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Characterization of Phytochemicals and Antioxidant Activities of a Purple Tomato (*Solanum lycopersicum* L.)

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ABSTRACT: A newly developed nongenetically modified purple tomato V118 was investigated for its phytochemical compositions and antioxidant activities. A highly efficient and sensitive UPLC method was developed for both the phenolics and carotenoids, which showed that in addition to the phytochemicals commonly known for tomatoes, V118 had a unique composition of anthocyanins. The total carotenoid content of V118 was 234.78 μg/g dry weight (DW), and the total phenolic content was 659.11 mg GAE/100 g DW. The antioxidant activities of the lipophilic extract as measured by the PCL and ORAC-L assays were 30.11 μmol TE/g DW and 11.97 μmol TE/g DW, respectively, while the hydrophilic extracts as determined by the ORAC-H and FRAP assays were 323.23 μmol TE/g DW and 54.95 μmol AAE/g DW, respectively. The LC-MS study showed three major anthocyanins, which were mainly acylglycosides of petunidin and malvidin. This study showed that purple tomatoes such as V118 possess additional phytochemicals like anthocyanins, which can potentially have added health benefits.

KEYWORDS: purple tomato, phytochemical, carotenoids, anthocyanins, flavonoids, phenolics, UPLC, antioxidant activity

INTRODUCTION

The tomato (*Solanum lycopersicum*), as a fresh or processed food, possesses a high nutritional value, due to its rich and diverse micronutrients: carotenoids, phenolics, and vitamins C and E.[1,2] Recent epidemiological studies have suggested that regular consumption of tomatoes can play an important role in preventing cancer and cardiovascular problems; thus, much attention has been given to the phytochemicals with antioxidant activities in tomatoes.[3,4]

Commonly consumed tomatoes are best known for their rich content of lycopene, a carotenoid known for many beneficial health effects.[5] While phytochemicals including carotenoids and phenolic compounds have been reported and attributed to the antioxidant potential,[6] pigments such as anthocyanins do not normally exist in tomato fruit. Anthocyanins are found in the blue, red, or black fruits and vegetables such as blueberries, raspberries, and eggplants.[6−8] Anthocyanins are strong antioxidants that play a role in self-protection against biotic and abiotic stresses for the plant itself, and because of the unique pigmentation characteristics, they can also be useful in chemotaxonomy.[9] The antioxidant properties of anthocyanins are also considered to contribute to the health benefits. Studies have shown that anthocyanins are rapidly absorbed at the stomach level and can reach the brain of rats after oral intake of an extract of *Vitis vinifera* grapes.[10,11]

Interests in the health benefits of anthocyanins have instigated research projects on anthocyanin-rich tomatoes. A genetically modified purple tomato highly enriched with anthocyanins has been developed by the ectopic expression of two selected transcription factors from the ornamental flower snapdragon.[12,13] This genetically engineered tomato has also been found to prolong the life of cancer-susceptible mice, suggesting that they have additional health-promoting effects.[12] The phytochemical compositions, particularly the anthocyanins, and their biosynthetic pathways have been reported.[13] However, genetically modified (GM) food crops may still face strong resistance from consumers in most countries.[14] One of the best known GM foods is the fresh FLAVR SAVR tomato and its processed product (paste), which failed to gain market and consumer acceptance because of the safety concerns.[15]

Purple colored fruits do exist in some wild tomato species; however, such tomatoes have not been as popular as other colored tomatoes largely because some wild purple tomatoes also happen to contain small amounts of the poisonous alkaloid tomatine.[16] The Solanaceae (nightshade) family of plants, to which the tomato belongs, contains both poisonous as well as edible members. Because of the significant roles of anthocyanins in maintaining human health and preventing diseases, efforts have been made in breeding purple tomatoes for food with enhanced human health benefits. Insofar, only one less known variety has been planned to be released by Oregon State University;[17] however, nothing has been announced. A similar breeding program to develop tomatoes rich in anthocyanins has been initiated in Ontario. This program is directed toward developing high-anthocyanin tomatoes suitable for processing, in contrast to the programs at Oregon State University and at Sakata Seed America, which are developing cultivars suitable for fresh consumption. As a result, a purple breeding line V118, in a processing tomato genetic background, was obtained independently through traditional cross-breeding (Figure 1). The ultimate goal of our program is to produce a commercial variety that

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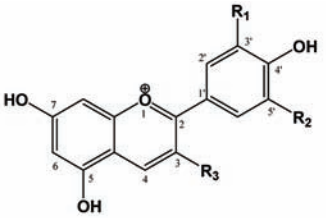

	R_1	R_2	R_3
Peak 9[a] (pet 3-*O*-caf-rut-5-*O*-glc)[b]	-OH	$-OCH_3$	-*O*-caf-rut-5-*O*-glc
Peak 10 (pet 3-*O*-*p*-coum-rut-5-*O*-glc)	-OH	$-OCH_3$	-*O*-*p*-coum-rut-5-*O*-glc
Peak 11 (mal 3-*O*-*p*-coum-rut-5-*O*-glc)	$-OCH_3$	$-OCH_3$	-*O*-*p*-coum-rut-5-*O*-glc

[a] Peak's numbers refers to Fig 3.
[b] Abbreviations used: pet, petunidin; mal, malvidin; caf, caffeoyl; rut, rutinoside; *p*-coum, para-coumaroyl; glc, glucoside.

Figure 1. Fruits of the whole and cut purple tomato (V118) and the major anthocyanins identified as follows: pet, petunidin; mal, malvidin; caf, caffeoyl; rut, rutinoside; *p*-coum, para-coumaroyl; glu, glucoside.

contains anthocyanins as well as other phytochemical antioxidants such as carotenoids of a conventional tomato. Tomatoes with high concentrations of both carotenoids and anthocyanins can potentially have stronger antioxidant activities and thus better help to reduce the risk of cancer and heart disease.[18,19]

Other than the GM purple tomato whose flavonoids, particularly the anthocyanin compositions, are known,[13] the phytochemical profile of the newly developed purple varieties or breeding lines through traditional breeding such as that of our V118, have not been reported. Accordingly, the objectives of this study were to identify and quantify the total and individual antioxidant phytochemicals, particularly carotenoids, anthocyanins, and other phenolics, and to find out how these phytochemical antioxidants contribute to the total antioxidant activity of the purple tomato V118.

MATERIALS AND METHODS

Plant Material. The purple tomato breeding line V118 used in this study was grown in the processing tomato breeding plots of the University of Guelph Ridgetown Campus, on a Brookston silt loam soil near Ridgetown, Ontario, Canada. This accession was a collection of one of the authors (S.L.). Transplants were set in the field in May 2009, in single rows, with 45 cm plant spacing within the row, and rows spaced 150 cm apart. The plots were maintained according to a standard processing tomato production management system with the exception that fungicides were not applied.

Four subsamples of at least 10 ripe fruits were collected randomly from each plot in September, 2009. All tomatoes were harvested at commercial maturity. All tomatoes were washed with tap water, cut into pieces, and ground with a commercial blender (7011, Waring Laboratory Science, USA) in order to obtain a homogeneous thin pulp. A known amount of about 30 g of this pulp was freeze-dried (Bulk tray dryer, Labconco, USA) and ground into fine powder. These materials were stored in polyethylene tubes at −80 °C prior to analysis.

Chemicals and Reagents. Protocatechuic acid, chlorogenic acid, gentistic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, naringenin, all-*trans*-lycopene, 1,3,5-tri(2-pyridyl)-2,4,6-triazine (TPTZ), L-ascorbic acid, gallic acid, Folin−Ciocalteu phenol reagent, fluorescein, Trolox, 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO, USA). All-*trans*-lutein, all-*trans*-β-carotene, delphinidin, cyanidin, petunidin, peonidin, and malvidin standards were purchased from Indofine (Belle Mead, NJ, USA). Sodium acetate, ferric chloride hexahydrate, sodium phosphate monobasic, sodium phosphate dibasic, and HPLC grade solvents, including methanol, methyl *tert*-butyl ether (MTBE), glacial acetic acid, formic acid, and ethanol, were purchased from Caledon Laboratories (Georgetown, Ont., Canada). Dimethyl sulfoxide (DMSO) was purchased form Fisher Scientific (Fair Lawn, NJ, USA). All other chemical reagents used were of analytical grade.

Colorimetric Study. Instrumental measurements of color were conducted at room temperature with a Minolta Chromameter (Chroma Meter CR-200; Minolta Camera Co. Ltd., Osaka, Japan) by placing the tomato powder in a 15 mm thick and transparent plastic cell without cover and by using a black plate as the background to standardize the measurements.[20] The chromameter consisted of an 8 mm diameter measuring area, and diffuse illumination/viewing was utilized. The tristimulus values of CIE L^*, a^*, b^* readings were calibrated against a standard white plate. CIE 1976 uniform color space was taken into account for the colorimetric analysis. Within the CIELAB uniform space, a psychometric index of lightness, L^* (ranging from 0, black, to 100, white), and two color coordinates, a^* (which takes positive values for the direction of redness and negative values for the direction of the complement green) and b^* (positive for yellowness and negative for blueness), are defined.[21] The data of each measurement are the average of triplicate measures on equidistant points of the sample.

Sample Extraction. The carotenoids of the purple tomato were extracted by using ethanol−hexane (4:3, v/v) as described in our recent publication.[22] The extract was evaporated to dryness under a gentle stream of nitrogen and redissolved in 1 mL of mobile phase B (methanol−MTBE−water 90:5:5, v/v/v) for UPLC analysis and antioxidant activity assays. All procedures were performed in dim lighting. Anthocyanins and phenolics were extracted from the dried powder of the whole fruit of the purple tomato by aqueous methanol. In brief, the freeze-dried tomato powder (2 g) was accurately weighed and transferred into a 50 mL tube containing 30 mL of 0.1% HCl (v/v) in 80% methanol.[6,23] The extraction was carried out on a rotary shaker (Scientific Industries Inc., USA) overnight (ca. 15 h; 400 rpm) at room temperature.[24,25] The mixture was centrifuged at 4000 rpm for 5 min (Eppendorf centrifuge 5810R, Brinkman Instruments Inc., Westbury, NY). The extraction was repeated three times, and the supernatants were combined, topped up to 90 mL, filtered through a 0.2-μm PTFE

membrane filter (VWR International, ON, Canada), and used as crude extract for further purification of anthocyanins and analyses of the total phenolic content, phenolic composition, and antioxidant activities. Hydrochloric acid was added to the extraction solvent to prevent degradation of the anthocyanins.[26,27] Further purification such as by the use of solid phase extraction is needed for the analysis of anthocyanins because there were other impurities such as amylose and proteins[28] in these extracts. Samples were extracted in triplicate.

Hydrolysis. Twenty milliliters of crude extract and 5 mL of 6 M HCl were mixed in a 40 mL tube tightly sealed with a screw cap, flushed with nitrogen, and then incubated in a shaking water bath at 90 °C for 2 h to hydrolyze the anthocyanins.[29] The samples were allowed to cool down and then centrifuged at 2000 rpm for 5 min. The supernatant was subjected to UPLC analysis.

Anthocyanin Purification by Solid Phase Extraction. Solid phase extraction (SPE) was used for anthocyanin purification (both before and after hydrolysis) prior to instrumental analysis. Because the purple tomato contains a relatively low content of anthocyanins, SPE can also serve as a means of concentration. The purification of anthocyanins was accomplished using Strata-X 33 μm Polymeric Reversed-Phase cartridges (Phenomenex, USA).[30] The cartridge was activated by passing 2 mL of MeOH solution acidified with formic acid (5%) and equilibrated with 2 mL of water−formic acid (95:5). The crude extract was concentrated to ca. 1/5 of its original volume by a rotary evaporator, then 5 mL of this concentrated solution was carefully loaded on to the cartridge, which was then washed with 10 mL of water−formic acid (95:5) to remove sugars, protein, and substances that could interfere with the analysis of anthocyanins. The elution of the anthocyanins was performed by the addition of 2 mL of methanol−formic acid (95:5). The eluate was dried under nitrogen and redissolved in water−formic acid (95:5) to a final volume of 500 μL.

UPLC Analyses of Phytochemical Antioxidants. *Carotenoids.* All-*trans*-carotenoids and *cis*-isomers were separated by UPLC in 15 min using a newly developed method.[22] *cis*-Carotenoids were identified by the characteristic UV/vis spectral pattern, maximum absorption wavelength, hypsochromic shift, and the Q-ratio values.[22]

Phenolics and Anthocyanins. The UPLC system Accela (Thermal Technologies Co. Ltd., USA) was equipped with a diode array detector (DAD) and an Ezchrom workstation for data processing. Separation was done in a Phenomenex Kinetex XB-C_{18} 1.7 μm column (100 × 2.1 mm, Phenomenex, Torrance, CA, USA) with a Phenomenex C_{18} guard column (4 × 3 mm, Phenomenex, Torrance, CA, USA). The column was thermostatically controlled at 30 °C, and the flow rate was set to 300 μL/min. The mobile phase consisted of two solvents: methanol−water−formic acid (A, 95:2:3, v/v/v) and water−methanol−formic acid (B, 95:2:3, v/v/v). The solvent gradient in volumetric ratios was as follows: 0−3 min, 10−30% A; 3−9 min, 30−40% A; 9−11 min, 40−50% A; 11−12 min, 50−100% A, and held at 100% A for 1 additional min. There was a 2 min postrun, which brings it back to the starting conditions. The UV−visible absorbance of the peaks were collected between 200 and 620 nm using DAD and monitored at three wavelengths (280, 320, and 520 nm). Quantification of protocatechuic acid, chlorogenic acid, gentistic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, and naringenin was done using data collected at 320 nm,[1] while that of the anthocyanins (petunidin and malvidin) was done using signals at 520 nm. Standard solutions were prepared separately by dissolving 10 mg of each compound in 5 mL of DMSO and then topped up to 100 mL in a volumetric flask with methanol (final concentration 100 μg/mL). All 8 phenolic and 2 anthocyanin compounds were quantified with external standards by using respective standard curves generated from serial dilutions of 10, 20, 40, 60, 80, and 100 μg/mL.

Method Validation Procedure. The newly developed UPLC methods for purple tomato phenolics and anthocyanins were validated in terms of sensitivity, linearity, precision, and accuracy according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines.[31] The limit of the detection (LOD) and limit of quantification (LOQ) were determined by injecting a series of dilute solutions with known concentrations. The recovery and intraday and interday respectabilities were determined according to Suo et al.[10]

LC-MS Analysis. LC-MS experiments were carried out using a Finnigan LCQ DECA ion trap mass spectrometer (ThermoFnnigan, San Jose, CA, USA) equipped with electrospray ionization (ESI) source. Separation was done using the same binary solvent system as that in the UPLC method but a different gradient program: 0−3 min, 0−35% A; 3−15 min, 35−40% A, 15−30 min, 40−60% A; 30−32 min, 60−100% A, then the gradient was held at 100% A for an additional 2 min. There was a 4 min postrun for reconditioning the column. The flow rate was set to 0.8 mL/min. The UV−visible absorbances of the peaks were collected between 190 and 800 nm. Positive ion mode was selected for data collection. Before sample analysis, the instrument was tuned by using a cyanidin standard to reach its optimum performance. As a result, the shear gas and auxiliary flow rates were set at 96 and 3 (arbitrary units), respectively. The capillary voltage was 32.5 kV, and its temperature was controlled at 350 °C. The entrance lens voltage was fixed at −58.0 V, and the multipole RF amplitude was at 770 V. The ESI needle voltage was 5 kV. The tube lens offset was 55.0 V, the multipole lens 1 offset was −4.40 V, and the multipole lens 2 offset was −8.00 V. The electron multiplier voltage was set at −1030 V for ion detection.

Determination of Total Phenolic Content. The total phenolic content (TPC) of the extract was estimated using a method by Wang et al.[32] Briefly, 25 μL of gallic acid standard or purple tomato extract was mixed with 125 μL of Folin−Ciocalteu reagent in 96-well microplates and allowed to react for 10 min at room temperature. Then 125 μL of saturated sodium carbonate (Na_2CO_3) solution was added and allowed to stand for 30 min at room temperature before the absorbance of the reaction mixture was read at 765 nm using a visible-UV microplate kinetic reader (EL 340, Bio-Tek Instruments Inc., Winooski, VT, USA).[32] The results were expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). All samples were tested in triplicate.

Antioxidant Assays. *Photochemiluminescence (PCL) Assay.* The PCL assay was performed on a Photochem system (Berlin, Germany). The PCL method is based on the photoinduced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide. Because the superoxide anion is a deleterious byproduct of oxygen metabolism responsible for the most important damage related to reperfusion injuries, the values obtained by the PCL method directly relate to health properties of a given food ingredient.[6] Complete reagent kits were purchased from the manufacturer. For lipid-soluble substances, the assay was a mixture of 2.3 mL of reagent 1 (sample solvent), 0.2 mL of reagent 2 (reaction buffer), and 25 μL of diluted reagent 3 (luminol) and reagent 4 (Trolox) for the calibration curve. To measure the antioxidant activity of a sample, reagent 4 was simply replaced by a sample solution. In this system, luminol is used as a photosensitizer, which generates superoxide radicals and a chemiluminogenic probe for free radicals.[33] The antioxidant activities are quantified based on their inhibitory effect on luminescence generation and are expressed as micromoles of Trolox equivalent per gram of DW tomato. Sample solutions were diluted 10 times in order for the readings to fit to the range of the standard curve.

Oxygen Radical Absorption Capacity Assay for Lipophilic Extracts (ORAC-L). Trolox was used as the standard and fluorescein as the fluorescent probe. Samples and Trolox were made in a 7% (w/v) randomly methylated β-cyclodextrin (RMCD) solvent to ensure the solubility of the lipophilic antioxidant in the reaction mixture.[34] The 7% RMCD solvent was made in a 50% acetone−water mixture (v/v) and was shaken for 1 h at room temperature on an orbital shaker at 200 rpm

prior to use. The sample solution was ready for analysis after further dilution with 7% RMCD. AAPH, a water-soluble azo compound, was used as a peroxyl radical generator. Briefly, 25 μL of blank, Trolox standard, or purple tomato extract (in triplicate) was mixed with 200 μL of fluorescein (0.0868 nM) solution and incubated for 30 min at 37 °C. Before the injection of 25 μL of 153 mM AAPH, the fluorescence was measured every minute for about 120 min until it reached zero (excitation wavelength, 485 nm; emission wavelength, 528 nm) in a Bio-Tek Fluorescence Spectrophotometer equipped with an automatic thermostatic holder (PL_X 800, Bio-Tek Instruments Inc., Winooski, VT, USA). A calibration curve was constructed daily by plotting the calculated differences of the area under the fluorescein decay curve between the blank and the sample for a series of standards of Trolox solutions (6.25, 12.5, 25, 50, and 100 μmol/L). The results were expressed as micromoles of Trolox equivalent (TE) per gram of dry weight tomato (μmol TE/g DW).[35]

ORAC Assay for Hydrophilic Extracts (ORAC-H). The ORAC assay for the hydrophilic extract of tomato (ORAC-H) was conducted according to Ou et al.[36] The protocols were the same as those described above for the ORAC-L assay except that a 75 mM phosphate buffer (pH 7.4) instead of 7% (w/v) RMCD is used.

Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay was determined according to the method of Benzie and Strain,[37] which was modified for the 96-well microplate reader.[38] The FRAP assay measures the ability of the antioxidants in tomato extracts to reduce the ferric-tripyridyl-triazine (Fe^{3+}-TPTZ) complex to the blue colored ferrous form (Fe^{2+}) that absorbs light at 593 nm. Briefly, a standard or sample extract (10 μL) was mixed with 300 μL of ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6; 10 mM TPTZ in 40 mM HCl; and 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1 (v/v/v)) and added to the wells. The plate was incubated at 37 °C for the duration of the reaction. The absorbance readings were taken at 593 nm at 30 min using a visible−UV microplate kinetic reader (EL 340, Bio-Tek Instruments, Inc., Winooski, VT, USA). Six concentrations of 25, 50, 100, 200, 400, and 800 μmol/L were used to prepare the standard curve of L-ascorbic acid. The antioxidant activities are expressed as micromoles of ascorbic acid equivalent (AAE) per gram of dry weight tomato (μmol AAE/g DW). Stronger absorption therefore indicates a higher reducing power of the phytochemical, thus, higher antioxidant activity.

Statistical Analysis. Quantitative data are presented as mean values with respective standard deviations of three independent extractions. All statistical analyses were performed with Statistix for Windows, version 9.0 (Analytical Software, Tallahassee, FL, USA).

RESULTS AND DISCUSSION

Colorimetric Study. To systematically study the color quality of the tomatoes, the CIE L^*-a^*-b^* system (International Commission on Illumination, Vienna), which has been favored by the USA food industry for measuring color of food products, was used. The values of L^*, a^*, and b^* were 60.63 ± 0.08, 29.08 ± 0.11, and 27.07 ± 0.06, respectively, for the purple tomato V118. These values were different from those of a typical commercial tomato H5108 F1 (L^*, a^*, and b^* were 52.30, 23.89, and 18.75, respectively), which was grown in the same experimental field in the same year.

UPLC Profiles of Phytochemical Antioxidants. A UPLC method was successfully established for both the carotenoids and the phenolics[22] (Figures 2 and 3). These methods have shortened the run time to 1/3−1/4 (15 min for carotenoids and 13 min for phenolics, respectively) of that of a conventional HPLC method; however, the separation was comparable to that of the latter methods.[1,8,39]

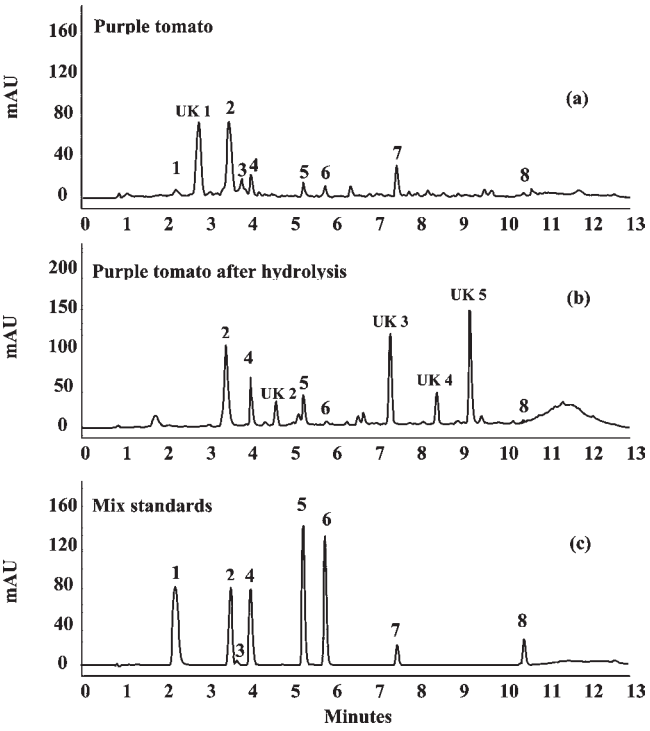

Figure 2. UPLC chromatograms of phenolic compounds in the purple tomato before (a) and after (b) hydrolysis and mixed standards (c) at 320 nm. Peaks: 1 = protocatechuic acid; 2 = chlorogenic acid ; 3 = gentistic acid ; 4 = caffeic acid; 5 = *p*-coumaric acid; 6 = ferulic acid; 7 = rutin; 8 = naringenin; and uk = unknown.

Details of the UPLC method for carotenoids have been described in our recent report.[22] All three major all-*trans* carotenoids and the eight phenolic compounds commonly found in tomatoes were identified in V118 by matching the retention times, UV−visible spectra, and mass spectrometric data with those of the corresponding standards and by cochromatography of samples spiked with the standards. Concentrations of the top three carotenoids in V118, lycopene, β-carotene, and lutein were similar to those in the 20 tomato cultivars in our previous study.[22] In terms of the phenolic compounds, there was an unknown peak UK1 (at 2.767 min, Figure 2a), which might be hydrolyzed to unknown peaks UK2−UK5 in Figure 2b. The unknown phenolic peak UK1 in Figure 2a is not unique to the purple tomato V118; it has been found in other varieties of tomato as well.[40,41] By comparing the chromatographic profiles and the eluting patterns, peak UK1 of V118 is highly likely the same as peak 35 as reported by Gómez-Romero et al.[40,42] This major peak was identified by others as caffeic acid-*O*-hexoside based on UV spectral (220 nm, 292 nm) and mass spectrometric data (m/z 341 $[M - H]^-$, m/z 179 $[M - H - hex]^-$, and 135 $[M - H - hex - CO_2]^-$).[40,42] Peak UK1 of this study also had two similar UV absorption bands (230 nm and 295 nm; Figure 4), and our MS data also gave the fragment ions at m/z 179 and m/z 341. Furthermore, we found that UK1 disappeared after acid hydrolysis giving rise to significantly increased peak heights of caffeic acid (at 4.033 min) and the new peaks UK2−UK5 (Figures 2a and 2b). On the basis of the above information, we concluded that UK1 in V118 was indeed a caffeic acid hexoside. The hydrolysis of the crude extract had also produced UK2−UK5 whose retention times were generally longer possibly due to the loss of highly hydrophilic

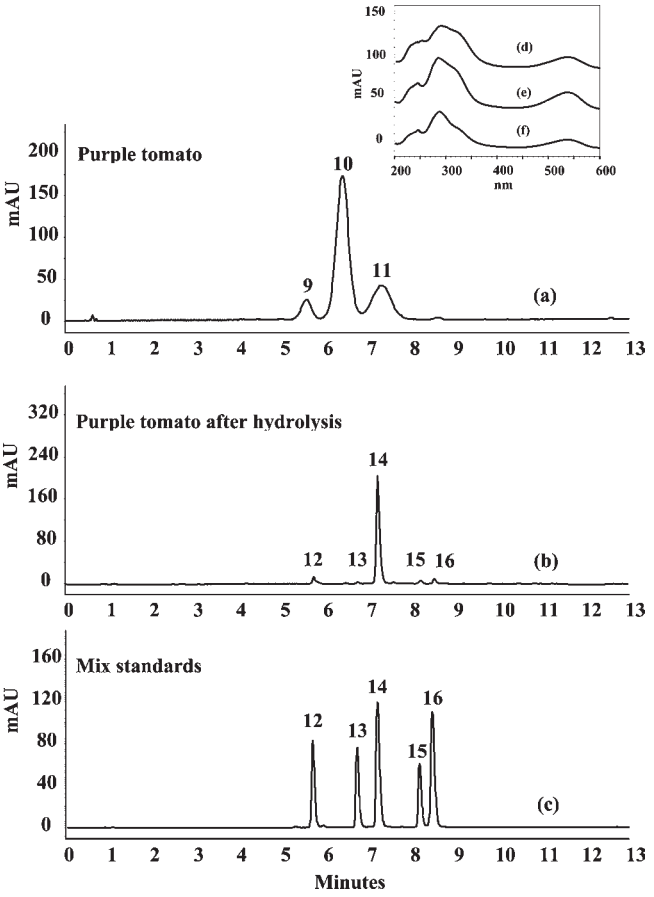

Figure 3. UPLC chromatograms of anthocyanins in the purple tomato before (a) and after (b) hydrolysis and mixed standards (c) at 520 nm. Peaks: 12 = delphinidin; 13 = cyanidin; 14 = petunidin; 15 = peonidin; 16 = malvidin. Insets: Absorption spectra of peak 9 (d), peak 10 (e), and peak 11 (f). Peak numbers correspond to those in Table 1.

glycosidic moieties (Figure 2b).[22] The absorption spectra of all unknown compounds (UK1−UK5) had typical features of a phenolic compound but were different from each other and did not match with any known phenolics commonly found in tomato (Figure 4). The UPLC profile of the hydrolyzed V118 extract was different from that reported by Luthria et al.,[40] which showed that the main phenolic acids in the hydrolysate of tomato were caffeic acid, *p*-coumaric acid, and ferulic acid. However, no phenolic profile of the crude extract was reported by these authors. Further studies need to be done for the identification of these unknown phenolics of V118. Anthocyanins were monitored at 520 nm with the same method, and three major peaks were detected (Figure 3).

Validation of the UPLC Methods. The UPLC method for the carotenoids was validated in our previous report.[22] For the phenolic compounds, the method was validated vigorously for sensitivity, linearity, precision, and accuracy in the detection of the 13 compounds (8 phenolics and 5 anthocyanidins). Correlation between the concentration and the peak area was highly linear ($R^2 > 0.99$) between 0.20 and 100 μg/mL. The LOD and LOQ values were less than 0.25 and 0.79 μg/mL, respectively; the relative standard deviation (RSD) for intraday and interday was less than 1.86% and 6.68%, respectively. The recovery studies for the quantified compounds were observed in the range of 92.6−105.2%. These data validate the UPLC method as being

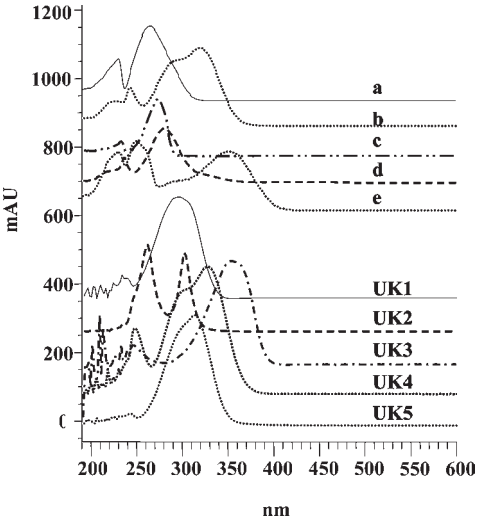

Figure 4. Absorption spectra of standards gallic acid (a), caffeic acid (b), catechin (c), phloretin (d), and rutin (e), and unknown peaks UK1−5. The UK peak numbers correspond to those found in Figure 2.

suitable for analysis of all phenolic compounds found in the purple tomato.

Identification of Anthocyanins. Anthocyanins from a traditionally cross-bred purple tomato have not been reported elsewhere. The UPLC chromatogram of the phenolic extract of the purple tomato revealed three major anthocyanin peaks (Figure 3a, 520 nm). The UV/vis spectra of the 3 anthocyanin peaks (Figure 3a) showed stronger absorption in the UV region (around 300 nm) as compared to that in the visible region, typically at 520 nm (Figures 3d−f). The characteristic absorption near 300 nm of the 0.1 AF3intrinsic anthocyanins (Figure 3a) suggest that they may be mono- or diacylated glycosides.[43] Further hydrolysis of the crude extract showed that all these 3 peaks disappeared, and 5 new peaks (peaks 12−16, Figure 3b) were produced. The UV/vis spectrum of the predominant anthocyanidin (Peak 14, Figure 3b) was found to match perfectly with that of the petunidin standard, together with its retention time (Figure 3c). Peak 14 was therefore tentatively identified as petunidin. Similarly, the other 4 minor anthocyanidin peaks 12, 13, 15, and 16 (Figure 3b) were temporarily assigned as delphinidin, cyanidin, peonidin, and malvidin, respectively.

While the most prevalent glycosylation in anthocyanins is with glucose, other sugar units including rhamnose, galactose, xylose, and arabinose are also present in anthocyanins. In addition, many anthocyanins have sugar residues acylated with aromatic acids, such as *p*-coumaric, caffeic, and ferulic acids . Table 1 gives the molecular ions and major fragments of the anthocyanin peaks of the original extract and its hydrolysate by LC-MS. The LC-ESI-MS experiment was conducted in the positive ion mode as this is considered the best for anthocyanins, which are positively charged.[44] LC-MS confirmation of the anthocyanidins in the hydrolysate was relatively straightforward as not only the retention times and UV/vis spectra of all peaks matched well with those of the respective standards,[43] but the molecular ions of peaks 12−16 in Figure 3b, m/z 303 $[M]^+$, 287 $[M]^+$, 317 $[M]^+$, 301 $[M]^+$, and 331 $[M]^+$, were the same as those of the respective standards, namely, delphinidin, cyanidin, petunidin, peonidin, and malvidin, respectively (Table 1). Among the anthocyanidins, petunidin was the predominant aglycone (91.9%), and the rest of

Table 1. MS Data of the Three Major Anthocyanin Peaks in the Crude Extract of V118

peak[a]	retention time (min)	proposed anthocyanins[b]	molecular ion $[M]^+$ (m/z)	fragment ions[c] (m/z)
9	5.550	pet 3-*O*-caf-rut-5-*O*-glc	949	**317**, 479, 787
10	6.400	pet 3-*O*-(*p*-coum)-rut-5-*O*-glc	933	**317**, 479, 771, 787
11	7.267	mal 3-*O*-(*p*-coum)-rut-5-*O*-glc	947	**331**, 493, 785, 801

[a] Peak numbers refer to Figure 3. [b] Abbreviations used: pet, petunidin; mal, malvidin; caf, caffeoyl; rut, rutinoside; *p*-coum, *para*-coumaroyl; and glc, glucoside. [c] Aglycone ions are in bold.

Table 2. Carotenoid Contents of the Purple Tomato V118[a]

compd	contents (μg/g DW)	compd	contents (μg/g DW)
di-*cis*-lutein	0.02 ± 0.02	di-*cis*-lycopene	0.34 ± 0.02
all-*trans*-lutein	1.31 ± 0.04	9 or 9′-lycopene	2.30 ± 0.35
di-*cis*-lutein	0.16 ± 0.01	all-*trans*-lycopene	174.04 ± 6.47
13 or 13′-lutein	0.50 ± 0.03	5 or 5′-lycopene	3.59 ± 0.34
9 or 9′-lutein	0.55 ± 0.05	di-*cis*-β-carotene	1.38 ± 0.15
di-*cis*-lutein	0.07 ± 0.01	di-*cis*-β-carotene	0.85 ± 0.03
di-*cis*-lutein	0.05 ± 0.03	all-*trans*-β-carotene	40.41 ± 3.75
15 or 15′-β-carotene	1.04 ± 0.05	9 or 9′-β-carotene	2.91 ± 0.57
di-*cis*-lycopene	0.56 ± 0.04	total lutein	2.66 ± 0.17
15 or 15′-lycopene	1.26 ± 0.14	total lycopene	185.01 ± 8.90
13 or 13′-lycopene	2.28 ± 0.09	total β-carotene	47.11 ± 4.32
di-*cis*-β-carotene	0.64 ± 0.03	total *cis*-carotenoids	19.01 ± 2.15
13 or 13′-β-carotene	0.51 ± 0.05	total *trans*-carotenoids	215.77 ± 12.72
		total carotenoids	234.78 ± 14.04

[a] Values are the mean ± SD, $n = 3$.

the minor aglycones accounted for only 9.1% of the total anthocyanidins.

Peak 9 of Figure 3a was characterized by ions at m/z 949 $[M]^+$, 787 $[M - \text{glucose}]^+$, 479 $[M - \text{glucose} - \text{caffeoyl}]^+$, and 317 $[M - \text{glucose} - \text{caffeoyl} - \text{rutinoside}]^+$; the molecular and fragment ions of peak 10 were m/z 933 $[M]^+$, 771 $[M - \text{glucose}]^+$, 479 $[M - \text{glucose} - \text{2-}p\text{-coumaroyl}]^+$, and 317 $[M - \text{glucose} - \text{2-}p\text{-coumaroyl} - \text{rutinoside}]^+$; and those of peak 11 were m/z 947 $[M]^+$, 785 $[M - \text{glucose}]^+$, 493 $[M - \text{glucose} - \text{2-}p\text{-coumaroyl}]^+$, and 331 $[M - \text{glucose} - \text{2-}p\text{-coumaroyl} - \text{rutinoside}]^+$. These fragmentation patterns, together with the molecular weights and the molecular ions of the anthocyanidin standards as detected in the LC-MS experiment (Table 1), helped the final identification of the three major anthocyanin peaks at 2.717, 3.167, and 3.717 min (Figure 3a) to be petunidin-3-*O*-caffeoyl-rutinoside-5-*O*-glucoside, petunidin-3-*O*-(*p*-coumaryl)-rutinoside-5-*O*-glucoside, and malvidin-3-*O*-(*p*-coumaryl)-rutinoside-5-*O*-glucoside, respectively (Table 1 and Figure 1), which had been reported in the purple potato previously (*Solanum tuberosum*, another *Solanaceae* plant).[45] The molecular ions and the fragmentation patterns of the peaks in the purple tomato and potato extracts were the same.[45] The anthocyanin composition of the non-GM purple tomato of this study was different from that of the GM purple tomato, which has been reported to contain mainly delphinidin 3-(coumaroyl)-rutinoside-5-glucoside and petunidin 3-(coumaroyl)-rutinoside-5-glucoside by Eugenio Butelli et al.[13] To the best of our knowledge, this is the first report of the identities of the major anthocyanins in a non-GM purple tomato.

Concentrations of Phytochemical Antioxidants. Concentrations of the individual and total phytochemical antioxidants, namely, the carotenoids and phenolics in V118, are shown in Tables 2 and 3. The total carotenoid content was 234.78 μg/g dry weight (DW), similar to the average amount reported for tomatoes (132−583 μg/g DW);[1] however, it is near the highest concentration of the 20 tomato varieties we recently studied that were grown in the same experimental field.[22] Lycopene was the dominant carotenoid (185.01 μg/g DW) in V118, followed by β-carotene (47.11 μg/g DW) and lutein (2.66 μg/g DW). About 8.1% of the total carotenoids in V118 were *cis*-carotenoids, a lower number than that of most of the tomato varieties studied.[22]

The total phenolic contents of V118 was determined by the FC method and expressed as the gallic acid equivalent (GAE) and by UPLC and expressed as the total phenolic index (TPI), which is the sum of the concentrations of all phenolics detected.[46] The total phenolic content was 659.11 mg GAE/100 g DW, which was similar to what has been reported for other tomato varieties (from 290 to 500 mg GAE/100 g DW).[45] The TPI was 307.00 mg/100 g DW in V118 (Table 3). The discrepancy between the TPC and the TPI values can be caused by the incomplete quantification of all peaks in the UPLC method and the potential interferences by other components in the TPC method.[46]

The major phenolic groups in V118 were the phenolic acids and flavonoids. Chlorogenic acid (65.56 mg/100 g DW) was the main phenolic acid (except UK1) followed by *p*-coumaric acid, gentistic acid, ferulic acid, caffeic acid, and protocatechuic acid, and rutin (52.39 mg/100 g DW) and naringenin (12.82 mg/100 g DW).

Table 3. Phenolic Contents of the Purple Tomato V118[a]

phenolic compounds	contents (mg/100 g DW)
protocatechuic acid	8.95 ± 0.16
chlorogenic acid	65.56 ± 2.69
gentistic acid	15.25 ± 0.76
caffeic acid	13.65 ± 0.83
p-coumaric acid	15.68 ± 0.74
ferulic acid	14.51 ± 0.99
rutin	52.39 ± 1.05
naringenin	12.82 ± 0.39
unknown 1[b]	83.17 ± 2.17
pet 3-*O*-caf-rut-5-*O*-glu[c]	1.88 ± 1.02
pet 3-*O*-*p*-coum-rut-5-*O*-glu	16.97 ± 5.18
mal 3-*O*-*p*-coum-rut-5-*O*-glu[d]	6.17 ± 3.48
TPI[e]	307.00 ± 16.72
TPC[f]	659.11 ± 23.28

[a] Values are the mean ± SD, $n = 3$. [b] Concentrations of unknown compounds are expressed as milligrams of caffeic acid equivalent/100 g dry weight. [c] Concentrations of petunidin-based anthocyanins are expressed as milligrams of petunidin equivalent/100 g dry weight. [d] Concentrations of the malvidin-based anthocyanin is expressed as milligrams of malvidin equivalent/100 g dry weight. [e] TPI: total phenolic index (sum of individual phenolic concentrations). [f] TPC: total phenolic content (mg GAE/100 g dry weight).

They were the main individual flavonoids. In the present UPLC method, all 8 phenolic compounds commonly found in traditional tomatoes[40−42] were also found in the purple tomato V118. The main phenolic compounds reported by Raffo et al. and Esra Capanoglu et al. were chlorogenic acid, naringenin, and rutin (2.67, 1.84, and 6.61 mg/100 g DW and 16.7, 2.2, and 16.9 mg/100 g DW, respectively), which were all found in the purple tomato of the present study except at higher concentrations (65.56, 12.82, and 52.39 mg/100 g DW, respectively, Table 3).[47,48] A significant portion of the total phenolics are from the unknown compound (UK1) whose concentration was pooled and expressed as the caffeic acid equivalent in Table 3.

Anthocyanin was another major group of flavonoids in V118. Among the 3 major anthocyanin peaks detected in the anthocyanin fraction of the V118 extract, petunidin-3-*O*-(*p*-coumaryl)-rutinoside-5-*O*-glucoside was the predominant one that accounted for approximately 67.8% of the total anthocyanins (by peak area) (Figure 3a). The total anthocyanin content in V118 was 72.31 mg/100 g, which included 9.04, 50.18, and 13.09 mg/100 g DW of petunidin-3-*O*-caffeoyl-rutinoside-5-*O*-glucoside, petunidin-3-*O*-(*p*-coumaryl)-rutinoside-5-*O*-glucoside, and malvidin-3-*O*-(*p*-coumaryl)-rutinoside-5-*O*-glucoside, respectively. The concentrations of these glycosides were calculated based on the equimolecular conversion using standard curves generated from their respective aglycones, i.e., petunidin and malvidin. This concentration was less than that of blueberry (2820 mg/100 g DW);[49] however, tomatoes are consumed in many food preparations and in larger quantities than seasonal and small fruits like blueberries. A purple variety of tomato such as the V118 of our study may have as many health implications as blueberry, especially when breeding effort is made to further increase the anthocyanin content.

Antioxidant Activities. Different methods were used to evaluate the antioxidant activity of the purple tomato V118, as no single chemical assay can accurately quantify the contribution of the lipophilic and hydrophilic components to the total antioxidant action of the plant food.[43] The antioxidant activities of the lipophilic extract (containing mainly carotenoids) were determined by the PCL and ORAC-L assays, and were found to be 30.11 μmol TE/g DW and 11.97 μmol TE/g DW, respectively. The hydrophilic extract (containing mainly phenolics) was subjected to the ORAC-H and FRAP assays, which gave 323.23 μmol TE/g DW and 54.95 μmol AAE/g DW, respectively (Table 4).

Table 4. Antioxidant Activities of the Purple Tomato V118 As Evaluated by PCL, ORAC-H, ORAC-L, and FRAP Assays

	antioxidant activity
PCL	30.11 ± 1.36 μmol TE/g DW
ORAC-L	11.97 ± 0.21 μmol TE/g DW
ORAC-H	323.23 ± 3.58 μmol TE/g DW
FRAP	54.95 ± 1.05 μmol AAE/g DW

The antioxidant activity of V118 as measured by PCL (30.11 μmol TE/g DW) was consistent with results reported for the traditional tomato varieties (6.01−48.83 μmol TE/g DW).[22] The ORAC-L value was 11.97 μmol TE/g DW, which is a little lower than the ORAC-L values of the traditional Bush tomato (18.6 μmol TE/g DW).[34] The FRAP value of V118 was 54.95 μmol AAE/g DW, slightly higher than what has been reported for other tomatoes (48.6 μmol AAE/g DW),[9] while the ORAC-H value for the purple tomato was 323.23 μmol TE/g DW, which is 2-folds higher than that of the ORAC-H values of the traditional tomato cultivar *San Marzano* (140 μmol TE/g DW).[50]

The hydrophilic fraction contributed 96.4% and the lipophilic fraction 3.6% to the total ORAC (sum of ORAC-L and ORAC-H values). The high values of ORAC-H are possibly due to the phenolic compounds that display high antioxidant activity, which has been similarly reported for the Bush tomato previously (2.0% for the ORAC value of lipophilic fraction and 98% for the hydrophilic fraction).[34] This result suggests that phenolic compounds are the major contributor to the antioxidant activity of purple tomato.

In conclusion, the purple tomato V118 is an excellent source of phytochemical antioxidants such as carotenoids and phenolics. V118 retains the phytochemical profile of a typical tomato variety that is rich in carotenoids (mainly lycopene) and nonanthocyanin phenolics (mainly chlorogenic acid). However, it is a unique tomato cultivar for its anthocyanin contents, which also gave V118 the distinctive L^*, a^*, and b^* values. As far as we know, no anthocyanins for a non-GM tomato variety have been reported and identified using the method we report herein. Anthocyanins are strong antioxidants, and anthocyanin-rich foods have been implicated in many beneficial health effects to humans. Tomato, arguably the most popular vegetable in the world, is already known as being a part of a healthy diet. A tomato cultivar such as V118 with additional phytochemical antioxidants like anthocyanins will undoubtedly contribute more to the health of humans.

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ABBREVIATIONS USED

UPLC, ultra-performance liquid chromatography; GAE, gallic acid equivalent; PCL, photochemiluminescence; ORAC, oxygen radical absorption capacity; TE, Trolox equivalent; FRAP, ferric reducing antioxidant power; AAE, ascorbic acid equivalent; LC-MS, liquid chromatography−mass spectrometry

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