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Original Research Article

Protein, free amino acid, phenolic, β-carotene, and lycopene content, and antioxidative and cancer cell inhibitory effects of 12 greenhouse-grown commercial cherry tomato varieties

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ABSTRACT

The content of water, free amino acids, amino acid metabolites, crude protein, the carotene pigments β-carotene and lycopene, and 9 characterized and 2 incompletely characterized individual phenolic (flavonoid) compounds of 12 greenhouse-grown cherry tomato varieties of various colors (green, yellow, orange, red, and black) was determined using HPLC and LC/MS methods. The phenolic content of the cherry tomatoes per unit weight is 3-4 times greater than reported values for large-sized tomatoes. Antioxidative effects using the ABTS and FRAP assays and cancer-cell-inhibiting effects against 2 normal (Chang liver and Hel299 lung) and 3 human cancer (lung A549; liver HepG2; and cervical HeLa) cell lines using the MTT cell viability assay were also determined. Lycopene inhibited all the cell lines, but showed strong activity against the cervical carcinoma and the lung cancer cells. The tomato extracts showed inhibition at the higher doses. The HeLa cervical carcinoma cell line was most inhibited by the pure compounds, and the HeLa or the HepG2 cells lines were the most inhibited by the tomato extracts. The results demonstrate wide-ranging differences as well as similarities in the content of nutritional and bioactive compounds in cherry tomatoes, and suggest that such knowledge can benefit consumers.

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1. Introduction

Tomatoes, the fruit of the species *Solanum lycopersicum*, are a good source of nutrients and bioactive compounds (Friedman, 2002, 2013; Frusciante et al., 2007). The nature and concentration of synthesized compounds are influenced by agricultural practices, environmental factors, variety, and ripeness (Davies and Hobson, 1981; Sánchez-Rodríguez et al., 2012). There is a large variation between cultivars with respect to both fruit size and color, which could affect their beneficial properties. Lenucci et al. found significant differences among 14 cherry tomato cultivars and four cultivars of high-pigment tomato hybrids in the composition of lycopene, β-carotene, α-tocopherol, vitamin C, and total phenolic and flavonoids contents, with the high-pigmented cultivars showing a very high lycopene content, suggesting high variability in the tomato germplasm (Lenucci et al., 2006). Breeding programs have had some success in transferring antioxidant parameters from wild-type species into commercial cultivars (Kavitha et al., 2014). Tomato grafting has shown to both increase and decrease the content of phenolic compounds (Sánchez-Rodríguez et al., 2012).

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These observations suggest the need for a better understanding of how bioactivity of tomatoes relates to their composition.

To help meet this need, the present study aims to increase our understanding of the distribution of free amino acids, proteins, the pigments β-carotene and lycopene, and phenolic compounds, as well as antioxidative and cancer-cell-inhibiting effects, among a diverse collection of cherry tomato varieties. Because composition can vary significantly under different environmental conditions (Mitchell et al., 2007; Sánchez-Rodríguez et al., 2012), the tomatoes in this study were grown under the same conditions. We measured composition using HPLC and LC/MS methods, antioxidative activities by the ABTS and FRAP assays, and effects on the growth of normal and cancer cells by the MTT assay.

2. Materials and methods

2.1. Materials

Quercetin-3-rutinoside (Q3R, lot BCBB6172, 95.3%) and chlorogenic acid (3-caffeoylquinic acid, 3-CQA, lot 27H1006, 95%) were obtained from Sigma (St. Louis, MO, USA), lycopene (lot 081M5160V, ≥90%, from tomatoes), β-carotene, purum (lot BCBH8425V, ≥97%) and naringenin (NG, lot BCBC7827V, 95%) from Aldrich (Milwaukee, WI, USA), naringenin chalcone (NGC, lot ASB-00014207-136, 98%) from ChromaDex Inc. (Laguna Hills, CA, USA), ABTS (>98%) from Tokyo Kasei Kogyo (Japan), gallic acid (lot AGM01, ≥98%) and tripyridyltriazine (TPTZ, lot FHL01, 98%) from Tokyo Chemical Industry (Japan). All other reagents were from Sigma.

Human normal liver (Chang) and lung cell lines (Hel299), lung cancer (A549), hepatoma (HepG2), and cervical carcinoma (HeLa) cells were from the American Type Culture Collection (ATCC, Rockville, MD, USA) and the Korean Cell Line Bank (KCLB, Seoul, Korea). RAW264.7 and L929 cells used for the TNF-α studies were obtained from Riken BRC Cell Bank (Tsukuba, Japan). Cell culture reagents were obtained from Gibco BRL (Life Technologies, Cergy-Pontoise, France). Dulbecco's modified Eagle's medium (DMEM), actinomycin D, lipopolysaccharide (LPS) from *E. coli* O127, and murine recombinant Tumor Necrosis Factor-α (TNF-α) were obtained from Wako Pure Chemical Industries (Osaka, Japan), Eagle's minimum essential medium (MEM) from Nissui Pharmaceutical (Tokyo, Japan), and FBS and RPMI 1640 medium from Gibco BRL (Grand Island, NY, USA).

HPLC-grade acetonitrile and formic acid were from J.T. Baker (Phillipsburg, NJ, USA) and Aldrich (Milwaukee, WI, USA), respectively. The solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and degassed in an ultrasonic bath before use.

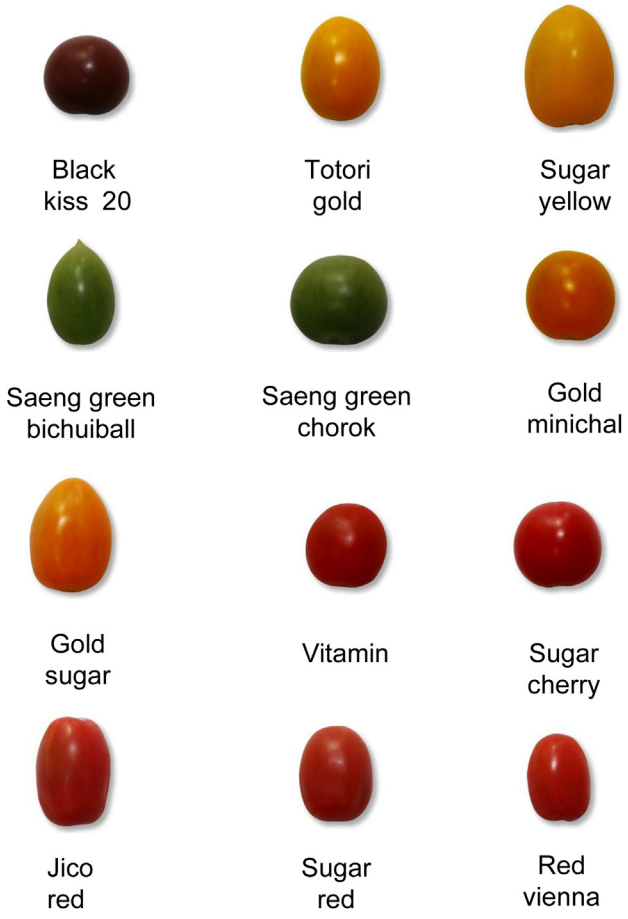

Fig. 1. Photograph of 12 cherry tomatoes evaluated in the present study.

2.2. Sample preparation

2.2.1. Sampling of tomato fruits

Twelve commercial varieties of Korean cherry tomato fruits were used in this experiment. Tomato seeds were planted on January 15, 2012 in a greenhouse of the Buyeo Tomato Experiment Station, Chung-Nam, Korea and harvested on May 2, 2012 at their optimum maturity stage for market (Fig. 1). Temperatures in the greenhouse were set from 25 to 28 °C during the day and from 13 to 18 °C during the night. Fruits were collected, weighed, and measured for size as shown in Table 1. Each tomato sample consisted of randomly selected 20 uniform-sized fresh fruits. After removal of the calyx, the fruits were cut into 4–5-mm thick slices.

Table 1
Dimensions, weight, and water and protein ($N \times 6.25$) content of 12 greenhouse-grown cherry tomato fruits.[a]

Variety (cultivar)	Color	Length (mm)	Width (mm)	Weight (g/fruit)	H_2O (%)	Protein (g/100 g dry wt)	Protein (g/100 g fresh wt)
Black Kiss 20	Red black	30.09 ± 0.46^{1}	32.89 ± 0.69^{1}	18.61 ± 0.91^{1}	$90.45 \pm 0.13^{1,2,3}$	$11.92 \pm 0.36^{1,2,3,4}$	$1.14 \pm 0.04^{1,2,3}$
Totori Gold	Yellow	37.83 ± 0.71^{2}	$28.81 \pm 0.91^{2,3}$	17.1 ± 1.3^{1}	88.62 ± 0.20^{4}	8.27 ± 0.55^{5}	$0.94 \pm 0.06^{1,3}$
Sugar Yellow	Yellow	44.0 ± 1.6^{3}	32.22 ± 0.43^{1}	$24.94 \pm 0.50^{2,3}$	$90.41 \pm 0.55^{1,2,3}$	14.2 ± 3.0^{1}	$1.07 \pm 0.29^{1,2,3}$
Saeng Green Bichuiball	Green	40.9 ± 1.7^{4}	$26.80 \pm 0.69^{2,4}$	17.36 ± 0.27^{1}	$90.81 \pm 0.18^{1,3}$	$13.18 \pm 0.47^{1,2}$	$1.21 \pm 0.04^{1,2}$
Saeng Green Chorok	Green	$34.21 \pm 0.24^{5,6}$	37.0 ± 1.9	27.22 ± 0.18^{2}	$90.01 \pm 0.10^{1,2,5}$	$12.87 \pm 0.48^{1,2,3}$	1.29 ± 0.05^{2}
Gold Minichal	Orange	$31.91 \pm 0.39^{1,5,7}$	32.24 ± 0.34^{1}	18.59 ± 0.08^{1}	$90.19 \pm 0.04^{1,2,3}$	$10.34 \pm 0.24^{2,3,4,5}$	$1.01 \pm 0.02^{1,2,3}$
Gold Sugar	Yellow	$42.15 \pm 0.89^{3,4}$	32.1 ± 1.0^{1}	$22.92 \pm 0.78^{3,4}$	$90.99 \pm 0.30^{1,3}$	$10.14 \pm 0.25^{3,4,5}$	$0.91 \pm 0.02^{3,4}$
Vitamin	Red	30.45 ± 0.39^{1}	29.26 ± 0.10^{3}	14.29 ± 0.28^{5}	$90.26 \pm 0.32^{1,2,3}$	$9.68 \pm 0.36^{4,5}$	$0.94 \pm 0.04^{1,3}$
Sugar Cherry	Red	30.9 ± 1.1^{1}	31.87 ± 0.89^{1}	18.7 ± 1.3^{1}	$89.73 \pm 0.22^{2,5}$	$9.82 \pm 0.19^{4,5}$	$1.01 \pm 0.02^{1,2,3}$
Jico Red	Red	$42.16 \pm 0.85^{3,4}$	$28.91 \pm 0.19^{3,4}$	21.4 ± 1.0^{4}	$89.02 \pm 0.28^{4,5}$	$9.80 \pm 0.06^{4,5}$	$1.08 \pm 0.01^{1,2,3}$
Sugar Red	Red	$36.3 \pm 1.5^{2,6,8}$	$28.50 \pm 0.85^{3,4}$	17.0 ± 1.1^{1}	91.16 ± 0.67^{3}	$10.40 \pm 0.28^{2,3,4,5}$	$0.92 \pm 0.02^{3,4}$
Red Vienna	Red	$33.7 \pm 1.1^{7,8}$	24.38 ± 0.57	12.7 ± 1.3^{5}	88.55 ± 0.54^{4}	$9.67 \pm 0.35^{4,5}$	$1.11 \pm 0.041^{2,4}$

Values within columns sharing a common superscript number are not significantly different ($p < 0.05$).
[a] Length, width, weight and protein value are average ± SD ($n = 3$).

The slices, including the seeds and gelatinous fluid, were then immersed in liquid nitrogen to fully freeze, then lyophilized (model PVTFD 10R, Ilsinbiobase Co., Ltd., Korea). Water content was determined by weighing the sample before and after freeze-drying.

The dried samples were ground briefly in a mortar and then in a Wiley mill to pass a 20-mesh screen. Because dried tomatoes are very hydroscopic, to avoid possible effects of moisture, light, temperature, and oxygen the samples were hermetically sealed in a desiccator containing P_2O_5 and stored in a dark freezer at −25 °C. The desiccator was brought to room temperature before removal of the sample.

2.2.2. Extraction of amino acids and phenolic compounds from cherry tomatoes

A sample of each powder (44.9–75.4 mg, depending on availability) was placed into a 25 mL volumetric flask and brought up to volume with 80% methanol in water. The flask was then placed into an ultrasonic bath for 60 min at 30 °C. The sample was centrifuged at 18,000 × *g* for 10 min at 1 °C. The supernatant was then passed through a 0.45 μm nylon filter (Millipore, Bedford, MA, USA). Spiking experiments showed that between 94.9–98.4% of phenolics were recovered. The filtrate was used for HPLC analysis of amino acids and phenolic compounds as well as for assaying antioxidative and cell-inhibiting effects.

2.2.3. Extraction of carotenoids

The extraction of carotenoids from tomatoes was adapted from the method of Nagata and Yamashita (Nagata and Yamashita, 1992). Each powder (44.9–75.4 mg, depending on availability) was placed into a 5 mL glass vial to which was added acetone–hexane solution (2 mL, 4:6, v/v). The vial was then placed into an ultrasonic bath for 60 min at 30 °C. The solution was centrifuged at 18,000 × *g* for 10 min at 1 °C under darkness. The pellet was similarly re-extracted. The combined filtrates were dried under reduced pressure. The residue was dissolved in the acetone-hexane solution (2 mL). The extract was kept in the dark prior to analysis by HPLC. Spiking experiments showed recovery of 91.3 and 95.6% of lycopene and β-carotene, respectively. Before testing for biologic activity, the extracts were solubilized in DMSO, then added to the aqueous systems. Although these precautions were taken to help keep the samples in solution, it is possible that the reaction system may not be homogeneous.

2.3. Tomato component analysis

2.3.1. Crude protein content

The total N content of each freeze-dried tomato powder (~1.5 g) was determined in duplicate by a Kjeltec 2300 Analyzer Unit (Foss, Sweden) according to the manufacturer's instructions. Protein content/100 g of dry wt. = $N \times 6.25$.

2.3.2. Amino acids and amino acid metabolites by HPLC

We determined the free amino acid and metabolite content of the cherry tomatoes in a single run. The analysis was carried out using an ion exchange chromatography method described previously (Choi et al., 2010, 2011a). Briefly, an aliquot (10 μL) of the filtrate obtained from the above extraction was injected into a Hitachi model L-8800 amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan) with a column packed with Hitachi custom ion-exchange resin 2622 (4.6 i.d. × 60 mm, particle size = 5 μm). Lithium citrate buffer and ninhydrin flow rates for post column reaction were 0.35 and 0.30 mL/min, respectively. The column temperature was 30–70 °C and the reaction coil temperature, 135 °C.

2.3.3. Content of phenolic compounds by HPLC

HPLC was carried out on a Shimadzu Prominance LC-20A (Shimadzu, Kyoto, Japan), which consisted of a CMA-20A controller, a DGU-20A3 degasser, two LC-20AD solvent delivery modules, an SIL-20AC autosampler, a CTO-20A column oven, and an SPD-M20A photodiode array (PDA) detector. The flow rate was 0.8 mL/min at 30 °C. Peaks were monitored at 190–400 nm and calibration was performed at 340 nm. An aliquot (20 μL) was injected directly into an Inertsil ODS-3V (5 μm, 4.6 mm × 250 mm) HPLC column (GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of the following gradient modes: 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). Each sample was extracted three times and analyzed in triplicate.

2.3.4. Identification of phenolic compounds by liquid chromatography-mass-spectrometry (LC-MS)

LC was carried out on an Agilent Technologies (Santa Clara, CA, USA) 1200 series binary LC system with a photodiode array detector monitored at 190–400 nm. The LC was coupled with a 3200 Q Trap LC-MS/MS system (Applied Biosystems Inc., Foster City, CA, USA). An aliquot (20 μL) of the extract was directly injected into an Inertsil ODS-3V (5 μm, 4.6–250 mm) HPLC column (GL Sciences Inc., Torrance, CA, USA). The mobile phase, column temperature, and flow rate were the same as those of the above-described HPLC system. 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 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 the test samples were compared with peak areas of known amounts of standard phenolic compounds 3-caffeoylquinic acid, quercetine-3-rutinoside, naringenin chalcone, and naringenin.

2.3.5. Analysis of lycopene and β-carotene by HPLC

The HPLC system used was a Shimadzu HPLC (Kyoto, Japan), consisting of a LC-10AD_{VP} pump, a SIL-10ADvp auto sampler, a CTO-10ASvp column oven, a SPDM 10Avp photodiode array (PDA) detector and a CBM-20A system controller. Data collection and integration were accomplished using a Shimadzu LC solution. The detector was set at 470 nm and an autosampler cooled to 4 °C. A reversed-phase C_{18} column, particle size (5 μM), packed with an Inertsil ODS-3V, 250 mm × 4.6 mm i.d. (GL Sciences Inc.) 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 peaks from β-carotene and lycopene.

2.4. Bioactivities

The supernatant (500–1000 μL) obtained from the above extraction was placed in a 10 mL vial and dried at 30 °C under reduced pressure. Each residue was weighed and then dissolved in DMSO (f.c. 100 mg/mL) and then diluted to a concentration of 1.25–10 μg/mL with phosphate buffer (0.1 M, pH 7.4). This diluted sample was used for antioxidant assays.

The inhibition of the ABTS and FRAP free radical was calculated using the following equation: ABTS and FRAP scavenging effect (%) = $(1 - A\ (\text{sample})/A\ (\text{blank}) \times 100)$, where

A (blank) is the absorbance of the control reaction (containing all reagents except the test sample) and A (sample) is the absorbance of the test sample. The antioxidant activity was expressed as the IC_{50}, defined as the concentration of sample that inhibited formation of ABTS and FRAP radicals by 50%. Details for each assay follow.

2.4.1. Antioxidative activity by scavenging of the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging activity was determined according to the method of Re et al. (1999). $ABTS^{\bullet+}$ cation radical was produced by the reaction between ABTS (7 mM) in H_2O and potassium persulfate (2.45 mM), stored in the dark at room temperature for 24 h. Before usage, the $ABTS^{\bullet+}$ solution was diluted with phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 ± 0.02 at 732 nm. The $ABTS^{\bullet+}$ solution (990 μL) was added to the sample (10 μL). After 1 min, the percentage inhibition at 732 nm was calculated for each concentration relative to a blank absorbance. The antioxidant potential of the sample, in terms of μg/mL, was determined from a standard curve plotted using Trolox.

2.4.2. Antioxidative activity by the ferric reducing/antioxidant power (FRAP) assay

The reduction by antioxidants in the sample of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}) was determined by the method of Benzie and Strain (Benzie and Strain, 1996). The FRAP reagent was freshly prepared by mixing of acetate buffer (100 mL, 300 mM, pH 3.6), TPTZ solution (10 mL, 10 mM TPTZ in 40 mM/HCl), $FeCl_3 \cdot 6H_2O$ (10 mL, 20 nM) in a ratio of 10:1:1 with distilled water (12 mL) at 37 °C. The FRAP reagent (1.8 mL), deionized water (180 μL) and the sample (60 μL) were then added to a test tube, which was incubated at 37 °C for 4 min. The absorbance was measured at 593 nm using FRAP working solution as a blank. The sample was diluted if necessary to bring the absorbance reading in the range between 0 and 2.0. The antioxidant potential of the sample in terms of mmol Fe^{2+}/mg, was determined from a standard curve plotted using the $FeSO_4 \cdot 7H_2O$.

2.4.3. MTT assay for growth inhibition of cells

The MTT assay that differentiates dead from living cells was adapted from the literature (Alley et al., 1988). The cell lines chosen for this study are the same as those in cited previous publications from this laboratory. The cells were maintained in α-MEM or RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 incubator. The β-carotene and lycopene were dissolved in DMSO (100 mg/mL), while the other samples were dissolved in DMSO (10%, 10 mg/mL). Samples were stored at 4 °C. Cells were seeded into a 96-well plate at a density of 1×10^4 cells/well and incubated for 24 h. Next, cells were treated with three concentrations (10, 50, 100 μg/mL) of pure compounds and five concentrations (10, 50, 100, 200, and 300 μg/mL) of tomato extract samples 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 (100 μL) was added to each well. The absorbance (A) was then read at 540 nm using a microplate reader (Bio-Rad Co., Hercules, CA, USA). The decrease in A measures the decrease in the number of viable cells calculated by using the following formula:

$$\%\,\text{inhibition of cells} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100.$$

2.5. 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, percentage inhibition of cells, with the aid of SigmaPlot 11 (Systat Software, Inc., San Jose, CA, USA). 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 Sigma Plot 11 software.

Statistical differences were determined by analysis of variation (ANOVA) followed by Holm–Sidak post hoc tests, and correlations by the Pearson correlation constant, using the Sigma Plot 11 software (Systat, Chicago, IL, USA). Statistical significance is defined as $p < 0.05$.

3. Results and discussion

3.1. Cherry tomato descriptive and content analysis

3.1.1. Colors, dimensions, water, and protein content of 12 cherry tomato fruits

Fig. 1 shows photographs of 12 greenhouse-grown cherry tomato cultivars evaluated in this study and Table 1 lists the colors, length and width dimensions, weight, moisture, and protein content of these tomatoes. The chosen tomatoes represent a full range of the colors and sizes found in commercial tomatoes, including fully ripe green, orange, yellow, red, and black colored tomatoes. The data show that: (a) the weight of fruit ranged from 12.7 g (Red Vienna) to 27.22 g (Saeng Green Chorok) or a 2.1-fold variation from lowest to highest weights; (b) the range of moisture content was narrow, from 88.55% (Red Vienna) to 91.16% (Sugar Red); and (c) the 'crude' protein content, which consists of free and protein-bound amino acids, ranged from 8.27 (Totori Gold) to 14.2 (Sugar Yellow) g/100 g dry wt. On a fresh basis, the protein content ranged from 0.91 (Gold Sugar) to 1.29 g/100 g (Saeng Green Chorok), a smaller variation than protein on a dry basis.

3.1.2. Free amino acids and N-containing secondary metabolites

Free amino acids from plants can have a dual role in the diet. As with any protein, they represent a source of nitrogen and of nutritionally essential amino acids. They are notable in that they quickly interact with the palate, greatly affecting flavor. They are also more bioavailable after consumption than protein-bound amino acids and can react with other components of the diet. For example, free amino acids can react with free sugars during heating to produce browning products. One such browning product, acrylamide, is potentially toxic (Friedman and Levin, 2008).

During ripening, tomato pepsidases hydrolyze a significant amount of protein into free amino acids (Sorrequieta et al., 2010). In our previous work (Choi et al., 2010), we found that the free amino acid content increased rapidly during the breaker stage and was highest when the tomato was about one-half red. Activities of amino acid-metabolizing enzymes during ripening may lead to changes in amino acid profile (Sorrequieta et al., 2010). In tomatoes, it appears that free amino acids are a major contributor to protein nutrition.

Table 2 reports the free amino acid and amino acid metabolite content of the cherry tomato samples in the order of HPLC elution position. Saeng Green Chorok, followed by Saeng Green Bichuiball, has the highest levels of total free amino acids. We also calculated the contribution of total free amino acids to the total protein determined from the Kjeldahl nitrogen reported in Table 1. The percentage of total amino acids as free amino acids ranged from 28% (Sugar Yellow) to 56% (Saeng Green Chorok).

L-Glu was the most abundant free amino acid, followed by L-Gln. Free L-Glu apparently increases significantly during tomato ripening (Boggio et al., 2000; Choi et al., 2010; Sorrequieta et al., 2010). Boggio et al. (2000) and Sorrequieta et al. (2010) tracked a number of nitrogen metabolizing enzymes in ripening fruit and

Table 2
Concentration of 20 free amino acids and 9 amino acid metabolites (in bold font) in 12 cherry tomato fruit samples.[a]

Name	Black Kiss 20	Totori Gold	Sugar Yellow	Saeng Green Bichuiball	Saeng Green Chorok	Gold Minichal	Gold Sugar	Vitamin	Sugar Cherry	Jico Red	Sugar Red	Red Vienna
p-Ser	21.53 ± 0.02	21.8 ± 1.9	21.00 ± 0.90	24.86 ± 0.47	23.07 ± 0.57	23.52 ± 0.11	19.32 ± 0.26	19.94 ± 0.43	19.85 ± 0.04	20.14 ± 0.93	20.39 ± 0.13	21.15 ± 0.93
o-Pea	38.7 ± 4.8	tr	tr	36.28 ± 0.69	52.5 ± 3.8	37.11 ± 0.41	28.18 ± 0.52	23.0 ± 1.4	38.4 ± 1.8	18.37 ± 0.11	nd	23.25 ± 0.96
L-Asp	394.4 ± 3.2	380.5 ± 4.4	529.7 ± 7.0	496.8 ± 1.8	577.9 ± 5.2	437.85 ± 0.43	574 ± 12	327.4 ± 2.2	441.2 ± 6.2	395.3 ± 1.3	510.0 ± 4.9	455 ± 13
L-Thr	72.61 ± 0.33	35.78 ± 0.62	33.44 ± 0.58	104.32 ± 0.17	88.2 ± 1.1	58.88 ± 0.28	42.6 ± 1.0	50.1 ± 1.4	43.04 ± 0.64	53.77 ± 0.29	43.89 ± 0.11	45.2 ± 2.2
L-Ser	135.31 ± 0.51	57.90 ± 0.95	53.94 ± 0.94	112.46 ± 0.37	110.7 ± 1.1	86.57 ± 0.52	51.4 ± 1.4	61.6 ± 1.1	55.7 ± 1.6	105.73 ± 0.47	62.88 ± 0.81	57.8 ± 2.5
L-Asn	26.44 ± 0.25	21.84 ± 0.25	21.55 ± 0.23	42.03 ± 0.13	35.48 ± 0.29	30.95 ± 0.02	28.74 ± 0.71	25.94 ± 0.48	22.56 ± 0.52	27.89 ± 0.27	27.49 ± 0.28	37.01 ± 0.94
L-Glu	1608.0 ± 8.2	1767.0 ± 1.1	2016 ± 22	2879.0 ± 9.0	3551 ± 30	2423.3 ± 5.9	2524 ± 57	1539 ± 24	2326 ± 23	1415.6 ± 6.2	2223 ± 18	1702 ± 46
L-Gln	729.2 ± 2.0	556.81 ± 0.83	526.9 ± 5.6	1282.74 ± 0.04	1086.3 ± 6.8	806.53 ± 0.74	863 ± 20	761.7 ± 8.9	611.1 ± 4.3	897.5 ± 5.7	802.2 ± 6.6	1062 ± 27
L-Pro	90.6 ± 3.0	114 ± 38	131.4 ± 8.2	77.0 ± 6.3	144.6 ± 5.4	137 ± 17	143 ± 18	133 ± 12	104 ± 42	127.13 ± 0.04	43.11 ± 0.99	86 ± 44
L-Gly	19.48 ± 0.57	10.0 ± 1.5	10.87 ± 0.25	16.59 ± 0.11	17.16 ± 0.31	14.99 ± 0.01	8.7 ± 2.5	10.11 ± 0.52	8.0 ± 3.5	10.4 ± 1.1	10.58 ± 0.49	5.54 ± 0.08
L-Ala	82.4 ± 1.8	51.0 ± 4.6	83.8 ± 3.2	52.52 ± 0.24	89.1 ± 1.9	90.3 ± 2.1	43.9 ± 6.3	56.8 ± 2.2	47.2 ± 4.4	43.45 ± 0.69	46.19 ± 0.22	nd
L-Cit	10.26 ± 0.19	nd	nd	nd	nd	nd	nd	nd	nd	2.5 ± 3.5	5.21 ± 0.40	49.25 ± 0.01
L-Val	61.35 ± 0.01	34.13 ± 0.44	37.01 ± 0.73	54.03 ± 0.38	57.58 ± 0.61	46.45 ± 0.14	36.42 ± 0.28	37.19 ± 0.38	34.06 ± 0.43	46.31 ± 0.44	38.35 ± 0.63	38.85 ± 0.81
L-Cys	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
L-Met	13.7 ± 1.3	13.1 ± 1.8	10.35 ± 0.28	11.76 ± 0.35	15.85 ± 0.18	16.1 ± 1.5	11.3 ± 1.2	13.5 ± 1.1	11.4 ± 1.7	15.7 ± 1.7	10.34 ± 0.17	11.89 ± 0.93
L-Ile	44.30 ± 0.76	20.3 ± 7.3	15.81 ± 0.16	49.91 ± 0.28	39.5 ± 1.2	25.67 ± 0.28	19.58 ± 0.42	26.74 ± 0.18	21.23 ± 0.18	29.11 ± 0.03	17.13 ± 0.19	17.86 ± 0.74
L-Leu	42.9 ± 1.3	tr	tr	64.87 ± 0.81	66.98 ± 0.39	43.66 ± 0.20	37.1 ± 1.3	35.37 ± 0.25	30.02 ± 0.88	36.65 ± 0.09	24.50 ± 0.98	26.47 ± 0.77
L-Tyr	20.3 ± 1.1	nd	nd	23.9 ± 2.9	24.10 ± 0.11	tr	nd	20.2 ± 2.8	14.8 ± 1.5	16.4 ± 1.6	nd	nd
L-Phe	107.63 ± 0.56	44.85 ± 0.01	81.35 ± 0.86	127.02 ± 0.19	162.99 ± 0.18	119.82 ± 0.30	106.20 ± 0.74	126.2 ± 1.1	108.93 ± 0.75	107.58 ± 0.22	70.1 ± 2.2	79.4 ± 1.3
β-Ala	27.8 ± 2.0	nd	nd	15.9 ± 3.2	18.2 ± 1.9	tr	nd	tr	tr	19.1 ± 6.5	nd	tr
GABA	644.4 ± 2.6	352.07 ± 0.94	195.2 ± 2.2	735.1 ± 2.3	730.2 ± 5.2	490.8 ± 1.9	247.7 ± 5.7	367.8 ± 6.6	285.3 ± 1.9	397.3 ± 2.1	282.8 ± 3.2	273.0 ± 7.4
Trp	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
EtNH2	10.96 ± 0.11	10.5 ± 1.2	12.23 ± 0.40	10.23 ± 0.06	9.8 ± 1.8	11.86 ± 0.38	11.1 ± 1.4	10.7 ± 1.2	11.13 ± 0.10	11.52 ± 0.32	12.23 ± 0.79	10.08 ± 0.86
Hyl	29.24 ± 0.47	30.29 ± 0.59	29.62 ± 0.00	27.37 ± 0.13	29.7 ± 1.1	30.51 ± 0.06	28.69 ± 0.02	28.90 ± 0.11	29.9 ± 1.1	30.20 ± 0.54	28.65 ± 0.02	30.29 ± 0.07
L-Lys	45.03 ± 0.15	25.58 ± 0.06	29.77 ± 0.76	44.86 ± 0.29	49.39 ± 0.26	45.85 ± 0.44	33.65 ± 0.49	39.2 ± 1.2	36.4 ± 1.0	34.54 ± 0.10	31.06 ± 0.03	30.3 ± 1.1
1Me-His	24.20 ± 0.76	nd	nd	34.5 ± 1.2	30.67 ± 0.82	nd	10.85 ± 0.06	nd	nd	15.89 ± 0.84	12.85 ± 0.00	11.94 ± 0.29
L-His	39.10 ± 0.71	18.7 ± 1.2	23.09 ± 0.01	46.78 ± 0.30	48.0 ± 3.0	36.70 ± 0.69	24.60 ± 0.97	23.6 ± 1.2	26.99 ± 0.13	29.46 ± 0.88	23.42 ± 0.40	24.39 ± 0.86
L-Car	66.1 ± 3.7	75.5 ± 9.7	81.0 ± 2.8	47 ± 12	58.0 ± 8.3	71 ± 10	74 ± 19	81.29 ± 0.59	88.9 ± 3.8	73.03 ± 0.57	53.6 ± 4.7	76.7 ± 2.3
L-Arg	48.8 ± 2.1	22.70 ± 0.22	32.88 ± 0.36	81.5 ± 2.1	66.8 ± 2.7	52.82 ± 0.86	44.7 ± 1.6	42.6 ± 1.5	33.71 ± 0.11	47.23 ± 0.98	32.1 ± 1.5	35.2 ± 1.4
Sum essential amino acids	426.6 ± 2.2	192.4 ± 7.6	230.8 ± 1.5	503.5 ± 1.1	528.4 ± 3.5	393.1 ± 1.8	311.4 ± 2.5	351.9 ± 2.7	312.0 ± 2.4	353.1 ± 2.0	258.7 ± 2.5	274.3 ± 3.4
Sum all amino acids	3581 ± 10	3174 ± 39	3637 ± 26	5568 ± 12	6232 ± 32	4473 ± 18	4592 ± 65	3330 ± 29	3977 ± 49	3439.7 ± 9.0	4016 ± 20	3715 ± 71
Sum metabolites	873.1 ± 6.9	490 ± 10	339.1 ± 3.7	941 ± 13	952 ± 11	665 ± 10	419 ± 20	546.9 ± 7.0	473.5 ± 4.7	588.0 ± 7.8	415.8 ± 5.7	495.7 ± 7.9
Sum of all free N containing compounds	4454 ± 12	3664 ± 40	3976 ± 26	6509 ± 17	7184 ± 34	5138 ± 21	5012 ± 68	3877 ± 30	4450 ± 49	4028 ± 12	4432 ± 21	4210 ± 71
% of N (Table 1) in free form	37 ± 1	44 ± 3	28 ± 6	49 ± 2	56 ± 2	50 ± 1	49 ± 1	40 ± 2	45 ± 1	41.1 ± 0.3	43 ± 1	44 ± 2

[a] Amino acid abbreviation follow IUPAC standard; values are average (mg/100 g dry wt) ± SD (each powder sampled in duplicate); nd, not detected; tr, trace. The LOD of amino acids; Asp:13 ng ~Pro:29 ng. Sum essential (indispensable) AA = sum of His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. Sum all AA = sum 20 amino acids. Sum metabolites = sum of *p*-Ser, *o*-Pea, L-Cit, *β*-Ala, GABA, $EtNH_2$, Hyl, Me-His, and L-Car.

found that their activity was consistent with L-Glu accumulation. Content of L-Glu is significant to tomato flavor, as it imparts the characteristic umami taste (Sorrequieta et al., 2010). In addition, L-Asp, also important for tomato flavor (Fuke and Konosu, 1991), and γ-amino-butyric acid (GABA; 4-aminobutyric acid) were present in significant amounts.

GABA, a non-protein amino acid synthesized from L-Glu, is often an important component of the free amino acid pool in plants. GABA levels are reported to increase in response to stress (Shelp et al., 1999), and may be a signaling molecule in plants in addition to acting as a neurotransmitter in mammals (Bouché and Fromm, 2004). The content in tomatoes is highest just before the breaker growth stage, and then decreases (Akihiro et al., 2008; Choi et al., 2010). Our results confirm that tomatoes are a good source of GABA (Saito et al., 2008).

The L-Pro, L-Phe, and L-Ser values ranged between 1 and 4% of the total free amino acids. The remaining amino acids were present at levels less than 2% with minor exceptions. L-Cit, β-Ala, L-Tyr, and Met-His were absent from many samples and neither L-Cys nor Trp was found in any of the samples.

Protein quality is contingent on a balance of the essential amino acids His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. Lys is nutritionally limiting in cereals, Met in legumes, and Trp and Lys in corn. Presence of these three amino acids in tomatoes can potentially balance their lack in the mentioned foods (Mercer et al., 1989).

Fig. 2 compares the essential amino acid profiles of: (1) the average free amino acids of all cherry tomatoes in this study; (2) a standard reference complete protein (Institute of Medicine, 2002); and (3) the standard analysis of tomato from the USDA National Nutrient Database (U.S. Department of Agriculture: Agricultural Research Service, 2012). In our samples, Leu, Lys, and Trp were the most limiting amino acids. Leu and Lys but not Trp were also limiting in the USDA data bank. In fact, we found no free Trp in any of the samples. This is consistent with a previous study in which free Trp content was found in some samples but not others (Choi et al., 2011b). In that study, Saeng Green Chorok contained free Trp, whereas the same variety in this study did not, indicating that factors other than variety may influence free Trp content of tomatoes.

3.1.3. Phenolic compounds in cherry tomatoes

Cherry tomatoes are a good source of phenolic compounds, but the variation in the content of these compounds by variety is not well documented. Cherry tomatoes contain about 3–4 times the flavonoid content of standard sized tomatoes (U.S. Department of Agriculture: Agricultural Research Service, 2011). This is not unexpected because cherry tomatoes have more surface area per unit volume than standard sized tomatoes and because the majority of phenolics are believed to be in the epicarp (Adato et al., 2009). When tomato mutants lack phenolics in the epicarp, the fruit is pink instead of red (Ballester et al., 2010). Apparently, the yellow color of the flavonoids contributes to the typical yellow and orange hues of the fruit, suggesting some association between tomato color and phenolic content (Adato et al., 2009). The bright carotenes present in the flesh contribute to the dominant color. In the present study, we identified the individual phenolic compounds and determined their levels in samples of 12 cultivars of variable color grown and harvested under identical environmental conditions.

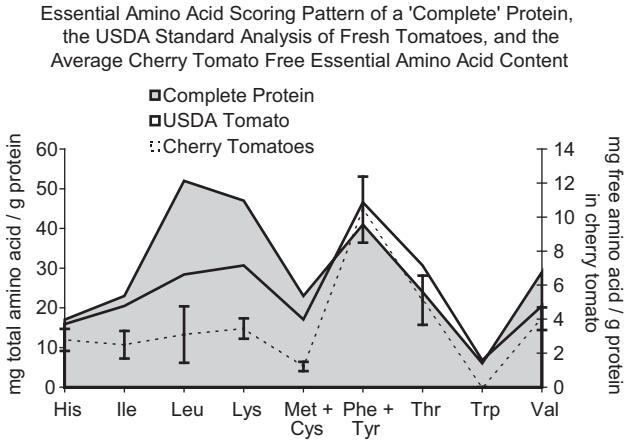

Fig. 2. Comparison of the average cherry tomato free essential amino acid content with an optimal complete protein (Institute of Medicine, 2002), and the USDA standard analysis of tomato (U.S. Department of Agriculture: Agricultural Research Service, 2012).

Fig. 3 shows a chromatogram of the cherry tomato phenolics separated by HPLC. Chromatographic retention times (Rt), UV/Vis spectra, and MS and MS/MS data are shown in Table 3. From these results, as well as from those reported in other studies (Ferreres et al., 2005; Ozga et al., 2007; Beelders et al., 2012), we identified the following compounds: 3-caffeoylquinic acid (chlorogenic acid), 5-caffeoylquinic acid (neochlorogenic acid), 3,4-di-O-caffeoylquinic (isochlorogenic) acid, 3,4,5-tri-caffeoylquinic acid, quercetin-3-apiosylrutinoside, quercetin-3-rutinoside (rutin), quercetin-3,7,4′-triglucoside, naringenin, and naringenin chalcone (phloretin). The HPLC chromatograms and the MS spectra also showed the presence of two caffeic acid–hexose isomers (Table 3) that we were not able to completely characterize. Fig. 4 shows the structures of the characterized compounds.

Table 4 lists the content of individual and total phenolics in cherry tomatoes separated by HPLC (Fig. 3). The last column in Table 4 lists the total (sum) of phenolics for each sample. The range (in mg/100 g dry wt) is from 64.6 for the Saeng Green Chorok sample to 440.0 for the Sugar Red sample or a striking 6.8-fold variation from the lowest to the highest value. Naringenin chalcone is the most abundant phenolic in all but the Saeng cherry tomato samples, in which none was detected. These two Saeng green cherry tomato samples had the lowest total phenolic content. The other samples had 30–70% of their phenolics as naringenin chalcone, with the higher content in Sugar Yellow, Gold Sugar, and Sugar Red samples. 3-Caffeoylquinic acid and quercetin-3-rutinoside were the next most abundant phenolics. Only small amounts of caffeic acid hexose isomer (I) were found, and naringenin was found in only trace amounts or not at all. Although high levels of naringenin have previously been reported in tomatoes, it is believed that naringenin chalcone is either converted to naringenin during certain extraction processes (Krause and Galensa, 1992), or that because the chalcone and naringenin have similar chromatographic elution positions, they are confused for each other during analysis (Slimestad and Verheul, 2011). Besides naringenin chalcone levels, which ranged between 0 and 70%, the caffeic hexose isomer (I) varied the most, from 0.6% in Sugar Yellow to 21% in Saeng Green Chorok samples (from 2.1 to 13.6 mg/100 g).

3.1.4. Lycopene and β-carotene content of 12 cherry tomatoes

In addition to phenolic-type antioxidants, tomatoes also contain the antioxidative carotenes, lycopene and β-carotene. Lycopene is responsible for the red and β-carotene for the orange color in the tomato pericarp. Carotenes accumulate in ripening tomatoes during the process of differentiation of chlorophyll-containing chloroplasts into carotene-containing chromoplasts (Egea et al., 2010). Various mutations in this pathway may result in tomatoes of different colors; lacking the ability to synthesize carotenes or to degrade chlorophyll (Paran and van der Knaap, 2007). Purple and black tomatoes produce carotenes, but do not fully degrade chlorophyll, leading to a dark brownish fruit color

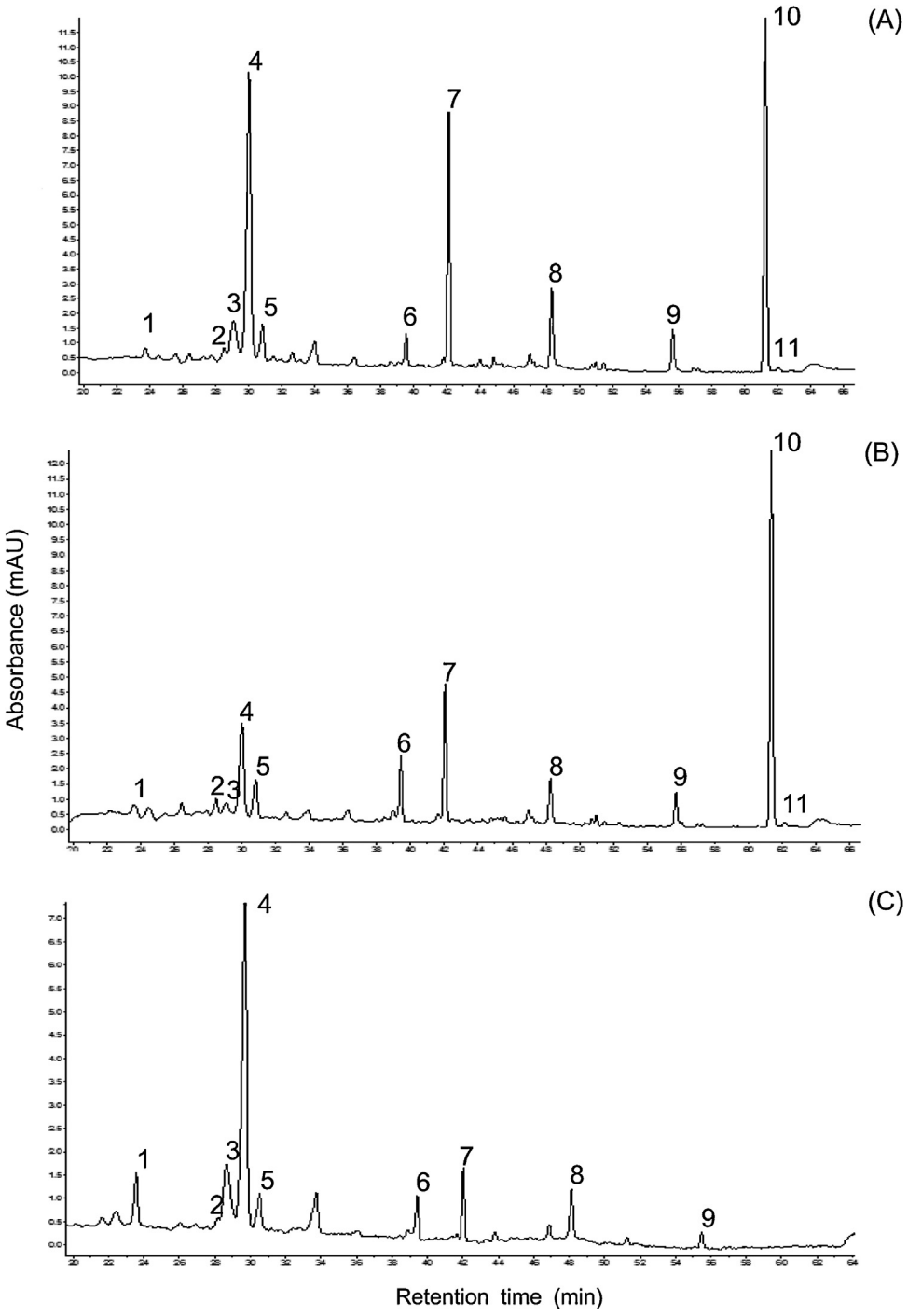

Fig. 3. HPLC chromatograms of phenolic compounds in Totori Gold (A), Sugar Red (B), and Saeng Green Bichuiball (C).

Table 3
Phenolic compounds identified by LC-PDA, MS, and MS/MS in the pulp extracts of 12 varieties of cherry tomato fruits.

Peak No. on HPLC	Retention time (min)	UV/vis maxima (nm)	$[M-H]^-$ (m/z)	MS/MS fragments	Identification
1	24.51 ± 0.01[a]	292, 244	341.2	179.2, 135.0	Caffeic acid-hexose isomer (I)(CH I)
2	28.79 ± 0.02	316, 248	341.3	221.3, 179.2, 135.0	Caffeic acid-hexose isomer (II) (CH II)
3	29.27 ± 0.01	326, 248	353.3	191.2	5-Caffeoylquinic acid (5-CQA)
4	30.97 ± 0.01	326, 248	353.0	191.0	3-Caffeoylquinic acid (3-CQA)
5	31.86 ± 0.01	326, 248	353.2	273, 204, 191.0	Caffeoylquinic acid isomer (CQAI)
6	41.15 ± 0.01	354, 254	741.1	300.2	Quercetin-trisaccharide (QTS)
7	43.63 ± 0.16	354, 256	609.1	300.1	Quercetin-3-rutinoside (Q3R)
8	50.23 ± 0.02	328, 250	515.4	354.0, 173.2	Di-caffeoylquinic acid (di-CQA)
9	57.72 ± 0.03	328, 250	677.1	353.0, 173.2	Tri-caffeoylquinic acid (tri-CQA)
10	63.15 ± 0.03	366, 250	271.1	151.1, 119.0	Naringenin chalcone (NGC)
11	63.90 ± 0.01	288, 251	271.1	151.1	Naringenin (NG)

[a] Average ± SD (n = 3).

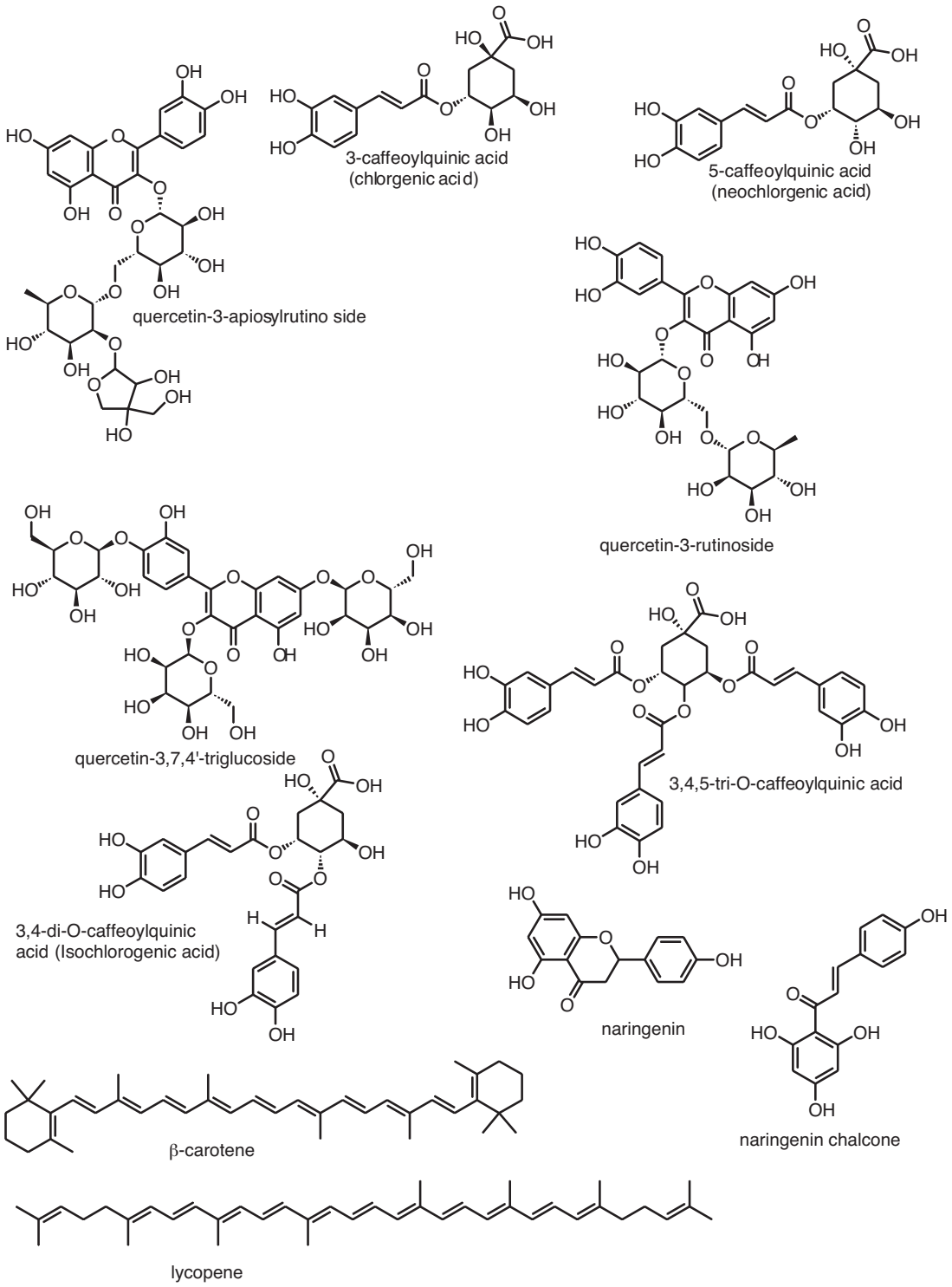

Fig. 4. Structures of characterized compounds in cherry tomato extracts.

from the combined green, red, and orange pigments (Barry and Pandey, 2009). Several recently developed cultivars (not evaluated in this study) are bright purple due to a high anthocyanin content that results from crossing tomato with the related anthocyanin-containing species, *Solanum chilense* (Jones et al., 2003; Gonzali et al., 2009).

The carotene range (Table 5, β-carotene + lycopene in mg/100 g dry wt) in green and yellow tomatoes was 0.96–9.9, and in orange, red, and red-black, 27.8–79.2. We ranked the cherry tomatoes based on color as follows: green (1), yellow (2), orange (3), red (4), and red-black (5). Color rank was correlated with lycopene ($r = 0.84$, $p < 0.05$) but not with β-carotene content. None of the green or yellow varieties contained more than a trace of lycopene. We could not predict the β-carotene content in the red tomatoes because the dark red lycopene concealed the contribution of the lighter-colored orange β-carotene. Two of the three yellow

Table 4
Concentration of phenolic compounds in the extracts of 12 samples of cherry tomato fruits.

Tomato samples	CHI	CHII	5-CQA	3-CQA	CQA1	Q3AR	Q3R	Di-CQA	Tri-CQA	NGC	NG	Sum
Black Kiss 20	9.6 ± 0.1	1.7 ± 0.0^{1,2,3}	2.3 ± 0.0^{1,2}	25.8 ± 0.0^{1}	7.2 ± 0.0^{1}	11.0 ± 0.1	23.7 ± 0.1	4.3 ± 0.1^{1}	2.5 ± 0.1	40.6 ± 0.2	tr	128.7 ± 0.3
Totori Gold	3.2 ± 0.1	1.8 ± 0.2	2.5 ± 0.1^{1}	71.1 ± 0.2	9.1 ± 0.1^{2,3}	9.1 ± 0.0^{1}	60.0 ± 0.1	11.1 ± 0.0	5.3 ± 0.1^{1}	240.4 ± 0.6	tr	413.6 ± 0.7
Sugar Yellow	2.1 ± 0.0	2.3 ± 0.2	4.6 ± 0.1	25.3 ± 0.2	8.7 ± 0.5^{2,4}	19.3 ± 0.1	36.0 ± 0.1	5.7 ± 0.1	4.8 ± 0.1^{2}	257.4 ± 0.6	nd	366.2 ± 0.9
Saeng Green Bichuiball	10.3 ± 0.0	2.0 ± 0.1^{1,4}	1.0 ± 0.1	57.6 ± 0.6	5.3 ± 0.4^{5}	9.4 ± 0.1	11.4 ± 0.1	4.1 ± 0.0^{1}	1.3 ± 0.1	nd	nd	102.4 ± 0.8
Saeng Green Chorok	13.6 ± 0.0	1.1 ± 0.2	2.3 ± 0.1^{1,2}	16.3 ± 0.0	5.4 ± 0.0^{5}	6.1 ± 0.2	17.6 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	nd	nd	64.6 ± 0.3
Gold Minichal	8.8 ± 0.1	2.9 ± 0.1	5.1 ± 0.1	33.7 ± 0.2	11.9 ± 0.1	9.0 ± 0.1^{1,2}	40.3 ± 0.1^{1}	8.4 ± 0.1	6.3 ± 0.0	69.9 ± 0.6	tr	196.3 ± 0.7
Gold Sugar	2.7 ± 0.0	2.1 ± 0.0^{2,5,6}	3.2 ± 0.0^{3,4}	26.4 ± 0.0^{2}	9.5 ± 0.0^{3}	8.8 ± 0.0^{2,3}	28.2 ± 0.1	6.4 ± 0.0	4.9 ± 0.0^{2}	218.3 ± 0.4	nd	310.5 ± 0.4
Vitamin	5.0 ± 0.1^{1}	1.6 ± 0.2^{4,7}	2.2 ± 0.3^{2}	47.7 ± 0.2	8.0 ± 0.4^{6}	8.0 ± 0.2	42.1 ± 1.5	7.2 ± 0.2	3.8 ± 0.1^{3}	131.7 ± 0.4	tr	257.3 ± 1.7
Sugar Cherry	6.0 ± 0.1	1.8 ± 0.1^{5}	3.0 ± 0.0^{3,5}	26.3 ± 0.1^{1,2}	7.3 ± 0.1^{1}	10.3 ± 0.1	40.2 ± 0.2^{1}	6.0 ± 0.0^{2}	3.8 ± 0.1^{3}	59.3 ± 0.2	tr	163.9 ± 0.4
Jico Red	7.5 ± 0.1	1.7 ± 0.2^{6,8}	3.4 ± 0.1^{4}	62.2 ± 0.2	8.4 ± 0.0^{4,6}	8.6 ± 0.1^{3}	40.7 ± 0.2^{1}	9.3 ± 0.1	5.2 ± 0.1^{1}	82.0 ± 0.1	nd	229.0 ± 0.4
Sugar Red	3.8 ± 0.1	2.0 ± 0.1^{3,7,8}	2.9 ± 0.0^{5}	40.8 ± 0.1	8.7 ± 0.3^{2,4}	6.5 ± 0.1	52.8 ± 0.2	7.9 ± 0.2	4.8 ± 0.2^{2}	309.7 ± 1.4	nd	440.0 ± 1.5
Red Vienna	4.9 ± 0.1^{1}	2.3 ± 0.1	4.0 ± 0.0	36.3 ± 0.4	8.1 ± 0.2^{4,6}	7.4 ± 0.0	49.5 ± 0.3	6.1 ± 0.1^{2}	4.4 ± 0.1	127.4 ± 0.5	tr	250.3 ± 0.8

Values within columns sharing a common superscript number are not significantly different ($p < 0.05$). Listed values are averages of triplicate analyses ($n = 3$) of a single powder (mg/100 g of dry wt) ± SD. nd, not detected. tr, trace. LOD: caffeic acid, 15.30 ng; chlorogenic acid, 3.06 ng, naringenin, 12.45 ng; and quercetin-3-rutinoside, 5.32 ng. The value of CHI, CHII, 3CQA, 5CQA, CQAI, di-CQA and tri-CQA are expressed as 3-CQA. QTS is expressed as Q-3-R. NGC is expressed as NG. *Abbreviations*: CHI, caffeic acid-hexose isomer (I); CHII caffeic acid-hexose (II); 3-CQA, 3-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid; CQAI, caffeoylquinic acid isomer; QTS, quercetin-trisaccharide; Q3R, quercetin-3-rutinoside; di-CQA, dicaffeoylquinic acid; tri-CQA, tricaffeoylquinic acid; NGC, naringenin chalcone; NG, naringenin.

Table 5
Lycopene and β-carotene content in 12 cherry tomato fruits.[a]

Tomato sample	Lycopene	β-Carotene
Black Kiss 20	54.20 ± 0.94	3.75 ± 0.07
Totori Gold	tr	5.4 ± 1.7
Sugar Yellow	nd	1.63 ± 0.02
Saeng Green Bichuiball	nd	1.25 ± 0.01
Saeng Green Chorok	nd	0.96 ± 0.04
Gold Minichal	41.7 ± 1.0	1.94 ± 0.01
Gold Sugar	2.82 ± 0.09	7.03 ± 0.60
Vitamin	37.0 ± 5.3	2.51 ± 0.00
Sugar Cherry	64.4 ± 4.4	2.94 ± 0.05
Jico Red	36.40 ± 0.65	2.09 ± 0.01
Sugar Red	76.7 ± 1.2	2.53 ± 0.03
Red Vienna	26.06 ± 0.02	1.75 ± 0.01

[a] Values are averages of duplicate analyses ($n = 2$) of a single powder (mg/100 g dry wt) ± SD; tr, trace; nd, not detected. The LOD of lycopene; 1.13 ng and carotene; 1.99 ng.

samples contained the highest amount of β-carotene, followed by the black cherry tomato sample. The green cherry tomatoes contained the lowest levels.

3.2. Bioactivities

3.2.1. Antioxidant activities of six pure tomato compounds

Table 6 shows the quantitative antioxidative effects in terms of IC_{50} values of the six commercially available pure tomato compounds determined by two methods (ABTS and FRAP). The ABTS IC_{50} values (in μM) ranged from 3.58 (highest activity) for lycopene to 9.57 for quercetin-3-rutinoside (lowest activity) or a 2.7-fold variation. The FRAP IC_{50} values (in μM Fe^{2+}/μg) ranged from 0.15 (naringenin) and 0.17 (β-carotene) to 6.3 (3-caffeoylquinic acid).

The relative values for the six compounds determined by the FRAP method seem quite different from those determined by the ABTS assay. Statistical analysis showed that the results from the two methods did not correlate. These results imply that the two methods measure different antioxidative effects.

3.2.2. Antioxidative effects of cherry tomato extracts

Table 7 shows that ABTS IC_{50} values for 12 cherry tomato powders ranged from 94.0 μg/mL for the Saeng Green Bichuiball sample to 379.5 μg/mL for the Sugar Yellow sample. The corresponding range of the FRAP IC_{50} values (in μmol Fe^{2+}/g) was much narrower, ranging from 19.5 (Saeng Green Chorok) to 32.4 (Totori Gold). These FRAP and the ABTS results for the extracts were also not statistically correlated. The FRAP was correlated with tomato extract content of tri-caffeoylquinic acid ($r = 0.828$) and di-caffeoylquinic acid ($r = 0.881$). These results show that (a) the trends in the antioxidative effects determined by the two methods are not parallel; and (b) excluding the Saeng Green Bichuiball sample, the range of the ABTS IC_{50} values for the other 11 samples is quite narrow.

3.2.3. Cell-growth-inhibiting effects of pure tomato compounds

Table 8 shows the inhibitory effects of three concentrations of each test substance evaluated by the MTT cell viability assay. Concentration dependence was determined by testing significantly different results between doses by ANOVA. IC_{50} values were calculated for samples displaying dose–response inhibition and for

Table 6
Antioxidant activity (IC_{50}), measured using ABTS and FRAP methods, of four tomato phenolic and two pigmented compounds.[a]

Compound	ABTS value IC50 (μg/mL)	FRAP value IC50 (mmol Fe^{2+}/mg)
3-Caffeoylquinic acid	7.97 ± 0.11	6.3 ± 1.5
Naringenin	9.01 ± 0.19	0.15 ± 0.03^{1}
Naringenin chalcone	4.72 ± 0.09	1.66 ± 0.13^{1}
Quercetin-3-rutinoside	9.57 ± 0.17	3.91 ± 0.12
Lycopene	3.58 ± 0.63^{1}	0.33 ± 0.01^{1}
β-Carotene	4.18 ± 0.55^{1}	0.17 ± 0.03^{1}

Values within columns sharing a common superscript number are not significantly different ($p < 0.05$).
[a] Values are average ± SD ($n = 3$).

Table 7
Antioxidant activity measured by ABTS and FRAP in 12 cherry tomato extracts.[a]

Tomato sample	ABTs value, IC_{50} (μg/mL)	FRAP value (μmol Fe^{2+}/g)
Black Kiss 20	310.9 ± 2.9^{1}	24.29 ± 0.11^{1,2}
Totori Gold	335.3 ± 4.2^{2}	32.38 ± 0.12^{3}
Sugar Yellow	379.5 ± 5.4	28.11 ± 0.28^{4}
Saeng Green Bichuiball	94.0 ± 2.1	23.15 ± 0.57^{1}
Saeng Green Chorok	323.4 ± 1.8	19.5 ± 1.7^{5}
Gold Minichal	313.7 ± 4.7^{1}	32.16 ± 0.52^{3}
Gold Sugar	347 ± 3^{3}	22.82 ± 0.76^{1,5}
Vitamin	358.7 ± 1.4^{4}	25.61 ± 0.55^{1,2}
Sugar Cherry	353.6 ± 2.4^{3,4}	24.16 ± 0.87^{1,2}
Jico Red	333.7 ± 2.7^{2}	30.6 ± 3.7^{3,4}
Sugar Red	334.6 ± 2.5^{2}	27.76 ± 0.84^{2,4}
Red Vienna	356.05 ± 0.87^{4}	27.52 ± 0.45^{2,4}

Values within columns sharing a common superscript number are not significantly different ($p < 0.05$).
[a] Values are averages of triplicate analyses ($n = 3$) of a single tomato powder ± SD.

Table 8
Inhibitory effects of four tomato phenolics and two pigments against normal liver (Chang), normal lung (Hel299), lung cancer cell (A549), liver cancer (HepG2), and cervical carcinoma (HeLa) cells determined by the MTT assay.[a]

Compounds	Conc. (μg/mL)	Growth inhibition (%)					IC_{50}				
		Chang	Hel299	A549	HepG2	HeLa	Chang	Hel299	A549	HepG2	HeLa
3-Caffeoylquinic acid	10	-7.8 ± 5.3	7.63 ± 0.31^{1}	-12.1 ± 4.9^{1}	-8.1 ± 4.9^{1}	0.09 ± 0.63^{1}	SG	NDR	NDR	Weak	Weak
	50	-13.38 ± 0.60^{1}	8.74 ± 0.19^{1}	-15.5 ± 5.0^{1}	-5.7 ± 4.5^{1}	0.87 ± 0.45^{1}					
	100	-15.6 ± 2.6^{1}	8.4 ± 1.1^{1}	-16.6 ± 3.6^{1}	6.2 ± 2.0	4.30 ± 0.59					
Naringenin	10	-17.0 ± 6.6	0.3 ± 5.7	-2.3 ± 1.4	14.8 ± 3.4	5.4 ± 1.6	SG	Weak	SG	Weak	94.1 ± 5.9
	50	-44.1 ± 3.2^{2}	10.8 ± 1.2^{2}	-14.8 ± 1.7	21.6 ± 1.0^{2}	39.5 ± 5.0					
	100	-36.0 ± 1.1^{2}	10.5 ± 1.1^{2}	-34.5 ± 3.2	20.80 ± 0.90^{2}	51.0 ± 4.2					
Naringenin chalcone	10	-30.2 ± 2.5^{3}	3.6 ± 2.4^{3}	-15.05 ± 0.53^{3}	12.11 ± 0.76	-2.95 ± 0.35	SG	NDR	NDR	Weak	84.2 ± 3.3
	50	-47.9 ± 3.2^{4}	7.91 ± 0.54^{3}	-30.3 ± 8.0^{3}	17.5 ± 3.4^{3}	33.44 ± 0.92					
	100	$-38.4 \pm 5.9^{3,4}$	8.4 ± 3.2^{3}	-21 ± 11^{3}	20.4 ± 1.3^{3}	59.4 ± 1.9					
Quercetin-3-rutinoside	10	-4.9 ± 1.9	6.5 ± 3.4^{4}	19.4 ± 3.5^{4}	13.7 ± 1.1^{4}	21.2 ± 4.9^{4}	Weak	NDR	Weak	Weak	Weak
	50	5.7 ± 2.9^{5}	6.7 ± 2.8^{4}	19.4 ± 2.5^{4}	12.5 ± 1.4^{4}	25.0 ± 2.2^{4}					
	100	8.0 ± 3.0^{5}	8.46 ± 0.58^{4}	32.9 ± 3.0	20.49 ± 0.87	36.3 ± 2.6					
Lycopene	10	34.8 ± 1.7	45.0 ± 4.9	54.82 ± 0.60	38.8 ± 1.3	64.1 ± 2.4	30.84 ± 0.35^{1}	21.0 ± 3.4	9.12 ± 0.44^{2}	27.79 ± 0.31^{1}	7.8 ± 1.2^{2}
	50	67.0 ± 1.7	77.9 ± 1.3	63.4 ± 1.5	64.78 ± 0.65	74.69 ± 0.11					
	100	86.7 ± 1.1	89.19 ± 0.09	66.5 ± 1.1	88.82 ± 0.83	89.31 ± 0.38					
β-Carotene	10	-2.8 ± 3.5^{6}	-6 ± 16^{5}	7.0 ± 3.2^{5}	-0.2 ± 2.1	14.2 ± 2.6^{5}	NDR	NDR	NDR	>100	NDR
	50	-1.2 ± 1.1^{6}	-1.9 ± 5.7^{5}	7.1 ± 1.8^{5}	2.61 ± 0.57	17.3 ± 2.3^{5}					
	100	1.2 ± 1.4^{6}	15.1 ± 3.4^{5}	7.6 ± 2.3^{5}	6.27 ± 0.20	19.09 ± 0.69^{5}					

Values within a column sharing a superscript number are not significantly different ($p < 0.05$). Values within a row sharing a superscript number are not significantly different ($p < 0.05$).

[a] Listed values are expressed as average ± SD ($n = 3$). NDR = no dose response, i.e. the inhibition of the cells by the three doses was not significantly different; SG = stimulated growth; Weak inhibition designated when 2 of the 3 doses were not significantly different.

Table 9
Inhibitory effects of 12 cherry tomato extracts against normal liver (Chang), normal lung (Hel299), lung cancer (A549), liver cancer (HepG2), and cervical carcinoma (HeLa) cells determined by the MTT assay.[a]

Tomato sample	Conc. (μg/mL)	Growth inhibition (%)				
		Chang	Hel299	A549	HepG2	HeLa
Black Kiss 20	10	-2.9 ± 2.1^{1}	5.68 ± 0.52^{1}	3.0 ± 1.1^{1}	7.8 ± 2.9^{1}	-6.9 ± 7.7
	50	1.0 ± 2.9^{1}	$10.2 \pm 3.1^{1,2}$	5.0 ± 2.8^{1}	12.5 ± 4.4^{1}	10.2 ± 2.6^{1}
	100	1.0 ± 1.4^{1}	$11.46 \pm 0.67^{1,2}$	15.74 ± 0.71^{2}	14.5 ± 5.6^{1}	18.9 ± 2.5^{1}
	200	16.1 ± 1.9	$15.8 \pm 3.0^{1,2}$	$22.3 \pm 4.2^{2,3}$	27.8 ± 1.2^{2}	32.4 ± 5.6^{2}
	300	23.2 ± 3.5	16.5 ± 6.6^{2}	26.6 ± 3.7^{3}	26.9 ± 2.6^{2}	33.5 ± 1.3^{2}
Totori Gold	10	-6.7 ± 5.6^{2}	-10.6 ± 1.5^{3}	-1.6 ± 1.6^{4}	22.1 ± 6.3^{3}	-0.9 ± 5.8
	50	-0.6 ± 1.7^{2}	$-7.2 \pm 3.3^{3,4}$	$1.01 \pm 0.50^{4,5}$	$31.7 \pm 6.3^{3,4}$	26.0 ± 2.3^{3}
	100	0.90 ± 0.74^{2}	-1.2 ± 2.6^{4}	$5.2 \pm 1.6^{5,6}$	$41.6 \pm 5.2^{4,5}$	$33.4 \pm 3.1^{3,4}$
	200	16.0 ± 4.4	5.9 ± 3.3^{5}	$5.4 \pm 1.5^{5,6}$	$38.8 \pm 5.9^{4,5}$	$34.1 \pm 2.8^{3,4}$
	300	26.7 ± 1.3	11.4 ± 2.7^{5}	10.0 ± 3.2^{6}	48.1 ± 3.6^{5}	35.9 ± 1.0^{4}
Sugar Yellow	10	-8.7 ± 2.9^{3}	-5.0 ± 5.3^{6}	-8.4 ± 2.0	-0.4 ± 5.7^{6}	**3.1 ± 1.6**[b]
	50	$-3.5 \pm 6.0^{3,4}$	-6.6 ± 3.0^{6}	1.74 ± 0.75^{7}	-4.3 ± 3.9^{6}	**12.7 ± 3.2**
	100	1.5 ± 1.8^{4}	$-1.9 \pm 2.0^{6,7}$	6.6 ± 2.0^{7}	2.10 ± 0.26^{6}	**18.4 ± 3.4**
	200	13.7 ± 4.8	$-1.5 \pm 4.7^{6,7}$	14.4 ± 3.9^{8}	26.7 ± 3.6^{7}	**25.3 ± 2.6**
	300	23.4 ± 2.1	7.6 ± 3.0^{7}	14.9 ± 1.6^{8}	35.1 ± 4.9^{7}	**30.3 ± 1.1**
Saeng Green Bichuiball	10	10.90 ± 0.03	7.3 ± 1.0	26.1 ± 3.2	28.4 ± 4.6^{8}	45.0 ± 4.4^{3}
	50	20.8 ± 3.6^{5}	24.5 ± 1.1^{8}	42.29 ± 0.94^{9}	32.6 ± 2.2^{8}	46.1 ± 3.1^{3}
	100	$22.1 \pm 2.7^{5,6}$	31.1 ± 3.0^{8}	43.9 ± 3.8^{9}	48.2 ± 3.8^{9}	47.9 ± 1.1^{3}
	200	19.2 ± 2.4^{5}	$31.0 \pm 5.8^{8,9}$	44.4 ± 2.0^{9}	43.8 ± 5.2^{9}	48.98 ± 0.50
	300	28.1 ± 3.3^{6}	41.8 ± 7.0^{9}	48.8 ± 3.1^{9}	49.2 ± 4.6^{9}	49.2 ± 4.3
Saeng Green Chorok	10	22.6 ± 1.1^{7}	24.98 ± 0.36	17.9 ± 5.7^{10}	10.6 ± 3.4	39.3 ± 2.2^{4}
	50	25.4 ± 9.0^{7}	37.9 ± 4.5^{10}	$28.8 \pm 6.4^{10,11}$	22.30 ± 0.68^{10}	40.1 ± 2.5^{4}
	100	28.5 ± 6.0^{7}	41.2 ± 2.3^{10}	$37.3 \pm 8.9^{11,12}$	$29.6 \pm 7.5^{10,11}$	46.5 ± 1.6^{5}
	200	29.0 ± 2.2^{7}	42.4 ± 5.0^{10}	$41.4 \pm 2.2^{11,12}$	34.1 ± 2.0^{11}	49.9 ± 2.7^{5}
	300	34.2 ± 4.3^{7}	47.9 ± 5.0^{10}	46.12 ± 0.12^{12}	33.8 ± 2.7^{11}	51.2 ± 3.1^{5}
Gold Minichal	10	5.1 ± 5.7^{8}	-1.4 ± 5.3^{11}	1.3 ± 1.5^{13}	25.38 ± 0.86^{12}	2.02 ± 0.44^{6}
	50	5.6 ± 3.2^{8}	-1.2 ± 2.2^{11}	1.8 ± 1.6^{13}	27.7 ± 2.8^{12}	6.8 ± 3.9^{6}
	100	-8.8 ± 4.0	-2.2 ± 1.9^{11}	3.3 ± 2.1^{13}	29.06 ± 0.34^{12}	10.8 ± 3.4^{6}
	200	22.2 ± 5.3^{9}	21.1 ± 5.2^{12}	15.3 ± 2.0^{14}	28.75 ± 0.62^{12}	26.1 ± 8.2^{7}
	300	29.0 ± 4.1^{9}	26.0 ± 4.3^{12}	16.0 ± 1.1^{14}	29.5 ± 2.0^{12}	30.4 ± 5.1^{7}
Gold Sugar	10	-4.0 ± 2.1^{10}	3.8 ± 1.0^{13}	-1 ± 15^{15}	17.7 ± 4.7^{13}	10.1 ± 4.8^{8}
	50	-3.1 ± 1.2^{10}	4.2 ± 1.7^{13}	4.3 ± 2.0^{15}	$26.7 \pm 3.4^{13,14}$	$16.1 \pm 3.4^{8,9}$
	100	-1.3 ± 1.3^{10}	4.9 ± 3.0^{13}	6.3 ± 2.8^{15}	$32.7 \pm 3.5^{14,15}$	$23.5 \pm 3.7^{9,10}$
	200	15.1 ± 2.6	15.4 ± 1.3^{14}	15.8 ± 3.4^{15}	42.9 ± 6.6^{15}	$27.4 \pm 1.6^{10,11}$
	300	28.8 ± 1.3	17.0 ± 4.5^{14}	15.95 ± 0.31^{15}	46.2 ± 7.5^{15}	35.5 ± 3.5^{11}
Vitamin	10	-9.7 ± 1.3	3.0 ± 1.8	-4.8 ± 2.6^{16}	9.2 ± 1.0^{16}	7.6 ± 3.6^{12}
	50	-0.93 ± 0.88^{11}	9.1 ± 2.3^{15}	-5.1 ± 3.6^{16}	11.0 ± 2.5^{16}	12.4 ± 4.1^{12}
	100	0.2 ± 3.9^{11}	9.9 ± 1.2^{15}	$-1.7 \pm 3.5^{16,17}$	13.9 ± 4.8^{16}	20.5 ± 7.1^{12}
	200	21.7 ± 4.6^{12}	8.3 ± 1.9^{15}	$1.9 \pm 1.9^{16,17}$	30.6 ± 3.0^{17}	28.8 ± 1.5^{13}
	300	22.3 ± 4.8^{12}	11.06 ± 0.36^{15}	5.9 ± 5.4^{17}	36.3 ± 3.9^{17}	37.4 ± 3.5^{13}
Sugar Cherry	10	-7.8 ± 1.3	1.1 ± 1.4^{16}	-6.0 ± 3.9^{18}	11.6 ± 3.5^{18}	3.5 ± 1.1
	50	-4.16 ± 0.93^{13}	$3.4 \pm 2.9^{16,17}$	-1.5 ± 4.0^{18}	$17.1 \pm 1.3^{18,19}$	11.3 ± 1.3
	100	-3.8 ± 1.4^{13}	$7.0 \pm 2.2^{17,18}$	0.0 ± 1.5^{18}	26.1 ± 1.5^{19}	20.8 ± 3.7
	200	17.86 ± 0.40	9.35 ± 0.34^{18}	18.4 ± 1.4^{19}	40.27 ± 0.98^{20}	30.2 ± 1.4^{14}
	300	24.8 ± 1.3	14.8 ± 1.5	25.1 ± 2.2^{19}	43.0 ± 7.6^{20}	31.7 ± 2.3^{14}
Jico Red	10	-4.37 ± 0.76^{14}	6.2 ± 1.2^{19}	-1.8 ± 6.6^{20}	-0.2 ± 3.2^{21}	-13.9 ± 6.2
	50	-3.97 ± 0.49^{14}	$12.1 \pm 3.6^{19,11}$	-1.9 ± 5.3^{20}	1.03 ± 0.34^{21}	10.7 ± 3.4^{15}
	100	-1.1 ± 2.0^{14}	$15.6 \pm 3.0^{19,20}$	-0.9 ± 4.6^{20}	10.1 ± 1.0	$21.8 \pm 5.8^{15,16}$
	200	14.5 ± 2.4	12.2 ± 6.7^{19}	0.1 ± 3.7^{20}	26.7 ± 1.6	23.3 ± 4.4^{16}
	300	29.7 ± 1.8	24.2 ± 4.4^{20}	11.8 ± 3.1^{20}	31.7 ± 1.5	$23.3 \pm 3.7^{15,16}$
Sugar Red	10	-1.8 ± 2.7^{15}	3.4 ± 1.1^{21}	-8.65 ± 0.63^{21}	-2.3 ± 1.0	-6.1 ± 5.9^{17}
	50	-0.5 ± 1.1^{15}	3.7 ± 2.0^{21}	-8.9 ± 2.0^{21}	14.3 ± 1.0^{22}	-0.2 ± 7.2^{17}
	100	0.9 ± 5.3^{15}	5.25 ± 0.10^{21}	-13.3 ± 2.5^{21}	14.5 ± 2.6^{22}	-0.2 ± 4.0^{17}
	200	31.20 ± 0.47^{16}	5.21 ± 0.60^{21}	6.3 ± 4.4^{22}	29.2 ± 2.5	22.6 ± 2.8^{18}
	300	31.3 ± 1.6^{16}	6.3 ± 2.6^{21}	7.7 ± 3.6^{22}	35.5 ± 3.5	26.2 ± 2.6^{18}
Red Vienna	10	-3.0 ± 3.7^{17}	3.3 ± 2.3^{22}	-8.8 ± 1.7^{23}	29.93 ± 0.32^{23}	-4.0 ± 2.0
	50	$1.2 \pm 2.2^{17,18}$	$7.40 \pm 0.91^{22,23}$	-4.7 ± 3.6^{23}	30.4 ± 3.9^{23}	14.7 ± 3.8^{19}
	100	3.62 ± 0.69^{18}	$10.0 \pm 3.0^{22,23}$	-5.2 ± 6.3^{23}	30.9 ± 1.0^{23}	$17.4 \pm 1.3^{19,20}$
	200	20.79 ± 0.30^{19}	10.7 ± 3.5^{23}	5.8 ± 1.7^{24}	42.8 ± 7.2^{24}	21.12 ± 0.64^{20}
	300	21.6 ± 3.0^{19}	19.6 ± 2.8	7.7 ± 2.8^{24}	45.8 ± 4.0^{24}	28.6 ± 2.4

Values within a column, sharing a superscript number are not significantly different ($p < 0.05$).
[a] Listed value is expressed as average ± SD ($n = 3$).
[b] Values in bold indicate that the five doses resulted in dose–response inhibition. Negative values indicate cell growth and positive values, inhibition of growth.

which the highest dose inhibited at least 50% of the cells. Only lycopene inhibited all the cell lines. Additionally, the IC_{50} values for lycopene were lower (activity was higher) than any of the other compounds. The HeLa cervical carcinoma cell line was the most susceptible to inhibition by the pure compounds, although there was no dose-response by β-carotene. The HepG2 normal liver cell line was mildly inhibited by all the compounds.

As noted in the Methods section, there is some uncertainty about the values for the hydrophobic compounds lycopene and carotene in this aqueous system. Indeed, the reproducibility of the β-carotene sample is poor, possibly due to this effect; however, it is quite good for lycopene, indicating that lycopene may be better solubilized.

3.2.4. Cell-growth-inhibiting effects of cherry tomato extracts

Table 9 shows the results of the cell-growth-inhibiting effects of the 12 tomato extracts tested against the same cell lines mentioned above. Although all cherry tomato extracts inhibited to some extent all cancer cell lines at the highest concentration (300 μg/mL), IC_{50} values were not calculated because none of the extracts caused dose–response inhibition of at least 50% of the cells at the doses tested. For the 300 μg/mL dose of each extract, the greatest inhibition was seen either with the HeLa cervical or the HepG2 liver cancer cell lines, depending on sample. For the liver cells, the extracts were considerably more active against the cancer than the normal cells. However, this was not the case for the lung cells. There was little difference in the effect of the extracts on the lung cancer compared to the normal lung cells.

These results indicate variable and unpredictable cellular inhibitory effects of the tomato extracts based on their composition. Thus, only the effect of the total tomato extract should be considered, because no one component would likely be responsible for the observed activity of the extracts.

4. Conclusions

Tomato extracts varied considerably in their levels of measured components. Variation in the protein content and in the amino acid profile can affect the nutritious value of the tomato. The crude protein content of the cherry tomato powders, which ranged from 8.3 to 14.2 g/100 g dry wt., is similar to that of cereals such as rice and wheat, suggesting that dry tomato powders with the highest protein content might contribute to dietary protein, and highlighting the need to evaluate their nutritional quality. But despite the fact that Trp is listed as being relatively abundant in cherry tomatoes (U.S. Department of Agriculture: Agricultural Research Service, 2012), free Trp was not found in any of our cherry tomato samples, thus affecting the quality of the protein. Phenolic and carotenoid compounds also varied many-fold, suggesting there is a range of healthfulness among different varieties.

The amino acid metabolite GABA, which has garnered attention due to its role as a neurotransmitter as well as other possible biological functions (Watanabe et al., 2002), is the most prevalent of the metabolites in the 12 cherry tomato cultivars, suggesting that these tomatoes are a good source of GABA. However, as with the other metabolites, the effect of dietary intake is still largely unknown. The concentration of the acrylamide precursor free L-Asp was relatively low in most samples, suggesting that adding fresh or processed cherry tomatoes to other food before 'cooking' will not significantly increase the heat-induced formation of toxic acrylamide. Cancer cells, especially the cervical and the liver carcinoma cells, were susceptible to inhibition by all the tomato extracts. Their preventive and therapeutic efficacy against human cancers merits study.

In conclusion, because both genetic and environmental factors govern the biosynthesis of bioactive tomato compounds (Gautier et al., 2008; Rossi et al., 2008; Juroszek et al., 2009; Prudent et al., 2009,2010; Carli et al., 2011; Barbagallo et al., 2013; Hallmann et al., 2013; Mazzucato et al., 2013; Iglesias et al., 2014), the large differences in composition and bioactivities observed in the present study among the 12 cherry tomato samples grown under identical environmental conditions appear to be governed by genetic factors.

References

Adato, A., Mandel, T., Mintz-Oron, S., Venger, I., Levy, D., Yativ, M., Domínguez, E., Wang, Z., De Vos, R.C.H., Jetter, R., Schreiber, L., Heredia, A., Rogachev, I., Aharoni, A., 2009. Fruit-surface flavonoid accumulation in tomato is controlled by a SLMYB12-regulated transcriptional network. PLoS Genetics 5, e1000777, http://dx.doi.org/10.1371/journal.pgen.1000777.

Akihiro, T., Koike, S., Tani, R., Tominaga, T., Watanabe, S., Iijima, Y., Aoki, K., Shibata, D., Ashihara, H., Matsukura, C., Akama, K., Fujimura, T., Ezura, H., 2008. Biochemical mechanism on GABA accumulation during fruit development in tomato. Plant and Cell Physiology 49, 1378–1389.

Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., Boyd, M.R., 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Research 48, 589–601.

Ballester, A.-R., Molthoff, J., de Vos, R., Hekkert, B.L., Orzaez, D., Fernández-Moreno, J.-P., Tripodi, P., Grandillo, S., Martin, C., Heldens, J., Ykema, M., Granell, A., Bovy, A., 2010. Biochemical and molecular analysis of pink tomatoes: deregulated expression of the gene encoding transcription factor SLMYB12 leads to pink tomato fruit color. Plant Physiology 152, 71–84.

Barbagallo, R.N., Di Silvestro, I., Patanè, C., 2013. Yield, physicochemical traits, antioxidant pattern, polyphenol oxidase activity and total visual quality of field-grown processing tomato cv. Brigade as affected by water stress in Mediterranean climate. Journal of the Science of Food and Agriculture 93, 1449–1457.

Barry, C.S., Pandey, P., 2009. A survey of cultivated heirloom tomato varieties identifies four new mutant alleles at the green-flesh locus. Molecular Breeding 24, 269–276.

Beelders, T., Sigge, G.O., Joubert, E., De Beer, D., De Villiers, A., 2012. Kinetic optimisation of the reversed phase liquid chromatographic separation of rooibos tea (*Aspalathus linearis*) phenolics on conventional high performance liquid chromatographic instrumentation. Journal of Chromatography A 1219, 128–139.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Analytical Biochemistry 239, 70–76.

Boggio, S.B., Palatnik, J.F., Heldt, H.W., Valle, E.M., 2000. Changes in amino acid composition and nitrogen metabolizing enzymes in ripening fruits of *Lycopersicon esculentum* Mill. Plant Science 159, 125–133.

Bouché, N., Fromm, H., 2004. GABA in plants: just a metabolite? Trends in Plant Science 9, 110–115.

Carli, P., Barone, A., Fogliano, V., Frusciante, L., Ercolano, M.R., 2011. Dissection of genetic and environmental factors involved in tomato organoleptic quality. BMC Plant Biology 11.

Choi, S.-H., Ahn, J.-B., Kozukue, N., Levin, C.E., Friedman, M., 2011a. Distribution of free amino acids, flavonoids, total phenolics, and antioxidative activities of jujube (*Ziziphus jujuba*) fruits and seeds harvested from plants grown in Korea. Journal of Agricultural and Food Chemistry 59, 6594–6604.

Choi, S.-H., Kim, H.-R., Kim, H.-J., Lee, I.-S., Kozukue, N., Levin, C.E., Friedman, M., 2011b. Free amino acid and phenolic contents and antioxidative and cancer cell-inhibiting activities of extracts of 11 greenhouse-grown tomato varieties and 13 tomato-based foods. Journal of Agricultural and Food Chemistry 59, 12801–12814.

Choi, S.-H., Lee, S.-H., Kim, H.-J., Lee, I.-S., Nobuyuki, K., Levin, C.E., Friedman, M., 2010. 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. Journal of Agricultural and Food Chemistry 58, 7547–7556.

Davies, J.N., Hobson, G.E., 1981. Constituents of tomato fruit – the influence of environment, nutrition, and genotype. CRC Critical Reviews in Food Science and Nutrition 15, 205–280.

Egea, I., Barsan, C., Bian, W., Purgatto, E., Latché, A., Chervin, C., Bouzayen, M., Pech, J.-C., 2010. Chromoplast differentiation: current status and perspectives. Plant and Cell Physiology 51, 1601–1611.

Ferreres, F., Sousa, C., Justin, M., Valentão, P., Andrade, P.B., Llorach, R., Rodrigues, A., Seabra, R.M., Leitão, A., 2005. Characterisation of the phenolic profile of *Boerhaavia diffusa* L. by HPLC-PAD–MS/MS as a tool for quality control. Phytochemical Analysis 16, 451–458.

Friedman, M., 2002. Tomato glycoalkaloids: role in the plant and in the diet. Journal of Agricultural and Food Chemistry 50, 5751–5780.

Friedman, M., 2013. Anticarcinogenic, cardioprotective, and other health benefits of tomato compounds lycopene, alpha-tomatine, and tomatidine in pure form and in fresh and processed tomatoes. Journal of Agricultural and Food Chemistry 61, 9534–9550.

Friedman, M., Levin, C.E., 2008. Review of methods for the reduction of dietary content and toxicity of acrylamide. Journal of Agricultural and Food Chemistry 56, 6113–6140.
Frusciante, L., Carli, P., Ercolano, M.R., Pernice, R., Di Matteo, A., Fogliano, V., Pellegrini, N., 2007. Antioxidant nutritional quality of tomato. Molecular Nutrition & Food Research 51, 609–617.
Fuke, S., Konosu, S., 1991. Taste-active components in some foods: a review of Japanese research. Physiology and Behavior 49, 863–868.
Gautier, H., Diakou-Verdin, V., Bénard, C., Reich, M., Buret, M., Bourgaud, F., Poëssel, J.L., Caris-Veyrat, C., Génard, M., 2008. How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? Journal of Agricultural and Food Chemistry 56, 1241–1250.
Gonzali, S., Mazzucato, A., Perata, P., 2009. Purple as a tomato: towards high anthocyanin tomatoes. Trends in Plant Science 14, 237–241.
Hallmann, E., Lipowski, J., Marszalek, K., Rembialkowska, E., 2013. The seasonal variation in bioactive compounds content in juice from organic and non-organic tomatoes. Plant Foods for Human Nutrition 68, 171–176.
Iglesias, M.J., López, J.G., Collados Luján, J.F., Ortiz, F.L., Pereznieto, H.B., Toresano, F., Camacho, F., 2014. Effect of genetic and phenotypic factors on the composition of commercial marmande type tomatoes studied through HRMAS NMR spectroscopy. Food Chemistry 142, 1–11.
Institute of Medicine, 2002. Protein and Amino Acids. National Academy of Sciences, Washington, DC, pp. 589–768.
Jones, C.M., Mes, P., Myers, J.R., 2003. Characterization and inheritance of the anthocyanin fruit (Aft) tomato. Journal of Heredity 94, 449–456.
Juroszek, P., Lumpkin, H.M., Yang, R.-Y., Ledesma, D.R., Ma, C.-H., 2009. Fruit quality and bioactive compounds with antioxidant activity of tomatoes grown on-farm: comparison of organic and conventional management systems. Journal of Agricultural and Food Chemistry 57, 1188–1194.
Kavitha, P., Shivashankara, K.S., Rao, V.K., Sadashiva, A.T., Ravishankar, K.V., Sathish, G.J., 2014. Genotypic variability for antioxidant and quality parameters among tomato cultivars, hybrids, cherry tomatoes and wild species. Journal of the Science of Food and Agriculture 94, 993–999.
Krause, M., Galensa, R., 1992. Determination of naringenin and naringenin-chalcone in tomato skins by reversed phase HPLC after solid phase extraction. Zeitschrift Für Lebensmittel-Untersuchung und -Forschung 194, 29–32.
Lenucci, M.S., Cadinu, D., Taurino, M., Piro, G., Dalessandro, G., 2006. Antioxidant composition in cherry and high-pigment tomato cultivars. Journal of Agricultural and Food Chemistry 54, 2606–2613.
Mazzucato, A., Willems, D., Bernini, R., Picarella, M.E., Santangelo, E., Ruiu, F., Tilesi, F., Soressi, G.P., 2013. Novel phenotypes related to the breeding of purple-fruited tomatoes and effect of peel extracts on human cancer cell proliferation. Plant Physiology and Biochemistry 72, 125–133.
Mercer, L.P., Dodds, S.J., Smith, D.I., 1989. Dispensable, indispensable and conditionally indispensable amino acid ratios in the diet. In: Friedman, M. (Ed.), Absorption and Utilization of Amino Acids, vol. 1. CRC Press, Boca Raton, FL, pp. 1–13.
Mitchell, A.E., Hong, Y.J., Koh, E., Barrett, D.M., Bryant, D.E., Denison, R.F., Kaffka, S., 2007. Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. Journal of Agricultural and Food Chemistry 55, 6154–6159.
Nagata, M., Yamashita, I., 1992. Simple method simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaishi 39, 925–928.
Ozga, J.A., Saeed, A., Wismer, W., Reinecke, D.M., 2007. Characterization of cyanidin- and quercetin-derived flavonoids and other phenolics in mature saskatoon fruits (*Amelanchier alnifolia* Nutt.). Journal of Agricultural and Food Chemistry 55, 10414–10424.
Paran, I., van der Knaap, E., 2007. Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. Journal of Experimental Botany 58, 3841–3852.
Prudent, M., Bertin, N., Génard, M., Muños, S., Rolland, S., Garcia, V., Petit, J., Baldet, P., Rothan, C., Causse, M., 2010. Genotype-dependent response to carbon availability in growing tomato fruit. Plant, Cell and Environment 33, 1186–1204.
Prudent, M., Causse, M., Génard, M., Tripodi, P., Grandillo, S., Bertin, N., 2009. Genetic and physiological analysis of tomato fruit weight and composition: influence of carbon availability on QTL detection. Journal of Experimental Botany 60, 923–937.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26, 1231–1237.
Rossi, F., Godani, F., Bertuzzi, T., Trevisan, M., Ferrari, F., Gatti, S., 2008. Health-promoting substances and heavy metal content in tomatoes grown with different farming techniques. European Journal of Nutrition 47, 266–272.
Saito, T., Matsukura, C., Sugiyama, M., Watahiki, A., Ohshima, I., Iijima, Y., Konishi, C., Fujii, T., Inai, S., Fukuda, N., Nishimura, S., Ezura, H., 2008. Screening for g-aminobutyric acid (GABA)-rich tomato varieties. Journal of the Japanese Society for Horticultural Science 77, 242–250.
Sánchez-Rodríguez, E., Ruiz, J.M., Ferreres, F., Moreno, D.A., 2012. Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. Food Chemistry 134, 775–782.
Shelp, B.J., Bown, A.W., McLean, M.D., 1999. Metabolism and functions of gamma-aminobutyric acid. Trends in Plant Science 4, 446–452.
Slimestad, R., Verheul, M., 2011. Properties of chalconaringenin and rutin isolated from cherry tomatoes. Journal of Agricultural and Food Chemistry 59, 3180–3185.
Sorrequieta, A., Ferraro, G., Boggio, S.B., Valle, E.M., 2010. Free amino acid production during tomato fruit ripening: a focus on L-glutamate. Amino Acids 38, 1523–1532.
U.S. Department of Agriculture: Agricultural Research Service, 2011. 03/07/2012. USDA Database for the Flavonoid Content of Selected Foods, Release 3. Retrieved from http://www.ars.usda.gov/nutrientdata (25.04.13).
U.S. Department of Agriculture: Agricultural Research Service, 2012. 03/07/2012. USDA National Nutrient Database for Standard Reference, Release 25. Retrieved from http://www.ars.usda.gov/ba/bhnrc/ndl (25.04.13).
Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., Hayasaki, H., 2002. GABA and GABA receptors in the central nervous system and other organs. International Review of Cytology 213, 1–47.