Fatty Acid Composition and Tocopherol Content of Drought Stressed Florunner Peanuts

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ABSTRACT

Drought stress has been heavily investigated for its effects on production efficiency and yield, but less attention has been given to its effects on peanut quality. Peanuts (Florunner cv) were stressed for 30 days, by withholding irrigation and using shelters, at the pre-flowering period (stressl), pod formation period (stress2), and maturation period (stress3). Fatty acid composition, oleic linoleic ratio (O/L), computed iodine value (IV) and tocopherol content of Florunner peanuts were investigated. The fatty acid composition acid composition, O/L ration, IV, alpha-tocopherol (α-T) and gamma-tocopherol (γ-T) were significantly affected by drought stress and peanut grade. As peanuts increased in size regardless of stressing period, long chain saturated fatty acids [arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0)], eicosenoic acid (20:1), (O/L), and α-tocopherol decreased significantly. Stressing peanuts during the maturation period is most detrimental to peanut stability, decreasing O/L ratio and increasing IV.

Key Words: *Arachis hypogaea* L., peanut grade, drought stress, fatty acids, O/L ratio, IV, tocopherol

Drought conditions cause low yields and poor grade peanuts (4,21), decrease subsequent germination (15), and increase incidence of aflatoxin contamination (5). The *Arachis hypogaea* L. cv. Florunner is more sensitive to drought at the seed filling phase compared to the pod-initiation phase (16), while cv. Valencia is most sensitive at the late flowering and pod formation period (17). Stressing cv Robut33-1 during the pre-flowering phase increases the yield 13-19% (12). Late-season drought is more detrimental to final pod yield than early-season stress (22). Mid-season drought (65-100 days after planting) decreases the yield and increases the portion of immature pods (13).

A peanut seed contains approximately 50% of its weight as oil. Generally the oleic and linoleic acid proportions together make up 80% of the fatty acid in peanut oil (1). Several factors affect the quantity of the individual fatty acids in peanut oil, including variety, seasonal variation, the part of the seed analyzed, abnormalities such as disease and insect damage (3), genotype (14), production location and/or temperature condition under which the crop is grown (18), maturity (19, 26), and the market grade (11). Peanut storage qualities and nutritional quality are both dependent on the relative proportions of the saturated and unsaturated fatty acids that make up the oil. From a nutritional standpoint, a high polyunsaturated fatty acid content is desirable in lowering plasma cholesterol level and low-density lipoprotein, which may reduce the risk of coronary heart disease and atherogenesis (8). Fats containing high percent oleic acid also may be beneficial in lowering blood cholesterol level (7). The total amount of unsaturation is inversely proportional to the keeping quality of the oil, oxidative rancidity increases with increased level of the polyunsaturated fatty acids which cause associate odors and flavors (20). The iodine value (IV), which provides a measure of the degree of oil unsaturation, and the ratio of oleic to linoleic acid (O/L) have been commonly used as a means of predicting shelf-life and measuring stability of the oil. Higher (O/L) ratios and lower IV generally suggest better stability and longer shelf-life (23, 25).

Peanuts are a good source of tocopherols, which are natural antioxidants (1). Total tocopherol content in almond, pecan, and macadamia kernels decreased during storage, as a result of inhibitory function during autoxidation (6).

Drought stress has been heavily investigated for its effect on production efficiency and yield, but less attention has been given to its effect on the quality of the peanuts. The objectives of this experiment were to investigate the effect of drought stress on the fatty acid composition, tocopherol content and stability of Florunner peanuts.

Materials and Methods

Peanuts, cv. Florunner were planted in rainfall protected plots of Tifton loamy sand (fine-loamy, siliceous, thermic plinthic (Kandidult) at the Coastal Plain Experiment Station, Tifton, GA. Treatments consisted of 30-day periods of water stress beginning 20 days after planting (DAP) at the vegetative growth or pre-flowering period (stress1), 50 DAP at the pegging down or pod formation period (stress2), and 80 DAP at seed filling or nut maturation period (stress3) in addition to a control or adequately-watered treatment (irrigated). Plots were maintained using recommended cultural practices (9). Automated portable rainout shelters built by Kvien and Branch (10) were positioned near the plots and automatically traveled over the plots whenever rain was detected during each drought stress period. Harvest date for each plot was determined by the hull-scrape method. Peanut were shelled and sized into three runner market-grades using standard shellers screen grades by Federal-State graders at Farmer Fertilizer and Milling in Colquitt, GA. The three grades were No. 1 (seed passing through a 71.4 mm slotted screen but riding a 63.5 mm), Medium (fall through 83.3 mm and ride a 71.4 mm), and Jumbo (ride a 83.3 mm).

Fatty acids analysis

Oil extraction and transesterification of fatty acids to their methyl esters by boron trifluoride were done according to AOAC methods (2). Fatty acid methyl esters were separated using a Varian Model 3700 Gas Chromatograph (Varian Associates, Inc. Palo Alto, CA) equipped with a (180 x 0.64 x 0.20 cm) glass column packed with GP 5% DEGS-PS on Supelcoport (100/20 mesh) (Supelco, Inc. Bellefonte, PA). Column temperature was programmed from 70 C (held for 5 min) to 190 C (held for 25 min.) at 10 C/min. The flame ionization detector temperature was 250 C and the injector temperature was 220 C. The carrier gas (nitrogen) had a flow rate of 20 mL/min. A Shimadzu CR601 chromatopac integrator (Shimadzu Corporation, Kyoto, Japan) was used to record the retention time and peak area of fatty acids. A standard fatty acid methyl esters mixture (All Tech Associates Inc., Applied Science Lab.. Deerfield, IL) was used to identify and fatty acids in the samples.

(O/L) and (IV) were computed as follows:

O/L ratio = % oleic (18:1)/linoleic (18:2)

IV= (% oleic x 0.8601) + (% linoleic x 1.7321 + (% eicosenoic x 0.7854)

Tocopherol analysis

Samples were ground using a Krups fast-touch (Type #203) coffee mill (Robert Krups, Closter, NJ) for 30 sec. A portion of the ground sample was then mixed with anhydrous sodium sulfate at a ratio of 1:4 (peanut:sodium sulfate) (w/w) and blended until mixed thoroughly. Duplicate 2.0 g

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Table 1. Analysis of variance mean squares x 10 for fatty acids, O/L, IV, α-T, and γ-T of four drought stress periods and three market grades Florunner peanuts.

		Fatty acids											
Source	df	16:0	18:0	20:0	22:0	24:0	18:1	18:2	20:1	O/L	IV	α-T	γ-T
Stress (S)	3	51.8**	1.6**	5.9*	69.4**	36.2**	276.4**	647.8**	9.8**	3.1**	1054.5**	12.9**	83.5**
Error A (Rep/S)	6	2.2	0.05	1.4	18.4	11.2	7.7	63.9	2.0	0.2	190.0	0.1	0.9
Grade (G)	2	18.8**	1.6**	54.3**	78.1**	21.3*	8.9	284.6**	7.8**	0.7**	804.7**	163.3**	108.6**
G x S	6	4.9*	0.01	11.2**	29.0*	12.7*	37.9*	49.8**	4.1**	0.03	280.1**	22.5**	125.1**
Error B (Rep/S/G)	52	1.6	0.03	2.6	13.9	5.2	12.3	15.0	1.1	0.02	79.4	0.9	1.8

*, ** Indicate 0.05 and 0.01 significance levels, respectively

samples were extracted with hexane containing (0.01%) butylated hydroxytoluene (BHT), using soxhlet extraction for 4 hr. (24). The volume of the extract was recorded to be used as a dilution factor when calculating tocopherol content.

HPLC separation and quantitation

Chromatographic separation of tocopherols were performed on a 0.25m x 4mm (5μm) LiChrosorb Si60 column (Rainin Instrument Co. Inc., Woford, MA.) with a mobile phase of 2-propanol (1.0% v/v) in hexane (HPLC grade) at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45μm Nylon 66^R membrane filter (Rainin Instrument Co. Inc., Woford, MA) and degassed immediately before used. A Shimadzu LC-6A (Shimadzu Corporation, Kyoto, Japan) pump was used and sample injections were made with a 100 μL Valco loop injection valve (Millipore Waters Chromatography Division, Milford, MA). A Perkin-Elmer 650-15 Fluorescence Spectrophotometer (Hitachi, Norwalk, CT) set at 290 nm excitation wavelength and 330 nm emission wavelength was used as a detector. A Hewlett-Packard 3392A integrator (Hewlett-Packard Co., Avondale, PA) was used for determination and quantitation of the tocopherols in the peanut samples. Tocopherol standards (α-T, β-T, γ-T, and δ-T) were obtained from Henkel Corporation, La Grange, IL. Tocopherol peaks were identified by comparison of retention time to these standards. Recovery studies were performed by spiking peanut samples with 20, 25, and 10 μg, of α-T, γ-T, and δ-T respectively before soxhlet extraction (24). The amount of α-T, γ-T, and δ-T were determined after spiking. The percent recovery of tocopherol was calculated as:

% Recovery = [C/(A +B)] x 100

Where: C = μg tocopherol found in spiked peanut sample

A = μg tocopherol found in original peanut sample

B = μg tocopherol added to the peanut sample

Results from the recovery studies showed that 100% of the added α-T and γ-T, and 90% of added δ-T were found in the extract.

Results and Discussion

The fatty acid composition, O/L ratio, IV, α-T and γ-T were significantly affected by drought stress. All the parameters except oleic acid (18:1) were significantly affected by the peanut grade (Table 1). Peanut grade x drought stress interaction (G x S) was significant for α-T, γ-T, IV, and all fatty acids except stearic acid (18:0) and O/L ratio (Table 1).

Smaller size seed (No. 1) had higher percentages of long chain saturated fatty acids (arachidic acid (20:0),l behenic acid (22:0), and lignoceric acid (24:0)], and eicoseonic acid (20:1) than larger size seed (Jumbo) (Table 2). The larger seeds usually are more mature and have shown to have higher O/L ratios and lower IV (23, 25), but the results of this study showed that the smaller size seed had higher O/L ratio and lower IV (table 2). Larger size seed had lower α-T and higher γ-T compared to smaller size seed (Table 2). Peanuts had low β-T and δ-T which range from 0.27 to 0.36 mg/100 g and from 0.56 to 0.65 mg/100 g for β-T and δ-T respectively.

Peanut stressed at nut maturation (Stress3) had higher

Table 2. Fatty acid composition, O/L, computed IV, and tocopherol content of three market grades of Florunner peanuts across four drought stress periods.

Fatty acid (%)	Market grade		
	No.1	Medium	Jumbo
Palmitic (16:0)	15.14b*	15.65a	15.59a
Stearic (18:0)	0.19c	0.30b	0.35a
Arachidic (20:0)	4.41a	4.24a	3.51b
Behenic (22:0)	6.84a	5.91b	5.80b
Lignoceric (24:0)	3.34a	2.91b	2.77b
Oleic (18:1)	40.72a	41.00a	40.63a
Linoleic (18:2)	26.95c	27.73b	29.10a
Eicosenoic (20:1)	2.28a	2.27a	1.96b
(O/L) ratio	1.52a	1.48b	1.41c
IV	81.71c	83.29b	85.36a
α-T (mg/100g)	12.76a	10.66b	10.03c
γ-T (mg/100g)	13.27b	15.27a	15.31a

* means followed by the same letter within each row are not significantly different at P = 0.05.

Table 3. Fatty acid composition, O/L, computed IV, and tocopherol content of drought stressed Florunner peanuts across three market grades.

Fatty acid (%)	Treatment			
	Irrigated	Stress1	Stress2	Stress3
Palmitic (16:0)	15.12b*	15.09b	15.38b	16.24a
Stearic (18:0)	0.34a	0.33a	0.31a	0.14b
Arachidic (20:0)	3.89b	3.97b	4.31a	4.03a
Behenic (22:0)	5.90b	6.68a	6.71a	5.44b
Lignoceric (24:0)	2.89ab	3.37a	3.35a	2.42b
Oleic (18:1)	41.70a	41.74a	40.59b	39.11c
Linoleic (18:2)	27.74b	27.27b	27.07b	30.63a
Eicosenoic (20:1)	2.27a	2.42a	2.13ab	1.87b
(O/L) ratio	1.50b	1.59a	1.50b	1.28c
IV	83.92b	81.41b	81.80b	86.68a
α-T (mg/100g)	10.93c	10.67c	11.74a	11.26b
γ-T (mg/100g)	15.54a	13.46b	13.75b	15.72a

* means followed by the same letter within each row are not significantly different at P = 0.05.

Table 4. Effect of peanut grade on fatty acid composition, O/L, computed IV, and tocopherol content of stressed Florunner peanut.

	Fatty acid (%)											
Peanut Grade	16:0	18:0	20:0	22:0	24:0	18:1	18:2	20:1	O/L	IV	α-T (mg/100 g)	γ-T
Irrigated #												
No.1	14.80a	.25b	4.05a	5.75a	2.65a	42.77a	27.40a	2.15a	1.57a	85.93a	12.21a	12.54b
Medium	15.27a	.35a	3.93a	5.50a	2.70a	41.82a	27.85a	2.43a	1.50b	86.12a	10.53b	17.41a
Jumbo	15.30a	.42a	3.70a	6.45a	3.32a	40.52b	27.98a	2.22a	1.45b	84.46b	10.05b	16.68a
Stress1 #												
No.1	14.72b	.22b	4.30a	7.63a	3.83a	41.52a	25.12c	2.57a	1.65a	81.24b	12.97a	10.93b
Medium	15.13ab	.33a	3.93b	6.48b	3.30a	41.98a	26.30b	2.38b	1.60b	83.53a	8.66b	13.07b
Jumbo	15.42a	.38a	3.68b	5.92b	2.97b	41.72a	27.40a	2.30b	1.52c	85.15a	10.39b	16.38a
Stress2 #												
No.1	14.77b	.23b	4.60a	8.00a	4.17a	39.98a	26.03b	2.13a	1.52a	81.15b	12.74a	11.42c
Medium	15.72a	.35b	4.43a	5.95b	2.67b	41.22a	27.35a	2.18a	1.51a	84.53a	12.65a	15.94a
Jumbo	15.67a	.42a	3.90b	6.18b	3.22ab	40.57a	27.83a	2.08a	1.46b	84.73a	9.85b	13.89b
Stress3 #												
No.1	16.27a	.07b	4.68a	5.97a	2.72a	38.60a	29.27b	2.28a	1.32a	85.69b	13.13a	18.21a
Medium	16.48a	.15a	4.65a	5.70a	2.42a	38.98a	29.42b	2.07a	1.33a	86.11b	10.81b	14.66b
Jumbo	15.97a	.20a	2.77b	4.65b	2.13a	39.73a	33.20a	1.26b	1.20b	92.67a	9.84c	14.31b

 # Treatment
*** Means followed by the same letter within each column for each treatment are not significantly different at p = 0.05.**

percentage of palmitic acid (16:0), linoleic acid (18:2) and lower percentage of stearic acid (18:0), oleic acid (18:1) and eicosenoic acid (20:1) compared to the control peanuts (irrigated). Maturation stress increased IV and α-T while decreased O/L ratio. Stress1 and Stress2 increased behenic acid (22:0), and lignoceric acid (24:0) while decreased IV and α-T significantly. While Stress1 increased O/L ratio, Stress2 increased α-T significantly (Table 3).

Regardless of stressing period, as peanut increased in size the long chain saturated fatty acids percentages, eicosenoic acid, O/L ratio, and α-T decreased while linoleic acid (18:2) and IV increased significantly (Table 4).

In Summary, small size seed (No. 1) exhibit the characteristics which contributes to greater stability and longer shelf life. Maturation stress is most detrimental to the peanut quality and stability. Tocopherols, natural antioxidant, should always be considered as a parameter when predicting peanut stability and shelf-life.

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