3	0.1 ± 0.0^{1}	1.7 ± 0.3^{1}	1.8 ± 0.3^{1} (17.0)	2.8 ± 0.2^{1}	31.7 ± 5.1^{1}	34.5 ± 5.1^{1} (11.3)	0.8	9.5
S4	0.4 ± 0.0^{1}	3.2 ± 0.1	3.6 ± 0.1 (8.0)	2.8 ± 0.1^{1}	22.5 ± 1.8^{1}	25.3 ± 1.8^{1} (8.0)	2.9	23.6
S5	1.4 ± 0.1^{2}	10.7 ± 0.7	12.1 ± 0.7 (7.0)	2.7 ± 0.1^{1}	20.5 ± 1.4^{1}	23.2 ± 1.4^{1} (7.6)	3.8	28.9
S6	1.2 ± 0.1^{2}	11.9 ± 0.7	13.1 ± 0.7 (9.9)	1.4 ± 0.1^{1}	14.8 ± 0.8^{1}	16.2 ± 0.8^{1} (10.6)	3.4	35.5
S7	2.3 ± 0.3	21.5 ± 0.3	23.8 ± 0.4 (9.3)	1.5 ± 0.2^{1}	13.8 ± 1.0^{1}	15.3 ± 1.0^{1} (9.2)	5.7	52.0
S8	nd[c]	tr[c]	tr	nd	tr	tr	nd	nd
S9−S11	nd	nd	nd	nd	nd	nd	nd	nd

[a] Average ± SD ($n = 3$). Values with shared superscript numbers in columns are significantly the same, $p < 0.01$. [b] B/A ratios are shown in parentheses. [c] nd, not detected; tr, trace.

Inhibition of Cancer Cells. Normal liver (Chang) and lung (Hel299) cells and three cancer cell lines (U937 lymphoma, A549 lung, HeLa cervical) were treated with four concentrations (1, 10, 50, and 100 μg/mL) of the glycoalkaloid-containing precipitates isolated from seven green tomatoes listed in **Table 1** (**Table 7**). The normal lung Hel299, lung cancer A549, and cervical cancer HeLa cell lines were highly susceptible to inhibition at all of the concentrations tested. Because IC_{50} values were below the concentrations tested, it was impossible to statistically correlate rates of inhibition with any other tomato attribute. The normal liver Chang and the lymphoma U937 cell lines were inhibited to a much lesser extent than the other cells.

Significance for Plant Physiology and the Diet. The results of the present study indicate that significant changes in composition of free amino acids, chlorophylls, carotenoids, phenolics, and glycoalkaloids occur in the tomato as it ripens. The small, immature tomatoes have particularly high levels of phenolics and glycoalkaloids, in part because of their high solids content. The high content of these compounds would contribute to making the fruit unpalatable, perhaps giving the seeds time to mature. Some of the most significant changes in composition occur at the breaker stage, as the tomato just begins to turn from green to red. At this stage, free amino acid levels reach a maximum, chlorophyll content decreases significantly, carotenoids start to appear, phenolic compounds isomerize and start to decrease, and glycoalkaloids are nearly completely degraded. The observed changes suggest that it may be possible to select tomato fruit with maximum content of beneficial components.

Glycoalkaloid-rich extracts of the green tomatoes inhibited several cell lines, including lung cancer (A549) and cervical carcinoma (HeLa) cells. Although the extracts merit study for their ability to ameliorate human cancers, they also inhibited normal lung (Hel299) cells. They were less active against histiocytic lymphoma (U937) cells and normal liver (Chang) cells. These observations and the oral feeding studies mentioned in the Introduction suggest that although tomatine appears to be cytotoxic when exposed directly to cells, it may not be toxic to whole organisms when consumed orally. In this context, it is important to note that widely consumed red tomatoes grown in the mountains of the Andes contain high levels of tomatine (*23*, *45*). Moreover, it may be possible to target delivery of tomatine to tumors without adversely affecting normal cells, as was reported for the cancer drug doxorubicin (*46*, *47*). Previously, we found that the cancer drug doxorubicin was less effective than tomatine in inhibiting cancer cells (*19*).

These considerations suggest a potential benefit to developing high-tomatine red tomatoes by suppressing the genes in the tomato

Table 7. Inhibitory Effect by Seven Green Tomato Extracts against Normal Liver Cells (Chang), Normal Lung Cells (Hel299), Histiocytic Lymphoma Cells (U937), Lung Cancer Cells (A549), and Cervical Carcinoma Cells (HeLa) Determined by the MTT Assay

	IC_{50} (μg/mL)				
growth stage	liver cell (Chang)	lung cell (Hel299)	histiocytic lymphoma cell (U937)	lung cancer cell (A549)	cervical carcinoma cell (HeLa)
S1	2.7	<1[a]	1.7	<1	<1
S2	3.2	<1	1.2	<1	<1
S3	1.0	<1	2.2	<1	<1
S4	5.0	<1	3.4	<1	<1
S5	3.4	<1	4.3	<1	<1
S6	14.4	<1	<1	<1	<1
S7	6.4	<1	<1	<1	<1

[a] Because the inhibition at all four concentrations was above 50% and was not concentration-dependent, we could not calculate exact IC_{50} values and can only estimate the values to be <1 μg/mL.

plant that govern the synthesis of enzymes that degrade tomatine during postharvest ripening of tomatoes (*48*). On the basis of our earlier studies on the occurrence and inheritance of tomatine in wild potato cultivars (*49*, *50*), it may also be possible to create health-promoting high-tomatine potatoes. Because tomatine is also reported to inhibit viruses (*51*, *52*), high-tomatine red tomatoes may increase resistance of tomato plants against the yellow curl virus, the cause of the most devastating plant disease in the world (*53*). In conclusion, assessment of the variation of micronutrient and secondary metabolite contents of tomato fruit during ripening allows selection of tomatoes with maximum content of health-promoting constituents.

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