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Commercial Runner Peanut Cultivars in the United States: Tocopherol Composition

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Tocopherols in commercially grown normal, mid- and high-oleic Runner peanuts from 2005 and 2006 were quantified to give accurate vitamin E contents. Tocopherols were extracted from raw peanuts by a direct solvent extraction procedure using 10% ethyl acetate in hexanes that provided percent recoveries of 105.4, 101.2, 103.9, and 102.8 for α-tocopherol (T), β-T, γ-T, and δ-T, respectively. No significant ($P > 0.05$) differences were noted in total tocopherol levels in normal- (22.4 mg/100 g), mid- (23.9 mg/100 g), and high-oleic (22.4 mg/100 g) Runner peanuts. α-T levels did vary significantly among the Runner cultivars classified by their oleic acid content (mid, 11.7 mg/100 g; normal, 10.9 mg/100 g; high, 9.8 mg/100 g). Cultivar effects were highly significant ($P < 0.001$) for α-, β-, γ-, and δ-T and total tocopherol contents, whereas production year effects were highly significant for α- and β-T levels. Year × cultivar interactions were not significant ($P > 0.05$). Cluster analysis segregated the cultivars into two major groups represented by lower α-T and higher γ-T levels (cluster A) and high α-T and low γ-T levels (cluster B) ($P < 0.05$). The mean α-T level in Runner peanuts (151 samples) was 10.5 ± 1.5 mg/100 g, which is 26.7% greater than the imputed value for peanuts, all types (NBD 16087) provided by the USDA National Nutrient Database for Standard Reference.

KEYWORDS: Runner peanuts; tocopherols; chemical composition; vitamin E; health benefits; USDA National Nutrient Database for Standard Reference

INTRODUCTION

In the United States, Runner, Virginia, Spanish, and Valencia market-type peanuts (*Arachis hypogaea* L.) are commercially grown. Runner cultivars, comprising normal-, mid-, and high-oleic varieties, are primarily produced in Georgia, Alabama, Florida, and Oklahoma and constitute the largest proportion of the total crop in the United States. Vitamin E, consisting of α-, β-, γ-, and δ-tocopherols as well as tocotrienols, is a fat-soluble vitamin that generally functions as a potent antioxidant via chain-breaking reactions during the peroxidation of unsaturated lipids (*1*). It is considered to be a significant health-promoting component of peanuts and tree nuts and affords health benefits to those who routinely consume nuts (*2*−*4*). Because peanuts and peanut butter are the most commonly consumed "nut," comprising over two-thirds of the nuts eaten in the United States, their contribution of vitamin E to the diet from a food source is significant (*5*). Knekt et al. (*6*) as well as Kushi et al. (*7*) demonstrated that the tocopherol content in food is inversely associated with mortality from cardiovascular disease. As a quencher of free radical damage, tocopherols also play a putative role in the prevention of Alzheimer's disease and cancer (*8*).

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Even though peanuts are recognized as an excellent source of vitamin E, published data on the vitamin E content of raw peanuts are variable and difficult to interpret. Most published studies reporting vitamin E levels in raw peanuts have been based on experimental growth conditions that do not reflect commercial production using present-day agronomic practices. Data often represent peanuts from unknown cultivars, cultivars no longer of commercial production significance, small sample sizes, or peanut samples of mixed types (e.g., Runner and Virginia). Currently, the USDA National Nutrient Database for Standard Reference, release 22 (*9*), reports only imputed values for α-tocopherol (α-T) in raw, Virginia peanuts (NBD 16095) and for raw, all-types peanuts (NBD 16087) of 6.56 and 8.33 mg/100 g, respectively. No data are provided for Runner peanuts or for other tocopherols (i.e., β-, γ-, and δ-T).

Of all of the natural forms of tocopherols and tocotrienols, α-T has the greatest biological activity as measured by the traditional rat sterility (fetal resorption) test. Therefore, accurate food compositional data for α-T are essential to correctly assess dietary intake of vitamin E, because human requirements are based on 2*R*-stereoisomers of α-T. Studies also suggest that γ-T does not get the respect it deserves as a nutrient. γ-Tocopherol scavenges reactive nitrogen oxide species (RNOS) to produce 5-nitro γ-T from nitrogen dioxide or the highly reactive peroxynitrite radicals

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generated in vivo from phagocytes during inflammation (*10*−*12*). It acts as an anti-inflammatory agent, inhibits protein kinase C activity, and aids in cell signaling. Specifically, γ-T and its physiological metabolite, 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC), inhibit cyclooxygenase-catalyzed prostaglandin E2 formation (*13*, *14*). Barreira et al. (*15*) suggested that γ-T and γ-CEHC may have anti-inflammatory properties similar to those of nonsteroidal anti-inflammatory drugs, whereas α-T is much less effective in this regard.

One of the earliest studies that reported the complete tocopherol profile (α-, β-, γ-, and δ-T) of raw peanuts showed that total tocopherol levels in five Runner cultivars ranged from 19.5 to 34.2 mg/100 g by Soxhlet extraction using hexane containing 0.01% (w/v) BHT (*16*). Cultivar differences were significant ($P < 0.05$); however, patterns of tocopherols were consistent among the cultivars. α-Tocopherol levels showed considerable variation, ranging from 7.5 to 12.9 mg/100 g. The sum of α- and γ-T constituted 80−90% of the total tocopherol determined in peanuts; tocotrienols were absent (*16*).

Chun et al. (*17*) provided tocopherol profiles for Runner peanuts composed of undetermined cultivars based on the direct solvent extraction procedure used in this study. Twenty-seven samples collected over two years had tocopherol levels of 11.0, 0.4, 10.3, 0.8, and 22.5 mg/100 g for α-, β-, γ-, and δ-T and total tocopherols, respectively. Although details of the extraction procedure were not given, Isleib et al. (*18*) noted tocopherol levels in peanut kernels quite similar to those reported by Chun et al. (*17*) in a mixed sample of peanuts consisting of nine cultivars (i.e., six Runner and three Virginia). In normal peanuts, tocopherol levels were found to be 10.9, 0.33, 8.5, 0.5, and 20.2 mg/100 g, respectively, for α-, β-, γ-, and δ-T and total tocopherols. Significantly lower levels of α-T and total tocopherols and significantly higher levels of γ-T and δ-T were determined in a mixed sample of high-oleic peanuts consisting of four Runner cultivars and one Virginia cultivar. Reported levels in the high-oleic sample were 9.0, 0.3, 9.2, 0.6, and 19.1 mg/100 g for α-, β-, γ-, and δ-T and total tocopherols, respectively.

Studies by Jonnala et al. (*19*) and Davis et al. (*20*) provide somewhat conflicting data compared to the above cited work. The Jonnala et al. (*19*) study used saponification and reported considerably lower vitamin E levels in five high-oleic Runner cultivars grown in Oklahoma. α-Tocopherol levels ranged from 6.8 to 7.4 mg/100 g, and total tocopherol levels ranged from 12.5 to 14.3 mg/100 g based on raw kernel content. The greatest α-T and total tocopherol quantities reported by Jonnala et al. (*19*) represent 79.8 and 75.5% of the mean levels reported by Isleib et al. (*18*) for high-oleic peanuts. Data collected by Davis et al. (*20*) suggested that high-oleic peanut oils possessed greater concentrations of total tocopherols than non-high-oleic counterparts. Unfortunately, Davis et al. (*20*) did not present the compositional data necessary to convert the oil concentrations to a kernel weight basis. Total tocopherols in the peanut oils ranged from 25.5 mg/100 g (C99-R) to 42.5 mg/100 g (AT-201), which represents high cultivar variability. The peanut oils were screw expeller oils and did not take in account complete oil extraction from the kernels.

In addition to cultivar variation, vitamin E levels in peanuts vary by market type (*21*), growing conditions (*16*), production year (*22*), geographic area of production (*22*), and stage of maturity (*16*).

Too often, food databases contain limited or dated compositional data with respect to nutrient and bioactive contents. Availability of improved cultivars and rapid acceptance by farmers, together with modification of production methods, require that compositional data on agricultural commodities be updated in a timely manner to ensure that they are representative of the commodity. This situation is certainly applicable to peanuts grown in the United States. At the request of the peanut industry, our study was designed to re-examine and update compositional information of peanuts, including the levels of key bioactives, grown in the United States. This paper (only a portion of the complete study) reports on the tocopherol (vitamin E) composition of normal-, mid-, and high-oleic Runner peanut cultivars. To ensure the best possible data, a sampling program was designed and completed by the industry to provide U.S. cultivars in current production by accepted agricultural practices. In our opinion, the strength of this research initiative lies in the care and detail that went into the sampling effort of the peanut cultivars. Data resulting from the analyses of 151 Runner peanut samples distinguishes this work from all other peanut tocopherol investigations. Findings from the research will be submitted to the USDA for consideration of inclusion in their Nutrient Database for Standard Reference, as there are currently no data for Runner peanuts (i.e., both normal- and high-oleate genotypes) and only an imputed vitamin E value exists for raw peanuts, all types (NBD 16087).

MATERIALS AND METHODS

Collection of Samples. Runner-type peanut samples ($n = 151$) composed of six normal-oleic, one mid-oleic (i.e., Tamrun OL01), and three high-oleic cultivars from the 2005 and 2006 production years were provided for this study by The Peanut Institute, the USDA−ARS National Peanut Laboratory, and personnel from the peanut industry. In brief, the sampling effort involved the development of a uniform sampling plan that accurately defined major Runner cultivars grown by U.S. peanut farmers. On the basis of sheller input and seed sales to reflect present-day percentages of peanuts in the U.S. market, the cultivars for study were chosen. After selection of these cultivars, seed growers were identified in each of the three peanut-growing regions (i.e., southeast, southwest, and Virginia/Carolina). All samples were field-grown and chosen to accurately reflect cultivars produced by farmers in 2005 and 2006. Peanuts were taken from seed wagons after initial drying to a moisture content between 8 and 10%. An official sample was then pulled from each wagon and graded. The sheller obtained the back half of the official-grade sample and subdivided the sample down to three pounds. This sample was then cleaned using a grade-room Farmerstock cleaner and forwarded to the USDA−ARS National Peanut Research Laboratory in Dawson, GA, for further processing. The samples were shelled and then sent to the Department of Food Science and Technology, University of Georgia, Athens, GA (UGA). After their arrival at UGA, the peanuts were packaged in labeled vacuum pouches (Prime Source, Kansas City, MO) with a vacuum system (Henkelman 600, Henkelman, 's-Hertogenbosch, The Netherlands) to prevent their degradation. The vacuum-packaged peanuts were stored at −40 °C until analyzed.

Chemicals. Hexanes, ethyl acetate, and isopropanol were of HPLC grade and purchased from Fisher Scientific Co. (Suwanee, GA). Anhydrous $MgSO_4$ powder was acquired from Mallinckrodt Baker Inc. (Phillipsburg, NJ). Butylated hydroxytoluene (BHT), ethanol, and tocopherol standards (α-, β-, γ-, and δ-T) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Quantification of Tocopherols. *Direct Solvent Extraction*. The direct solvent extraction method for extracting vitamin E from raw peanuts, based on a technique developed by Landen but modified by Lee et al. (*23*), was employed. Each sample was assayed in triplicate. Approximately 20 g of raw peanuts was ground in a coffee mill (model Tipo 203, Krups, New York, NY) for 10 s with intermittent pulses. One gram of ground sample was accurately weighed into a 125 mL round-bottom glass tube to which 4 mL of 80 °C deionized water was added. The contents were mixed with a stainless steel spatula and then sonicated for 1 min to facilitate solubilization/extraction. Ten milliliters of isopropanol containing 0.01% (w/v) BHT, 5 g of anhydrous $MgSO_4$ powder, and 25 mL of extracting solvent (ethyl acetate/hexanes, 10:90, v/v) containing 0.01% (w/v) BHT, were added and mixed with the spatula. The mixture was blended using a homogenizer (model 300A, Pro Scientific Inc.,

Monroe, CT) at 5600 rpm for 90 s and filtered through a medium-porosity glass filter using a vacuum bell jar filtration unit (Kontes Glass Co., Vineland, NJ). The filter cake was transferred back to the 125 mL round-bottom glass tube for a second extraction. This time, 5 mL of isopropanol containing 0.01% (w/v) BHT and 25 mL of the extracting solvent were added, homogenized, and filtered as described. The pooled filtrates were transferred to a 100 mL volumetric flask and diluted to the mark with the extracting solvent. The solution was passed through a 0.45 μm nylon membrane filter (GE Osmonics Labstore, Minnetonka, MN). A 1.0 mL aliquot of the extract was evaporated under a stream of nitrogen. The residue was redissolved in 1 mL of the mobile phase prior to injection in the HPLC. All extraction steps were carried out under yellow light to prevent oxidation of the tocopherols.

HPLC Quantitation. The vitamin E extracts were injected into an HPLC system consisting of a Shimadzu LC-6A pump equipped with an RF-10A$_{XL}$ fluorescence detector (Shimadzu Corp., Columbia, MD), a SpectraSERIES AS 100 autosampler (Thermo Separation Products, Inc., San Jose, CA), a Waters 746 Data Module integrator (Waters Corp., Milford, MA), a normal-phase LiChrosorb Si 60 column (4 mm × 250 mm, 5 μm particle size; Hibar Fertigsäule RT, Merck, Darmstadt, Germany) connected to a LiChroCART 4-4 guard column packed with LiChrospher Si 60 (5 μm), and an isocratic mobile phase comprising 0.85% (v/v) isopropanol in hexanes at a flow rate of 1.0 mL/min. Before use, the mobile phase was vacuum filtered through a 0.45 μm nylon membrane filter (MSI, Westboro, MA) and degassed by stirring under vacuum. The excitation and emission wavelengths for the fluorescent determination of tocopherol isomers were 290 and 330 nm, respectively. Twenty microliters of sample extract and the tocopherol standard solution were injected per run.

Standard Preparation. Standards were prepared as described by Lee et al. (*23*). Briefly, ~50 mg of α-, β-, and δ-T and 100 mg of γ-T were dissolved in 25 mL of hexanes. For a purity check, 1 mL of each tocopherol solution was pipetted into a 25 mL volumetric flask and evaporated to dryness under a stream of nitrogen. The residue was diluted to volume with 95% (v/v) ethanol. The absorbance of the solution was measured with a DU-62 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) in a quartz cuvette. Extinction coefficients ($E^{1\%}_{1cm}$) of the tocopherol homologues, first published by Scott (*24*) and presented by Eitenmiller et al. (*25*), at λ_{max} of 294, 297, 298, and 298 nm for α-, β-, γ-, and δ-T, respectively, were used to determine percent purity. The purity of each standard was calculated with the equation

$$\% \text{ purity} = (A \times 10/C)/E^{1\%}_{1cm}$$

where A is the absorbance, C is the concentration (mg/mL), and $E^{1\%}_{1cm}$ is the extinction coefficient at λ_{max} for each tocopherol.

The purities of α-, β-, γ-, and δ-T standards were found to be 99.07, 82.47, 98.71, and 89.16%, respectively. On the basis of the purity check, concentrations in the stock standard solutions of α-, β-, γ-, and δ-T solutions were 1.96, 1.65, 3.65, and 1.80 mg/mL, respectively. For the daily working standard, stock solutions were diluted with an appropriate amount of the mobile phase containing 0.01% (w/v) of BHT to give concentrations of 1.96, 0.26, 1.46, and 0.18 μg/mL for α-, β-, γ-, and δ-T, respectively.

Calculation. Concentrations of the tocopherols were calculated from each peak area determined by the Waters 764 integrator (Millipore Corp., Cary, NC) based on the fluorescence response of each tocopherol in a 20 μL injection of the working standard stock solution.

Method Validation. Recovery was determined on the basis of AOAC guidelines (*26*). Ground, raw peanut kernels and Standard Reference Material (SRM) 2387 Peanut Butter [National Institute of Standards and Technology (NIST), Gaithersburg, MD] were spiked with known levels of the tocopherols. Final spiking levels per gram of sample were 21.17, 14.74, 39.43, and 19.48 μg for α-, β-, γ-, and δ-T, respectively. Recovery was calculated with the equation (*26*)

$$R\,(\%) = \frac{C_s - C_p}{C_a} \times 100$$

where R (%) is the percent recovery of added standard, C_s is the tocopherol content in the spiked sample, C_p is the tocopherol concentration in the unspiked sample, and C_a is the amount of tocopherol standard added.

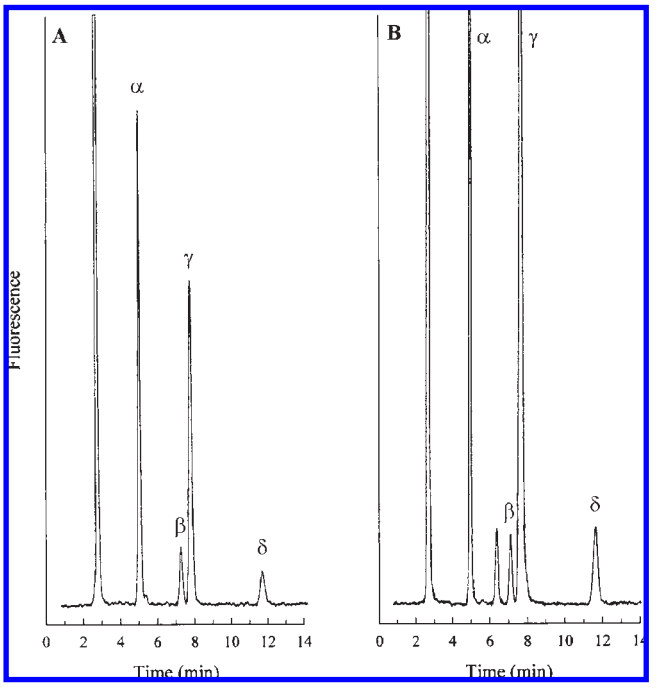

Figure 1. HPLC chromatograms with fluorescence detection of (**A**) commercially available tocopherol standards and (**B**) tocopherol isomers detected in a Runner peanut sample.

Table 1. Recoveries of Tocopherols from Raw Runner Peanuts and the Peanut Butter Standard Reference Material (SRM 2387)

	recovery (%)	
tocopherol	raw peanuts[a] (n = 15)	SRM 2387[b] (n = 5)
α-T	105.4 ± 5.2	100.5 ± 1.0
β-T	101.2 ± 4.9	97.0 ± 2.5
γ-T	103.9 ± 4.4	99.0 ± 2.8
δ-T	102.8 ± 4.5	105.8 ± 2.6

[a] Values (mean ± SD) are based on 15 replicate analyses from 3 cultivars (i.e., Georgia Green [n = 5] for normal, Tamrun OL01 [n = 5] for mid-oleic, and Flavorunner-458 [n = 5] for high-oleic cultivars). [b] Values (mean ± SD) are based on 5 replicate analyses.

Table 2. Accuracy and Repeatability Precision (% RSD$_r$) for the Analysis of Tocopherols in the Peanut Butter Standard Reference Material (SRM 2387)

	contents (mg/100)				
tocopherol	certified value[a]	analytical value[b]	bias	% of certified value	% RSD$_r$[c]
α-T	10.8 ± 1.1	10.0 ± 0.7	0.8	92.6 ± 6.5	7.0
β- + γ-T[d]	10.0 ± 1.9	9.7 ± 0.1	0.3	97.0 ± 1.0	1.0
δ-T	1.0 ± 0.3	1.2 ± 0.1	0.2	120.0 ± 10.0	8.3

[a] The certified reference values for the tocopherols in SRM 2387 were derived from the combination of results provided by NIST and collaborating laboratories. [b] Values (mean ± SD) are based on five replicate analyses. [c] Repeatability precision (% RSD$_r$). [d] NIST provided the sum of β- and γ-T, because the reference values were obtained by a reversed-phase high-performance liquid chromatography which cannot separate β- and γ-T.

Accuracy and bias were evaluated by assaying SRM 2387 and comparing the analytical values to the certified reference values. Bias is the difference between the analytical value from the certified reference value. Interday precision (i.e., relative repeatability of the standard deviation, % RSD$_r$) was evaluated using SRM 2387 according to published protocols (*27*). All of the assays were replicated five times.

Statistical Analysis. Vitamin E contents were reported as means ± standard deviation (SD). Differences of the means of each peanut cultivar

Table 3. Tocopherol Contents in Normal-, Mid-, and High-Oleic Cultivars (Milligrams per 100 g)[a]

type	α-T	β-T	γ-T	δ-T	total
normal-oleic (n = 81)	10.9 ± 1.4 b[b]	0.33 ± 0.14 a	10.4 ± 2.4 b	0.85 ± 0.40 a	22.4 ± 3.3 a
mid-oleic (n = 15)	11.7 ± 1.0 a	0.35 ± 0.07 a	11.2 ± 0.8 ab	0.68 ± 0.10 ab	23.9 ± 1.4 a
high-oleic (n = 55)	9.8 ± 1.3 c	0.26 ± 0.07 b	11.7 ± 1.4 a	0.66 ± 0.08 b	22.4 ± 1.7 a
total (n = 151)	10.5 ± 1.5	0.31 ± 0.12	10.9 ± 2.0	0.76 ± 0.31	22.5 ± 2.7

[a] n = number of samples; data represent the mean ± SD of each sample assayed in triplicate. [b] Means with different letters indicate significant differences among normal-, mid-, and high-oleic peanut cultivars by Tukey's multiple test (P < 0.05).

Table 4. Tocopherol Contents in Runner Peanut Cultivars (Milligrams per 100 g)[a]

cultivar	α-T	β-T	γ-T	δ-T	total
normal					
Georgia Green					
2005 (n = 22)	12.0 ± 1.2a[b]	0.3 ± 0.1b	10.9 ± 3.1	1.2 ± 0.4a	24.5 ± 3.5a
2006 (n = 22)	10.6 ± 1.4b	0.5 ± 0.1a	9.8 ± 1.8	0.9 ± 0.3b	21.8 ± 3.4b
mean ± SD	**11.3 ± 1.5AB**[c]	**0.4 ± 0.2A**	**10.3 ± 2.6BC**	**1.1 ± 0.4A**	**23.1 ± 3.7A**
Tamrun 96					
2005 (n = 5)	11.0 ± 1.1	0.2 ± 0.1	11.8 ± 0.5b	0.6 ± 0.1	23.6 ± 0.9
2006 (n = 3)	10.2 ± 0.5	0.3 ± 0.0	12.9 ± 0.3a	0.6 ± 0.0	23.9 ± 0.4
mean ± SD	**10.7 ± 1.4AB**	**0.2 ± 0.1C**	**12.2 ± 0.1AB**	**0.6 ± 0.1B**	**23.8 ± 0.6A**
C99-R					
2005 (n = 9)	10.2 ± 1.2	0.2 ± 0.1b	10.8 ± 2.3a	0.7 ± 0.2a	21.8 ± 2.7
2006 (n = 15)	10.0 ± 1.0	0.3 ± 0.0a	8.5 ± 1.1b	0.5 ± 0.1b	19.2 ± 1.3b
mean ± SD	**10.1 ± 1.0B**	**0.3 ± 0.1BC**	**9.3 ± 2.0C**	**0.6 ± 0.2B**	**20.2 ± 2.3B**
Georgia-01R					
2005 (n = 1)	11.3	0.3	12.8	1.2	25.7
Georgia-03L					
2006 (n = 1)	7.8	0.4	11.0	0.9	20.1
AP-3					
2006 (n = 3)	11.4 ± 0.9	0.3 ± 0.0	13.0 ± 0.7	0.7 ± 0.1	25.2 ± 0.9
mid-oleic					
Tamrun OL01					
2005 (n = 2)	13.5 ± 0.1a	0.2 ± 0.0b	11.1 ± 1.1	0.8 ± 0.1	25.6 ± 1.2
2006 (n = 13)	11.4 ± 0.7b	0.4 ± 0.1a	11.2 ± 0.8	0.7 ± 0.1	23.6 ± 1.3
mean ± SD	**11.7 ± 1.0A**	**0.4 ± 0.1AB**	**11.2 ± 0.1ABC**	**0.7 ± 0.1B**	**23.9 ± 1.4A**
high-oleic					
Tamrun OL02					
2005 (n = 5)	11.8 ± 0.9a	0.1 ± 0.0b	11.3 ± 1.1	0.7 ± 0.1	24.0 ± 1.8
2006 (n = 17)	10.3 ± 0.8b	0.3 ± 0.0a	10.7 ± 1.4	0.7 ± 0.1	22.0 ± 2.0
mean ± SD	**10.7 ± 1.0AB**	**0.3 ± 0.1BC**	**10.8 ± 1.3ABC**	**0.7 ± 0.1B**	**22.4 ± 2.1AB**
Flavorunner-458					
2005 (n = 3)	11.5 ± 0.5	0.1 ± 0.0b	10.9 ± 0.2	0.6 ± 0.1	23.1 ± 0.7
2006 (n = 3)	10.7 ± 0.8	0.4 ± 0.0a	11.2 ± 0.2	0.7 ± 0.1	23.0 ± 0.8
mean ± SD	**11.1 ± 0.8AB**	**0.3 ± 0.1BC**	**11.1 ± 0.3ABC**	**0.7 ± 0.1B**	**23.0 ± 0.7AB**
Georgia-02C					
2005 (n = 3)	9.4 ± 0.6	0.1 ± 0.0b	13.0 ± 0.6	0.8 ± 0.0a	23.3 ± 1.2
2006 (n = 24)	8.6 ± 0.7	0.3 ± 0.0a	12.5 ± 1.0	0.6 ± 0.1b	22.1 ± 1.5
factorial analysis[d]					
cultivar[e]	***	***	***	***	***
year[f]	***	***	NS	*	**
cultivar × year[g]	NS[h]	NS	NS	NS	NS
mean ± SD	**8.7 ± 0.7C**	**0.3 ± 0.1BC**	**12.6 ± 0.9A**	**0.7 ± 0.1B**	**22.2 ± 1.5AB**

[a] n = number of samples; data represent the mean ± SD of each sample assayed in triplicate. Boldface mean is the mean of all samples for each cultivar for production years 2005 and 2006. [b] Small case letters that are different for production years within a cultivar indicate that the means are significantly different by Tukey's multiple-range test (P < 0.05). [c] Means (n = 7) for production years 2005 and 2006 with different capital letters indicate significant differences of each cultivar except for Georgia-01R, Georgia-03L, and AP-3 (P < 0.05). [d] A two-way factorial analysis was used. [e] All cultivars (n = 7) except for Georgia-01R, Georgia-03L, and AP-3. [f] Production years (n = 2; 2005 and 2006). [g] The interaction between cultivar × production year. [h] NS, not significant; *, **, and *** correspond to significant at P < 0.05, 0.01, and 0.001, respectively.

were determined by Tukey's multiple-range test at $P < 0.05$ using Statistical Analysis System (SAS) software, version 9.0. Cultivar, production year, and cultivar × production year effects were evaluated by a two-way factorial analysis of variance. Georgia 01-R, Georgia-03L, and AP-3 cultivars were eliminated from this factorial analysis and the comparison between cultivars because only one production year was available. The experimental factorial design contained 7 × 2 levels for each of two variables, cultivar and production year. Cultivar and production year interactions were determined at $P < 0.05$, 0.01, and 0.001, respectively.

Cluster analysis was performed to identify relative relationships among Runner cultivars. Hierarchical cluster analysis (HCA) employing the unweighted pair-group method using arithmetic average (UPGMA) was carried out according to the method of Romesburg (*28*). The UPGMA clustering method is the most popular one because it can be employed with any similarity. The hierarchical algorithm represents the nested grouping of patterns and similarity levels at which groupings change (*28*). From 1.0 on similarity, a value for the nearest-neighbor distance at which two clusters from a preceding level merged was calculated. HCA was completed using the XLSTAT for Windows program.

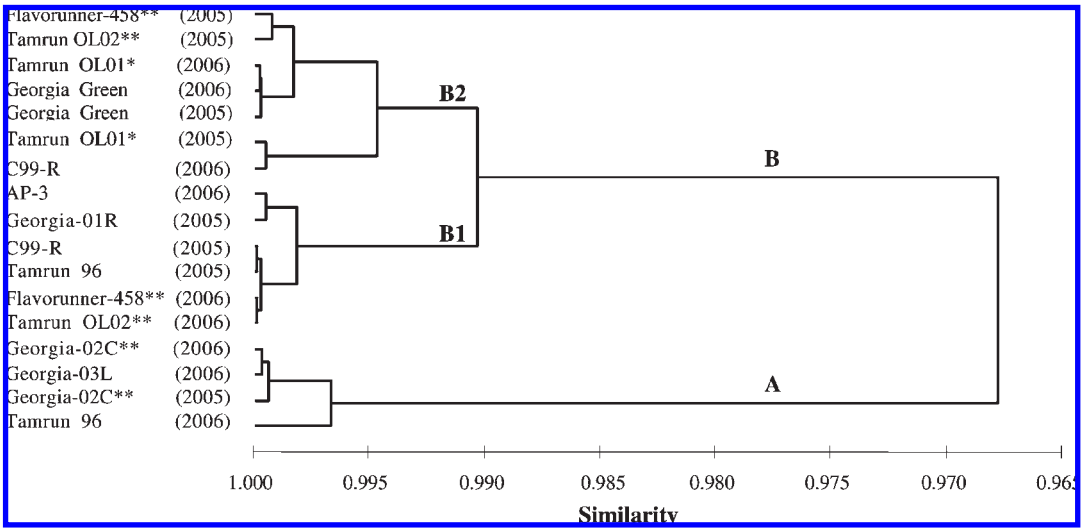

Figure 2. Dendrogram of Runner cultivars based on their means of tocopherol compositions. * and ** represent mid- and high-oleic cultivars.

RESULTS AND DISCUSSION

Figure 1A depicts the HPLC chromatogram for the α-, *β*-, *γ*-, and *δ*-T commercial standards, which eluted at retention times of 4.90, 7.17, 7.75, and 11.84 min, respectively. **Figure 1B** shows an HPLC chromatogram of the tocopherol isomers detected in a Runner peanut sample from this study. In Dr. Eitenmiller's laboratory, palm oil samples are routinely analyzed for their tocopherol and tocotrienol contents. Lack of signals for α-, *β*-, *γ*-, and *δ*-tocotrienol at expected retention times confirmed that they were absent from the peanut samples or at extremely low levels below the detection limit of the system.

Method Validation. Recoveries (%) of tocopherols from raw Runner peanuts and the peanut butter standard reference material from NIST (SRM 2387) are given in **Table 1**. Percent recoveries for the tocopherols ranged from 101.2 to 105.4 for raw peanut kernels and from 97.0 to 105.8 for SRM 2387. **Table 2** presents accuracy determined as percent of the certified tocopherol values for SRM 2387. These values were 92.6 ± 6.5, 97.0 ± 1.0, and 120.0 ± 10.0 for α-, *β*- + *γ*-, and *δ*-T, respectively, indicating very close agreement between the analytical values and the certified values. Small, positive bias values were apparent (0.8, 0.3, and 0.2) for α-, *β*- + *γ*-, and *δ*-T, respectively. Evaluating accuracy by comparing generated analytical data to accepted values from a certified standard has been reported in other works (*29*, *30*); unfortunately, this is not the norm as most studies fail to perform such evaluations. On the basis of five injections, linearity tests of the standard curves were completed over the ranges of 0.0−39.20, 0.0−5.24, 0.0−29.20, and 0.0−3.61 ng in 20 *μ*L injection volumes for α-, *β*-, *γ*-, and *δ*-T, respectively. Excellent linear relationships ($r^2 > 0.999$) were observed for the fluorescent response of each tocopherol. Limit of detection (LOD) values were 0.15, 0.14, 0.16, and 0.11 ng/20 *μ*L injection for α-, *β*-, *γ*-, and *δ*-T, respectively, whereas the respective limit of quantitation (LOQ) values were 0.28, 0.26, 0.28, and 0.13 ng/20 *μ*L injection.

Tocopherols in Normal-, Mid-, and High-Oleic Runner Cultivars. Tocopherol levels in normal-, mid-, and high-oleic cultivars collected in 2005 and 2006 are presented in **Table 3**. Normal cultivars included Georgia Green ($n = 44$), Tamrun 96 ($n = 8$), C99-R ($n = 24$), Georgia-01R ($n = 1$), Georgia-03L ($n = 1$), and AP-3 ($n = 3$). The mid-oleic group contained Tamrun OL01 ($n = 15$), and the high-oleic group consisted of Tamrun OL02 ($n = 22$), Flavorunner-458 ($n = 6$), and Georgia-02C ($n = 27$). Mean vitamin E (i.e., α-tocopherol [T] levels) in the normal-, mid-, and high-oleic cultivars examined were 10.9 ± 1.4, 11.7 ± 1.0, and 9.8 ± 1.3 mg/100 g, respectively. These values represent ca. 30, 40, and 17% increases, respectively, from the imputed value listed in the USDA National Nutrient Database for Standard Reference (*9*) for raw peanuts, all types (NBD 16087). The overall means for the individual tocopherols in Runner peanuts ($n = 151$) were 10.5 ± 1.5 for α-T, 0.31 ± 0.12 for *β*-T, 10.9 ± 2.0 for *γ*-T, and 0.76 ± 0.31 mg/100 g for *δ*-T, respectively. Total tocopherol levels in normal-, mid-, and high-oleic cultivars were 22.4 ± 3.3, 23.9 ± 1.4, and 22.4 ± 1.7 mg/100 g, respectively. Differences in total tocopherol contents were not significant ($P > 0.05$) among the three groups. For all samples, α-T and *γ*-T were the dominant E vitamers, comprising ca. 95% of the total vitamin E present in the kernel. The mid-oleic cultivar, Tamrun OL01, had significantly higher α-T content than normal and high-oleic cultivars ($P < 0.05$). Normal cultivars had significantly greater α-, *β*-, and *δ*-T contents than high-oleic cultivars, whereas the *γ*-T content was significantly lower ($P < 0.05$). Higher *γ*-T levels in high-oleic peanut oils compared to normal peanut oils have been reported by Yamaki et al. (*31*) and Isleib et al. (*18*). The Isleib et al. (*18*) study, although based on mixed samples of Runner and Virginia types, showed *γ*-T levels to be significantly higher ($P < 0.01$) in the oil from the high-oleic cultivars. The Yamaki et al. (*31*) study compared the high-oleic Runner SunOleic cultivar to a Virginia-type sample of unspecified cultivars. For α-T, the mid-oleic cultivar, Tamrun OL01, had a significantly higher level ($P < 0.05$) of 11.7 mg/100 g compared to normal- (10.9 mg/100 g) and high-oleic cultivars (9.8 mg/100 g).

Tocopherol levels in the 10 Runner peanut cultivar samples from the 2005 and 2006 production years are given in **Table 4**. Total tocopherol contents ranged from 20.1 mg/100 g (Georgia-03 L) to 25.7 mg/100 g (Georgia-01R). On the basis of data from the two crop years, the mean total tocopherol level of 20.2 mg/100 g found in C99-R was significantly lower than mean levels found in the other cultivars ($P < 0.05$). Significant ($P < 0.05$) differences in total tocopherol contents between the 2005 and 2006 production years were noted for Georgia Green and C99-R.

For the individual tocopherols, cultivar differences were observed, but specific patterns of variation were not apparent (**Table 4**). For α-T, the mean levels ranged from 7.8 mg/100 g (Georgia-03L) to 11.7 mg/100 g (Tamrun OL01). The mean α-T content of Georgia-02C (8.7 mg/100 g) was significantly lower

than that found in the other cultivars. Only Georgia-03L, which was not included in the statistical analysis because only one sample from 2006 was available, possessed a lower α-T level (7.8 mg/100 g). Year-to-year variation was also noted in α-T contents for Georgia Green, Tamrun OL01, and Tamrun OL02 ($P < 0.05$).

Mean γ-T levels ranged from 9.3 mg/100 g (C99-R) to 12.6 mg/100 g (Georgia-02C). Year-to-year variation was noted in γ-T levels for Tamrun 96 and C99-R ($P < 0.05$). β-Tocopherol levels were found at levels between 0.1 and 0.5 mg/100 g. Such low levels of β-T are, however, typical of most foods. Except for Georgia Green and Georgia-01R, δ-T was present at levels below 1.0 mg/100 g.

Factorial analysis (**Table 4**) revealed that cultivar ($n = 7$) effects were strongly significant ($P < 0.001$) for all tocopherols. Strongly significant ($n = 2$) year effects were noted for α- and β-T ($P < 0.001$). Year × cultivar interactions were not significant ($P > 0.05$).

Cluster Analysis. Figure 2 depicts the dendrogram representing cultivar clusters grouped by similarities in tocopherol profiles. Runner cultivars were grouped into clusters A and B at a similarity level of ~0.967. The mean level of α-T for the cultivars in cluster B (11.0 mg/100 g) was significantly greater than the mean of cluster A cultivars (8.9 mg/100 g) ($P < 0.05$). Conversely, for γ-T, the mean for cluster A (12.6 mg/100 g) was significantly higher than the mean of cluster B (10.5 mg/100 g) ($P < 0.05$). Because γ-T is the biosynthetic precursor for α-T through the action of γ-tocopherol methyltransferase (γ-TMT) in the plant kingdom, one expects these relationships to exist (*32*). Shintani and DellaPenna (*33*) showed that γ-TMT is a primary determinant of the tocopherol composition of seed oils.

Within cluster B, the cultivars in subcluster B1 contained a significantly higher ($P < 0.05$) mean γ-T content (11.2 mg/100 g) compared to the mean of subcluster B2 cultivars (10.2 mg/100 g). Again, as noted for the relationship between α-T and γ-T levels shown for clusters A and B, the mean α-T level for cultivars in subcluster B2 (11.2 mg/100 g) was significantly higher ($P < 0.05$) than the mean for subcluster B1 (10.4 mg/100 g), demonstrating that as α-T content increases, γ-T content decreases. The relative order of the clusters based on the mean α-T and γ-T levels clearly shows the biosynthetic relationship between α-T and γ-T (γ-T, cluster A > cluster B1 > cluster B2; α-T, cluster B2 > cluster B1 > cluster A).

To summarize, the composition of tocopherols was assessed in commercial Runner peanuts obtained from a well-designed sampling plan that provided cultivars representative of those in current production by accepted farming practices. Cultivar effects were highly significant for the tocopherols and for total tocopherol levels. Cluster analysis segregated the cultivars according to α- and γ-T levels. The study clearly indicates that the combination of analytical data with chemometric techniques can be used by peanut breeders to give more information on peanut cultivars than is possible with the experimental data alone. The mean α-T level of 10.5 mg/100 g determined in Runner peanuts indicates that peanuts and peanut products provide on average 25^{+}% higher levels of vitamin E to the consumer than indicated by the value currently reported in the USDA Nutrient Databank of Standard Reference, release 22 (*9*). The typical daily intake of vitamin E by a U.S. resident (ca. 10 mg) is below the Recommended Daily Allowance (RDA) of 22.5 IU or 15 mg of α-tocopherol. Therefore, consuming peanuts and peanut-containing foods on a daily basis is a healthy choice in trying to meet the RDA for vitamin E.

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