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Influences of Harvest Date and Location on the Levels of β-Carotene, Ascorbic Acid, Total Phenols, the in Vitro Antioxidant Capacity, and Phenolic Profiles of Five Commercial Varieties of Mango (*Mangifera indica* L.)

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Mango (*Mangifera indica* L.) is a tropical fruit grown worldwide with excellent nutritional value and widely attributed health-promoting properties. Extensive studies have been made of the high concentrations of phenolic antioxidants in mango peels, seeds, and leaves, yet less is known about the phenolic antioxidants of mango fruit pulp. Five varieties of mangoes from four countries were evaluated with multiple harvests over 1 year to compare the β-carotene, ascorbic acid, and total phenolic contents and antioxidant capacities of the fruit pulp and to compare the phenolic profiles of the individual varieties. To minimize ripeness variability, only soft fruit (0.5−1 N compression) with a minimum of 10% soluble solids were used for these measurements. Ascorbic acid ranged from 11 to 134 mg/100 g of pulp puree, and β-carotene varied from 5 to 30 mg/kg among the five varieties. Total phenolic content ranged from 19.5 to 166.7 mg of gallic acid equivalents (GAE)/100 g of puree. The varieties Tommy Atkins, Kent, Keitt, and Haden had similar total phenolic contents, averaging 31.2 ± 7.8 mg GAE/100 g of puree, whereas the variety Ataulfo contained substantially higher values. Similar trends were observed in the DPPH radical scavenging activities among the five varieties. In contrast, the country of origin and harvest dates had far less influence on these parameters. Ataulfo mangoes contained significantly higher amounts of mangiferin and ellagic acid than the other four varieties. Large fruit-to-fruit variations in the concentrations of these compounds occurred within sets of mangoes of the same cultivar with the same harvest location and date.

KEYWORDS: Mangiferin; ellagic acid; gallotannins; tropical fruit; polyphenol; vitamin C; *Mangifera indica* L.; antioxidant; HPLC-MS

INTRODUCTION

Mangoes are a traditional, tropically grown fruit with wide consumer appeal and are now imported into the U.S. on a large scale. Mangoes are a rich source of dietary fiber, vitamin C, and phenolic compounds (*1*−*4*), and there have been numerous studies on the nutritional value and health-promoting properties of this fruit (*5*−*13*). Of particular interest are the high concentrations of phenolic compounds in mango, especially in the peels and kernels. These phenolic compounds include xanthone-*C*-glycosides, gallotannins, benzophenones, flavonol glycosides, 5-alkyl- and 5-alkenylresorcinols, and many other miscellaneous phenols (*1*, *4*, *11*, *14*−*18*), and a number of these classes of compounds represent potentially new, value-added coproducts for this crop. However, in contrast to the peel and kernel, mango pulp is generally depleted of many of these compounds, with the exceptions of high concentrations of gallotannins and other miscellaneous minor-occurring phenols (*4*, *17*). Key interest exists in elaborating the roles of these polyphenolic compounds, and other antioxidants, in the purported health benefits of mango pulp.

The antioxidant capacity of tropical fruit such as mangoes is taking greater importance in evaluations of fruit quality and in marketing for human health benefits. Although the antioxidants of mangoes have been the subjects of earlier studies, complete information is still lacking about the influences of cultivar type, production practices, and harvest locations and dates on antioxidant capacities in mango pulp (*4*, *16*, *19*−*22*). In this study, five of the main varieties imported into the United States, harvested in four countries and at different dates, were evaluated over 1 year of harvests to compare the β-carotene, ascorbic acid, total phenolic, and in vitro antioxidant capacities of these fruits and to compare the phenolic profiles of the individual varieties.

MATERIALS AND METHODS

Mango Samples. Fruit of the mango cultivars Kent, Tommy Atkins, and Keitt were obtained from commercial orchards in Mexico, Peru, Brazil, and Ecuador during 2006−2007. Haden mangoes were obtained

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only from Peru and Mexico, and Ataulfo mangoes were obtained solely from Mexico. The four harvests of Ataulfo mangoes were made at different orchards throughout Mexico. For the other mango varieties, the same orchards were used for the multiple harvests in each country. Harvests consisted of sampling from 20 trees within an orchard for each harvest date. Fruit were shipped at the hard green stage to Lane, OK, to minimize effects of shipping on final fruit quality and subsequently brought to full ripeness by holding at 20−23 °C and 90% RH. Flesh firmness was determined by removing peel on one shoulder (about 3 cm^2) of each of 20−40 fruit, and the compression was determined with a force gauge (6 kg) fitted with an 8 mm flat compression head. Fruit were then completely peeled and sliced, the kernel was removed, and slices were placed in a weigh boat. Two slices (50 g) were placed in small freezer bags and stored at −80 °C for ascorbic acid analysis. Juice from the slices was placed on a digital refractometer (Atago, PR100) to determine soluble solids content. The remaining slices were pureed with a blender and then with a homogenizer (Brinkmann, Inc.) for 30 s at medium speed. One aliquot of puree (50 mL) was immediately frozen at −80 °C for phenolic analysis, and another aliquot was used to measure pH and then was frozen at −80 °C for carotenoid analysis. To minimize fruit maturity as a variable, the criteria of 10−13% soluble solids content (SSC), a pH of 3.8−4.3, and a firmness of < 1 N were used to select a subset of 10−12 fruit for each variety and harvest. These fruit were analyzed individually to determine β-carotene concentrations, in vitro antioxidant capacity (DPPH radical scavenging), and the total phenolic (Folin−Ciocalteu) and ascorbic acid contents, as well as to characterize the phenolic compound profiles of each variety harvested at different locations and with different harvest dates.

β-Carotene Extraction. About 0.6−1 g of thawed mango puree was weighed in brown glass vials, mixed with 10 mL of 90% ethanol, and sonicated for 10 min. Acetone (5 mL), hexane (10 mL), and 0.02 g BHT were added, and the mixture was placed on an orbital shaker for 15 min at medium speed (about 2 rpm). Subsequently, 3 mL of deionized water was added and the mixture was shaken for 5 min and then allowed to settle for 5 min. The hexane layer (5 mL) of each sample was transferred to a corresponding vial containing 5 mL of methanol with 10% KOH. Vials were capped and placed on an orbital shaker for 3 h. A 3 mL portion of the hexane layer was transferred to another vial containing 10 mL of deionized water, the mixture was shaken, and the process was repeated twice to remove KOH. Duplicate samples of the hexane layer were filtered using 0.45 μm PTFE syringe filters (Daigger, Vernon Hills, IL) into 2 mL amber crimp-top vials (Daigger, Vernon Hills, IL).

Ascorbic Acid Extraction. Mango slices were placed into 5% metaphosphoric acid and ground with a mortar and pestle while partially frozen. Purees were centrifuged at 7000 rpm and 4 °C for 15 min. Supernatants were held on ice and used to determine total ascorbic acid.

Phenolic Compounds Extractions. Three replicates of 20 g of mango pulp puree from individual fruit were accurately weighed and homogenized with 100 mL of acetone using a Kinematica Polytron Homogenizer (Brinkmann Instruments, Inc.) at medium speed for 20 s. The homogenate was passed through a sintered glass funnel, and the remaining colorless residue was similarly homogenized with methanol and filtered. The combined acetone and methanol filtrates of each replicate sample were evaporated under vacuum at 40 °C with a Rotovapor R-114 (Buchi Corp.) to approximately 30 mL and extracted three times with 40 mL portions of hexane. The aqueous phase was concentrated at 40 °C in a SpeedVac Rotary Concentrator SVC-200H (Savant Instruments, Inc.) to approximately 30 mL, and the final volume was measured. Aliquots of the sample extracts were passed through a 0.45 μm PTFE membrane syringe filter (Titan 44525-NP, Sun SRI) and stored at −20 °C. For microplate assays the filtered sample extracts were diluted 1:10 in deionized water prior to use.

Measurement of Ascorbic Acid and β-Carotene. Total ascorbic acid content was determined by measuring the absorbance at 525 nm using a spectrophotometer (Shimazdu UV 160) following the method of Hodges et al. (*23*). β-Carotene was determined by HPLC following the method of Craft (*24*). Samples in 2 mL vials were loaded onto a high-performance liquid chromatograph (HPLC) equipped with an autosampler, photodiode array detector, and integration software (Hewlett-Packard 1100, Wilmington, DE). Analyses of the carotenoids were achieved with a YMC C_{30} carotenoid column (4.6 × 250 mm) (Waters, Milford, MA) using a tertiary gradient as described previously (*25*). Injection volumes of 0.1 mL were used for samples and standards. Standards (β-carotene, phytoene, phytofluene, violaxanthin) obtained from Sigma (St. Louis, MO) and from CaroteNature (Geneva, Switzerland) were used to verify peaks and calculate concentrations following the method of Craft (*24*).

Measurement of Total Phenols. The Folin−Ciocalteu assay as described by Singleton et al. (*26*) was modified for use with a Synergy HT-I 96-well microplate reader and KC4 version 3.4 software (Bio-Tek Instruments, Inc.). Large decreases in the levels of ascorbic acid occurred during extract preparation (see above), and during the storage at −20 °C preceding the total phenolic measurements by the Folin−Ciocalteu assay, the ascorbic acid levels decreased to less than 1 mg of ascorbic acid/100 g of puree in most samples. However, no changes were observed in the HPLC chromatograms of the phenolic constituents in the processed stored samples compared to those of freshly prepared extracts (data not shown). Standards and sample extracts were diluted 1:10 in deionized water with further dilution of samples as needed in final volumes of 100 μL per well. In each well 25 μL of Folin−Ciocalteu phenol reagent (2 N Sigma F-9252 diluted 1:1 with deionized water) was added to 10−40 μL of sample dilution and mixed. At less than 8 min prior to the addition of Folin−Ciocalteu phenol reagent, 100 μL of sodium carbonate solution (75 g/Liter) was added to each well and mixed. After reaction in the dark for 2 h, absorbance readings were taken at 760 nm. Calibration curves of gallic acid (0.25−2.5 μg/100 mL) were used to calculate milligrams of GAE of each sample per 100 g of puree. As indicated above, ascorbic acid contributed negligibly to the Folin−Ciocalteu measurements.

Measurement of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity. The DPPH radical scavenging assay as described by Yamaguchi et al. (*27*) was modified for use with a Synergy HT-I 96-well microplate reader and KC4 version 3.4 software (Bio-Tek Instruments, Inc.). In the microplate wells 200 μL of a 1:10 dilution of DPPH stock solution (1.0 mM in absolute alcohol) was combined with 10, 20, 30, and 40 μL of freshly prepared sample extract (diluted 1:10 or other appropriate dilution in deionized water). The reaction mixtures were shaken at room temperature in the dark and the reactions run to completion for 3 h. The reduction of absorbance at 517 nm was monitored as the DPPH radical scavenging activity. Standard curves were run with gallic acid, and reduction levels of the samples were converted to milligrams of GAE per 100 g of sample puree. Also, for a number of representative samples, time courses of DPPH radical scavenging were recorded at 1 min intervals for a total of 30 min. Slopes of the linear portions of the time courses were compared to the values of slopes obtained with known amounts of gallic acid standards. Comparisons of slopes of mango extract samples and gallic acid standards allowed a kinetic comparison of radical scavenging activities of the samples expressed as milligrams of GAE/100 g of mango puree. Similar to the Folin−Ciocalteu measurements, the DPPH radical scavenging measurements contained negligible contributions from ascorbic acid.

HPLC and LC-MS Analysis. Previously published HPLC−photodiode array (PDA) methods for the analysis of mango phenols were used with modifications (*1*, *17*). The chromatographic separation of phenolic compounds was performed with a Varian ProStar high-performance liquid chromatograph (Varian, Walnut Creek, CA), equipped with a Varian ProStar 335 PDA detector and a Varian Model 410 autosampler. Data processing was performed with Varian LC Workstation version 6.41. Compound separations were accomplished using a 150 × 3.0 mm (i.d.) Discovery RP Amide C16 5 μm column (Supelco, Belefonte, PA). Elution conditions included a two-solvent gradient composed initially of 2% acetic acid/acetonitrile (90:10, v/v) and then increased in acetonitrile concentrations in linear gradients to 83:17 (v/v) over 25 min, to 80:20 (v/v) over 25 min, to 60:40 (v/v) over 20 min, remained at 60:40 (v/v) for 5 min, and finally lowered to 90:10 (v/v) over 10 min at a flow rate of 0.75 mL min^{-1}. Chromatograms were recorded at 280 and 330 nm, and spectra were scanned from 230 to 600 nm. Calculations of the gallotannin contents in the mango extracts were made using the UV absorbance of the gallotannin HPLC peaks converted to gallic acid equivalents by use of an integrated peak area/microgram conversion factor measured at 280 nm for gallic acid.

LC-MS analyses were performed with a Waters Alliance HPLC (Waters, Medford, MA) connected in series with a Waters 996 PDA detector and a Waters/Micromass ZQ single quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. The majority

of phenols, occurring as gallotannins (gallic acid conjugates), were optimally detected by MS with negative ESI, whereas the detection of mangiferin and ellagic acid conjugates was optimally achieved with positive ESI. Quantitative analyses of mangiferin and ellagic acid conjugates were made using single ion response (SIR) measurements recorded at m/z 423 and 303, representing the mangiferin and ellagic acid aglycone protonated fragment ions, respectively. Data handling was done with MassLynx software version 3.5 (Micromass, division of Waters Corp., Beverly, MA). Post column split to the PDA and mass ZQ detector was 10:1. MS parameters were as follows: ionization mode, ES^+; capillary voltage, 3.0 kV; extractor voltage, 5 V; source temperature, 100 °C; desolvation temperature, 225 °C; desolvation N_2 flow, 465 L h^{-1}, cone N_2 flow, 70 L h^{-1}; scan range, m/z 150−1600; scan rate, 1 scan s^{-1}; cone voltages, 20 and 40 eV.

Statistical Analysis. Each harvest location and date for each of the five mango varieties consisted of 12 fruit which were individually analyzed for β-carotene, ascorbic acid, total phenol, DPPH radical reduction, and gallotannin contents. Among the purees and constituent extracts, the data sets comprised 8−12 individually measured replicates. Data were analyzed by analysis of variance (ANOVA) with the XLSTAT software (Addinsoft, Paris, France). Separation of means was performed with the Fisher least significant difference test with $\alpha = 0.05$.

RESULTS

Ascorbic Acid and β-Carotene Contents. The predominant antioxidants in mango pulp are carotenoids (mainly β-carotene), ascorbic acid, and polyphenolic gallotannins. The average β-carotene and ascorbic acid levels in the pulp of the five mango varieties harvested in different locations and at different dates are given in **Table 1**. Tommy Atkins contained the lowest average β-carotene content (4.9 ± 1.5 mg/kg of FW), while the Ataulfo contained the highest (26.1 ± 4.4 mg/kg of FW). Haden, Kent, and Keitt contained average β-carotene contents (6.8 ± 3.3, 12.9 ± 5.1, and 10.4 ± 2.4 mg/kg of FW, respectively) closer to Tommy Atkins than to Ataulfo. For a number of specific harvests (Tommy Atkins/Mexico, Haden/Peru, Kent/Mexico, Kent/Peru, and Keitt/Mexico) higher β-carotene content occurred in fruit harvested at the later harvest dates, but this did not occur for the other remaining fruit collections. For Haden and Kent mangoes, generally higher β-carotene values occurred at certain harvest dates for fruit grown in Mexico in comparison to other locations. No other correlations were observed for the other mango varieties.

The average ascorbic acid levels over all harvest locations and dates were 19.3 ± 4.8, 24.7 ± 7.9, 25.6 ± 4.9, 31.0 ± 5.2, and 125.4 ± 6.4 mg/100 g of puree in the Tommy Atkins, Keitt, Kent, Haden, and Ataulfo varieties, respectively (**Table 1**). The range of ascorbic acid concentrations in the Haden and Tommy Atkins samples were similar to values obtained elsewhere by HPLC (∼13−20 mg/100 g of FW) (*3*, *21*). The elevated ascorbic acid levels in Ataulfo are similar to the levels in Ubá (77.7 mg/100 g of FW), a variety similar to Ataulfo (*3*). For a number of specific harvests (Tommy Atkins/Brazil and Ecuador, Haden/Mexico, Kent/Mexico, and Ataulfo/Mexico) no significant changes occurred in the ascorbic acid between the earlier and later harvest dates. For other specific harvests (Kent/Mexico, Haden/Peru, and Tommy Atkins/Mexico) there were significantly decreased ascorbic acid levels in the later harvested fruit. In contrast, elevated ascorbic acid levels occurred in the later harvested Kent/Peru and Haden/Mexico. These results suggest that there is no consistent trend in the ascorbic acid content in mangoes in early and late harvested fruit. No effects of harvest locations on ascorbic acid content for any of the varieties were observed.

In Vitro Antioxidant Capacity. The in vitro antioxidant capacities of the mango purees were quantified by DPPH radical reduction expressed as milligrams of gallic acid equivalents (GAE)/100 g of fresh weight (FW) puree (**Table 2**). The measurements for each of these assays were allowed to run to completion and, therefore, contained no kinetic component (relative reactivity) in their values. In the DPPH assays no significant differences occurred for any of the mango varieties relative to their harvest location or date, nor among Tommy Atkins, Kent, Keitt, and Haden. For these cultivars the average total DPPH reduction capacity was 21.0 ± 6.5 mg of GAE/100 g of puree with a range of 11.3−35.2 mg of GAE/100 g of puree. The striking exception was Ataulfo, which contained an average of 104.2 ± 33.5 mg of GAE/100 g of puree.

For a number of the mango puree samples, the in vitro antioxidant activity was also measured by comparing of the rates of DPPH reduction by mango puree extracts relative to gallic acid. These kinetic measurements of DPPH reduction (**Table 2**) (also expressed as mg of GAE/100 g of puree) were typically close in value to those for the end point measurements for Tommy Atkins, Kent, Keitt and Haden. Interestingly, the average ratio of the end point/kinetic DPPH reduction values for these four varieties (1.12 ± 0.43) was substantially lower than the average ratio observed for Ataulfo (2.28 ± 1.17), and this suggests that there may be differences in the antioxidant compositions of Ataulfo pulp compared to other mango varieties.

Total Phenolic Content. The same trends observed for the ascorbic acid and DPPH antioxidant capacity, relative to cultivar type, also occurred in the values for the total phenolic measurements (**Table 2**). The varieties Tommy Atkins, Kent, Keitt, and Haden had similar total phenolic contents, where the average of all four varieties equaled 31.2 ± 7.8 mg of GAE/100 g of puree, with a range of 19.5−49.4 mg of GAE/100 g of puree. The

Table 1. β-Carotene (mg/kg of FW) and Ascorbic Acid (mg/100 g of FW) in Mango Puree[a]

cultivar	location	harvest date	β-carotene	ascorbic acid (A)
Tommy Atkins	Brazil	9-29-06	6.90d,e,f	15.5a,b
		10-26-06	3.08a	17.9b,c,d
		11-15-06	4.01a,b,c,d	17.5b,c
Tommy Atkins	Ecuador	11-17-06	5.73a,b,c,d,e	21.9c,d,e,f,g
		12-11-06	4.43a,b,c,d	17.3a,b,c
		1-23-07	3.19a,b	21.7c,d,e,f,g
Tommy Atkins	Peru	2-15-07	5.07a,b,c,d,e	20.6b,c,d,e,f
Tommy Atkins	Mexico	5-01-06	3.85a,b,c	29.6h,i,j,k
		5-23-06	6.65c,d,e	19.1b,c,d,e
		6-02-06	6.92d,e,f	11.5a
Haden	Peru	1-20-07	3.60a,b,c	31.3 h,i,j,k
		2-15-07	6.27b,c,d,e	23.6d,e,f,g
Haden	Mexico	5-08-06	10.72g	27.6g,h,i,j
		5-22-06	3.85a,b,c	33.8k,l
		6-02-06	9.77f,g	38.8l
Kent	Mexico	6-16-06	10.68g	31.6i,j,k
		7-07-07	18.05h	20.2b,c,d,e
		7-25-06	39.02l	29.9h,i,j,k
		8-11-06	19.35h,i	27.8g,h,i,j
Kent	Peru	1-26-07	5.59a,b,c,d,e	18.5b,c,d
		2-15-06	11.16g	26.7f,g,h,i
Kent	Ecuador	1-20-07	12.30g	24.9e,f,g,h
Keitt	Mexico	7-07-06	7.67e,f	33.4j,k,l
		7-25-06	12.02g	22.6c,d,e,f,g
		8-29-06	11.46g	17.9b,c,d
Ataulfo	Mexico	5-18-06	29.14k	125.1m
		5-08-06	30.69k	120.5m
		6-16-06	22.04i,j	121.3m
		6-02-06	22.58j	134.5n

[a] Means followed by the same letter in a column are not significantly different by Fisher's least significant difference test at $\alpha = 0.05$. Each replicate contained eight individually analyzed samples.

Table 2. In Vitro Antioxidant Capacity (DPPH Radical Reduction) (mg of GAE/100 g of FW), Total Phenolic Content (mg of GAE/100 g of FW), and Gallotannin Content (mg/100 g of FW) of Mangoes Harvested at Different Locations and Dates[a]

			DPPH			
sample	source	harvest date	end point	kinetic	phenolic (Folin)	gallotannin
Tommy Atkins	Mexico	5-01-06	16.1a,b,c	11.9 ± 3.1	21.6a,b	54 ± 30
		5-23-06	18.5a,b,c,d	NR	20.1a,b	56 ± 19
		6-02-06	21.1a,b,c,d	NR	30.1a,b	160 ± 80
Tommy Atkins	Brazil	9-29-06	14.5a,b	NR	23.6a,b	53 ± 17
		10-26-06	11.7a,b	NR	19.5a	27 ± 9
		11-15-06	11.3a	NR	28.1a,b	99 ± 32
Tommy Atkins	Ecuador	11-17-06	12.3a,b	NR	23.6a,b	53 ± 41
		12-11-06	NA	NA	NA	NA
		1-23-07	24.7c,d,e	29.4 ± 2.8	47.9b	159 ± 46
Tommy Atkins	Peru	2-15-07	34.1e	24.0 ± 0.9	30.6a,b	96 ± 38
Haden	Mexico	5-08-06	21.6a,b,c,d	13.5 ± 4.0	27.7a,b	81 ± 14
		5-22-06	35.2e	NR	27.2a,b	85 ± 54
		6-02-06	25.8c,d,e	13.5 ± 2.7	39.5a,b	130 ± 88
Haden	Peru	1-20-07	24.8c,d,e	29.4 ± 5.4	76.3c,d	NR
		2-15-07	15.4a,b,c	14.7 ± 2.2	32.2a,b	93 ± 45
Kent	Mexico	6-16-06	17.8a,b,c,d	NR	35.2a,b	97 ± 63
		7-07-07	23.8b,c,d,e	NR	31.9a,b	105 ± 55
		7-25-06	26.2d,e	NR	28.7a,b	61 ± 26
		8-11-06	26.9d,e	19.7 ± 1.7	34.2a,b	114 ± 69
Kent	Ecuador	1-20-07	13.3a,b	31.9 ± 3.3	34.1a,b	91 ± 39
	Peru	1-15-06	NR	NR	NR	NR
		2-15-06	22.9b,c,d	29.9 ± 2.8	49.4b,c	50 ± 22
Keitt	Mexico	7-07-06	22.2b,c,d	NR	36.1a,b	101 ± 36
		7-25-06	22.9b,c,d	18.3 ± 3.7	26.0a,b	64 ± 24
		8-29-06	25.9c,d,e	40.1 ± 9.5	27.3a,b	53 ± 14
Ataulfo	Mexico	5-08-06	83.9g	38.1 ± 4.9	99.5d,e	342 ± 123
		5-18-06	145.2i	37.1 ± 9.4	99.3d,e	325 ± 109
		6-02-06	116.9h	61.1 ± 25.6	130.8f	554 ± 112
		6-16-06	70.9f	63.5 ± 17.3	107.7e	708 ± 618

[a] Means followed by the same letter in a column are not significantly different by Fisher's least significant difference test at $\alpha = 0.05$. Each replicate contained 8−12 individually analyzed samples. NR = not recorded.

phenolic contents of Tommy Atkins and Haden were similar to values reported elsewhere for these varieties (*3*, *21*). Ataulfo contained substantially higher average total phenolic contents (109.3 ± 14.8 mg of GAE/100 g of puree), similar to that of the related Ubá mango (208.7 mg of GA/100 g of FW) (3). Haden mangoes harvested in Peru on 1-20-07 (76.3 mg of GAE/100 g of puree) contained significantly higher phenolic content compared to the fruit harvested on 2-15-07 (32.2 mg of GAE/100 g of puree). In all of the other fruit harvests, no significant differences in the total phenolic contents occurred in relation to either harvest location or date. In most cases, the total phenolic contents were in close agreement with the in vitro antioxidant levels due to the minimal ascorbic acid contributions in these measurements.

Gallotannin Content and HPLC Profiles of Phenols. Profiles of the phenolic compounds in the pulp of the five mango varieties, measured by reversed-phase HPLC, were consistent with the occurrence of hydrolyzable gallotannins as the major chemical species. The hydrolyzable gallotannin conjugates occurred with elution times greater than 40 min in **Figure 1**. Similar gallotannin profiles occurred for all five mango varieties, although numerous differences occurred in earlier eluting compounds (data not shown), including, in part, low concentrations of tetragalloyl glucose (*17*), methylgallate, gallic acid, and conjugates of ellagic acid and mangiferin. Peak assignments of the gallic acid conjugates in the HPLC profiles were based on the characteristic UV and negative electrospray ionization (ESI)−mass spectra (*4*, *17*).

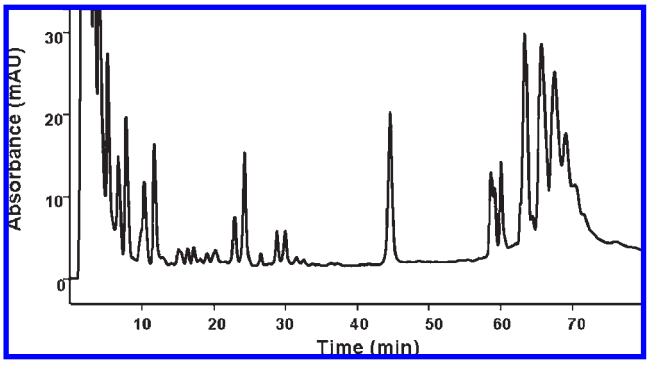

Figure 1. HPLC (A_{280}, nm) of mango puree extract. Compounds eluted after 40 min comprised mainly gallotannins.

The major peak at 44 min in **Figure 1** is due to the elution of pentagalloyl glucose; peaks between 58 and 61 min are due to isomers of hexagalloyl glucose, and the remaining peaks are due to the elutions of hexa-, octa-, and nonagalloyl glucose, sequentially. Trace levels of higher galloyl content oligomers were also observed by negative ion ESI-MS detection in the later eluting portions of the HPLC chromatograms (data not shown).

Levels of gallotannins in the mango puree extracts were quantified by measuring the total integrated peak areas for the gallotannins in the 40−75 min portions of HPLC chromatograms

Table 3. Levels (μg/g of Fresh Weight Puree) of Mangiferin and Ellagic Acid in Selected Sets of Mango Purees[a]

cultivar	source	harvest date	mangiferin	ellagic acid
Haden	Mexico	5-23-06	46.5 ± 26.8	101.6 ± 112.5
		7-26-06	28.1 ± 14.4	49.2 ± 32.5
Keitt	Mexico	7-07-06	2.8 ± 5.2	92.4 ± 41.3
		7-25-06	trace	trace
Kent	Ecuador	1-20-06	trace	trace
Kent	Mexico	6-16-06	17.1 ± 10.5	340.6 ± 170.8
		7-25-06	3.8 ± 6.1	39.4 ± 45.7
Kent	Peru	1-26-07	110.2 ± 116.3	2385.5 ± 1193.4
Tommy Atkins	Mexico	5-01-06	trace	trace
		7-13-06	182.7 ± 197.3	112.1 ± 97.2
Ataulfo	Mexico	5-08-06	227.4 ± 204.3	25.7 ± 9.2
		5-18-06	252.4 ± 130.1	72.5 ± 34.8
		6-02-06	749.2 ± 404.3	126.4 ± 38.2
		6-16-06	996.1 ± 882.3	187.1 ± 88.3

[a] Data are expressed as mean ± standard deviation. Each replicate contained 8−12 individually analyzed samples.

recorded at 280 nm (see above) and by calculating a milligrams of gallotannin/100 g of FW puree value by using the peak area$_{280nm}$/μg conversion factor of gallic acid. As shown in **Table 2**, no consistent trends occurred in the gallotannin contents in the mango varieties relating to harvest locations. However, significantly higher gallotannin contents occurred for the later harvest dates in a number of harvest locations. Examples are seen in the Tommy Atkins mangoes harvested in Mexico (54−160 mg/100 g of FW), Brazil (53−99 mg/100 g of FW), and Ecuador (53−159 mg/100 g of FW), and the Haden mangoes harvested in Mexico (81−130 mg/100 g of FW). Nonsignificant trends toward higher total phenolic contents, measured by the Folin−Ciocalteu assay, were similarly observed for these samples (**Table 2**). Although the four sets of Ataulfo mangoes were collected at different locations, similar sharply higher gallotannin levels occurred in the fruit collected at the later two harvest dates.

Levels of two minor-occurring phenolic compounds, mangiferin and ellagic acid, were also measured for select samples in the five mango cultivars (**Table 3**). Levels of mangiferin in the two later harvest dates (6-02-06 and 6-16-06) for Ataulfo were much higher than in the fruit harvested at the earlier two dates (5-08-06 and 5-18-08). As evidenced by the large standard deviation values, large fruit-to-fruit variations in mangiferin content occurred within each set. Large fluctuations were also observed for both mangiferin and ellagic acid within the sets of the Haden, Keitt, Kent, and Tommy Atkins mango cultivars (**Table 3**). For three pairs of samples (Haden/Mexico, 5-23-06 and 7-26-06), (Keitt/Mexico, 6-16-06 and 7-25-06), and (Kent/Mexico, 6-16-06 and 7-25-06), the contents of ellagic acid were much lower at the later harvest dates than at the earlier harvest dates. However, the opposite was true for the multiple harvests for Tommy Atkins/Mexico and Ataulfo/Mexico. A dramatically higher level of ellagic acid (2385 μg of ellagic acid/g of puree) for Kent (Peru, harvested 1-12-07) was observed relative to the much lower levels measured at other harvest dates and locations.

DISCUSSION

The results of this study show that only typically minor differences in the β-carotene, ascorbic acid, and total phenolic contents and in vitro antioxidant capacities occur among the Haden, Kent, Keitt, and Tommy Atkins mangoes (four of the main commercial varieties of mango imported into the U.S.). Consistent trends in the ascorbic acid, total phenolic contents, and DPPH radical scavenging activities influenced by harvest locations for these mango varieties were not observed. If significant differences had been observed among these four varieties, differences in cultivation practices, i.e. rootstock selections, climate, etc. may have been attributing factors. Differences in the levels of these main antioxidants in these four similar varieties compared to those in the Ataulfo mangoes were observed, and these differences were significantly larger than the differences attributable to different harvest dates and countries of origin. Also relating to these observations were the large standard deviations in the average concentrations of the main antioxidants as shown in the data in **Tables 1 − 3**, and these large fruit-to-fruit differences, for all practical purposes, mask whatever minor trends that may possibly occur as a result of harvesting the fruit in different locations and dates. For this reason, this analysis of the mango fruit was not continued a second year.

Finally, while Ataulfo is unusual in its high antioxidant capacity, there are probably other cultivars and selections in the worldwide germplasm population that are similar to this cultivar, such as Ubá from Brazil. In these varieties, the influences of the higher ascorbic acid and gallotannin concentrations on taste perceptions are unclear, but the high average ascorbic acid levels in Ataulfo mangoes are still low relative to the fruit's citric acid content (data not shown) and thus are not likely to significantly contribute to the mango taste. More likely are possible influences of the high concentrations of the gallotannins, compounds known for their potent astringency (*28*), on taste attributes of Ataulfo and similar mango varieties. The influences of these polyphenolic compounds on mango taste remain to be evaluated.

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