I.B. Hashim, P.E. Koehler\* and R.R. Eitenmiller

Department of Food Science and Technology, University of Georgia, Athens, Georgia 30602

Alpha-, beta-, gamma-, and delta-tocopherol ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T and  $\delta$ -T) were determined in peanut (*Arachis hypogaea* L.) cultivars Florunner, Sunrunner, Southern Runner, AT-127, GK-7, NC-9 and GK-3. There were significant differences in tocopherol content among runner- and virginiatype peanut cultivars, and these differences were affected by the degree of maturation.  $\alpha$ -T decreased with maturation for all of the cultivars except Florunner.  $\gamma$ -T increased with maturation for GK-7, NC-9 and GK-3 but decreased for Florunner. For the mature nuts, Florunner and NC-9 had the highest levels of  $\alpha$ -T while GK-3 and Southern Runner had the lowest levels. GK-7 and Sunrunner had the highest levels of  $\gamma$ -T, while the lowest levels were in Florunner and GK-3.

KEY WORDS: Arachis hypogaea L., cultivar, genotype, maturity, tocopherol.

Peanut (Arachis hypogaea L.) seed is an important oil crop throughout the world. Vitamin E is a naturally occurring antioxidant in edible oils and fats, mainly consisting of alpha-tocopherol ( $\alpha$ -T), beta-tocopherol ( $\beta$ -T), gammatocopherol ( $\gamma$ -T) and delta-tocopherol ( $\delta$ -T) (1). All natural tocopherols are derived from plant sources and concentrate in the seeds. Tocopherols act in the same manner as synthetic antioxidants by delaying or preventing oxidation of oils by interrupting the formation of free radicals (2). The various tocopherols differ in their biological activities and their ability to protect oils from oxidative rancidity. While  $\alpha$ -tocopherol is the most biologically active form of natural Vitamin E (3), the relative antioxidative effectiveness of the tocopherols increases in this order:  $\alpha$ -T,  $\beta$ T,  $\gamma$ T and  $\delta$ T (4–6).

Peanuts are a good source of tocopherols (7). Sturm *et al.* (5) determined tocopherols in peanut oil from 17 varieties of three genotypes grown under the same conditions and found that runner varieties are the richest source for  $\alpha$ -T,  $\gamma$ T and  $\delta$ -T, while Spanish varieties had the lowest  $\alpha$ -T. Green (8) studied tocopherols during maturation of maize, wheat, barley and pea plants. The effect of maturation has not been previously examined in peanuts. The objectives of this study were to determine the variation among tocopherols and their level in different peanut cultivars at various maturity stages.

# MATERIALS AND METHODS

Shelled peanuts from eight cultivars of two genotypes, runner [Florunner (Flo-R), Sunrunner (Sun-R), Southern Runner (South-R), GK-7 and AT127] and virginia (NC-7, NC-9 and Gk-3), were obtained from the Coastal Plain Experiment Station (Tifton, GA; 1990 crop). All cultivars were grown under the same standard cultural practices and conditions. From each cultivar, three maturity stages [immature (Im-M), middle mature (Mid-M) and mature (Mat)] were obtained and stored at -10 °C until used for tocopherol analysis.

Extraction for tocopherol analysis. Samples were ground in a Krups fast-touch (Type # 203) coffee mill (Robert Krups, Closter, NJ) for 30 s. A portion of the ground sample was then mixed with sodium sulfate anhydrous powder at a ratio of 1:4 [peanut/sodium sulfate (w/w)] and blended until mixed thoroughly. Duplicate 2.0-g samples were extracted by Soxhlet with hexane containing 0.01% butylated hydroxytoluene. The extractions were done in a dark chamber for four hours. The extract was used directly for tocopherol analysis (9). The volume of the extract was used as a dilution factor for calculating tocopherol content.

High-performance liquid chromatography (HPLC) separation and quantitation. A 25 cm  $\times$  4 mm (5  $\mu$ m) LiChrosorb Si60 column (Rainin Instrument Co. Inc., Woford, MA) with a mobile phase of 2-propanol (1.0%, vol/vol) in hexane (HPLC grade) at a flow rate of 1.0 mL/min was used for tocopherol analysis. The mobile phase was filtered through a 0.45  $\mu$ m Nylon 66<sup>R</sup> membrane filter (Rainin Inc.) and degassed immediately before use. A Shimadzu LC-6A pump (Kyoto, Japan) was used, and sample injections were made with a 100-L Valco loop injection valve (Rheodyne, Cotati, CA). The detector was a Perkin-Elmer 650-15 fluorescence spectrophotometer (Hitachi, Norwalk, CT) at 290 nm excitation and 330 nm emission wavelength. A Hewlett-Packard 3392A integrator (Hewlett-Packard Co., Avondale, PA) was used to quantitate tocopherol levels from the detector output. Tocopherol standards ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T and  $\delta$ -T) were obtained from Henkel Corporation (La Grange, IL). Tocopherol peaks were identified by retention time relative to the standards. Recovery was assessed by spiking 20, 25 and 10  $\mu$ g of  $\alpha$ -T,  $\gamma$ -T and  $\delta$ -T, respectively, into 2.0 g sample before Soxhlet extraction (9). The  $\alpha$ -T,  $\gamma$ -T and  $\delta$ -T were determined and percent recovery was calculated. The concentration of each tocopherol was calculated from the peak area and corresponding standard peaks.

## **RESULTS AND DISCUSSION**

Figure 1 is a representative chromatogram of the normalphase HPLC tocopherol profile of peanut extract. This was an injection of a concentrated extract, which gave an off-scale peak of  $\alpha$ -T and  $\gamma$ -T to quantitate  $\beta$ -T and  $\delta$ -T. Significant differences were found within the tocopherol profile among maturity stages of runner-type peanut.  $\alpha$ -T decreased with maturation for all runner types except for Flo-R. Each cultivar has its own pattern during maturation; Flo-R increased with maturation while South-R and Sun-R decreased. AT-127 increased, then decreased. GK-7 decreased, then increased with maturation (Fig. 2). The  $\gamma$ -T increased with maturation for GK-7 while the middle mature stage of Flo-R and AT-127 showed the highest level, which was significantly different from the mature and the immature peanut. There were no significant

<sup>\*</sup>To whom correspondence should be addressed.



FIG. 1. Typical high-performance liquid chromatography tocopherol profile of peanuts.

differences among the different maturity stages for South-R and Sun-R (Fig. 3).

For virginia types,  $\alpha$ T decreased significantly with maturation of NC-7, but there was no significant difference between the immature and middle mature peanuts. For GK-3, there was no significant difference between immature and mature peanuts, but the middle mature nuts were significantly different from the mature nuts. There were no significant differences among the maturity stages for NC-9 (Fig. 4). The  $\gamma$ T increased significantly with maturation for NC-9 and GK-3 but there was no significant difference between the middle mature and mature peanuts for either cultivar. There were no significant differences among the maturity stages for NC-7 (Fig. 5). These results agree with the findings of Green (8), that tocopherol patterns in maize, wheat, barley and pea plants change greatly during maturation.

The synthesis of  $\gamma$ -T is associated with seed formation or particularly seed maturation, because  $\alpha$ -T decreases with maturation while  $\gamma$ -T increases (except for Flo-R). We speculate that  $\gamma$ -T could be formed by process of demethyl-



FIG. 2. Changes in alpha-tocopherol (alpha-T) content of runner peanuts with maturity. Abbreviations: South-R, Southern Runner; Sun-R, Sunrunner; Flo-R, Florunner; Im-M, immature stage; Mid-M, middle mature stage; Mat, mature stage.



FIG. 3. Changes in gamma-tocopherol (gamma-T) content of runner peanuts with maturity. For abbreviations see Figure 2.

ation from  $\alpha$ -T in the seeds, which may improve seed stability.

For mature kernels, significant differences were found within the tocopherol profile between genotypes and among cultivars of the same genotype. Among runner types, Flo-R showed the highest level of  $\alpha$ -T and the lowest level of  $\gamma$ -T, while GK-7 showed the highest levels of both  $\gamma$ -T and  $\delta$ -T. For virginia types, NC-9 showed the highest levels of  $\alpha$ -T,  $\gamma$ -T and  $\delta$ -T while GK-3 showed the lowest levels of  $\alpha$ -T and  $\gamma$ -T and the highest value for  $\beta$ -T (Table 1).

Because  $\alpha$ T is the most biologically active form of vitamin E, the other tocopherols were converted to  $\alpha$ T by dividing  $\beta$ T,  $\gamma$ T and  $\delta$ T by 2, 10 and 40, respectively, to obtain the total  $\alpha$ T. From a nutritional point of view, NC-9 and Flo-R had the highest levels of vitamin E (total  $\alpha$ T), while South-R and GK-3 had the lowest levels. From the



FIG. 4. Changes in alpha-tocopherol (alphaT) content of virginia peanuts with maturity. For abbreviations see Figure 2.

stability point of view, GK-7 and Sun-R had the highest levels of  $\gamma$ T and  $\delta$ T while Flo-R and GK-3 had the lowest levels (Table 1).

Results from the recovery studies showed that 100% of the added  $\alpha$ -T and y-T, and 90% of added  $\delta$ -T were found in the extract.



FIG. 5. Changes in gamma-tocopherol content of virginia peanuts with maturity. For abbreviations see Figure 2.

### TABLE 1

Tocopherol Content of Mature Peanut Kernels (mg/100 g)<sup>a</sup>

Cultivar	a-T	<i>β</i> -T	γ-T	δ-T	Total a-T
Florunner	12.93a	0.31b,c	9.49f	0.36 <sup>e</sup>	14.05a,b
Sunrunner	10.72 <sup>b</sup>	0.31b,c	20.36b	0.89a,b	12.94b,c
GK-7	9,92b,c	0.28 <sup>c</sup>	22.97a	0.98a	12.39c,d
AT-127	9 <u>.51</u> b,c	0.31b,c	17.71 <sup>c</sup>	0.82 <sup>b</sup>	11.46d,e
Southern Runner	7.45 <sup>e</sup>	0.20d	11.27 <sup>e</sup>	0.53d	8.70g
NC-9	12.97a	0.37b	16.35 <sup>c</sup>	0.66 <sup>c</sup>	14.81 <sup>a</sup>
NC-7	8.89d,e	0.26c,d	13.28d	0.60c,d	10.37e, <b>f</b>
GK-3	8.09d,e	0.48 <sup>a</sup>	9.88e,f	0.60c,d	9.34 <sup>f</sup> ,g

<sup>a</sup>Values in the same column followed by the same letter are not significantly different at (P > 0.05). T, tocopherol.

#### ACKNOWLEDGMENTS

The authors thank Dr. Craig Kvein at the Department of Agronomy, Coastal Plain Experiment Station, Tifton, GA, for supplying the peanut samples. This work was supported in part by Georgia Agricultural Commodity Commission for Peanuts and by the Georgia Agriculture Experiment Station.

#### REFERENCES

- 1. Ball, G.F.M., and P.W. Ratcliff, J. Food Technol. 13:433 (1978).
- 2. Labuza, T.P., Crit. Rev. of Food Technol. 2:355 (1971).
- 3. Parrish, D.B., CRC Crit. Rev. Food Sci. Nutr. 13:161 (1980).
- 4. Griewahn, J., and F.B. Daubert, J. Am. Oil Chem. Soc. 25:26 (1948).
- 5. Sturm, P.A., R.M. Parkhurst and W.A. Skinner, Anal. Chem. 38:1244 (1966).
- 6. Cort, W.M., J. Am. Oil Chem. Soc. 51:321 (1974).
- 7. Ahmed, E.M., and C.T. Young, in *Peanut Science and Technology*, edited by H.E. Pattee, and C.T. Young, American Peanut Reseach Education Society, Inc., Yoakum, 1982, pp. 670.
- 8. Green, J., J. Sci. Food Agr. 9:801 (1958).
- 9. Yao, F., Ph.D dissertation, HPLC Quantification of Tocopherols in Pecans and Relationship of Tocopherol Levels During Storage to Pecan Quality, University of Georgia, Athens, 1990.

[Received September 9, 1992; accepted April 21, 1993]