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The influence of storage time on fatty acid, tocopherol and seed quality of peanut

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RESEARCH ARTICLE

Abstract

The seed size and storage time of peanuts is important, not only for marketing, but for storage and the production of by-products like peanut butter, as well as its use as a source of cooking oil used in confectionary products for human consumption. The purpose of this work was to investigate the effect of storage time and seed size grade on the quality of peanut seed in 2008 and 2009. Storage time had a negative effect on seed quality parameters such as oil content and its lipid components like fatty acids, α-tocopherol and δ-tocopherol. Each different seed size also had a significant impact on all fatty acids, oil content, and the proteins of the seed in both years with the exception of palmitoleic acid and arachidic acid. The results of this study indicated that there was a negative relationship between oleic acid and linoleic acid. Oil stability in grade-2 and grade-3 seed size was significantly decreased with extended storage time. Generally, both the α-tocopherol and δ-tocopherol values of each grade seed size decreased with extended storage time. The most suitable harvest time should be determined by taking the highest yield of peanut with grade-1 seed size, due to the fact that there are major differences in fatty acid components, oil content and the tocopherol level of different peanut seed size on plants. It was suggested that after harvest, peanut seeds should be sold and roasted without being stored.

Keywords: fatty acid, lipid oxidation, peanut, protein, tocopherol

1. Introduction

The growth habit of the peanut plant is indeterminate, therefore, at harvest time, the seeds on a single plant have various levels of maturity, which affect the peanut's composition, quality, and flavour (Sanders *et al*., 1982). The environmental factors that are most important to seed storage are relative humidity (RH) and temperature (McDonald, 2004); of these, the optimal conditions for peanut seed storage are 10 °C and 65% RH (Ketring, 1992). Differences in variety, changing environment conditions, and handling result in a range of flavour profiles in peanuts from various origins. Peanut seed quality can deteriorate rapidly during storage (Perez and Arguello, 1995). The factors that lead to undesirable peanut flavours include curing temperature, exposure time to excessive temperatures, moisture content, peanut size, and the stage of the peanut kernel maturity (Pattee *et al*., 1965). In addition, extended storage time increases peanut off-flavour development and decreases roasted peanut flavour (Bett and Boylston, 1992; Pattee *et al.,* 1999). However, seed size (width designed 5.95, 7.14, 7.94, 8.73 mm) and storage time significantly affect the carbohydrate levels of all peanuts, even if stored at 4 °C cold storage with 65% RH (Pattee *et al*., 1981). The data suggests that inferior quality is being introduced to the USA market because of the use of a 5.95 mm screen size as a minimum for grading USA no. 1 Virginia-type peanuts (Pattee *et al*., 2006). Research by Rucker *et al*. (1994) showed that pod density is highly correlated to kernel maturity and seed size distribution. As pod density increased, the maturity of the seed inside the pod also increased, and the percentage of jumbo and medium-sized kernels tended to increase while the percentage of size-one kernels decreased. Immature seeds with yellow or orange colour mesocarp have the highest carbohydrate level and changes in maturity distribution

(percentage of each maturity class) in commercial-sized seed grades can be determined on progressive harvest dates to evaluate any potential effects on the overall quality perception of lots which may be used in peanut butter or chopped nut products (Chiou *et al*., 1992; Kim and Hung, 1991; Vercellotti *et al*., 2007). Small pods and seeds can be used in animal diets.

According to Young *et al*. (1972) mature peanut seeds have a high stearic acid (18:0) and oleic acid (18:1) content, and are lower in linoleic (18:2) and other fatty acids, and contain a higher ratio of oleic/linoleic (O/L) acid than immature seeds. The O/L ratio is an important indicator of the stability of oil (Reed *et al*., 2002). The peanut seed has higher resistance to oxidative degradation when it contains high oleic acid; when the peanut is roasted, the flavour of the peanut seed remains intact. Talcott *et al*. (2005) implied that oxidative processes are typically accelerated by conditions of light, oxygen, water activity or exposure to high temperatures, and food procedures attempt to control many of these factors through the use of nitrogen-filled headspaces, vacuum packaging, and barriers to light. However, oxidation of polyunsaturated fatty acids, specifically linoleic (18:2) and linolenic (18:3) acids, still occurs, despite following strict handling practices. The oxidative stability of the raw peanuts showed a general tendency to decrease with seed size and storage time (Pattee *et al*., 2006).

α-Tocopherol is the form of vitamin E that is preferentially absorbed and accumulated in humans (Rigotti, 2007). Vitamin E is known for its antioxidant, immune-enhancing, anti-inflammatory, and antiplatelet aggregation effects. Vitamin E is used clinically to prevent cardiovascular disease, cancer, cataracts, complications of diabetes, and to improve immune functions. Peanuts are a good source of tocopherols (Jonnala *et al*., 2006). The tocopherol content of peanuts varies with the variety of peanut and the location of production; peanut oil mainly contains α-tocopherol (50-373 mg/kg) and δ-tocopherols (90-390 mg/kg) (Firestone, 1999). Since the price of peanuts is cheapest at harvest time, some farmers choose to store their peanuts for approximately 2 to 3 months in order to obtain a higher selling price. Farmers also sell peanut pods without classification. Therefore, this study investigated the changing fatty acid and seed quality capacities of different seed sizes in an NC-7 peanut cultivar, which is of high market value, during extended storage time.

2. Materials and methods

The peanut cultivar NC-7 was planted on 5 May 2008 and 7 May 2009 at the Crop Science Department of the Faculty of Agriculture in Adnan Menderes University, Aydın, Turkey (37° 39′ E 27° 52′ N in the West Aegean region of Turkey). Before being planted, the seeds were fertilised with 49.5 kg/ha nitrogen (N), 49.5 kg/ha phosphate (P_2O_5), and 49.5 kg/ha potassium (K_2O) by applying 330 kg/ha of 15-15-15 fertiliser to the field. A second dose of nitrogen of 49.5 kg/ha was provided with 150 kg/ha of ammonium nitrate (33%) at flowering time or during the second irrigation. Irrigation and weed control were applied to plots during the growing period when necessary. Each plot was mechanically dug, inverted and allowed to air-dry in the field for 7-13 days before harvest. The integrity and maturity of the pods was maintained and they were placed in mesh bags to cure with ambient air until mean seed moisture was 8-10%. After the peanut pods were harvested on 1 November 2008 and on 5 November 2009, they were placed in controlled storage at 5 °C and 60% relative humidity until they were analysed in 2008 and 2009. For storage, 5,000 g of peanuts were individually sealed in plastic bags to be removed at each sampling time and harvest time, after 1 and 2 months storage time, respectively. A 1,500 g sample of the sound mature kernel fraction from each replicate of each seed size grade was used. The pods were sorted into increasing maturity classes based on mesocarp colour designated by visual inspection as yellow, orange, brown and black. Colour class designations corresponded to classes previously described by Williams and Drexler (1981) and Williams *et al*. (1987). Kernel size distribution was determined using screens specified in the United States Department of Agriculture (USDA) grading procedures (USDA, 1993). Seed sizes were designed by 5.95, 7.14, 7.94, and 8.73 mm width. Seeds that remained on the sieve with a 8.73 mm diameter hole were classified as first (grade-1) class. Seeds that remained on the sieve with a 7.14 mm diameter hole were defined as second (grade-2) class. Seeds that remained on the sieve with 5.95 mm diameter hole were classified as third (grade-3) class.

Tocopherol analysis

The tocopherol content of peanut seeds was determined according to Katsanidis and Addis (1999). The individual tocopherol isomers were analysed using a normal phase HPLC column (Zorbax RX-SIL; Agilent Technologies, Santa Clara, CA, USA). The analytical separation of tocopherol isomers was achieved with an isocratic elution of hexane:isopropyl alcohol (99:1 v/v). Total run time and flow rate were 15 min. and 1.3 ml/min, respectively. The oil sample was dissolved in hexane (12.5 mg/ml) and filtered through a 0.2 mm filter (Iso-Discfilter; Supelco, Bellefonte, PA, USA). An external calibration curve was prepared for each tocopherol standard to calculate the amount of tocopherols present in the oil sample (Jonnala *et al*., 2006).

Peanut oil analysis

The oil of the peanuts was analysed using a Soxhlet device by modifying the method of Soxhlet (1879). The following steps of the method were followed for the determination

of oil content. Firstly, 250 ml balloons were tarred. A 10-g sample was taken from a ground sample and surrounded with filter paper. 150 ml hexane was wrapped and put into Soxhlet tubes. The Soxhlet tubes were immersed in the Soxhlet device for about 3-6 h, and then put into an oven for 30 min at 105 °C to root out the hexane. The oil ratio was calculated as a percentage.

Protein analysis

To accomplish this, one gram of the sample was placed in a digestion tube with 25 ml of concentrated sulphuric acid (H_2SO_4). Seven grams of potassium sulphate (K_2SO_4) and a metallic catalyst, usually copper, were then added. The digestion tube was placed into a digestion block where it was heated to boiling temperature. Digestion was usually completed after 1 h at 370 to 400 °C. Distillation involves the separation of ammonia nitrogen. About 100 ml of water was carefully added to all tubes, and cooled ~15 to 20 min to room temperature. This was accomplished by raising the pH with sodium hydroxide (33% NaOH; 125 ml) which changes the ammonium (NH_4^+) ion to ammonia (NH_3). It was possible to separate the nitrogen by distilling the ammonia and collecting the distillate in a suitable trapping medium. The most common method was titration of the ammonia with a standard solution of one-tenth normal hydrochloric acid (0.1 N HCl) in the presence of a mixed indicator. The mixed indicators (bromocresol green and methyl red) were available in the 4% boric acid solution. After this process the amount of nitrogen needed to be calculated and it was necessary to show how much was present in the sample. The ratio of protein was analysed using a modified version of the Kjeldahl method by AOAC (1990). This calculation can either be performed as a percentage of nitrogen or a percentage of protein. For percentage nitrogen (%N):

$$\%N = 0.014 \times N \times (V1 - V2) \times 100 / m \quad (1)$$

Where V1 is the volume of the HCl solution during titration (ml); V2 is in the control tube (ml); N is the concentration of the HCl solution used; and m is the sample weight (g).

The percentage protein (%P) was calculated as follows:

$$\%P = 6.25 \times \%N \quad (2)$$

Fatty acid analysis

The fatty acids present in the peanut oil were determined by GC-2010 gas-liquid chromatography. Firstly, 0.1 g peanut oil, 2 ml n-heptane and 0.2 ml potassium hydroxide were weighed and mixed. After this solution was mixed for 30 sec, there was a 30-min waiting period until the oil subsided. 100 µl was taken by micro syringe from the upper layer and then put in a GC-2010 chromatography device. The column used was a 50 m 0.25 mm i.d., 0.25 mm film BPX-70 biscyanopropyl-fused silica capillary column (SGE Inc., Austin, TX, USA). Helium carrier gas was used at a linear velocity of 22 cm/s and the split ratio was 40:1. The initial column temperature was 185 °C held for 20 min. The temperature ramp was 5 °C/min until 250 °C, held for 15 min. The fatty acid composition was calculated by the area percentage of each peak (Reed *et al.*, 2002).

Statistical analyses

Data were analysed as a 3×3 randomised block that included one peanut cultivar analysed at 3 seed size grades at 3 sampling times and at 1 storage temperature. Storage time was the main factor. Seed size grade was the subplot. Analysis of variance (ANOVA) was conducted using JMP software, version 5 (SAS Institute, 2002), with mean separation performed by the LSD test (P<0.05).

3. Results

The results of the ANOVA of this study are presented in Table 1 and 2, according to the mean of both years. Storage time had a statistically significant effect on oil content, α-tocopherol, δ-tocopherol, oleic/linoleic acid ratio, palmitic acid, heptadecanoic acid, stearic acid, behenic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, gadoleic acid, erusic acid and lignoceric fatty acids except for protein, arachidic acid, and heptadecenoic acid. Seed size also had a significant effect on all chemical components of seed in both years except for palmitoleic acid and arachidic acid (Table 1 and 2). Seed size × storage time interactions had effect on all traits except for protein, heptadecanoic acid, and arachidic acid (Table 1 and 2).

The highest oil ratio was in grade-1 seed size at all storage times. According to the mean of all storage times, the oil content of grade-1 seed size, which is more than that of the grade-2 and grade-3 seed size, was 45.52% (Table 3). Particularly after a 1-month storage time, the oil content of grade-2 and grade-3 seed size quickly decreased, whereas grade-1 seed size was stable in terms of oil content (Table 3). It was shown that the oil ratio of smallest seed size was decreased with the extended storage time. Although storage time and seed size × storage time interaction had no effect on protein of different seed size grade, it had an effect on the protein level of the peanut seed. The highest protein content was recorded in grade-3 seed size at 26.84% (Table 3). The lowest protein content was recorded in grade-1 seed size at 22.82% in the year 2008. The protein content of classified seed sizes decreased during storage time (Table 3). However, after harvest, the protein content of all different seed grades was not statistically changed during the storage time. There was no difference in the protein content of peanut seeds during storage time (Table 3). The α-tocopherol and δ-tocopherol compositions of different peanut seed size are shown in Table 3. The grade-2 seed

Table 1. Result of variance (calculated F-value) for the storage time and seed size classification of oil, proteins and fatty acids.

Variance source	df	Oil	Protein	Palmitic	Palmitoleic	Heptadecanoic	Heptadecenoic	Stearic	Oleic	Oleic/linoleic
Year	1	0.062ns	0.002ns	0.367ns	0.699ns	6.925*	2.211ns	2.386ns	0.009ns	0.229ns
Storage	2	6.268**	2.311ns	35.134**	6.122**	4.093*	1.351ns	8.063**	11.431**	13.126**
Year × Storage	2	6.210**	0.089ns	1.367ns	0.810ns	0.403ns	0.007ns	2.693ns	4.373*	0.132ns
Classification	2	76.222**	16.634**	7.843**	0.123ns	7.540**	17.301**	19.137**	316.498**	26.016**
Year × Classification	2	0.068ns	0.012ns	0.190ns	0.082ns	1.351ns	2.453ns	4.153*	0.002ns	1.895ns
Storage × Classification	4	11.408**	1.482ns	12.823**	3.175*	1.129ns	2.686*	35.857**	6.236**	6.547**
Year × Storage × Classification	4	0.638ns	0.069ns	0.499ns	1.593ns	0.751ns	0.512ns	3.652*	1.843ns	0.726ns

d.f. = degree of freedom; ns = not significant; * $P<0.05$; ** $P<0.01$.

Table 2. Result of variance (calculated F-value) for the storage time and seed size classification of fatty acids and tocopherols.

Variance source	df	Linoleic	Linolenic	Arachidic	Gadoleic	Behenic	Lignoceric	α-tocopherol	δ-tocopherol
Year	1	0.317ns	0.023ns	0.093ns	3.631ns	0.418ns	0.135ns	0.014ns	0.001ns
Storage	2	149.768**	7.411**	0.744ns	5.456**	27.382**	9.396**	73.851**	29.688**
Year × Storage	2	1.213	7.106**	0.177ns	4.466*	4.302*	1.018ns	1.552ns	0.281ns
Classification	2	129.590**	64.300**	1.941ns	121.906**	308.812**	8.967**	214.944**	34.539**
Year × Classification	2	22.275**	0.411ns	0.175ns	3.413*	0.496ns	0.753ns	0.005ns	0.003ns
Storage × Classification	4	38.436**	43.428**	0.750ns	69.255**	17.827**	11.692**	27.584**	12.242**
Year × Storage × Classification	4	28.077**	2.711*	0.222ns	3.573*	0.878ns	0.694ns	1.913ns	0.040ns

d.f. = degree of freedom; ns = not significant; * $P<0.05$; ** $P<0.01$.

size had the highest total α-tocopherol content (291.5 mg/kg oil). Both the α-tocopherol and δ-tocopherol values of all seed sizes were decreased throughout the storage time (Table 3). The grade-3 seed size had the highest total α-tocopherol content (309.67 and 311.41 mg/kg oil) in harvest time in both years. The highest α-tocopherol values in harvest time were determined to be 311.41 mg/kg oil in grade-3 seed size, while δ-tocopherol was determined by 188.33 mg/kg oil in grade-1 seed size without storage (Table 3). The storage time negatively affected the α-tocopherol and δ-tocopherol levels of peanut seeds. In particular, even though the values of α-tocopherol in the grade-2 seed size was increased suddenly in the two-month storage time after harvest time, the values of α-tocopherol in grade-1 and grade-3 seed sizes in this period were decreased with the extended storage time after the harvest (Table 3). The differences in the α-tocopherol and δ-tocopherol values between grade-1 and grade-3 in harvest time were very high, up to 40% percent, approximately (Tables 3). The highest O/L ratio was immediately determined by 1.78 of grade-1 seed size without storage after harvest (Table 3). Especially oil stability and lipid oxidation in grade-2 seed size was significantly decreased with extended storage time. The highest O/L ratio was obtained without storage after the harvest time (Table 3). The O/L ratio of each different seed size was significantly decreased with extended storage time (Table 3). Extended storage time and seed size might be expected to have an effect on stearic acid in both years, but the highest stearic acid was recorded in grade-3 seed size in harvest time (Table 4). But after storage, while the ratio of stearic acid was increased in grade-1 size and grade-2 size, the ratio of stearic acid of grade-3 seed size was decreased with storage (Table 4). When data from the two years were combined, linolenic acid, which is polyunsaturated fatty acid, was increased with extended storage time in each different seed size. The lowest linolenic acid was determined

Table 3. Effect of storage time on oil (%), protein (%), oleic/linoleic acid ratio, α-tocopherol and δ-tocopherol (mg/kg) of different peanut seed size.

Storage (time)	Classification (degree)	Oil (%)		Protein (%)		Oleic/linoleic (%)		α-tocopherol (mg/kg)		δ-tocopherol (mg/kg)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Harvest	1st grade	45.81	45.55	26.37	26.48	1.76	1.78	227.67	225.21	188.33	185.23
	2nd grade	42.77	42.57	23.84	23.80	1.64	1.62	293.67	286.98	130.33	126.33
	3rd grade	40.66	40.52	22.82	22.85	1.50	1.54	309.67	311.41	126.33	118.51
1 month	1st grade	44.98	44.83	24.95	25.02	1.72	1.72	205.67	199.98	184.00	178.00
	2nd grade	43.23	43.67	24.43	24.34	1.50	1.51	275.00	268.87	120.00	118.25
	3rd grade	41.46	41.49	24.59	24.32	1.43	1.41	296.67	294.42	114.33	108.63
2 months	1st grade	45.77	45.20	26.80	26.84	1.67	1.67	110.67	114.40	101.00	105.00
	2nd grade	41.50	41.54	24.44	24.52	1.53	1.48	306.00	301.32	98.23	103.43
	3rd grade	33.26	33.03	24.04	24.13	1.41	1.39	211.67	204.98	113.00	118.00
5% LSD	Y	ns		ns		ns		ns		ns	
	S	1.122		ns		0.046		11.305		11.316	
	C	1.153		0.855		0.046		11.615		11.626	
	Y × S	1.547		ns		ns		ns		ns	
	Y × C	ns		ns		ns		ns		ns	
	S × C	1.849		ns		0.080		18.626		18.643	
	Y × S × C	ns		ns		ns		ns		ns	

C = classification; ns = not significant; S = storage time; Y = year.

in harvest time. The difference between grade-1 and grade-3 was approximately 2-2.5% in terms of linolenic acid (Table 4). Averaged over the two years, the highest ratio of linolenic acid was obtained in the two-month storage time after the harvest. The palmitic acid level of the grade-3 size was higher than that of the grade-1 and grade-2 sizes with the extended storage time (Table 4). The ratio of palmitic acid obtained from each different seed size was increased during the storage time (Table 4). The highest linoleic acid ratio was obtained in the two-month storage time after harvest, while the highest oleic acid was immediately obtained after the harvest. Neither seed size nor storage time affected arachidic acid in 2008 and 2009 (Table 5). The ratio of gadoleic, behenic and lignoceric acids of each different seed size were increased generally with storage. The highest ratio of gadoleic, behenic and lignoceric acids were obtained in grade-3 seed size in the two-month storage time after the harvest (Table 5). The change in fatty acid composition during storage was illustrated by the fact that the highest oleic acid was 52.32% of grade-1 seed size in harvest time while it was 47.92% of grade-3 seed size at that time (Table 6). The oleic acid of all different seed sizes was statistically lowered with extended storage time; linoleic acid was significantly increased with extended storage time in both years (Table 6). After the harvest and during both storage times, the oleic acid ratio of grade-1 seed size was higher than that of the grade-2 and grade-3 seed size (Table 6). On the other hand, the linoleic acid ratio of the grade-3 seed size was statistically higher than that of the grade-2 and grade-1 seed size. The linoleic acid ratio of the biggest seed size grade (grade-1) was lowest during the storage times. In both years, it was determined that the linoleic acid ratio of all different seed sizes was increased by extended storage time (Table 6). Years × storage × seed size interaction significantly affected the linoleic acid ratio of peanut seed (Table 2)

When data from the two years were combined, the main effects of storage time and seed size were significant as well as the interaction between the treatments. When we look at the correlation coefficients in Table 7, classified seed sizes are significantly positively correlated with oil content, protein content, oleic acid, stearic acid, δ-tocopherol, while it was significantly negatively correlated with palmitic acid, linoleic acid, linolenic acid, gadoleic acid, behenic acid and α-tocopherol. It was found that there were significant positive correlations among palmitic acid, myristic acid and stearic acid. The correlation coefficients in Table 7 showed highly significant interaction between gadoleic acid, behenic acid, and lignoceric. Generally, oleic acid has a significantly negative correlation with linoleic acid, linolenic acid, gadoleic acid, behenic acid, lignoceric acid and α-tocopherol. On the other hand, it has a positive correlation with stearic acid and δ-tocopherol (Table 7). However, linoleic acid has a significantly positive correlation with linolenic acid, gadoleic acid, behenic acid, while it

Table 4. Effect of storage time on palmitic, palmitoleic, heptadecanoic, heptadecenoic, stearic fatty acids of different peanut seed size.

Storage (time)	Classification (degree)	Palmitic (C16:0) (%)		Palmitoleic (C16:1) (%)		Heptadecanoic (C17:0) (%)		Heptadecenoic (C17:1) (%)		Stearic (C18:0) (%)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Harvest	1st grade	9.23	9.29	0.100	0.110	0.050	0.050	0.020	0.022	2.45	2.41
	2nd grade	9.18	9.21	0.090	0.090	0.050	0.050	0.030	0.030	2.65	2.63
	3rd grade	8.80	8.81	0.090	0.090	0.050	0.060	0.030	0.030	2.70	2.72
1 month	1st grade	9.19	9.21	0.100	0.100	0.050	0.050	0.030	0.030	2.96	2.98
	2nd grade	9.62	9.60	0.100	0.100	0.040	0.040	0.020	0.020	2.73	2.72
	3rd grade	9.75	9.68	0.090	0.095	0.040	0.050	0.030	0.030	2.55	2.59
2 months	1st grade	9.30	9.34	0.100	0.110	0.050	0.050	0.030	0.020	2.87	2.88
	2nd grade	9.63	9.66	0.100	0.100	0.040	0.040	0.020	0.020	2.68	2.72
	3rd grade	10.03	10.08	0.110	0.100	0.050	0.050	0.030	0.030	2.57	2.55
5% LSD	Y	ns		ns		0.004		ns		ns	
	S	0.139		0.005		0.004		ns		0.052	
	C	0.143		ns		0.004		0.003		0.054	
	Y × S	ns		ns		ns		ns		ns	
	Y × C	ns		ns		ns		ns		0.076	
	S × C	0.230		0.007		ns		0.005		0.086	
	Y × S × C	ns		ns		ns		ns		0.114	

C = classification; ns = not significant; S = storage time; Y = year.

Table 5. Effect of storage time on linolenic, arachidic, gadoleic, behenic, lignoceric fatty acids of different peanut seed size.

Storage (time)	Classification (degree)	Linolenic (C18:3) (%)		Arachidic (C20:0) (%)		Gadoleic (C20:1) (%)		Behenic (C22:0) (%)		Lignoceric (C24:0) (%)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Harvest	1st grade	29.48	29.55	1.540	1.540	1.42	1.41	3.50	3.52	0.120	0.121
	2nd grade	30.26	30.30	1.520	1.510	1.31	1.31	3.30	3.31	0.140	0.140
	3rd grade	31.35	31.38	1.500	1.500	1.27	1.26	2.59	2.60	0.140	0.140
1 month	1st grade	30.34	30.41	1.400	1.450	1.00	1.03	2.50	2.50	0.110	0.111
	2nd grade	32.18	32.18	1.500	1.500	1.35	1.34	3.43	3.43	0.110	0.108
	3rd grade	32.83	32.85	1.530	1.540	1.62	1.62	4.28	4.30	0.150	0.150
2 months	1st grade	30.77	30.84	1.440	1.420	1.08	1.09	2.73	2.73	0.130	0.133
	2nd grade	32.25	32.30	1.550	1.540	1.44	1.41	3.78	3.80	0.160	0.160
	3rd grade	32.95	32.89	1.540	1.510	1.71	1.72	4.47	4.49	0.170	0.171
5% LSD	Y	ns		ns		ns		ns		ns	
	S	0.008		ns		0.046		0.118		0.012	
	C	0.007		ns		0.047		0.121		0.010	
	Y × S	0.012		ns		0.064		0.163		ns	
	Y × C	ns		ns		0.067		ns		ns	
	S × C	0.012		ns		0.076		0.194		0.016	
	Y × S × C	0.016		ns		0.101		ns		ns	

C = classification; ns = not significant; S = storage time; Y = year.

Table 6. Effect of storage time on oleic and linoleic fatty acids of different peanut seed size.

Storage (time)	Classification (degree)	Oleic (C18:1) (%)		Linoleic (C18:2) (%)	
		2008	2009	2008	2009
Harvest	1st grade	52.29	52.32	29.48	29.45
	2nd grade	49.51	49.56	30.26	30.21
	3rd grade	47.92	47.94	31.35	31.29
1 month	1st grade	52.18	52.10	30.34	30.41
	2nd grade	48.73	48.79	32.18	32.13
	3rd grade	46.85	46.92	32.83	32.80
2 months	1st grade	51.37	51.43	30.77	30.84
	2nd grade	48.16	48.24	30.34	32.25
	3rd grade	46.15	46.25	32.95	32.98
5% LSD	Y	ns		ns	
	S	0.395		0.191	
	C	0.405		0.197	
	Y × S	0.544		ns	
	Y × C	ns		0.278	
	S × C	0.650		0.315	
	Y × S × C	ns		0.417	

C = classification; ns = not significant; S = storage time; Y = year.

was negatively correlated with δ-tocopherol (Table 7). No correlation was found between storage and classified seed size (Table 7).

4. Discussion

After being harvested, the oil content of peanut seed was not changed after the 1-month storage time but was statistically changed after the 2-month storage time. Mean and standard deviation values of the oil content in our study were close to those reported by Grosso *et al*. (1994) and Dwivedi *et al.* (2000) but higher than the oil content values reported (28-40%) by Davis *et al*. (2008). It could be considered that grade-1 seed size has a higher oil content than grade-2 and grade-3 seed size because the seeds of grade-2 and grade-3 size have not fully matured at harvest time and the oil accumulation, which is the main storage reserve in the form of triacylglycerols, proteins, and carbohydrates in the form of starch, was not yet completed in grade-2 and grade-3 seed sizes. In particular, it was observed that the oil content of 1-grade seed size was not changed with storage time, which is why it is thought that the oil content of 1-grade seed size was stable at harvest time. Due to the fact that the seeds of grade-1 formed earlier than those of grade-2 and grade-3, the accumulation of protein in grade-1 seed size was higher than the others. The protein of each grade seed size was not significantly changed by storage time.

The predominant fatty acid was oleic acid (C18:1), which accounted for more than 50% of the fatty acids present in oils, similar to the results of Davis *et al.* (2008). The oleic acid ratio of 1-grade seed size was higher than 2-grade and 3-grade seed size with results similar to those of McNeill and Sanders (1998). The linoleic and linolenic acid levels of grade-3 seed size were significantly increased by extended storage time. These results correspond to those from other studies and show that the sum of oleic and linoleic acids accounts for almost 80% of the total fatty acids detected in peanut samples (Andersen and Gorbet, 2002). Young *et al*. (1972), How and Young (1983), Andersen *et al*. (1998), Dwivedi *et al*. (2000), Lopez *et al*. (2001), Baker (2002) and Asibuo *et al*. (2008) implied that the O/L ratio could be an important indicator for the stability and lipid oxidation of peanut oil which is expressed.

Trends observed for lipid oxidation in each peanut seed size grade were dependent on storage time and natural differences in fatty acid composition during storage. Free fatty acids may also have contributed to seed deterioration by disrupting membranes and/or through the toxicity of subsequent peroxidation products (Trawatha *et al*., 1995). Moreover, according to the literature, it seems that the oxidation of fatty acids becomes significant after an induction period during which antioxidants are destroyed (Sun *et al*., 2001; Zacheo *et al*., 2000). The correlation between oleic acid and linoleic acid was strong but negative (r=-0.68, P<0.001); that is, an increase in one fatty acid leads to a corresponding decrease in the other. In previous studies involving multiple peanut cultivars, a strong negative correlation (i.e. an inverse association) between oleic acid and linoleic acid in peanut lipids was also observed (Andersen and Gorbet, 2002; Shin *et al*., 2010). This negative relationship originates from the biochemical pathways of peanut development: in the peanut germplasm, palmitoyl-CoA is elongated to stearoyl-CoA followed by desaturation forming oleic acid. Then, the action of oleoyl-phosphatidylcholine desaturase (a Δ^{12}-fatty acid desaturase) synthesises linoleic acid from oleic acid (Baydar and Turgut, 1995; Patel *et al*., 2004). Palmitic acid was positively correlated with linoleic acid, which was similar to the results of Shin *et al.* (2010). Evidently, as the levels of stearic and oleic acids increase, there is also a greater chance of elongation to the minor fatty acids, arachidic and gondoic acids (Baydar and Turgut, 1995; Shin *et al*., 2010).

When data concerning the oil composition of the two years were combined in terms of the seed size, there were a change and deterioration in different seed sizes. Sanders *et al*. (1982) explained with studies on oil composition changes with peanut maturation that a greater potential for lipid degradation was indicated in immature peanuts. Thus, sized lots containing higher percentages of immature peanuts are more likely to exhibit lipid degradation and off-flavours such as 'painty' (McNeill and Sanders, 1998).

Table 7. Correlation coefficients between storage time and seed size classification and oil, protein, fatty acids and tocopherols.

	S	C	Oil	Pro	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0	α-toc	δ-toc
S	-	0.09	-0.02	0.21	0.58**	0.40**	0.38**	0.29*	0.19	-0.17	0.50**	0.33**	-0.11	0.13	0.24	0.50**	-0.45**	-0.47**
C	-	-	0.77**	0.60**	-0.29*	0.02	0.04	-0.22	0.35**	0.93**	-0.52*	-0.46**	-0.20	-0.63**	-0.88**	-0-36**	-0.59**	0.47**
Oil	-	-	-	0.37**	-0.51**	-0.16	-0.07	-0.24	0.35**	0.80**	-0.55**	-0.63**	-0.13	-0.66**	-0.81**	-0.40**	-0.211	0.32**
Pro	-	-	-	-	0.14	0.17	0.13	0.01	-0.11	0.51**	0.12	-0.02	-0.29*	-0.15	-0.47**	-0.14	-0.55**	0.192
C16:0	-	-	-	-	-	0.40**	0.06	0.11	-0.44**	-0.50**	0.83**	0.74**	0.03	0.69**	0.57**	0.46**	-0.07	-0.37**
C16:1	-	-	-	-	-	-	0.82**	0.71**	-0.42**	-0.05	0.38**	0.70**	-0.27**	0.39**	0.16	0.62**	-0.27**	-0.02
C17:0	-	-	-	-	-	-	-	0.78**	-0.33**	0.09	0.01	0.50**	-0-31*	0.20	-0.01	0.50**	-0.29*	0.20
C17:1	-	-	-	-	-	-	-	-	-0.35**	-0.13	0.07	0.56**	-0.27*	0.31*	0.19	0.54**	-0.16	0.03
C18:0	-	-	-	-	-	-	-	-	-	0.34**	-0.52**	-0.71**	0,09	-0.81**	-0.40**	-0.21	-0.29*	0.02
C18:1	-	-	-	-	-	-	-	-	-	-	-0.68**	-0.55**	-0.18	-0.70**	-0.94**	-0.43**	-0.50**	0.60**
C18:2	-	-	-	-	-	-	-	-	-	-	-	0.73**	0.10	0.75**	0.64**	0.42	0.16	-0.47*
C18:3	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.88**	0.68**	0.60*	0.02	-0.19
C20:0	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22	0.23	-0.09	0.24	-0.06
C20:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.80**	0.45**	0.31*	-0.24
C22:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.54**	0.43**	-0.54**
C24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.04	-0.39**
α-toc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.11
δ-toc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C = classification; ns = not significant; Pro = protein; S = storage time; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C17:0 = heptadecanoic acid; C17:1 = heptadecenoic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid; C18:3 = linolenic acid; C20:0 = arachidic acid; C20:1 = gadoleic acid; C22:0 = behenic acid; C24:0 = lignoceric acid; α-toc = α-tocopherol; δ-toc = δ-tocopherol.

* $P<0.05$; ** $P<0.01$

Although Garcia-Pascual *et al*. (2003) point out that there were no significant differences between the samples stored at 8 °C for 9 months and the others stored at 36 °C for 4 months, a statistically significant relationship between extended storage time and a reduction in α-tocopherol and δ-tocopherol content was found. This is in line with the findings of some researchers (Pascual *et al*. 2003; Sun *et al*., 2001). Pascual *et al*. (2003) concluded that tocopherols are in fact effective in quenching lipid peroxide radicals. In addition, some researchers explained that the oxidation of fatty acids becomes significant after an induction period during which antioxidants are destroyed (Zacheo *et al*., 2000). Pokorny *et al.* (2003) stated that tocopherols of Virginia peanut oil were fully decomposed after a 35-day storage period.

5. Conclusions

In summary, this two year study showed that storage negatively affects seed quality such as oil content, fatty acids components, α-tocopherol and δ-tocopherol, even when the conditions of storage are controlled. Long-term storage conditions can easily change the qualitative components of peanut seeds stored in uncontrolled conditions by farmers. α-tocopherol, which is vitamin E, was diminished by long-term storage. All of the changes that took place with raw peanut seed could be attributed to the facts that the carbohydrate levels of seeds may be changed by storage and different seed sizes contain different carbohydrates, which are sources of oil, fatty acids, tocopherol, etc in the seeds. This supports the findings of Ross and Mixon (1989) and Pattee *et al*. (1997, 2000), all of whom reported that the individual components of the peanut carbohydrate have been shown to change during maturation. Additionally, carbohydrates also change across the seed sizes and over storage time. If peanut seeds are used as an oil, they must not be stored. All the more so because the oleic/linoleic acid ratio, an important indicator for the stability and lipid oxidation of peanut oil, was decreased with long-term storage. The most suitable time to harvest should be determined by the highest yield of peanut crop when the peanut pod is ready to be shelled, described by the shell-out method (Williams and Drexler, 1981; Williams *et al*., 1987). The ratio of grade-1 seed size was approximately 71% in the seeds of peanut plants. Since grade-1 seed size was higher than grade-2 and grade-3 seed size in terms of oil quality, the ratio of grade-1 seed size should be elevated for high oil quality. All quality components such as fatty acids, oil content, protein content and the tocopherol level of peanut seeds could be most beneficial to peanut breeding programs to ensure the maintenance of flavour quality in future peanut varieties. After harvest, when seed moisture is 8-10%, peanut seeds should be sold without being stored.

References

Andersen, P.C. and Gorbet, D.W., 2002. Influence of year and planting date on fatty acid chemistry of high oleic acid and normal peanut genotypes. Journal of Agriculture and Food Chemistry 50: 1298-1305.

Andersen, P.C., Hill. K., Gorbet, D.W. and Brodbeck, B.V., 1998. Fatty acid and amino acid profiles of selected peanut cultivars and breeding lines. Journal of Food Composition and Analysis 11: 100-111.

Asibuo, J.Y., Akromah, R., Safo-Kantanka, O., Adu-Dapaah, H.K., Ohemeng-Dapaah, S., Agyeman, A., 2008. Chemical composition of groundnut, *Arachis hypogaea* (L) landraces. African Journal of Biotechnology 7: 2203-2208.

Association of Official Analytical Chemists (AOAC), 1990. Methods of analysis, 15th Ed. AOAC, Washington, DC, USA.

Baker, G.L., 2002. Flavor formation and sensory perception of selected peanut genotypes (*Arachis hypogea* L.) as affected by storage water activity, roasting, and planting date. PhD thesis. University of Florida, Gainesville, FL, USA. Available at: http://etd.fcla.edu/UF/UFE1000105/baker_g.pdf.

Baydar, H. and Turgut, K., 1995. The basics of metabolic and physiological oil quality breeding in plants. Akdeniz University Journal of Faculty Agriculture 8: 205-216.

Bett, K.L. and Boylston, T.D., 1992. Effect of storage on roasted peanut quality: descriptive sensory analysis and gas chromatographic techniques. In: St. Angelo, A.J. (ed.) Lipid oxidation in food. Journal of American Chemical Society, New York, NY, USA, pp. 322-343.

Chiou, R.Y.Y., Liu, J.C.D., Liu, C.P., Ferng, S. and Tsait, R.T., 1992. Characterization of peanut kernels as affected by harvest date and drying practices. Journal of Agriculture and Food Chemistry 40: 1536-1540.

Davis, J.P., Dean, L.O., Faircloth, W.H. and Sanders, T.H., 2008. Physical and chemical characterizations of normal and high-oleic oils from nine commercial cultivars of peanut. Journal of the American Oil Chemists' Society 85: 235-243.

Dwivedi, S.L., Nigam, S.N. and Rao, R.C.N., 2000. Photoperiod effects on seed quality traits in peanut. Crop Science 40: 1223-1227.

Firestone, D. 1999. Physical and chemical characteristics of oils. Fats and waxes. AOCS Press, Champaign, IL, USA.

Grosso, N.R., Lamarque, A.L., Maestri, D.M., Zygadlo, J.A. and Guzman, C.C., 1994. Fatty acid variation Runner peanut (*Arachis hypogaea* L.) among geographic localities from Cordoba (Argentina). Journal of the American Oil Chemists' Society 71: 541-542.

How, J.S.L. and Young, C.T., 1983. Comparison of fatty acid content of ımported peanuts. Journal Series of the North Carolina Agriculture Research Service 60: 5.

Jonnala, R.S., Dunford, N.T. and Dashiell, K.E., 2006. Tocopherol, phytosterol and phospholipid compositions of new high oleic peanut cultivars. Journal of Food Composition and Analyses 19: 601-605.

Katsanidis, E and Addis, P.B., 1999. Novel HPLC analysis of tocopherols, tocotrienols and cholesterol in tissue. Free Radical Biology and Medicine 27: 1137-1140.

Ketring, D.L., 1992. Physiology of oil seeds. X. Seed quality of peanut genotypes as affected by ambient storage temperature. Peanut Science 19: 72-77.

Kim, N.K. and Hung, Y.C., 1991. Mechanical properties and chemical composition of peanuts as affected by harvest date and maturity. Journal of Food Science 56: 1378-1381.

Lopez, Y., Smith, O.D., Senseman, S.A. and Rooney, W.L., 2001. Genetic factors ınfluencing high oleic acid contentin spanish market-type peanut cultivars. Crop Science 41: 51-56.

McDonald, M.B., 2004. Orthodox seed deterioration and its repair. In: Benech-Arnold, R.L. and Sanchez, R.J. (eds.) Handbook of seed physiology. Food Products Press and Haworth Reference Press, New York, NY, USA, pp. 273-296.

McNeill, K.L. and Sanders, T.H., 1998. Maturity effects on sensory and storage quality of roasted Virginia-type peanuts. Journal of Food Science 63: 366-369.

Pascual, G.P., Mateos, M., Carbonell, V. and Salazar, D.M., 2003. Influence of storage conditions on the quality of shelled and roasted almonds. Biosystems Engineering 87: 201-209.

Patel, M., Jung, S., Moore, K., Powell, G., Ainsworth, C. and Abbott, A., 2004. High-oleate peanut mutants result from a MITE insertion into the FAD2 gene. Theoretical and Applied Genetic 108: 1492-1502.

Pattee, H.E., Beasley, E.O. and Singleton, J.A., 1965. Isolation and identification of volatile components from high-temperature cured off flavor peanuts. Journal of Food Science 30: 388-392.

Pattee, H.E., Giesbrecht, F.G. and Isleib, T.G., 1999. Sensory attribute variation in low-temperature-stored roasted peanut paste. Journal of Agriculture and Food Chemistry 47: 2415-2420.

Pattee, H.E., Isleib, T.G. and Giesbrecht, F.G., 1997. Genotype-by environment interaction in the sweet and bitter attributes of peanut. Peanut Science 24: 117-123.

Pattee, H.E., Isleib, T.G., Giesbrecht, F.G. and McFeeters, R.F., 2000. Investigations into genotypic variations of peanut carbohydrates. Journal of Agriculture Food Chemistry 48: 750-756.

Pattee, H.E., Pearson, J.L., Young, C.T. and Giesbrecht, F.G., 2006. Changes in roasted peanut flavor and other quality factors with seed size and storage time. The Institute of Food Technologists 2: 455-456.

Pattee, H.E., Young, C.T. and Giesbrecht, F.G., 1981. Seed size and storage effects on carbonhydrates of peanut. Journal of Agriculture and Food Chemistry 29: 800-802.

Perez, M.A. and Arguello, J.A., 1995. Deterioration in peanut (*Arachis hypogaea* L. cv. Florman) seeds under natural and accelerated aging. Seed Science and Technology 23: 439-445.

Pokorny, J., Parkanyiova, L., Reblova, Z., Trojakova, L., Sakurai, H., Uematsu, T., Miyahara, M. and Yano, T., 2003. Changes on storage of peanut oils containing high levels of tocopherols and β-carotene. Czech Journal of Food Science 1: 19-27.

Reed, K.A., Sims, C.A., Gorbet, D.W. and O'Keefe, S.F., 2002. Storage water activity affects flavor fade in high and normal oleic peanuts. Food Research International 35: 769-774.

Rigotti, A., 2007. Absorption, transport, and tissue delivery of vitamin E. Molecular Aspects of Medicine 28: 423-36.

Ross, L.F. and Mixon, A.C., 1989. Changes in soluble carbohydrates in developing seeds from Florunner peanuts. Journal of Food Composition Analyses 2: 157-160.

Rucker, K.S., Kvien, C.K., Calhoun, K., Henning, R.J., Koehler, P.E., Ghate, S.R. and Holbrook, C.C., 1994. Sorting peanut by pod density to improve quality, kernel maturity distribution, and reduce aflatoxin. Peanut Science 21: 147-152.

Sanders, T.H., Lansden, J.A., Greene, R.L., Drexler, J.S. and Williams, E.J., 1982. Oil characteristics of peanut fruit separated by a non-destructive maturity classification method. Peanut Science 9: 20-23.

Shin, E.C., Craft, B.D., Pegg, R.B., Phillips, R.D. and Eitenmiller, R.R., 2010. Chemometric approach to fatty acid profiles in runner-type peanut cultivars by principal component analysis (PCA). Food Chemistry 119: 1262-1270.

Soxhlet, F., 1879. Die gewichtsanalytische Bestimmung des Milchfettes. Dinglers Polytechnisches Journal 232: 461-465.

Statistical Analysis System (SAS) Institute, 2002. SAS user's guide: statistics, 8th Ed. SAS Institute, Carry, NC, USA.

Sun, W., Kawano, Y., Shiomori, K., Yonekura, M., Mitani, H. and Hatate, Y., 2001. Auto-oxidation rate of linoleic acid and effect of antioxidants on the oxidation. Kagaku Kogaku Ronbunshu 27: 76-84.

Talcott, S.T., Duncan, C.E., Pozo-Insfran, D.D. and Gorbet, D.W., 2005. Olyphenolic and antioxidant changes during storage of normal, mid, and high oleic acid peanuts. Food Chemistry 89: 77-84.

Trawatha, S.E., Tekrony, D.M. and Hildebrand, D.F., 1995. Relationship of soybean seed quality to fatty acid and C6-aldehyde levels during storage. Crop Science 35: 1415-1422.

United States Department of Agriculture (USDA), 1993. Milled peanuts: inspection instructions. USDA Agricultural Marketing Service, Fruit and Vegetable Division, Washington, DC, USA.

Vercellotti, J.R., Sanders, T.H., Chung, S.Y., Bett, K.L. and Vinyard, B.T., 2007. Carbohydrate metabolism in peanuts during postharvest curing and maturation. Developments in Food Science 37: 1547-1578.

Williams, E. and Drexler, J.S., 1981. A non-destructive method for determining peanut pod maturity. Peanut Science 8: 134-141.

Williams, E.J., Ware, G.O., Lee, J.Y. and Drexler, J.S., 1987. Effect of pod maturity and plant age on the seed size distribution of Florunner peanuts. Peanut Science 14: 79-83.

Young, C.T., Mason, M.E., Matlock, R.S. and Waller, G.R., 1972. Effect of maturity on the fatty acid composition of eight varieties of peanuts grown at Perkins, Oklahoma in 1968. Journal of the American Oil Chemists' Society 49: 314-317.

Zacheo, G., Cappello, M.S., Gallo, A., Santino, A. and Cappello, A.R., 2000. Changes associated with postharvest ageing in almond seeds. LWT-Food Science and Technology 33: 415-423.