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Three-Year Comparison of the Content of Antioxidant Microconstituents and Several Quality Characteristics in Organic and Conventionally Managed Tomatoes and Bell Peppers

ALEXANDER W. CHASSY,[†] LINH BUI,[‡] ERICA N. C. RENAUD,[§] MARK VAN HORN,[#] AND ALYSON E. MITCHELL*,[†]

Department of Food Science and Technology and Department of Plant Sciences, One Shields Avenue, University of California Davis, Davis, California 95616; Masterfoods USA, 3250 East 44th Street, Vernon, California 90058; and Seeds of Change, 340 Highway 582, San Juan Pueblo, New Mexico 87566

Understanding how the environment and production and cultivation practices influence the composition and quality of food crops is fundamental to the production of high-quality nutritious foods. In this 3-year study, total phenolics, percent soluble solids, ascorbic acid, and the flavonoid aglycones quercetin, kaempferol, and luteolin were measured in two varieties of tomato (*Lycopersicon esculentum* L. cv. Ropreco and Burbank) and two varieties of bell peppers (*Capsicum annuum* L. cv. California Wonder and Excalibur) grown by certified organic and conventional practices in a model system. Significantly higher levels of percent soluble solids (17%), quercetin (30%), kaempferol (17%), and ascorbic acid (26%) were found in Burbank tomatoes (fresh weight basis; FWB), whereas only levels of percent soluble solids (10%) and kaempferol (20%) were significantly higher in organic Ropreco tomatoes (FWB). Year-to-year variability was significant, and high values from 2003 influenced the 3-year average value of quercetin reported for organic Burbank tomatoes. Burbank tomatoes generally had higher levels of quercetin, kaempferol, total phenolics, and ascorbic acid as compared to Ropreco tomatoes. Bell peppers were influenced less by environment and did not display cropping system differences.

KEYWORDS: Antioxidants; flavonoids; total phenolics; ascorbic acid quercetin; kaempferol; luteolin

INTRODUCTION

Consumer awareness of the relationship between foods and health, together with environmental concerns, has led to an increased demand for organically produced foods. Organic food sales have increased by about 20% per year since 1990 and were estimated at $10.4 billion in 2003 on the U.S. market alone (*1*). Consumer studies have shown multiple reasons for organic preferences, including environmental and socioeconomic concerns, opinions of taste, and the belief that organic foods are healthier (*2*, *3*). Reviews of over a 150 studies comparing the nutritional quality of conventionally and organically produced vegetables demonstrate inconsistent differences with the exception of higher levels of ascorbic acid (vitamin C) and less nitrate in organic products (*2*, *4*, *5*). However, these data are difficult to interpret because cultivar selection and agronomic conditions varied widely and different methods of sampling and analysis were used in the investigations cited. Additionally, the majority of these studies did not assess levels of secondary plant metabolites (e.g., phenolic antioxidants such as the flavonoids) because their role in human health was not yet appreciated (*5*).

In recent years there has been a growing effort aimed at understanding relationships between crop management and the antioxidant microconstituents of fruits and vegetables as these foods are the primary sources of flavonoids in the Western diet (*6*−*20*). Epidemiological studies suggest that flavonoids protect against cardiovascular disease (*21*) and, to a lesser extent, against cancer (*22*) and other age-related diseases such as dementia (*23*). Flavonoids are potent antioxidants (*24*, *25*), scavenge free radicals (*26*), induce several protective enzyme systems (*27*), and play key roles in many of the processes underlying vascular dysfunction and the development of atherosclerosis (*28*). Although polyphenols such as the flavonoids are implicated in the prevention of chronic disease, almost all attempts to assign health-promoting activity to the in vitro antioxidant action of any flavonoids in foods have been unsuccessful. Williams and Manach point to some of the difficulties in interpreting data

* Corresponding author [telephone (530) 752-7926; fax (530) 752-4759; e-mail aemitchell@ucdavis.edu].
[†] Department of Food Science and Technology, University of California.
[‡] Masterfoods USA.
[§] Seeds of Change.
[#] Department of Plant Sciences, University of California.

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from intervention studies of polyphenols including incomplete characterization of the test material, unvalidated biomarkers, and the lack of understanding of poyphenol bioavailability and metabolism (*29*). Recently, themes have shifted away from free radical scavenging and metal chelating properties of polyphenols toward understanding specific polyphenol/polyphenol metabolite interactions with biomolecules affecting cell signaling, membrane properties, and gene and protein expression.

The consumption of tomatoes (8.21 kg per capita in 2003) and especially tomato products (32.6 kg per capita 2003) in the Western diet is quite high (*30*). They are an important and significant source of vitamin C (19 mg/100 g of fresh weight), vitamin A (623 IU/100 g of fresh weight), and lycopene (3.0 mg/100 g of fresh weight). The main flavonoids found in tomatoes are quercetin, kaempferol, and naringenin, with quercetin levels ranging from 0.03−2.77 mg/100 g in fresh tomatoes to 4.77 mg/100 g in processed tomato products (*31*). Fresh and processed tomato products supply about 2.0 g of quercetin annually per capita.

Both conventional and organic agricultural practices include combinations of farming practices that can vary greatly depending upon region, climate, soil quality, occurrence and prevalence of pests and diseases, and farm management practices. These systems never reflect a steady-state condition, but change dynamically (*32*). Still, fundamental differences between organic and conventional production systems, particularly in soil fertility management, have the potential to affect the nutritive composition of plants and, in particular, secondary plant metabolites (*5*). Organic systems rely on the activity of a diverse soil ecosystem to make nitrogen (N) available to plants. Conventional farms utilize fertilizers containing inorganic nitrogen, which is directly available to plants. The ready availability of inorganic nitrogen has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids. According to the carbon/nutrient balance theory (CNB), growth rate (GR), and growth differentiation balance hypothesis (GDB), high nutrient availability leads to an increase in plant growth and development rates and biomass and a decreased allocation of resources toward the production of carbon-containing compounds such as starch, cellulose, and non-nitrogen-containing secondary metabolites [for a review see Stamp (*33*)]. Although genetics are the primary determinant of the composition of secondary plant metabolites, environment and phytopathogen stress also play key roles in the production of plant defense compounds (*34*, *35*). Recently, Toor et al. examined the influence of nutrient source on soluble solids, pH, titratable acidity, antioxidant components, and the antioxidant activity of greenhouse-grown tomatoes (*14*). Tomatoes were grown with mineral nutrient solutions (containing NH_4^+ and NO_3^-), chicken manure, and a grass−clover mulch. The mean total phenolic and ascorbic acid contents of tomatoes grown using the grass-clover mulch (29%) and chicken manure (17.6%) were higher than those of tomatoes grown with the mineral nutrient solutions and demonstrate that nutrient source can play a role in determining the levels of antioxidants in tomatoes.

There are currently several studies comparing the influence of crop management practices on a range of factors in fruits and vegetables including total phenolics (*6*, *11*, *13*−*15*, *17*), flavonoids or phenolic acids (*6*−*8*, *11*−*13*, *17*, *19*, *20*, *36*), carotenoids (*12*−*14*, *16*), vitamins C or E (*6*, *11*−*14*, *16*, *20*), polyphenol oxidase activity (*10*, *15*, *16*), and antimutagenic activity (*6*, *18*). Although many of these studies demonstrate higher levels of one or more of these compounds in organic produce, several also show variable or inconsistent effects. Drawing conclusions regarding the influence of crop management practices on nutrient quality from this data set is difficult for many reasons. For example, many comparisons were made using samples taken from unmatched farms experiencing different environmental conditions and pressures (*7*−*12*, *19*, *20*). Others were performed using organic and conventional plots located on the same farm (*6*, *13*, *15*−*17*), in glasshouses (*14*), or in plastic tunnels (*12*) where photosynthesis could be a limiting factor. The study of Verberic et al. is difficult to interpret because different cultivars of apples were compared in the organic and integrated cropping systems (*8*). In the study by Young et al., organic and conventional fertilizer regimens provided equivalent rates of nitrogen to the crops, and as the CNB model predicts, little difference was found in the composition of flavonoids between the systems with the exception of the organic pac choi plants, which experienced greater insect pressure (*17*). Most of these studies represent only one seasonal harvest, give little information on preplanting conditions (e.g., tillage, cover-crop, previous crop, etc.), and provide almost no information on the length of time the organic fields were under organic cultivation prior to the study. Additionally, some studies involve fruits (*8*, *10*, *11*−*16*, *18*−*20*), whereas others investigate vegetables (*7*, *9*, *17*).

Several of these studies demonstrate variability in the responses of individual phytochemicals (*12*−*17*). However, variability is expected as many of the phytochemicals compared have different biosynthetic pathways and endogenous functions and therefore would respond differently to agronomic and/or environmental pressures. For example, Caris-Veyrat et al. found higher levels of vitamin C and polyphenols in organic tomatoes grown in plastic tunnels, whereas dry matter levels of lycopene and naringenin were not statistically different (*12*). Similarly, studies have shown that organic cultivation had no consistent effect on the levels of phenolic compounds in strawberries compared to conventional cultivation (*6*, *19*). For example, organic Cavendish strawberries had statistically significant higher levels of total phenolics (TP) (16%) and ellagic acid (55%) than conventionally grown Cavendish strawberries, whereas the conventionally grown Honeoye variety had significantly higher TP (14%) and ellagic acid (10%) than the organically grown fruit (*6*). In the Finnish study (*19*), six varieties of strawberries were compared across 17 different farms in eastern Finland, with three varieties being grown organically. As environmental factors (e.g., location, soil type, etc.) were not controlled, inconsistent effects could be expected.

Contemporary knowledge of the effect, relative importance, and synergy of food constituents in the prevention of chronic disease and human health is very limited. In general, epidemiological studies indicate that increasing the total intake of fruits and vegetables in the Western diet is associated with a decreased risk of cardiovascular disease. However, differences in the concentration of individual components in foods cannot yet be used to draw conclusions regarding the effect of crop management on nutritional quality because biochemical mechanisms for individual components are not elucidated. Nevertheless, understanding relationships between crop management and food composition will be increasingly important as the relative significance and role of different food constituents in human health become better known.

The dynamic nature of agriculture makes adequately controlled comparisons of agronomic systems free from confounding influences experimentally challenging. The goal of the present study was to compare the content of antioxidant microconstituents and several quality characteristics in two

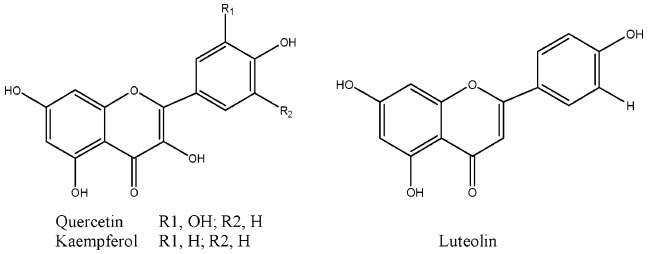

Figure 1. Structures for the flavonoid aglycone quercetin, kaemferol, and luteolin.

varieties of tomatoes (*Lycopersicon esculentum* L. cv. Ropreco and Burbank) and two varieties of bell peppers (*Capsicum annuum* L. cv. California Wonder and Excalibur) grown under organic and conventional conditions in a model system over a 3-year period. The levels of total phenolics, percent soluble solids, ascorbic acid, and the flavonoid aglycones of quercetin, kaempferol, and luteolin (peppers only) were monitored in fruit collected between 2003 and 2005. Structures for these flavonoids can be found in **Figure 1**.

MATERIALS AND METHODS

Chemicals. Acetone, sodium carbonate, metaphosphoric acid, methanol, and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). Folin reagent was purchased from the Sigma Chemical Co. (St. Louis, MO). *tert*-Butyl hydroquinone, trifluoroacetic acid (TFA), morin hydrate (3,5,7,2′,4′-pentahydroxyflavone; 95%), and quercetin dihydrate (3,5,7,3′,4′-pentahydroxyflavone; 99%) were from the Aldrich Chemical Co. (Milwaukee, WI). Luteolin (5,7,3′,4′-tetrahydroxyflavone) and kaempferol (3,5,7,4′-tetrahydroxyflavone) were purchased from the Indofine Chemical Co. (Hillsborough, NJ). Reagent grade, bacteria-free water was generated by a Barnstead E-pure four-module deionization system (Dubuque, IA).

Tomato and Pepper Cultivation. Tomato seeds (*L. esculentum* cv. 'Burbank' and 'Ropreco') and bell pepper seeds (*C. annuum* cv. 'California Wonder' and 'Excalibur') were provided by Seeds of Change (Santa Fe, NM). The seeds were planted in plastic seedling flats with organic or conventional soils. Seedlings were watered side-by-side in a greenhouse until transplantation to the organic and conventional fields. The certified organic and conventional fields were located 107 m apart from each other and were supplied by the same irrigation system. The organic fields were certified in 2003, yet were under organic management since 1978. Both plots were in mixed vegetable production (organic or conventional) for many years prior to the start of the experiment. The soils in both fields are classified as Reiff very fine sandy loam soil. For each species and in each field, there were six subplots, and each variety was randomly assigned to three subplots. Subplots were six rows wide and seven plants long with sampling restricted to the 20 nonperimeter plants in each subplot. All plants were grown in an identical format using matched drip irrigation in each row. Perimeter plants (outer row) were excluded from sampling.

Conventional tomato and bell pepper fields were fertilized prior to transplanting with 18, 54, and 13 kg/ha of mineralized nitrogen (N), phosphorus (P), and potassium (K), respectively. Additionally, conventional crops received applications of synthetic fertilizer nitrogen, ranging from 90 to 202 kg/ha for tomatoes and from 135 to 242 kg/ha for peppers. Organic fields received tilled Woolypod vetch (*Vicia dasycarpa*) cover crop with an average of 135 kg of N/ha (2003), no cover crop (2004), and lana vetch, Magnus pea, and Bell bean cover crop for 124 kg of N/ha (2005). Fields were treated with dairy manure compost (2003−2004) or California Organic Fertilizer (12−5−1) in 2004−2005 prior to transplanting for a total nitrogen addition of 225 kg of N/ha. Peppers received 135 kg of N/ha as blood meal during the growing season in 2003. Cover crops were seeded the previous fall, grew through winter, and in the spring were mowed and tilled into the soil with a disk several weeks prior to transplanting the cast crops. In 2003, all peppers were supplemented with 472 kg/ha gypsum. In 2004 and 2005, 1000 lb of gypsum was added to all fields prior to planting, and near harvest, an organic-compliant solubilized gypsum product was added through irrigation lines. Similarly, an organic-compliant lime spray was applied to all tomatoes for sunburn protection in 2003 and 2004.

Conventional crops in 2003 received Pyrellin in the greenhouse and permethrin in the fields to control aphids. Diazinon was used for insect control in 2003, 2004, and 2005 on peppers and in 2004 on tomatoes. Sulfur (active ingredient applied as a dust) was used in 2005 to combat russet mites in the tomatoes. The herbicide Devrinol was used in conventional fields after transplanting in 2004 and 2005. All pesticides were applied by using a manual spray tank.

Preparation of Plant Materials. Tomato plants are determinant. Fruits were picked when >95% of the fruit within the plot were judged to be red, upon visual inspection by an experienced individual. This is the most common means of determining harvest of California tomatoes. A sample (>3 kg) of fruit was harvested from each plant (with the exception of 2003 in which the whole plant was harvested). In all years, after harvest, tomatoes were sorted for freedom from defects and color uniformity to be "red" as defined by the USDA Standards for Grade of Fresh Tomatoes (7 CFR 51). Pepper samples were harvested after the first round of mature peppers had been thinned. On the specified dates, mature green peppers defined as being free from defect and yellow or red coloration were harvested. Composite samples were composed of >10 peppers each. Fruits were stored at 10−15 °C for a period of less than 4 days (2003) or 1 day (2004 and 2005) prior to washing and selection for freedom from defects and color uniformity. Fruit was sliced (FP150, Horbart Corp.), vacuum packaged (Ultravac 225, Koch Equipment), and frozen using a blast freezer (−40 °C). Samples were stored at −80 °C prior to analysis. Tomatoes were harvested on July 17−18, 2003, August 18, 2004, and August 30, 2005. Peppers were harvested on July 31, 2003, August 18, 2004, and September 30, 2005. To determine in-field variability (2003), fruits from three individual randomly selected primary plants within each subplot were combined and analyzed separately. In 2004 and 2005, fruits from three individual randomly selected primary plants within each subplot were combined to form a composite sample for analysis.

Soil, Temperature, and Solar Radiation Measurements. Randomly selected soil samples were taken from each subplot prior to planting, at midgrowth, and after harvest. Soil analyses (i.e., phosphorus, potassium, magnesium, calcium, sodium, hydrogen, pH, cation exchange capacity, soluble salts, nitrate, ammonium, total Kjeldahl nitrogen, zinc, manganese, iron, copper, and boron) were performed by A&L Western Agricultural Laboratories (Modesto, CA). Active and total bacterial and fungal biomass as well as hyphal diameter, protozoa, and specific nematode populations were monitored by Soil Foodweb, Inc. (Corvallis, OR). Daily maximum atmospheric temperature readings and net solar radiation calculations for Davis, CA, were acquired from the California Irrigation Management Info Server (CIMIS).

Percent Soluble Solids, Hunter Color, and Total Phenolics. Frozen samples were defrosted for 30 min and homogenized on high for 30 s (Waring Products, Inc., Torrington, CT). The percent soluble solids was determined using an RFM-80 temperature correcting refractometer (Bellingham + Stanley, Atlanta, GA), and results are expressed as °Brix. Hunter color values, expressed in the terms *L*, *a*, and *b*, were evaluated using a Hunter LabScan 5100 Colorimeter with Universal Software (Hunter Associates Laboratory, Inc.). Total phenolics were measured in 80% acetone extracts using a modified method of Singleton and Rossi (*37*) and corrected for contributions of ascorbic acid and reducing sugars as described in Ough and Amerine and Asami et al. (*11*, *38*).

Ascorbic Acid. The ascorbic acid content of the 2003 samples was measured using a Hewlett-Packard 1050 HPLC (Agilent, Palo Alto, CA). Breifly, a 50 g sample of frozen tomato was homogenized for 30 s in 100 mL of 2.5% metaphosphoric acid. The sample was centrifuged at 4000 rpm for 10 min and filtered through cheesecloth into a 250 mL volumetric flask. The solution was brought to volume, and a portion was filtered using a 25 μm syringe filter (Waters) prior to injection on the HPLC. Ascorbic acid was resolved using an isocratic method composed of 2% KH_3PO_4 at 0.5 mL/min on a 250 × 4.6 mm i.d., 5 μm Zorbax XDB-C18 (Agilent, Palo Alto, CA) equipped with a 12.5 × 4.6 mm i.d. 5 μm Zorbax XDB-C18 guard column, and monitoring was performed at 245 nm (*39*). In 2004 and 2005, HPLC analyses were

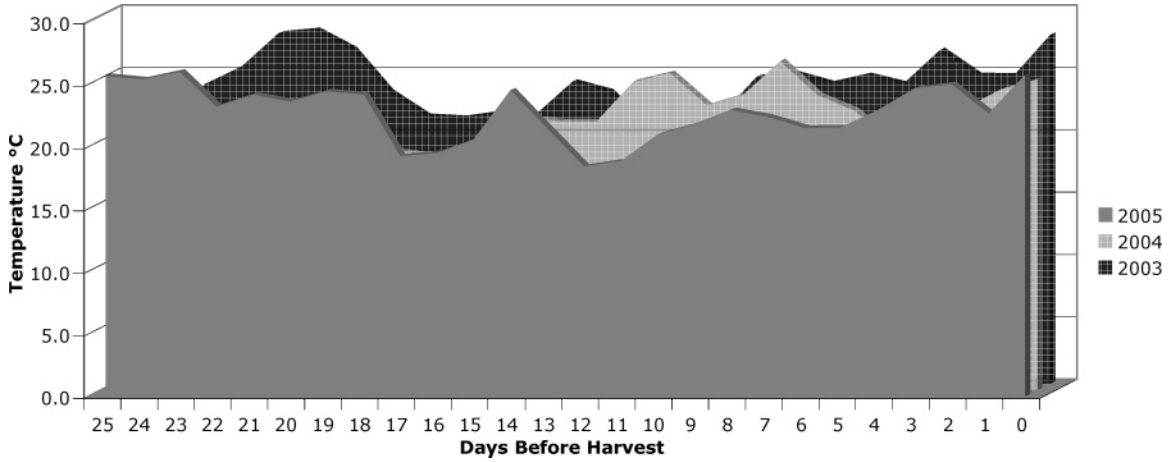

Figure 2. Average daily temperature prior to tomato harvest in 2003–2005.

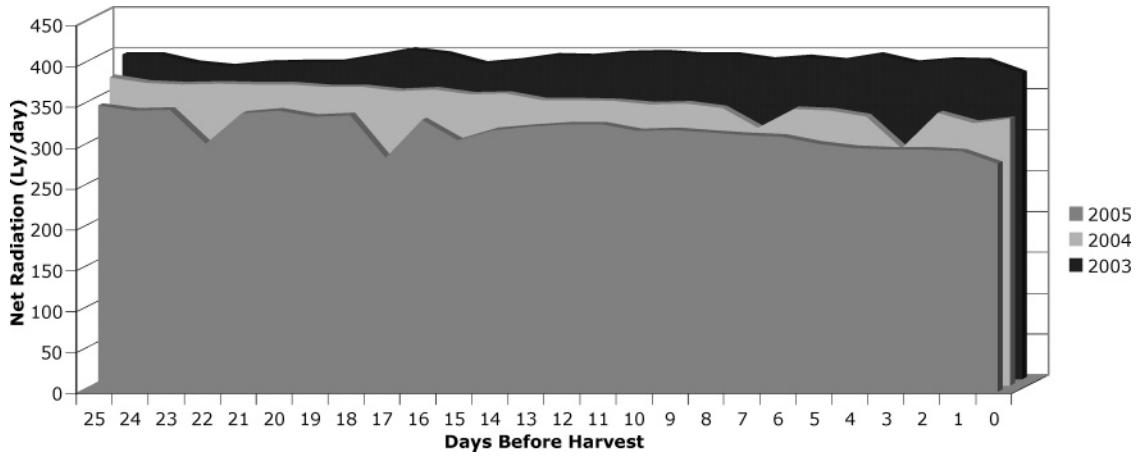

Figure 3. Daily net solar radiation prior to tomato harvest in 2003–2005. Langley (Ly) is a measure of solar radiation equal to 1 calorie per square centimeter.

performed using the same system with a slight modification to the mobile phase (0.05 M KH_3PO_4 adjusted to pH 2.6) and a flow rate of 1.0 mL/min (*40*). Quantification was achieved using ascorbic acid as an external standard.

Percent Solid Matter. Frozen material (tomato and pepper) was weighed and lyophilized to dryness (3−6 days) in a darkened chamber, and the percent solid matter was calculated from the ratio of wet material to dry. The resulting dry matter was stored at −80 °C prior to flavonoid analysis. Total phenolics and ascorbic acid measurements were made on a fresh weight basis (FWB), whereas flavonoid measurements were made on a dry weight basis (DWB). The annual average solids content of each cultivar of tomato and pepper in each agricultural treatment was used for DWB and FWB conversions.

Flavonoid Analysis. Flavonoid analysis followed the method of Merken and Beecher (*41*). Briefly, 1 g of pestle-pulverized, lyophilized tomato or bell pepper was combined with 100 mL of 1.6 g/L *tert*-butyl hydroquinone, 1.2 N hydrochloric acid, and 50% methanol (MeOH). To this mixture was added 0.5 mL of a 1 mg/mL standard of either luteolin (tomatoes) or morin (peppers) as an internal standard. Internal standard recovery was 92−93% for both morin and luteolin. Samples were refluxed for 4 h at 100 °C in 250 mL round-bottom flasks. Timed-hydrolysis studies, under the conditions of our system, resulted in <10% loss of quercetin between 2 and 4 h and a >25% increase in kaempferol levels. A 4 h reflux time in 1.2 N hydrochloric acid was therefore used as the optimal condition for maximal recoveries of quercetin, kaempferol, and luteolin (in peppers). An aliquot was removed, diluted 50:50 v/v with MeOH (200 μL), and filtered through a 0.5 mL 25 μm MC Ultrafree-MC filter (Millipore, Bedford, MA). Flavonoids were separated using a Hewlett-Packard 1090 HPLC equipped a variable wavelength diode-array detector and Chemstation LC 3D rev. A.08.03 software (Agilent, Palo Alto, CA) monitoring 370 nm. Reversed phase HPLC was performed using a 250 × 4.6 mm i.d., 5 μm Zorbax XDB-C18 column and a 12.5 × 4.6 mm i.d., 5 μm Zorbax XDB-C18 precolumn (Agilent). The mobile phase consisted of 0.05% TFA in water (solvent A), 0.05% TFA in MeOH (solvent B), and 0.05% TFA in acetonitrile (solvent C). Separations were effected by a series of linear gradients using a flow rate of 1.0 mL/min as follows: 90−85% A, 6−9% B, 4−6% C, 0−5 min; 85−71% A, 9−17.4% B, 6−11.6% C, 5−30 min; 71−0% A, 17.4−85% B, 11.6−15% C, 30−60 min. The linear ranges of quantitation for quercetin, luteolin, and kaempferol were 0.5−10, 0.25−10, and 0.1−10 μg/mL, respectively.

Statistical Analysis. Data were analyzed using SAS software version 9.1 (SAS Institute, Cary, NC). Specifically, a two-way analysis of variance (ANOVA) with main effects (cropping system, cultivar, and year) and all two- and three-way interactions were evaluated using the General Linear Model (PROC GLM) procedure. Fisher's protected least significant difference (LSD) was used for mean separation of cultivation method and cultivar by individual year ($P < 0.05$). For **Tables 2** and **4** the average values are reported as the average ± the standard deviation.

RESULTS

Soil samples, analyzed during the 3-year period, demonstrate that there were no gross differences or deficiencies in soil quality between the two cropping systems (data not shown). Weather data collected by CIMIS demonstrated higher daily maximum temperatures (**Figure 2**) and net solar radiation (**Figure 3**) during the 25-day period prior to harvesting in 2003 as compared to 2004 and 2005.

The *P* values of main and interaction effects for tomatoes are given in **Table 1**. The main factor year (Y) had the greatest

Table 1. Anova for Cropping System, Cultivar, and Growing Year for the 3-year comparison (2003–2005) of Ropreco and Burbank tomatoes

analysis	cropping system (CS)	cultivar (C)	year (Y)	CS × C	CS × Y	C × Y	CS × C × Y
soluble solids	<0.0001[a]	0.5407	0.0049	0.1056	<0.0001	0.0315	0.4922
quercetin (DWB)	0.0671	<0.0001	<0.0001	0.4487	<0.0001	<0.0001	0.3650
quercetin (FWB)	0.0022	0.0067	<0.0001	0.5650	<0.0001	0.0066	0.5891
kaempferol (DWB)	0.1259	0.0007	<0.0001	0.9569	0.433	0.4108	0.8913
kaempferol (FWB)	0.0017	0.0978	<0.0001	0.9529	0.884	0.0042	0.4972
total phenolics (FWB)	0.2779	0.1470	0.2271	0.4498	0.0011	0.7302	0.1416
total phenolics (DWB)	0.8096	0.0546	0.3186	0.5365	0.4471	0.0338	0.0003
ascorbic acid (FWB)	0.0052	0.0416	<0.0001	0.3193	0.0005	0.1369	0.9835
ascorbic acid (DWB)	0.2439	0.0006	<0.0001	0.1920	0.0159	0.9222	0.5200
Hunter *a*/*b*	0.0836	<0.0001	<0.0001	0.4606	0.7571	0.0824	0.6133

[a] Units expressed as *P* values for statistical significance.

Table 2. Soluble Solids, Antioxidant Microconstituents, and Hunter *a*/*b* Values in Burbank and Ropreco Tomatoes Grown from 2003 to 2005

		Burbank cultivar			Ropreco cultivar		
analysis		conventional	organic	% increase[a]	conventional	organic	% increase[a]
soluble solids (°Brix)	2003	4.0 ± 0.2 b[b]	6.0 ± 0.6 a		4.4 ± 0.3 b	5.7 ± 0.5 a	
	2004	4.8 ± 0.2 bc	5.4 ± 0.2 a		4.5 ± 0.1 c	5.0 ± 0.4 ab	
	2005	5.2 ± 0.3 b	5.1 ± 0.4 b		5.9 ± 0.1 a	5.4 ± 0.3 ab	
	av	*4.7* ± *0.6* b	*5.5* ± *0.5* a	**18**	*4.9* ± *0.8* b	*5.4* ± *0.4* a	**9**
quercetin (mg/100 g, DWB)	2003	68.7 ± 5.8 b	109.0 ± 27.4 a		37.7 ± 1.3 c	60.7 ± 6.4 bc	
	2004	21.1 ± 2.8 ab	17.6 ± 4.1 b		22.1 ± 1.4 ab	25.1 ± 2.3 a	
	2005	58.4 ± 14.3 ab	48.5 ± 11.0 b		47.4 ± 2.3 ab	33.0 ± 2.7 a	
	av	*49.4* ± *25.0* a	*58.4* ± *46.5* a	18	*35.7* ± *12.8* b	*39.6* ± *18.7* b	11
quercetin (mg/100 g, FWB)	2003	3.43 ± 0.29 bc	6.30 ± 1.58 a		2.18 ± 0.08 c	4.55 ± 0.48 b	
	2004	1.18 ± 0.15 bc	1.12 ± 0.26 a		1.15 ± 0.07 c	1.44 ± 0.13 b	
	2005	3.32 ± 0.81 a	2.84 ± 0.65 ab		3.20 ± 0.16 a	2.20 ± 0.18 b	
	av	*2.64* ± *1.27* b	*3.42* ± *2.64* a	**29**	*2.18* ± *1.03* b	*2.73* ± *1.62* b	25
kaempferol (mg/100 g, DWB)	2003	18.8 ± 2.3 b	19.0 ± 5.4 a		15.4 ± 5.0 b	17.0 ± 5.7 b	
	2004	27.0 ± 2.1 a	28.0 ± 1.9 a		21.6 ± 0.6 b	21.5 ± 0.4 b	
	2005	28.4 ± 0.7 a	31.9 ± 0.9 a		25.0 ± 2.9 b	28.6 ± 2.5 b	
	av	*24.7* ± *5.2* ab	*26.3* ± *6.6* a	6	*20.7* ± *4.9* c	*22.4* ± *5.8* bc	8
kaempferol (mg/100 g, FWB)	2003	0.94 ± 0.11 ab	1.10 ± 0.31 a		0.89 ± 0.29 c	1.28 ± 0.43 bc	
	2004	1.51 ± 0.12 b	1.78 ± 0.12 a		1.12 ± 0.03 c	1.23 ± 0.02 c	
	2005	1.61 ± 0.04 b	1.87 ± 0.05 a		1.69 ± 0.20 ab	1.91 ± 0.17 a	
	av	*1.35* ± *0.36* bc	*1.58* ± *0.42* a	**17**	*1.23* ± *0.41* c	*1.47* ± *0.38* ab	**19**
total phenolics (mg/100 g, FWB)	2003	30.7 ± 2.7 c	44.0 ± 7.5 a		34.4 ± 2.0 bc	38.1 ± 5.9 ab	
	2004	34.8 ± 2.5 c	36.7 ± 2.1 a		31.6 ± 1.9 bc	32.9 ± 2.5 ab	
	2005	40.6 ± 2.5 c	33.1 ± 7.6 a		37.3 ± 1.8 bc	33.6 ± 1.9 ab	
	av	*35.4* ± *5.0* c	*37.9* ± *5.6* a	7	*34.4* ± *2.9* bc	*34.9* ± *2.8* ab	1
total phenolics (mg/100 g, DWB)	2003	616 ± 54 ab	783 ± 179 a		595 ± 34 ab	502 ± 71 b	
	2004	624 ± 45 ab	578 ± 33 a		606 ± 37 ab	574 ± 44 b	
	2005	715 ± 44 a	564 ± 130 b		552 ± 26 b	744 ± 51 a	
	av	*652* ± *55* a	*642* ± *123* b	–2	*584* ± *29* b	*607* ± *124* a	4
ascorbic acid (mg/100 g, FWB)	2003	13.7 ± 2.0 b	25.7 ± 7.3 a		14.2 ± 1.5 b	23.8 ± 5.6 a	
	2004	16.0 ± 0.9 a	16.3 ± 1.2 a		11.2 ± 1.7 b	9.8 ± 1.3 b	
	2005	22.7 ± 1.9 a	24.2 ± 5.4 a		23.3 ± 0.8 b	22.1 ± 2.4 b	
	av	*17.5* ± *4.7* b	*22.1* ± *5.1* a	**26**	*16.2* ± *6.3* b	*18.6* ± *7.6* b	14
ascorbic acid (mg/100 g, DWB)	2003	275 ± 40 b	444 ± 126 a		246 ± 25 b	296 ± 110 b	
	2004	288 ± 17 a	257 ± 19 ab		215 ± 33 bc	170 ± 22 c	
	2005	400 ± 33 a	413 ± 91 ab		344 ± 12 bc	330 ± 35 c	
	av	*321* ± *69* ab	*371* ± *100* a	16	*268* ± *67* b	*265* ± *84* b	–1
Hunter *a*/*b*	2003	1.11 ± 0.11 b	1.07 ± 0.28 b		1.64 ± 0.12 a	1.40 ± 0.18 ab	
	2004	2.41 ± 0.16 b	2.33 ± 0.15 b		2.53 ± 0.12 a	2.50 ± 0.06 ab	
	2005	2.27 ± 0.22 b	2.22 ± 0.16 b		2.52 ± 0.06 a	2.39 ± 0.01 ab	
	av	*1.93* ± *0.71* b	*1.87* ± *0.70* b	–3	*2.23* ± *0.51* a	*2.10* ± *0.61* a	–6

[a] Boldface entries indicates significant differences. [b] Different letters within rows indicate statistical differences by protected LSD ($P < 0.05$).

Table 3. Anova for Cropping System, Cultivar, and Growing Year for the 3-Year Comparison (2003–2005) of California Wonder and Excalibur Bell Peppers

analysis	cropping system (CS)	cultivar (C)	year (Y)	CS × C	CS × Y	C × Y	CS × C × Y
solid matter	0.7734[a]	0.6489	<0.0001	0.3406	0.1897	0.5807	0.7959
soluble solids	0.3415	0.5444	0.0002	0.4929	0.0121	0.6648	0.6819
quercetin (DWB)	0.9915	0.9566	0.5351	0.8114	0.0706	0.8966	0.8697
quercetin (FWB)	0.9594	0.7426	0.0324	0.8039	0.1695	0.8049	0.8547
luteolin (DWB)	0.2718	0.5423	0.6424	0.6438	0.3715	0.1374	0.8807
luteolin (FWB)	0.1328	0.5064	<0.0001	0.6376	0.2848	0.1638	0.736
kaempferol (DWB)	0.9824	0.0041	0.0001	0.3896	0.1481	0.1268	0.9498
kaempferol (FWB)	0.5416	0.0048	<0.0001	0.5197	0.0086	0.0243	0.5146
total phenolics (FWB)	0.7082	0.6138	0.1556	0.0950	0.9318	0.2516	0.7662
total phenolics (DWB)	0.7394	0.8439	<0.0001	0.0641	0.5791	0.5238	0.5883
ascorbic acid (FWB)	0.5134	0.1666	<0.0001	0.3845	0.0287	0.0249	0.8597
ascorbic acid (DWB)	0.3554	0.1799	<0.0001	0.4736	0.0436	0.022	0.916

[a] Units expressed as *P* values for statistical significance.

Table 4. Interplot Variability (± SD) in Tomatoes (2003)

		organic		conventional	
analysis	subplot	Burbank	Ropreco	Burbank	Ropreco
soluble solids (°Brix)	1	6.2 ± 0.3	5.8 ± 0.3	3.9 ± 0.0	4.2 ± 0.9
	2	5.3 ± 0.6	6.1 ± 0.3	4.2 ± 0.1	4.2 ± 0.3
	3	6.4 ± 1.2	5.2 ± 0.4	4.0 ± 0.3	4.8 ± 0.2
quercetin (mg/100 g, DWB)	1	139.8 ± 36.7	55.8 ± 14.6	62.0 ± 26.5	37.0 ± 3.2
	2	87.4 ± 23.0	67.9 ± 8.9	72.0 ± 34.6	39.2 ± 6.7
	3	99.7 ± 53.3	58.3 ± 26.2	72.3 ± 19.5	36.8 ± 4.8
kaempferol (mg/100 g, DWB)	1	15.6 ± 7.9	11.4 ± 10.5	16.4 ± 3.1	18.2 ± 2.2
	2	16.3 ± 6.5	16.9 ± 6.0	20.9 ± 2.4	18.5 ± 2.3
	3	25.2 ± 5.1	22.8 ± 1.4	19.2 ± 0.9	9.6 ± 9.1
total phenolics (mg/100 g, FWB)	1	73.1 ± 7.2	50.2 ± 2.2	42.4 ± 3.1	44.5 ± 3.0
	2	52.7 ± 10.6	62.8 ± 2.1	42.2 ± 4.1	44.5 ± 1.0
	3	67.5 ± 16.3	52.4 ± 5.8	39.4 ± 6.8	47.5 ± 3.3
ascorbic acid (mg/100 g, FWB)	1	32.3 ± 4.4	25.7 ± 2.5	11.7 ± 6.3	12.9 ± 3.6
	2	26.9 ± 1.9	28.1 ± 1.4	13.8 ± 6.0	15.8 ± 2.2
	3	17.9 ± 0.8	17.5 ± 2.3	15.6 ± 5.9	14.0 ± 6.4

influence on the content of percent soluble solids, quercetin, kaempferol, and ascorbic acid and the Hunter *a*/*b* ratio. Cultivar (C) was significant ($P < 0.05$) for quercetin, kaempferol (DWB), ascorbic acid, and the Hunter *a*/*b* ratio. The cropping system (CS) was significant for percent soluble solids, quercetin (FWB), kaempferol (FWB), and ascorbic acid (FWB). No interactive effects were observed for cropping system and cultivar (CS × C). Interactive effects between cropping system and year (CS × Y) were significant for percent soluble solids, quercetin, TP (DWB), and ascorbic acid. Interactive effects between cultivar and year (C × Y) were significant for percent soluble solids, quercetin, TP (FWB) and ascorbic acid.

A LSD comparison of the statistical differences ($P < 0.05$) of the annual means and of the 3-year averages for tomatoes is given in **Table 2**. A comparison of the 3-year averages in Burbank tomatoes indicates soluble solids, quercetin (DWB), quercetin (FWB), kaempferol (FWB), kaempferol (DWB), TP (FWB), ascorbic acid (FWB), and ascorbic acid (DWB) were 18, 18, 29, 6, 17, 7, 26, and 16% higher in organic tomatoes. Whereas total phenolics (DWB) and Hunter *a*/*b* ratio were 2 and 3%, respectively, higher in the conventional Burbank tomatoes (**Table 2**). A similar trend was apparent in the Ropreco cultivar, for which levels of soluble solids, quercetin (DWB), quercetin (FWB), kaempferol (FWB), kaempferol (DWB), TP (FWB), TP (DWB), ascorbic acid (FWB), and ascorbic acid (FWB) were 9, 11, 25, 8, 19, 1, 4, and 14%, respectively, higher in organic tomatoes, whereas ascorbic acid (DWB) and the Hunter *a*/*b* ratio were 1 and 6%, respectively, higher in the conventional Ropreco tomatoes.

Burbank tomatoes generally had higher levels of quercetin, kaempferol, TP, and ascorbic acid as compared to Ropreco tomatoes.

The *P* values resulting from an ANOVA of the main and interaction effects for peppers are given in **Table 3**. The main factor year (Y) influenced the content of solid matter, soluble solids, quercetin (FWB), kaempferol, TP (DWB), and ascorbic acid. Cultivar was significant for only kaempferol. No main factor effect for cropping system was found.

DISCUSSION

The ascorbic acid levels measured in Burbank and Ropreco tomatoes are similar to levels previously reported (*42*). Ascorbic acid levels in bell peppers are lower than those reported by Lee and Kader; however, they are similar to levels reported in fresh-cut bell peppers (*42*, *43*). The levels of quercetin are similar to levels previously reported for tomatoes (*44*, *45*), whereas the levels of kaempferol (~2.74 mg/100 g of tomatoes) and luteolin (~1.97 mg/100 g of peppers) are higher than values reported (0.07 and 0.69 mg/100 g for kaempferol and luteolin, respectively) in the USDA flavonoid database (*31*). Higher levels of these flavonoids likely reflect longer refluxing times or different cultivars. The concentration of acid and the reflux time influence the hydrolysis rates of flavonoid glycosides (*46*). Optimization of our methodology indicated that a 4-h refluxing (1.2 M HCl) was required to obtain maximal hydrolysis of glycosides of both quercetin and kaempferol in tomatoes and quercetin and luteolin in peppers. Nearly complete hydrolysis of quercetin is achieved

Table 5. Soluble Solids, Solid Matter, and Antioxidant Microconstituents in California Wonder and Excalibur Bell Peppers Grown from 2003 to 2005

		California Wonder cultivar		Excalibur cultivar	
analysis		conventional	organic	conventional	organic
solid matter (%)	2003	11.71 ± 0.59	11.49 ± 0.56	11.87 ± 1.17	11.40 ± 0.12
	2004	6.42 ± 0.11	7.07 ± 0.21	6.41 ± 0.35	6.47 ± 0.30
	2005	6.98 ± 0.05	6.84 ± 0.28	7.07 ± 0.52	6.85 ± 0.02
	av	*8.37 ± 0.25*	*8.47 ± 0.35*	*8.45 ± 0.68*	*8.24 ± 0.15*
soluble solids (%)	2003	5.0 ± 0.1	4.6 ± 0.2	4.8 ± 0.6	4.7 ± 0.1
	2004	3.9 ± 0.2	4.5 ± 0.8	3.4 ± 0.2	4.5 ± 0.8
	2005	5.0 ± 0.3	4.8 ± 0.0	5.1 ± 0.2	4.8 ± 0.2
	av	*4.6 ± 0.2*	*4.6 ± 0.3*	*4.4 ± 0.3*	*4.7 ± 0.4*
quercetin (mg/100 g, DWB)	2003	36.7 ± 0.7	38.4 ± 18.5	32.3 ± 15.7	33.6 ± 10.2
	2004	48.5 ± 22.7	35.8 ± 21.1	56.3 ± 28.3	31.8 ± 13.3
	2005	27.6 ± 8.1	43.2 ± 18.4	27.7 ± 17.1	46.5 ± 19.8
	av	*37.6 ± 10.5*	*39.1 ± 19.3*	*38.8 ± 20.4*	*37.3 ± 14.4*
quercetin (mg/100 g, FWB)	2003	4.4 ± 0.1	4.4 ± 2.1	3.8 ± 1.9	3.8 ± 1.2
	2004	3.1 ± 1.5	2.5 ± 1.5	3.6 ± 1.8	2.1 ± 0.9
	2005	1.92 ± 0.57	2.96 ± 1.26	1.96 ± 1.21	3.18 ± 1.36
	av	*3.2 ± 0.7*	*3.3 ± 1.6*	*3.1 ± 1.6*	*3.0 ± 1.1*
luteolin (mg/100 g, DWB)	2003	24.7 ± 1.3	20.4 ± 2.8	27.1 ± 5.5	25.1 ± 2.1
	2004	23.4 ± 5.6	19.5 ± 2.2	27.3 ± 9.5	22.8 ± 2.3
	2005	24.3 ± 6.8	24.1 ± 2.9	18.8 ± 2.9	21.8 ± 6.4
	av	*24.1 ± 4.6*	*21.3 ± 2.6*	*24.4 ± 6.0*	*23.2 ± 3.6*
luteolin (mg/100 g, FWB)	2003	2.98 ± 0.15	2.35 ± 0.32	3.18 ± 0.64	2.87 ± 0.24
	2004	1.50 ± 0.36	1.38 ± 0.15	1.75 ± 0.61	1.47 ± 0.15
	2005	1.70 ± 0.47	1.65 ± 0.20	1.33 ± 0.20	1.49 ± 0.44
	av	*2.06 ± 0.33*	*1.79 ± 0.22*	*2.09 ± 0.48*	*1.94 ± 0.28*
kaempferol (mg/100 g, DWB)	2003	14.0 ± 0.4 ab[a]	12.9 ± 0.2 b	15.2 ± 1.0 a	15.3 ± 0.3 a
	2004	16.3 ± 1.5 ab	17.2 ± 2.8 b	16.0 ± 0.4 a	18.0 ± 1.5 a
	2005	16.7 ± 1.6 ab	15.6 ± 0.9 b	19.3 ± 2.3 a	18.7 ± 1.3 a
	av	*15.7 ± 1.2* ab	*15.2 ± 1.3* b	*16.8 ± 1.2* ab	*17.3 ± 1.0* a
kaempferol (mg/100 g, FWB)	2003	1.70 ± 0.05 ab	1.48 ± 0.03 b	1.79 ± 0.12 a	1.74 ± 0.04 a
	2004	1.04 ± 0.09 ab	1.22 ± 0.20 b	1.03 ± 0.03 a	1.16 ± 0.10 a
	2005	1.17 ± 0.11 ab	1.07 ± 0.06 b	1.37 ± 0.16 a	1.28 ± 0.09 a
	av	*1.30 ± 0.08* b	*1.26 ± 0.10* b	*1.40 ± 0.10* a	*1.39 ± 0.08* a
total phenolics (mg/100 g, FWB)	2003	71.2 ± 1.0 b	66.0 ± 2.5 b	54.9 ± 8.2 a	63.8 ± 3.0 a
	2004	61.3 ± 6.7 b	53.5 ± 14.5 b	54.1 ± 3.4 a	61.0 ± 7.4 a
	2005	54.4 ± 10.8 b	54.3 ± 18.7 b	56.3 ± 5.7 a	60.8 ± 5.0 a
	av	*62.3 ± 6.2* b	*57.9 ± 11.9* b	*55.1 ± 5.8* a	*61.9 ± 5.1* a
total phenolics (mg/100 g, DWB)	2003	588 ± 8 b	555 ± 21 b	446 ± 67 a	564 ± 26 a
	2004	955 ± 104 b	757 ± 205 b	845 ± 53 a	943 ± 115 a
	2005	780 ± 154 b	794 ± 274 b	796 ± 81 a	888 ± 73 a
	av	*774 ± 89* b	*702 ± 167* b	*696 ± 67* a	*798 ± 71* a
ascorbic acid (mg/100 g, FWB)	2003	31.9 ± 6.3 b	28.5 ± 1.9 b	28.6 ± 16.0 a	23.7 ± 16.6 a
	2004	28.3 ± 29.6 b	62.0 ± 28.9 b	7.7 ± 2.5 a	24.7 ± 5.9 a
	2005	99.7 ± 1.8 b	95.1 ± 7.2 b	114.5 ± 20.8 a	98.7 ± 2.9 a
	av	*53.3 ± 12.6* ab	*61.9 ± 12.7* a	*50.3 ± 13.1* ab	*49.0 ± 8.5* b
ascorbic acid (mg/100 g, DWB)	2003	264 ± 52 ab	248 ± 17 a	243 ± 136 ab	208 ± 145 b
	2004	441 ± 461 ab	877 ± 408 a	120 ± 39 ab	383 ± 92 b
	2005	1428 ± 26 ab	1390 ± 105 a	1619 ± 295 ab	1441 ± 42 b
	av	*711 ± 180* ab	*838 ± 177* a	*661 ± 157* b	*677 ± 93* b

[a] Different letters within rows indicate statistical differences by LSD ($P < 0.05$).

quickly (<2 h); however, it took 4−5 h to achieve nearly complete hydrolysis of kaempferol and luteolin in these samples. In the previous studies, a 2-h refluxing period was used (*44*, *45*).

Levels of soluble solids were higher in both cultivars of organic tomatoes as compared to their conventional counterparts in 2003 and 2004. Soluble solids in the Burbank tomatoes ranged from 4.0 to 5.2 °Brix (conventional) and from 5.1 to 6.0 °Brix (organic). In Ropreco tomatoes, soluble solids ranged from 4.4 to 5.9 °Brix (conventional) and from 5.4 to 5.7 °Brix (organic). On average, organic Burbank tomatoes were 17% higher and Ropreco tomatoes were 10% higher in soluble solids than their conventional counterparts. Higher °Brix values can influence the perceived sweetness and flavor of the tomatoes.

Annual comparisons demonstrate the importance of comparing individual factors in multiple years. For example, the organic

Burbank tomatoes from 2003 had high levels of quercetin (109 ± 27.4 mg/100 g, DWB) as compared to the range of values measured in both organic and conventional Burbank tomatoes (17.6−68.7 mg/100 g, DWB). The high level of quercetin obtained in 2003 for the organic Burbank tomatoes is responsible for the higher 3-year average values as no statistical difference was obtained in 2004 and 2005. This effect was also reflected in the levels of total phenolics measured in the 2003 organic Burbank tomatoes (783 ± 179 mg/100 g, DWB) as compared to the range of values measured in the other Burbank tomatoes (564−715 mg/100 g, DWB). In 2004, the values of quercetin (17.6−25.1 mg/100 g, DWB) were lower than the range of values in both organic and conventional tomatoes (33.0−68.7 mg/100 g, DWB). In Ropreco tomatoes, levels of quercetin (FWB) were statistically higher in 2003 in organic tomatoes and statistically higher in conventional tomatoes in 2005. In-field variability was determined in 2003 on composite samples taken from three individual tomato plants within each subplot. In-field variability was low (**Table 4**) and demonstrated that values are consistent among subplots within a given year.

In tomatoes, sun exposure has been demonstrated to positively correlate with increases in ascorbic acid and quercetin (*42*, *48*). Tomato color correlates with maturity, and the Hunter *a*/*b* ratio, which measures the relative amounts of red (*a*) and yellow (*b*), has been used as an indicator of maturity (*47*). In 2003, tomatoes demonstrated lower Hunter *a*/*b* ratios than in the subsequent years. However, as color was measured on homogenized samples, they likely reflect a difference in the distribution of color inside the tomato as the range of values for soluble solids, which also reflect maturity, did not indicate that these tomatoes were at a different stage of maturity. Tomato color may have been influenced by environmental conditions as the radiation index was higher in 2003 (**Figure 2**) as compared to 2004 and 2005.

Measurements of antioxidant microconstituents and solids in the two cultivars of bell peppers compared in this study indicate that the bell peppers were influenced less by environment and/or cropping system than tomatoes. In a previous study, Flores et al. demonstrated that nitrate fertilization did not influence levels of ascorbic acid, sugars, or total phenolic acids in red peppers (*C. annuum* L.) (*49*). Studies by Cipollini et al. indicate that nitrogen mainly affected glycoalkaloids and total phenolics in the leaves of *S. carolinense*, whereas the fruits maintain a relatively constant composition of these secondary plant metabolites, irrespective of the N supply (*50*). This suggests that compositional changes due to the crop management are likely to be substantially greater in leafy vegetables than in vegetables that are physiologically fruits, such as tomatoes and peppers. These results demonstrate the point that different crops will respond differently to agronomic pressures and nutrient bioavailability and indicate that generalized statements regarding the influence of copping systems on the antioxidant micronutrient composition of fruits and vegetables are not appropriate.

Genotype has the greatest influence on the phytochemical composition of fruits and vegetables. Significant interactions were noted in cultivar comparisons between Burbank and Ropreco tomatoes. On average, the Burbank tomatoes had higher levels of quercetin, kaempferol, total phenolics, and ascorbic acid than the Ropreco cultivar (**Table 2**). Conversely, the Hunter *a*/*b* ratio was lower in the Burbank cultivar as compared to the Ropreco cultivar and may reflect higher levels of carotenoids in this cultivar. In bell peppers, the levels of kaempferol (DWB) were higher in Excalibur peppers as compared to California Wonder peppers (**Table 5**).

The annual variability in levels of flavonoids, total phenolics, and ascorbic acid in the tomatoes compared in this study point to the importance of making multiple-year comparisons to establish the role of exogenous factors such as environment as well as the normal variation within a cultivar. The data are presented on a DWB for analytical comparisons and on a FWB for nutritional comparisons. For example, levels of kaempferol on average are 6% higher in organic Burbanks and 8% higher in organic Ropreco tomatoes on a DWB, whereas levels are 17% higher in organic Burbanks and 19% higher in organic Ropreco tomatoes on a FWB. These differences (FWB) show that on average a 40 g organic tomato would provide as much kaempferol as a 47 g conventional one of the same variety.

Large-scale, multiyear comparisons with carefully defined inputs and variables are still needed to evaluate whether agronomic practices (e.g., the influence of nitrogen source) influence antioxidant microconstituents in specific crops and cultivars. Moreover, to establish if organic foods have any nutritional advantage at the consumer level, multiregional, market basket studies need to be undertaken.

ABBREVIATIONS USED

FWB, fresh weight basis; DWB, dry weight basis; TP, total phenolics; LSD, least significant difference.

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