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Antioxidant Capacity and Lipid Characterization of Six Georgia-Grown Pomegranate Cultivars

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Six pomegranate (*Punica granatum*) cultivars were investigated for their antioxidant capacity and lipid profile. Total polyphenols were determined according to the Folin−Ciocalteau method. Major organic acids and phenolic compounds were analyzed by RP-HPLC. Two in vitro antioxidant assays, ferric reducing antioxidant power and Trolox equivalent antioxidant capacity, were used to assess antioxidant capacity. Total lipid was extracted according to the Folch method, and fatty acid methyl esters were determined by GC. Tocopherols and phospholipids were identified and quantified by NP-HPLC using a fluorescence detector for tocopherols and an evaporative light scattering detector for phospholipid analysis. Phytosterols were analyzed by GC. The predominant organic acid was citric acid followed by malic acid. The peel fraction had the highest total hydrolyzable tannins content (4792.3−6894.8 mg/100 g of FW). Overall, the highest antioxidant capacity was found in leaves followed by peel, pulp, and seed. Pomegranate seed had an average lipid content of 19.2% with punicic acid as the predominant fatty acid. Pomegranate seed had high contents of α-tocopherol (161.2−170.1 mg/100 g) and γ-tocopherol (80.2−92.8 mg/100 g).

KEYWORDS: Antioxidant capacity; fatty acids; organic acids; phospholipids; phytosterols; polyphenols; *Punica granatum*; tocopherols

INTRODUCTION

Phytochemicals, particularly antioxidants from natural sources such as fruits and vegetables, have gained popularity because many epidemiological studies have shown their protective properties against several chronic diseases such as cancer and cardiovascular diseases (*1*). Products and intermediates of oxidative stress pathways are associated with several chronic diseases. Oxidative stress is the disturbance of the pro-oxidant−antioxidant balance in favor of the former, leading to potential damage (*2*). Interest in natural antioxidants has increased during the past few decades because of the adverse effects shown by synthetic antioxidants and also due to the worldwide trend to avoid or minimize the use of artificial food additives (*3*). The most abundant antioxidants in fruits are polyphenols, vitamins, and carotenoids.

The lipid profiles of several fruits and their seeds have been characterized and various bioactive compounds isolated (*4*, *5*). Determination of the lipid classes of the sample may aid in deciding its application in food, health, and other industries. This in turn will help in identifying the potential of the crop as a mainstream agricultural product.

Pomegranate (*Punica granatum*), belonging to family Punicaceae, has been used in several traditional medicine systems. It is a rich source of various bioactive compounds demonstrating antioxidant and anti-inflammatory activities (*6*). Pomegranate seed oil comprising 12−20% of the total seed weight consists of approximately 80% conjugated octadecatrienoic fatty acids, mainly punicic acid (*6*). Highest antioxidant activity has been shown in pomegranate peel compared to pulp and seed fractions (*7*), which can be attributed to its high content of tannins, especially punicalagin isomers. Pomegranate juice is bright red in color due to its high content of flavonoids and anthocyanins. Pomegranate juice exhibits antiatherogenic activities (*8*). Leaves, flowers, bark, and roots also contain distinctive compounds having potent physiological effects.

Pomegranate is a minor fruit crop of Georgia, but with the current commercial and nutritional potentials, a detailed study may help in its cultivar selection and application. The peels and seeds, which are usually disposed of as waste material in many food-processing industries, could be a rich source of beneficial phytochemicals. From economic and environment points of view disposing of such wastes should be avoided. Recovery of bioactive compounds would help to make the recycling of wastes economically viable and also result in value addition to these minor crops. The main objective of this research was to compare six Georgia-grown pomegranate cultivars in terms of their organic acid contents, antioxidant capacities, and lipid profiles.

MATERIALS AND METHODS

Plant Material. Six pomegranate (*P. granatum*, Punicaceae) cultivars, R19, R26, Cvg-Eve, North, Crab, and Cranberry, along with their leaves were obtained from Ponder farm, a University of Georgia operated farm near Tifton, GA. Managed plant growth conditions were minimal. Irrigation was not performed, and only natural rainfall was utilized. No supplemental fertilizers were applied. They were planted in a loamy-sand soil (sand, 86%; silt, 7%; and clay, 7%). Some pruning was performed, but they were not managed in a commercial manner. They were planted from 1990 to 1993 and have been neglected until spring of 2008, when some

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minor pruning and a fungicide application were performed (although not registered for use on pomegranate). The samples were transported in ice coolers from the farm to the University of Georgia. All fruits were at ripe stage ready for fresh consumption.

Chemicals. Pure standards of succinic acid, DL-malic acid, oxalic acid, BHT, gallic acid, quercetin, ellagic acid, (+)-catechin, ferulic acid, *p*-coumaric acid, caffeic acid, (−)- epicatechin, Supelco 37 FAME mix, heptadecanoic acid, 14% boron trifluoride in methanol, 5β-cholestan-3β-ol, 5% DMDS in toluene (Sylon-CT), α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, L-α-phosphatidylethanolamine, Folin−Ciocalteu reagent, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), citric acid, and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO). 2,4,6-Tripyridyl-*s*-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Morris Plains, NJ). L-Ascorbic acid was from Mallinckrodt Baker Inc. (Phillipsburg, NJ) and $FeCl_3 \cdot 6H_2O$ from Fluka (Milwaukee, WI). Plant sterol mixture was purchased from Matreya LLC (Pleasant Gap, PA). BSTFA with 1% TMCS was purchased from Thermo Scientific (Rockford, IL) and L-α-phosphatidylcholine from Avanti Polar Lipids, Inc. (Alabaster, AL). Other solvents and chemicals were purchased from Sigma Chemical Co., J. T. Baker Chemical Co. (Phillipsburg, NJ), and/or Fischer Scientific (Norcross, GA).

Sample Preparation. The samples were washed with water and wiped dry completely. Five hundred grams of the fruits was divided into three fractions as peel, pulp (juice), and seeds for antioxidant capacity. Pulp was separated from the seeds by collecting the arils in a muslin cloth and gently pressing by hand. The leaves of each cultivar were also analyzed. For the lipid profile, 500 g of fruits was divided into two fractions, seeds and fruits (without the seed, i.e., peel and juice together). The fractions were ground using a mortar and pestle. All sample preparation was done under cool and dark conditions. All fractions were packaged in amber bottles, labeled, and stored at −80 °C after flushing with nitrogen until analyzed.

Dry Weight (DW) Determination. DW was determined following the guidelines of AOAC official method 967.03 (*9*). Sample dry weight [g/g of fresh weight (FW)] was calculated as shown in eq 1

$$DW = (c - a)/(b - a) \quad (1)$$

where a is the weight of the empty pan (g), b is the weight of the pan and fresh sample (g), and c is the weight of the pan and dried sample (g). All samples were analyzed in triplicates, and average values were reported.

Preparation of Hydrophilic and Lipophilic Fractions. Hydrophilic and lipophilic fractions were prepared using the method of Jimenez-Alvarez et al. (*10*). Two grams of each fruit fraction was weighed, and 10 mL of hexane was added to each. The samples were centrifuged for 10 min at 2500 rpm. After centrifuging, the supernatants were collected. This procedure was repeated once again, and the supernatant was dried under nitrogen and reconstituted in 10 mL of 95% ethanol. The lipophilic fractions were stored at −20 °C until analyzed. For the hydrophilic fraction, after all of the hexane had been removed from the residues, 5 mL of acetone/water/acetic acid (70:28:2, v/v/v) was added. A similar centrifugation step was followed as described above, and supernatants were collected. The volume was made up to 10 mL with acetone/water/acetic acid (70:28:2, v/v/v), and samples were stored at −20 °C until analyzed. These lipophilic and hydrophilic fractions (0.2 g/mL) were used for total polyphenol and antioxidant assays.

Major Organic Acids. One gram of fresh sample was mashed with 10 mL of 1 M HCl using a mortar and pestle. After flushing with nitrogen, the samples were centrifuged at 2000 rpm for 15 min and placed in a water bath at 90 °C for 30 min. The samples were then allowed to cool to room temperature, and the supernatant was filtered through a 0.45 μm membrane filter. Organic acids were analyzed and identified using a Hewlett-Packard (Avondale, PA) HP 1100 HPLC system with a diode array detector based on the method of Chen et al. (*11*). An Agilent Zorbax Eclipse XDB-C18, 3.5 μm, 4.6 × 150 mm, column and an isocratic mobile phase of 0.5% ammonium phosphate, pH adjusted to 2.8 with phosphoric acid, was used at a flow rate of 0.5 mL/min. The injection volume was 20 μL, and the column temperature was maintained at 40 °C. Detection was done at 214 nm. Triplicate determinations were made and averaged. Organic acids were identified on the basis of the retention times of the individual external standards. Quantification was based on external standards (10−1600 μg/mL).

Major Phenolic Compounds. Major phenolic compounds were determined following the method described by Pastrana-Bonilla et al. (*12*). One gram of fresh sample was mashed and diluted with 10 mL of 80% methanol in 6 N HCl. The samples were vortexed and placed in a water bath shaker at 60 °C and 200 rpm for 2 h. After cooling to room temperature, the supernatants were filtered through a 0.45 μm membrane filter and injected into a Hewlett-Packard HP 1100 HPLC system with a diode array detector. The column used was a Beckman Ultrasphere C18, 5 μm, 4.6 × 250 mm, with temperature set at 40 °C. The injection volume was 20 μL. The mobile phase at a flow rate of 1 mL/min consisted of solvent A, methanol/acetic acid/water (10:2:88, v/v/v); solvent B, acetonitrile; and, solvent C, water. A linear gradient was used as follows: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; and at 25 min, 30% solvent A and 70% solvent B, with a 5 min postrun of 100% solvent C. Detection was done at 260, 280, 320, and 360 nm. Identification was based on the retention times and characteristic UV spectra. Quantification was based on external standard curves. All analyses were performed in triplicate, and averages are reported.

For identification of punicalgin isomers MS-ESI (electrospray ionization) was performed on a PE Sciex API 1 (Perken Elmer, Waltham, MA) quadrupole mass spectrometer operated in the negative ESI mode. Twenty microliters of sample was injected with methanol as carrier. The carrier flow rate was 0.2 mL/h. Continuous mass spectra were recorded over the range 295−1150 m/z with 0.2 m/z steps.

Total Polyphenols (TPP). Total polyphenols were determined according to the Folin−Ciocalteu reagent method (*13*). To each 200 μL of sample were added 1 mL of 0.2 N Folin−Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution; the samples were mixed well and allowed to stand for 30 min at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV−vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). Quantification was based on the standard curve generated with 100−400 mg/L of gallic acid, and average results from triplicate determinations are reported as milligrams of GAE per 100 g of FW.

Antioxidant Capacity. *Ferric Reducing Antioxidant Capacity (FRAP) Assay*. The FRAP assay was carried out as described by Benzie and Strain (*14*) with slight modifications. Stock solutions of 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine solution in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$ were prepared. Working solution was prepared by mixing the stock solutions in a 10:1:1 ratio. The solution was maintained at 37 °C and pH 3.6. Then, 50 μL of sample was mixed with 2 mL of the working solution, and absorbance was read at 593 nm against an appropriate blank for 4 min. The change in absorbance was calculated and related to the standard curve generated with Trolox. Results were expressed as micromolar TE per g of FW. All assays were in triplicate, and averages are reported.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The antioxidant capacity was measured according to the radical cation decolorization assay based on the methods of Re et al. (*20*) and van den Berg et al. (*15*, *16*). Briefly, 7 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed and kept in the dark at room temperature for 12−16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. To each 50 μL aliquot of Trolox standard or sample was added 2 mL of diluted $ABTS^{\bullet+}$, and the absorbance was read for 6 min at 734 nm. Appropriate solvent blanks were also run in each assay. The percentage inhibition of absorbance was calculated and plotted as a function of Trolox concentration. TEAC values of samples were calculated on the basis of the standard curve and reported as micromolar TE per g of FW from the average of triplicate determinations.

Lipid Extraction. Total lipids were extracted using the Folch method (*17*). Solvent was removed under nitrogen, and sample was dried in a desiccator at room temperature. The constant weight was recorded, and the samples were stored at −80 °C in hexane with 0.005% BHT to prevent oxidation.

Fatty Acid Profile. Lipid samples were converted to fatty acid methyl ester following AOAC official method 996.01 (*18*) and analyzed with a Hewlett-Packard 6890 series II gas chromatograph (Agilent Technologies

Inc., Palo Alto, CA) using a Supelco SP-2560, 100 m × 25 mm × 0.2 μm column. Helium was the carrier gas at a flow rate of 1.1 mL/min at constant pressure. The injection volume was 1 μL, and a spilt ratio of 5:1 was used. Detection was with a flame ionization detector at 260 °C. The column was initially held at 150 °C for 3 min and then increased to 215 at 10 °C/min and held at 215 °C for 40 min. All samples were analyzed in triplicate, and average values are reported.

The position of the cis−trans−cis isomer was identified with a Shimadzu 300 UV−vis spectrophotometer (Shimadzu UV-1601) on the basis of the Sita Devi method (*19*). The fatty acid methyl esters (FAME) were purified by TLC on silica gel plates using hexane/diethyl ether (94:6, v/v) as developing solvent. Fatty acids isolated by TLC were dissolved in cyclohexane (10 μg/mL), and the absorbance was read at a wavelength range of 200−300 nm.

Identification of punicic acid was done with a Hewlett-Packard 5890 series gas chromatograph (Agilent Technologies Inc., Palo Alto, CA) coupled with MS. A Supelco SP-2560, 100 m × 25 mm × 0.2 μm, column was used. The injector and detector were maintained at 250 and 230 °C, respectively. The column was initially held at 150 °C for 3 min, then increased to 215 at 10 °C/min, held at 215 °C for 20 min, and further increased to 230 at 1.5 °C/min and held at 230 °C for 30 min. The pressure was maintained at 24 psi. Scans from 50 to 550 m/z were done. The mass spectrum of the sample was identified by computer comparison against a mass spectral library.

Phytosterols. The samples were saponified according to the Kim et al. (*20*) method. Fifty milligrams of sample was weighed, and 20 μL of internal standard solution (2 mg/mL of 5β-cholestan-3β-ol) was added. The samples were flushed with nitrogen, and 250 μL of saturated KOH and 2 mL of 3% pyrogallol in ethanol were added. The samples were placed in a water bath at 80 °C for 30 min. After the samples had cooled to room temperature, 5 mL of hexane and 4 mL of water were added, and the samples were vortexed. The upper hexane layer was recovered and dried completely under nitrogen. Derivatization was done by adding 50 μL of TMS/pyridine (1:1, v/v) and placing the samples in a water bath at 70 °C for 1 h to obtain better resolution and peak shape. The samples were dried under nitrogen and reconstituted in 0.5 mL of hexane; 1 μL of this solution was injected into the GC. A Hewlett-Packard 6890 series II gas chromatograph equipped with a FID was used. The column was an HP-5, 5% phenylmethyl siloxane from Agilent (30 m × 0.32 mm × 0.25 μm), and the carrier gas was helium with a flow rate of 1.5 mL/min at constant pressure. The injector and detector temperatures were maintained at 300 and 320 °C, respectively. The column was initially held at 260 °C and programmed to increase to 300 °C at the rate of 3 °C/min and held at 300 °C for 6.7 min. Identification of sterols was based on a plant sterol mixture, whereas quantification was based on an internal standard method as shown in eq 2 (*21*)

$$\text{phytosterol amount (mg/100 g)} = 100(\text{PA}_s)(m_{is})/(\text{PA}_{is})(m_s) \quad (2)$$

where PA_s is the sterol peak area, m_{is} is weight of the internal standard (mg), PA_{is} is the internal standard peak area, and m_s is the weight of the sample (g). All samples were analyzed in triplicate, and average values are reported.

Tocopherols. Tocopherols were analyzed according to the method described by Chun et al. (*22*). A 50−100 mg sample was weighed, and 6% ethanolic pyrogallol solution was added. The samples were vortexed, and 0.5 mL of 60% KOH solution was added. After flushing with nitrogen, the samples were kept in a shaking water bath for 30 min at 80 °C. The samples were allowed to cool to room temperature, and 2 mL of 2% NaCl solution was added. One milliliter of hexane/ethyl acetate (85:15, v/v) containing 0.005% BHT was added and vortexed. The upper hexane layer was recovered, and this extraction was repeated two to three times. The hexane fractions were pooled, dried under nitrogen, reconstituted in 2 mL of hexane, and filtered through a 0.45 μm membrane filter. A 50 μL aliquot was injected with the help of a 250 μL injection loop into a Hewlett-Packard HP 1090 HPLC system with a fluorescence detector with excitation set at 290 nm and emission at 330 nm. An Agilent Zorbax RX-SIL, 5 μm, 4.6 × 250 mm, silica column was used. The mobile phase consisted of hexane/isopropanol (99.3:0.7, v/v) with a flow rate of 1 mL/min. The tocopherols were identified by comparing their retention times with those of authentic standards. Tocopherol quantity was calculated on the basis of the standard calibration curves and reported as milligrams per 100 g from the average of triplicate determinations.

Table 1. Gradient Elution for Phospholipid Determination by HPLC-ELSD

time (min)	flow rate (mL/min)	isopropanol (vol %)	water (vol %)	hexane (vol %)
0.0	0.8	42.0	5.0	53.0
8.0	0.8	42.0	5.0	53.0
8.1	1.2	42.0	5.0	53.0
25.0	1.0	54.0	10.0	36.0
25.1	0.8	66.0	17.0	17.0
35.0	0.8	66.0	17.0	17.0
38.0	0.8	42.0	5.0	53.0
45.0	0.8	42.0	5.0	53.0

Table 2. Dry Matter (DM) Content of Cultivars (% FW)

part	cultivar	DM[a]
seed	R19	40.0 ± 0.1b
	R26	50.0 ± 0.1a
	Cvg-Eve	40.0 ± 0.1b
	North	40.0 ± 0.1b
	Crab	40.0 ± 0.1b
	Cranberry	30.0 ± 0.0c
pulp	R19	10.0 ± 0.0a
	R26	10.0 ± 0.0a
	Cvg-Eve	10.0 ± 0.0a
	North	10.0 ± 0.0a
	Crab	10.0 ± 0.0a
	Cranberry	10.0 ± 0.0a
peel	R19	20.0 ± 0.0b
	R26	30.0 ± 0.0a
	Cvg-Eve	20.0 ± 0.0b
	North	30.0 ± 0.0a
	Crab	20.0 ± 0.0b
	Cranberry	20.0 ± 0.0b
leaf	R19	30.0 ± 0.0b
	R26	30.0 ± 0.0b
	Cvg-Eve	40.0 ± 0.1a
	North	30.0 ± 0.0b
	Crab	40.0 ± 0.1a
	Cranberry	30.0 ± 0.0b

[a] Values are the average of triplicates ± standard deviation. Values with the same letter for each fruit part in the column are not significantly different at $p \leq 0.05$.

Phospholipids. To analyze phospholipids by HPLC-ELSD, solid phase extraction was used to separate neutral lipids, glycolipids, and polar lipids as described by Descalzo et al. (*23*). The sample was fractionated on a normal phase silica cartridge (Supelclean LC-Si, 500 mg, 3 mL) previously conditioned with 10 mL of chloroform. Sequential elution was carried out with 20 mL of chloroform, 5 mL of acetone, and 20 mL of methanol. The methanol extract containing the sample was dried under nitrogen and reconstituted in 1 mL of chloroform. The samples were filtered through a 0.2 μm membrane filter, and 10 μL was injected into the HPLC system. Phospholipid analysis was performed with a Hewlett-Packard HP 1100 HPLC system with ELSD (Sedex 55) according to the Zhang et al. (*24*) method with few modifications. The stationary phase was an Agilent Zorbax RX-SIL, 5 μm, 4.6 × 250 mm silica column, and the mobile phase consisted of a gradient elution of isopropanol, water, and hexane as shown in **Table 1**. Drift tube temperature was set at 60 °C, pressure at 2 bar, and gain at 5. Triplicate determinations were made and averaged. Quantification was based on the individual phospholipid standards (0.5−4 mg/mL).

Table 3. Major Organic Acids in Fruit Parts and Leaves (mg/100 g of FW)[a]

	cultivar	malic acid	citric acid	oxalic acid	ascorbic acid	succinic acid	total organic acids[b]
seed	R19	93.5 ± 0.7a	315.7 ± 1.0c	8.6 ± 0.2b	1.4 ± 0.4b	nd	419.3 ± 1.2bc
	R26	102.2 ± 2.6a	482.6 ± 5.7a	11.2 ± 0.4a	4.2 ± 0.2a	5.4 ± 0.4a	605.7 ± 7.8a
	Cvg-Eve	92.7 ± 2.3a,b	305.1 ± 32.8c	8.7 ± 0.7b	nd	nd	406.5 ± 6.7c
	North	83.0 ± 3.5b	243.1 ± 4.5d	8.6 ± 0.6b	nd	nd	334.7 ± 33.9d
	Crab	98.2 ± 3.7a	464.6 ± 34.5a	9.8 ± 0.6a,b	3.4 ± 0.3a	22.3 ± 0.5b	580.5 ± 18.1a
	Cranberry	100.4 ± 1.0a	357.7 ± 5.3b	9.2 ± 0.2b	3.3 ± 0.5a	4.0 ± 0.2b,c	474.6 ± 6.4b
pulp	R19	156.7 ± 4.5b,c	826.7 ± 5.4d	23.8 ± 0.3c,d	48.2 ± 0.3d	17.8 ± 0.6c,d	1073.2 ± 10.1d
	R26	183.4 ± 9.3a	1926.6 ± 33.7a	34.1 ± 0.5a	65.9 ± 1.3a	25.3 ± 0.4a	2235.3 ± 13.0 a
	Cvg-Eve	149.4 ± 2.5c	857.9 ± 12.7d	22.5 ± 0.7d	43.3 ± 1.3e	16.4 ± 0.5d	1089.5 ± 30.0d
	North	132.5 ± 2.2d	488.3 ± 13.0e	21.1 ± 1.4d	36.5 ± 0.6f	16.8 ± 0.4d	695.1 ± 8.2e
	Crab	164.1 ± 2.7b	1653.2 ± 47.4b	31.5 ± 0.7a	61.1 ± 2.4b	14.0 ± 1.1b	1932.2 ± 30.1b
	Cranberry	159.1 ± 3.8b	1204.7 ± 3.4c	26.8 ± 0.4b,c	55.8 ± 0.7c	18.5 ± 0.4c	1464.8 ± 5.5c
peel	R19	99.0 ± 1.0b	766.9 ± 3.2d	7.4 ± 0.4b	24.5 ± 0.3b	13.0 ± 0.9b	910.7 ± 2.4d
	R26	116.5 ± 2.4a	1678.2 ± 8.4a	9.3 ± 0.4a	37.3 ± 5.7a	16.5 ± 0.7a	1857.9 ± 28.5a
	Cvg-Eve	98.2 ± 4.1b	606.0 ± 5.1e	7.7 ± 0.6b	23.6 ± 1.8b,c	13.5 ± 0.3b	749.1 ± 11.8e
	North	93.0 ± 2.6b	507.0 ± 3.5f	6.6 ± 0.2b	20.3 ± 0.7c	12.7 ± 0.2b	639.6 ± 13.9f
	Crab	107.8 ± 5.8b	1339.5 ± 1.4b	8.7 ± 0.4a,b	30.9 ± 0.8a	13.0 ± 2.6a,b	1500.9 ± 50.9b
	Cranberry	108.5 ± 2.1a,b	985.5 ± 1.4c	7.5 ± 0.2b	28.9 ± 0.6a,b	14.4 ± 0.4a	1144.8 ± 3.7c
leaf	R19	71.2 ± 3.0a,b	130.2 ± 1.6c	28.6 ± 0.5c,d	12.5 ± 0.4b	12.8 ± 0.3c	255.4 ± 1.8d
	R26	78.1 ± 1.5a	177.3 ± 1.6a	38.8 ± 3.5a	16.0 ± 2.1a	14.2 ± 0.9a	324 ± 5.9a
	Cvg-Eve	65.0 ± 2.6b	132.4 ± 2.1c	27.6 ± 2.6d	9.6 ± 0.6e	13.2 ± 2.8a,b	247 ± 2.2de
	North	72.5 ± 2.1a	113.6 ± 7.6d	28.3 ± 3.1d	10.3 ± 1.1d,e	4.5 ± 0.3b,c	237.6 ± 2.0e
	Crab	77.1 ± 1.9a	161.8 ± 5.3a,b	32.5 ± 4.6b	14.8 ± 2.2a	14.0 ± 0.7a	300.2 ± 7.0b
	Cranberry	80.0 ± 2.4a	149.2 ± 23.1b	31.1 ± 0.7b,c	10.9 ± 3.8c,d	12.4 ± 0.8c	283.6 ± 3.2c

[a] Each value is the mean of triplicates ± standard deviation. nd, not detected. Values with the same letter for each fruit part in each column are not significantly different at $p \leq 0.05$. [b] Sum of organic acids identified.

Table 4. Individual Phenolic Compounds in Fruit Parts and Leaves (mg/100 g of FW)[a]

part	cultivar	hydrolyzable tannins[b]	caffeic acid	*p*-coumaric acid	ferulic acid	catechin	epicatechin	quercetin	total polyphenols[c]
seed	R19	36.3 ± 2.3a	2.8 ± 0.3a	1.4 ± 0.9b	0.8 ± 0.3b,c	nd	6.5 ± 0.3a	10.8 ± 1.4a	91.1 ± 1.1a
	R26	31.8 ± 1.0ab	2.5 ± 0.5a	1.3 ± 0.3b	0.7 ± 0.3c,d	nd	6.2 ± 0.5a	11.2 ± 1.2a	90.4 ± 0.8a
	Cvg-Eve	26.6 ± 0.5c	3.1 ± 1.2a	2.1 ± 0.2a,b	0.5 ± 0.0e	nd	5.8 ± 0.3a	10.6 ± 1.3a	87.4 ± 1.3ab
	North	22.8 ± 1.3d	2.1 ± 0.4a	2.8 ± 0.2a	0.5 ± 0.3d,e	nd	6.0 ± 0.6a	10.6 ± 1.0a	84.9 ± 1.0b
	Crab	30.7 ± 1.3b	3.4 ± 0.3a	3.2 ± 0.1a	1.1 ± 0.3a,b	nd	6.1 ± 0.3a	11.1 ± 1.4a	90.1 ± 1.5a
	Cranberry	36.6 ± 0.6a	3.2 ± 0.3a	3.6 ± 0.8a	1.3 ± 0.3a	nd	6.1 ± 0.4a	10.9 ± 1.7a	91.1 ± 1.4a
pulp	R19	103.1 ± 1.2a	14.4 ± 1.4a	8.1 ± 1.3a	2.0 ± 0.1a	101.2 ± 6.7a	11.7 ± 1.9a	77.1 ± 6.5a	173.4 ± 0.7a
	R26	99.5 ± 0.9a	13.8 ± 1.3a,b	7.7 ± 0.3a	1.7 ± 0.3b	96.4 ± 6.4b	10.9 ± 1.7a	75.8 ± 5.8a	167.5 ± 0.6b
	Cvg-Eve	80.9 ± 3.5b	12.5 ± 1.1c	7.1 ± 0.3a	1.4 ± 0.3b,c	88.6 ± 2.3d	10.8 ± 1.1a	69.8 ± 7.8c	161.5 ± 1.3c
	North	71.2 ± 2.2c	12.3 ± 1.2c	6.6 ± 0.6a	1.3 ± 0.2c	82.7 ± 5.3e	9.6 ± 1.6a	66.7 ± 5.7d	151.3 ± 1.4d
	Crab	84.3 ± 1.4b	13.6 ± 1.2b	7.5 ± 0.5a	1.8 ± 0.3a,b	92.3 ± 1.2c	10.5 ± 2.1a	72.1 ± 5.9b	161.6 ± 1.5c
	Cranberry	100.6 ± 1.3a	14.1 ± 1.4a	8.0 ± 0.1a	2.0 ± 0.3a	98.5 ± 4.5b	11.7 ± 0.7a	76.3 ± 5.3a	170.9 ± 0.9ab
peel	R19	5759.5 ± 61.3c	19.7 ± 2.7a	4.3 ± 0.9a	17.7 ± 2.3a	118.3 ± 6.8b,c	26.1 ± 3.4d,e	94.3 ± 8.3b	311.4 ± 0.8b
	R26	5185.9 ± 66.2d	19.2 ± 2.8a	4.1 ± 0.1a	17.5 ± 3.1a	115.8 ± 2.2c	26.7 ± 2.8c,d	94.8 ± 8.5a	303.7 ± 3.5c
	Cvg-Eve	6076.3 ± 24.8b	19.8 ± 2.0a	4.7 ± 0.9a	17.8 ± 0.3a	120.6 ± 5.7b	27.7 ± 2.4b,c	95.8 ± 8.6a,b	314.0 ± 1.1b
	North	4792.3 ± 35.5e	18.9 ± 2.7a	3.8 ± 0.3a	17.1 ± 0.3a	110.7 ± 7.7d	25.4 ± 2.8e	92.1 ± 8.5b	285.8 ± 2.5d
	Crab	6894.8 ± 12.6a	21.4 ± 3.8a	5.1 ± 0.3a	18.9 ± 1.6a	126.7 ± 7.1a	29.5 ± 3.1a	99.2 ± 7.2a	329.1 ± 1.3a
	Cranberry	6780.9 ± 18.4a	20.7 ± 2.2a	5.2 ± 0.3a	18.6 ± 3.0a	125.6 ± 6.3a	28.3 ± 3.5a,b	97.8 ± 7.2a	323.7 ± 1.6a
leaf	R19	6147.9 ± 89.5bc	22.3 ± 3.6a	16.7 ± 0.4a	11.5 ± 0.7a	42.7 ± 3.3a	61.4 ± 6.2a	33.8 ± 2.6a	344.2 ± 0.6e
	R26	6772.0 ± 39.0a	21.8 ± 3.5a	16.3 ± 1.1a	11.2 ± 0.3b	41.9 ± 8.3a	61.8 ± 5.4a	32.9 ± 2.4a,c	355.6 ± 1.1d
	Cvg-Eve	6240.6 ± 34.7b	22.7 ± 2.3a	16.8 ± 0.9a	10.9 ± 1.1c	42.3 ± 2.4a	60.3 ± 5.2a	32.7 ± 2.1c,d	344.0 ± 1.1e
	North	6060.6 ± 34.6c	22.1 ± 2.3a	17.2 ± 0.1a	11.1 ± 1.7b	42.8 ± 2.9a	60.7 ± 4.3a	32.4 ± 2.6d	370.4 ± 1.3b
	Crab	6954.3 ± 14.4a	23.5 ± 2.4a	16.1 ± 0.8a	10.8 ± 0.9c	41.7 ± 1.1a	61.3 ± 5.9a	33.4 ± 2.3a	380.9 ± 0.5a
	Cranberry	6782.7 ± 50.1a	23.3 ± 3.3a	17.1 ± 0.5a	11.3 ± 0.3a,b	42.1 ± 3.3a	61.5 ± 5.3a	33.2 ± 3.3a,b	363.0 ± 1.0c

[a] Each value is the mean of triplicates ± standard deviation. nd, not detected. Values with the same letter for each fruit part in each column are not significantly different at $p \leq 0.05$. [b] Hydrolyzable tannins include gallotannins, ellagic acid derivatives, and gallagyl tannins, which comprise mainly punicalagin isomers and punicalin. [c] Sum of hydrophilic and lipophilic fractions determined by Folin−Ciocalteau assay (mg of GAE/100 g of FW).

Statistics. All samples were analyzed in triplicate, and the results are expressed as average ± standard deviation. All statistical analyses were carried out using the Microsoft Excel software package (Microsoft Corp., Redmond, WA). Single-factor ANOVA and multiple-range test for variables was used to determine significant differences. Significance was determined at $p \leq 0.05$.

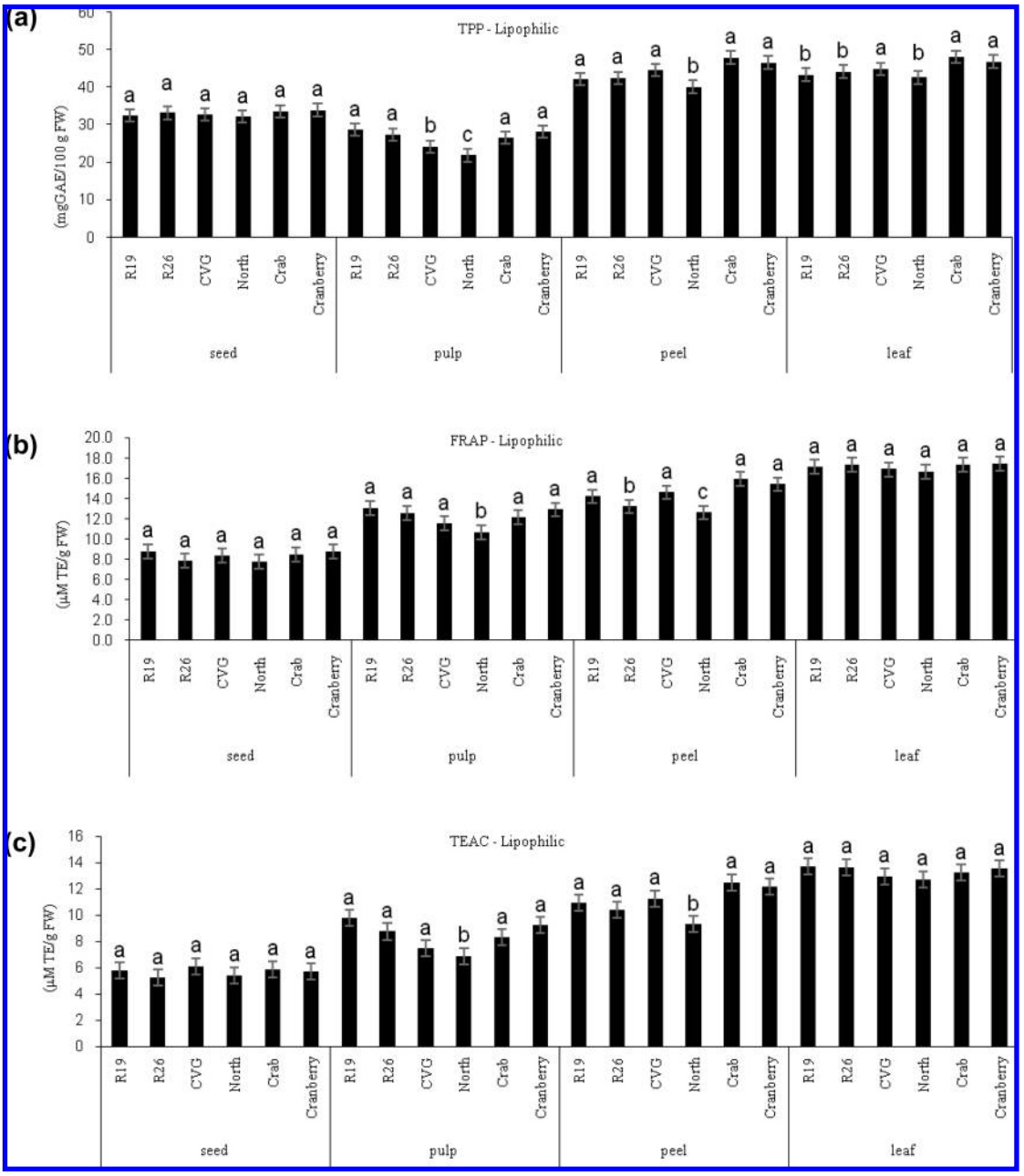

Figure 1. Total polyphenols (TPP) (**a**), FRAP (**b**), and TEAC (**c**) of lipophilic fractions. Values are the average of triplicates. Values with the same letter for each fruit part are not significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

The dry matter contents of different parts of the cultivars are shown in **Table 2**. Dry matter contents of different fractions were comparable within the cultivars. The highest dry matter content was found in seeds (40−50% of FW).

Table 3 shows the major organic acids in different pomegranate cultivars. Organic acids are of great importance to plants, humans, and commercial industries. Fruits and fruit products have a low pH, because they contain high levels of organic acids. Organic acids also influence the growth of microorganisms in fruits and their products and therefore have an effect on their shelf life. Another aspect of organic acids is their influence on the sensory properties and nutritional profile of juice products. Color is also related to the type and level of acids present. The predominant organic acid was citric acid followed by malic acid. This trend is similar to previously reported results by Melgarezo et al. (*25*). The reported average citric acid content in juice from arils ranged from 142 mg/100 g in sweet cultivars to 2317 mg/100 g in sour cultivars (*25*). In all of the cultivars, the pulp fraction had the highest organic acids content followed by peel, leaf, and seeds. Highest ascorbic acid was found in Crab pulp. Citric acid ranged from 113.6 mg/100 g of FW in North leaf fraction to 1926.6 mg/100 g of FW in R26 pulp. For total organic acid content the order of the cultivars was R26 > Crab > Cranberry > Cvg-Eve > R19 > North for pulp fractions. The total organic acid content varied depending on the sourness/sweetness of the cultivar.

Several phenolic compounds were identified in the samples. These were mainly phenolic acids such as caffeic, *p*-coumaric, and ferulic acids and flavonoids such as catechin, epicatechin, and quercetin, with the highest being hydrolyzable tannins as shown in **Table 4**. Hydrolyzable tannins include gallotannins, ellagic acid derivatives, and gallagyl tannins, which comprise mainly punicalagin isomers and punicalin. Two major peaks were obtained at 280 nm along with gallic and ellagic acids, which were further

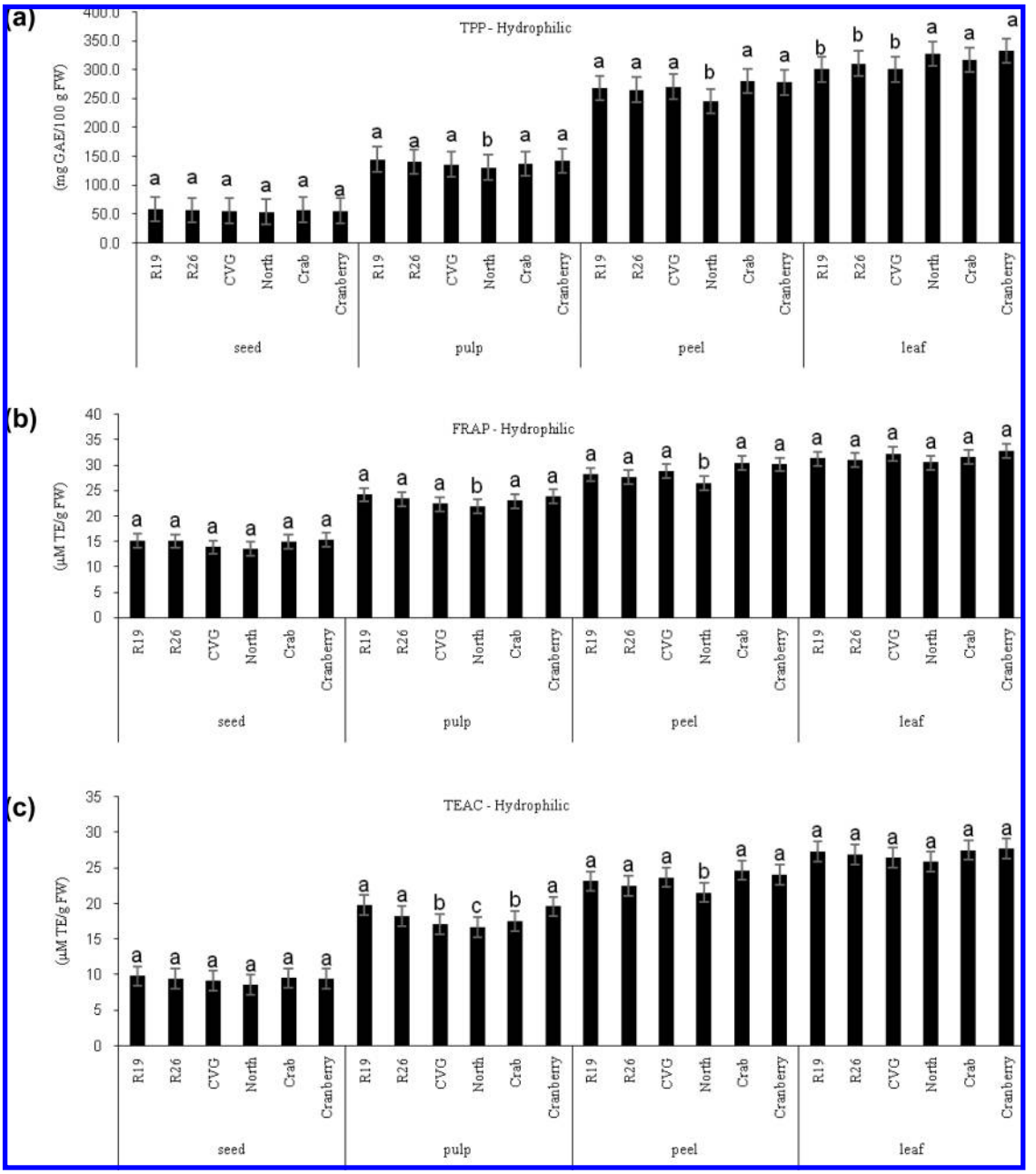

Figure 2. Total polyphenols (TPP) (**a**), FRAP (**b**), and TEAC (**c**) of hydrophilic fractions. Values are the average of triplicates. Values with the same letter for each fruit part are not significantly different at $p \leq 0.05$.

purified. MS-ESI analyses of the peaks indicated the presence of a quasimolecular parent ion at 1084 *m*/*z* (M-H) and a daughter ion at 601 *m*/*z* (gallagic acid), characteristic of punicalagin. Both peaks had the same UV spectra with maxima at 258 and 380 nm. These observations are in accordance with previous studies on pomegranate by Gil et al. (*26*). Gallic acid and its derivative, ellagic acid and its derivatives, and punicalgin isomers were quantified as total hydrolyzable tannins using HPLC-UV detection. Applicable external standards were used for quantification. The highest hydrolyzable tannins were found in leaves, followed by peels, and ranged from 22.8 mg/100 g of FW in North seed to 6954.3 mg/100 g of FW in Crab leaf as shown in **Table 4**. By comparison of the peel fractions of different cultivars, the following trend was observed for total hydrolyzable tannins: Crab > Cranberry > Cvg-Eve > R19 > R26 > North. Among the pulp fractions, the trend was R19 > Cranberry > R26 > Crab > Cvg-Eve > North. Leaves contained the highest amount of caffeic and *p*-coumaric acids. Among the flavonoids, epicatechin and quercetin were found in all of the samples, whereas catechin was absent in the seeds of all the cultivars. In pulp and peel fractions, the predominant flavonoid was catechin with the highest concentration in Crab peel (126.7 mg/100 g of FW). The presence of caffeic, ferulic, and *p*-coumaric acids, catechin, and quercetin in pomegranate juice has also been previously reported (*27*). The average concentration of epicatechin ranged from 6.1 mg/100 g of FW in seeds to 61.2 mg/100 g of FW in leaves (**Table 4**).

The total polyphenol content and FRAP and TEAC values are shown in **Figures 1** and **2**. Considerable difference was found in lipophilic and hydrophilic fractions of the samples, with the hydrophilic fraction showing higher TPP and antioxidant capacity. Among the lipophilic fractions, the highest concentration of TPP was found in Crab leaves (48.1 mg of GAE/100 g of FW). The lipophilic pulp fraction had the lowest TPP content

(**Figure 1a**). The highest FRAP value was found in Cranberry leaves, 17.4 μM TE/g of FW, and the lowest in North seed (7.8 μM TE/g of FW) (**Figure 1b**). TEAC values were lower than the FRAP values, with the lowest being in R26 seed (5.2 μM TE/g of FW) and the highest in R19 leaf (13.7 μM TE/g of FW) (**Figure 1c**). Among the hydrophilic fractions, the highest TPP content, FRAP, and TEAC values were found in Cranberry leaf (332.6 mg of GAE/100 g of FW and 32.8 and 27.7 μM TE/g of FW, respectively) (**Figure 2**). A higher correlation coefficient was found between TPP content and FRAP in hydrophilic fractions ($R^2_{\text{hydrophilic}} = 0.95$) compared to lipophilic fractions ($R^2_{\text{lipophilic}} = 0.50$). High correlation was found between TPP content and TEAC ($R^2_{\text{hydrophilic}} = 0.98$ and $R^2_{\text{lipophilic}} = 0.82$), indicating that the antioxidant capacity of pomegranate may be attributed to total polyphenols content and also that TEAC can be used for assessing both hydrophilic and lipophilic antioxidant capacities. In the case of antioxidant assays, a strong linear correlation coefficient was found in both hydrophilic and lipophilic fractions ($R^2_{\text{hydrophilic}} = 0.99$ and $R^2_{\text{lipophilic}} = 0.91$). Antioxidant capacity and polyphenol content were found to be strongly correlated to the visual appearance of the pulp and peel of the cultivars. North, which has the lowest total polyphenols, total phenolic content, and antioxidant capacity, is light pink in color with a whitish peel.

A comparison of pomegranate with other Georgia-grown crops and other fruits and fruit juices is shown in **Table 5**. Pomegranate pulp had a higher antioxidant capacity (26.5 μM TE/g of FW) than Georgia-grown blackberries (20.4 μM TE/g of FW) (*28*), muscadines (17.6 μM TE/g of FW) (*12*), southern highbush blueberries (14.8 μM TE/g of FW) (*28*), Vidalia onion (1.1 μM TE/g of FW) (*29*), and other fruits and juices in terms of TEAC. TEAC was used for comparison because it is the most common antioxidant assay and also our results showed that it is better suited for both hydrophilic and lipophilic fractions. Pomegranate peel (34.3 μM TE/g of FW) had the highest antioxidant capacity among all Georgia-grown crops and other common fruits such as apple (1.6 μM TE/g of FW) and strawberry (11.0 μM TE/g of FW) (*30*). Pomegranate pulp (26.5 μM TE/g of FW) has a higher antioxidant capacity compared to red wine (19.8 μM TE/g of FW) (*31*), which is considered to be one of the most common and a rich source of antioxidants. The commercial pomegranate juice showed higher antioxidant capacity (41.6 μM TE/g of FW) (*31*) than those determined in our laboratory because during processing extracts of peel and seeds are also incorporated into the juice, whereas in our laboratory the juice from the aril was gently hand-pressed using a muslin cloth. Moreover, ascorbic acid is also added to commercial pomegranate juice, contributing to high antioxidant capacity. Overall, pomegranate has a much higher antioxidant capacity than most of the commonly consumed fruits and fruit juices.

Total lipids and fatty acid compositions are given in **Tables 6** and **7**. The total lipid content plays an important role in several biochemical and nutritional studies. Not only the amount but also the type of fat has a profound effect on health. Determining the fatty acid profile of a substance helps in deciding its end use in both food and nonfood applications. Seeds had an average of 19.2%, and fruit fraction had 0.3% total lipids content. The total lipid contents of Cvg-Eve and North seeds were not significantly different from each other but were significantly different from the

Table 5. Comparison of Pomegranate with Other Georgia-Grown Crops and Other Fruits and Fruit Juices

fruit	total polyphenols (mg of GAE/100 g of FW)	TEAC (μM TE/g of FW)	ref
pomegranate seed[b]	89.2 ± 7.1[a]	15.0 ± 1.8[a]	
pomegranate pulp[b]	164.4 ± 6.4[a]	26.5 ± 2.1[a]	
pomegranate peel[b]	311.3 ± 10.8[a]	34.3 ± 1.9[a]	
Georgia-grown other main crops			
rabbiteye blueberries	556.1 ± 216.9	27.6 ± 5.3	*28*
southern highbush blueberries	399.3 ± 149.1	14.8 ± 8.2	*28*
blackberries	486.5 ± 97.1	20.4 ± 3.3	*28*
muscadine-purple (whole fruit)	247.7 ± 100.5	17.6 ± 7.1	*12*
Vidalia onion (var. Nirvana)	73.3 ± 1.1	1.1 ± 0.0	*29*
other fruits			
apple (Red Delicious)		1.6	*30*
banana		0.6	*30*
pineapple		9.9	*30*
cherry		2.7	*30*
watermelon		0.7	*30*
strawberry (cultivated)		11.0	*30*
fruit juices			
apple juice[c]		4.3 ± 0.3[g]	*31*
red wine[d]		19.8 ± 0.4[g]	*31*
pomegranate juice[e]		41.6 ± 1.8[g]	*31*
acai juice[f]		12.8 ± 0.4[g]	*31*

[a] Sum of hydrophilic and lipophilic fractions. [b] Average of all six cultivars ± standard deviation. [c] Dole apple juice (Pepsico, NY). [d] Merlot Beringer (Beringer Vineyards, Napa, CA). [e] POM Wonderful (POM Wonderful LLC, Los Angeles, CA). [f] Bolthouse Bom Dia Acai-Mangosteen (Bolthouse Juice Products LLC, Bakersfield, CA). [g] TEAC (μM TE/mL).

Table 6. Total Lipid Content and Fatty Acid Compositions of Pomegranate Seed (%)[a]

lipid	R19	R26	Cvg-Eve	North	Crab	Cranberry
total lipid	18.1 ± 0.3b	18.3 ± 0.4b	20.7 ± 0.4a	21.5 ± 1.1a	18.3 ± 0.3b	18.2 ± 0.3b
myristic	0.3 ± 0.1b	0.3 ± 0.3b	0.4 ± 0.0a	0.5 ± 0.2a	0.2 ± 0.0b	0.4 ± 0.0a,b
palmitic	4.2 ± 0.2a	4.8 ± 0.2a	4.4 ± 0.2a	4.1 ± 0.4a	3.2 ± 0.1b	2.8 ± 0.4b
palmitoleic	0.1 ± 0.0b	0.1 ± 0.0b	0.2 ± 0.0a	0.1 ± 0.0b	0.1 ± 0.0b	0.1 ± 0.0b
stearic	3.3 ± 0.2a	3.6 ± 0.3a	3.1 ± 0.1a,b	2.8 ± 0.0b,c	2.4 ± 0.3c	2.1 ± 0.6c
oleic	3.6 ± 0.3d	4.1 ± 0.1c	5.4 ± 0.2b	7.5 ± 0.1a	5.8 ± 0.2b	7.7 ± 0.2a
linoleic	3.2 ± 0.2d	3.8 ± 0.3c	3.5 ± 0.2c,d	4.9 ± 0.0b	3.3 ± 0.1d	5.8 ± 0.3a
linolenic	0.3 ± 0.1a	0.1 ± 0.0a	0.2 ± 0.0a	0.1 ± 0.0a	0.3 ± 0.0a	0.2 ± 0.0a
punicic	83.4 ± 0.5a	81.8 ± 0.4a	80.7 ± 0.5a,b	78.3 ± 0.7b	83.1 ± 0.3a	78.8 ± 0.7b
arachidic	0.6 ± 0.1a	0.8 ± 0.0a	0.6 ± 0.0a	0.3 ± 0.0b	0.5 ± 0.1a,b	0.4 ± 0.1b
lignoceric	0.8 ± 0.1b	0.7 ± 0.1b	0.8 ± 0.3b	0.8 ± 0.1b	0.6 ± 0.2b	1.3 ± 0.2a
nervonic	0.2 ± 0.0b	0.2 ± 0.0c	0.2 ± 0.0c	0.4 ± 0.0a	0.4 ± 0.1a	0.2 ± 0.0c
Σsaturated	9.3	10.3	9.4	8.6	7.0	7.1
Σunsaturated	90.8	90.1	90.2	91.3	93.0	92.8
saturated/unsaturated	0.1	0.1	0.1	0.1	0.1	0.1

[a] Each value is the mean of triplicates ± standard deviation. Values with the same letter in each row are not significantly different at $p \leq 0.05$.

Table 7. Total Lipid Content and Fatty Acid Compositions of Pomegranate Fruit (%)[a]

lipid	R19	R26	Cvg-Eve	North	Crab	Cranberry
total lipid	0.2 ± 0.0a	0.3 ± 0.0b	0.3 ± 0.0b	0.2 ± 0.0a	0.3 ± 0.0b	0.3 ± 0.0b
myristic	0.5 ± 0.1a	0.6 ± 0.3a	0.7 ± 0.1a	0.8 ± 0.1a	0.6 ± 0.3a	0.8 ± 0.3a
palmitic	27.6 ± 0.5b	28.1 ± 0.2a	27.8 ± 0.3a,b	26.8 ± 0.3c	28.3 ± 0.5a	28.6 ± 0.6a
palmitoleic	0.4 ± 0.3a	0.4 ± 0.1a	0.5 ± 0.2a	0.4 ± 0.0a	0.5 ± 0.1a	0.5 ± 0.1a
stearic	3.1 ± 0.3a	3.2 ± 0.3a	3.4 ± 0.4a	3.3 ± 0.1a	3.2 ± 0.3a	3.3 ± 0.3a
oleic	18.8 ± 0.7a	18.4 ± 0.6b	18.9 ± 0.3a	18.3 ± 0.3b	19.2 ± 0.4a	18.7 ± 0.5a,b
linoleic	46.7 ± 0.3a	47.2 ± 0.5a	45.7 ± 0.4a	45.3 ± 0.5a,b	45.8 ± 0.7a	45.0 ± 0.6b
linolenic	0.2 ± 0.1a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
punicic	nd	nd	nd	nd	nd	nd
arachidic	0.3 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.1a	0.3 ± 0.0a	0.3 ± 0.1a	0.3 ± 0.1a
lignoceric	1.4 ± 0.2a,b	1.6 ± 0.1a	1.5 ± 0.0a	1.3 ± 0.0b	1.5 ± 0.3a	1.6 ± 0.1a
nervonic	0.8 ± 0.2a	0.7 ± 0.2a	0.8 ± 0.1a	0.8 ± 0.1c	0.7 ± 0.2a	0.8 ± 0.0a
Σsaturated	33.1	33.9	33.9	32.7	34.1	34.8
Σunsaturated	66.9	66.9	66.1	65.0	66.4	65.2
saturated/unsaturated	0.5	0.5	0.5	0.5	0.5	0.5

[a] Each value is the mean of triplicates ± standard deviation. nd, not detected. Values with the same letter in each row are not significantly different at $p \leq 0.05$.

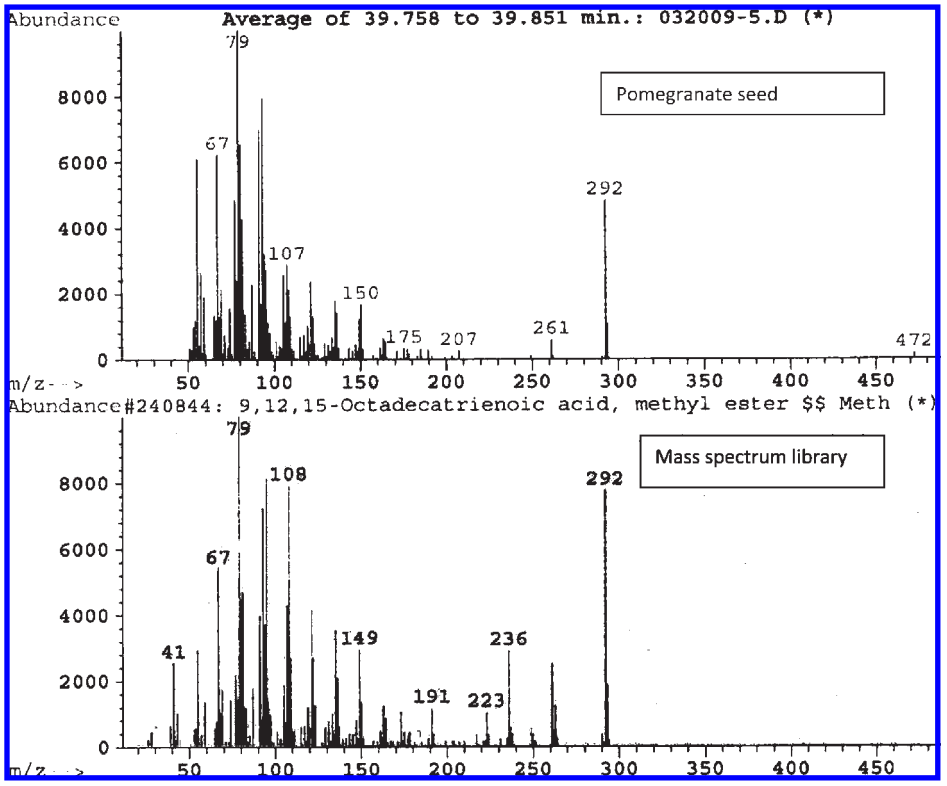

Figure 3. GC-MS spectrum of pomegranate seed fatty acids. Injection was done at 250 °C and detection at 235 °C. Pressure was maintained at 24 psi. Scan was done over the range 50−550 *m*/*z*. The mass spectrum of FAME was characterized by an abundant molecular ion at 292 *m*/*z*, which was identified as a methyl ester of octadecatrienoic acid isomer with the help of a mass spectrum library/database.

other four cultivars (**Table 6**). Comparing the fruit lipid content, no significant difference was observed between North and R19 cultivars, but they were significantly different from the other cultivars (**Table 7**). A weak correlation was found between total lipid content and FAMEs (R^2_{seed} = 0.36 and R^2_{fruit} = 0.48). The mass spectrum of FAME was characterized by an abundant molecular ion at 292 *m*/*z*, which was identified as a methyl ester of octadecatrienoic acid isomer (**Figure 3**). Furthermore, its UV spectra showed maxima at 265, 275, and 287 nm, which correspond to cis−trans−cis configuration (*19*). With the help of these results the compound was identified as punicic acid (9*cis*,11*trans*,13*cis*-octadecatrienoic acid), which was the predominant fatty acid in the seed lipids. This is in accordance with previously reported studies (*32*). The highest punicic acid content was in R19 cultivar (83.4%). Linoleic acid was the major fatty acid in fruit fractions, with the lowest in Cranberry (45.0%) and the highest content in R26 (47.2%). Fruit fractions also had higher palmitic and oleic acid contents than the seeds. The saturated/unsaturated ratio was the same in all of the seeds (0.1) and in the fruits (0.5).

Table 8 shows the tocopherol, phytosterol, and phospholipid contents. For phytosterols and tocopherols, the crude lipid samples were saponified before analysis followed by derivatization in the case of phytosterols to obtain better resolution and peak shape. Pomegranate seed had high contents of α-tocopherol (average = 167.3 mg/100 g) and γ-tocopherol (average = 84.6 mg/100 g).

Table 8. Tocopherol, Phytosterol, and Phospholipid Contents of Seeds and Fruits (mg/100 g)[a]

		tocopherols			phytosterols				phospholipids[b]	
part	cultivar	α	γ	δ	brassicasterol	campesterol	stigmasterol	β-sitosterol	PC	PE
seed	R19	161.2 ± 1.4d	81.1 ± 0.2c	20.7 ± 0.4b,c	2.1 ± 0.9a	22.8 ± 1.6b	32.9 ± 3.6c	333.4 ± 11.2a	5.8 ± 3.6e	15.6 ± 2.7d
	R26	170.1 ± 0.4a,b	80.2 ± 1.1c	20.3 ± 0.4c	2.2 ± 0.2a	17.9 ± 2.1e	38.1 ± 1.3b	314.3 ± 13.4a,b	18.2 ± 1.4c	10.2 ± 0.9e
	Cvg-Eve	168.1 ± 1.5b,c	88.2 ± 0.8b	23.1 ± 2.4a	1.5 ± 0.1b	19.5 ± 2.0c,d	27.8 ± 4.8d	290.1 ± 11.5b	23.1 ± 2.7b	62.2 ± 4.7b
	North	173.7 ± 1.3a	92.8 ± 0.3a	23.8 ± 1.2a	0.9 ± 0.2c	39.3 ± 1.8a	28.5 ± 1.5d	243.5 ± 10.2c	16.3 ± 3.2d	40.8 ± 2.6c
	Crab	168.2 ± 1.2b	82.4 ± 0.7c	21.4 ± 1.5b	nd	19.1 ± 1.1d	46.3 ± 8.1a	345.8 ± 11.0a	26.1 ± 2.1a	74.2 ± 3.7a
	Cranberry	162.4 ± 0.8c,d	81.4 ± 0.5c	20.6 ± 0.9c	nd	38.5 ± 2.5a	35.8 ± 5.1b,c	338.3 ± 12.3a	17.3 ± 2.2c,d	43.4 ± 2.1c
fruit	R19	1.1 ± 0.1d	nd	0.5 ± 0.0b	0.5 ± 0.1a	nd	18.1 ± 2.1a	32.7 ± 9.6e	5.7 ± 2.8b	11.5 ± 1.0b
	R26	3.4 ± 0.2b	0.1 ± 0.0a	1.1 ± 0.1a	0.6 ± 0.3a	nd	14.9 ± 1.6a,b	38.1 ± 10.2c	4.8 ± 1.1b	nd
	Cvg-Eve	0.6 ± 0.1e	nd	0.4 ± 0.0b,c	nd	nd	11.4 ± 5.6b,c	35.8 ± 5.7d	4.8 ± 1.0b	9.4 ± 3.8b
	North	4.1 ± 1.1a	0.1 ± 0.0a	0.1 ± 0.0c	0.3 ± 0.2a,b	nd	9.3 ± 1.1c	49.4 ± 8.3a	15.1 ± 4.1a	33.5 ± 3.9a
	Crab	4.0 ± 1.0a	0.2 ± 0.0a	0.7 ± 0.1a,b	nd	nd	10.9 ± 1.8c	47.4 ± 10.2a	5.8 ± 1.9b	nd
	Cranberry	2.8 ± 0.2c	0.2 ± 0.0a	0.1 ± 0.0c	nd	nd	11.7 ± 2.9b	41.2 ± 6.8b	3.7 ± 0.2c	nd

[a] Each value is the mean of triplicates ± standard deviation. nd, not detected. Values with the same letter for each fruit part in each column are not significantly different at $p \leq 0.05$. [b] PC, phosphatidylcholine; PE, phosphatidylethanolamine.

β-Tocopherol was not detected in any of the samples. The major phytosterols analyzed were brassicasterol, campesterol, stigmasterol, and β-sitosterol, as these are the most common naturally occurring phytosterols. Among them, β-sitosterol was the most abundant in all of the samples, ranging from 32.7 mg/100 g in R19 fruit to 345.8 mg/100 g in Crab seed. The phospholipids were analyzed by HPLC-ELSD. The lowest content of phosphatidylcholine (PC) was found in Cranberry fruit (3.7 mg/100 g) and the highest in Crab seed (26.1 mg/100 g). Crab seed had the highest content of phosphatidylethanolamine (PE) (74.2 mg/100 g) among all cultivars. Pomegranate seed oil contains higher phytosterols (**Table 8**) compared to crude soybean oil, which contains 183 mg/100 g β-sitosterol, 68 mg/100 g campesterol, and 64 mg/100 g stigmasterol (*33*). In terms of tocopherols, pomegranate seed oil contains much higher α-tocopherol (**Table 8**) than soybean oil (9−12 mg/100 g) and similar γ- and δ-tocopherols (**Table 8**). The γ- and δ-tocopherol contents previously reported in crude soybean oil were 74−102 and 24−30 mg/100 g, respectively (*34*).

The results of our research indicate that the byproducts of pomegranate processing, that is, peel and seeds, are rich sources of several high-value compounds with potential beneficial physiological activities. The rich bioactive profile of pomegranate makes it a highly nutritious and desirable fruit crop. Analyzing these cultivars in terms of their antioxidant capacity and lipid profile will also aid in proper cultivar selection, propagation, and commercialization.

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