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RESEARCH ARTICLE

Influence of fatty acid contents and biochemical changes during storage of groundnut varieties cultivated in Tamil Nadu, India

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Abstract

Groundnut varieties lose their physical and nutritional qualities during post-harvest storage, although their yield is high. In this study, we assessed the fatty acid profile and changes in their content and their influence on the seed quality of groundnut varieties (TMV 13, TMV 14, CO 6, VRI 8 and BSR 2) cultivated in Tamil Nadu, India, under normal storage conditions (with moisture content <8 %). In all varieties, the Electrical Conductivity (EC) of seed leachate, free fatty acids and lipid peroxidation were increased with age, accompanied by decreased levels of phytate content, germination percentage and vigor index. However, the seed weight, total oil and protein contents were not considerably altered up to 7 months in all varieties. Among the varieties, VRI 8, TMV 13 and TMV 14 had less upsurge of EC (44 - 47 % increase), an indicator of membrane integrity, after seven months, but CO 6 (88.67 % increase) and BSR 2 (86.89 %) showed high EC values. The oleate/linoleate ratio was high in VRI 8 (2:1) and low in BSR 2 (1.27:1). The varieties with high oleate, VRI 8 (50.68 %) and TMV 13 (49.28 %), can be stored (with >70 % germinability) for 5-6 months, whereas CO 6, with low oleate content (42.0 %), lost its germinability (below 65 %) and vigor within 3-4 months in ambient conditions. In addition, phytate, a suppressor of oxidative reactions, was relatively high in TMV 13 and TMV 14 and low in CO 6 after 5 months. Our study reveals that all varieties (VRI 8, TMV 13, TMV 14, BSR 2) with moisture content <8 %, except CO 6, can be stored for up to five months with minimum germination standards and without loss of oil and protein contents.

Keywords

fatty acid profile; groundnut; lipid peroxidation; seed germination; storage period

Introduction

Groundnut or peanut (*Arachis hypogaea*) is an economically and nutritionally important legume crop cultivated in arid and semi-arid areas worldwide. It is the most popular and vital food and oil seed crop due to the richness of oil (44 - 56 %), protein (20 - 30 %) contents and other health-promoting nutrients (1). There are many groundnut varieties, grouped into four major types (Virginia, Runner, Spanish and Valencia), cultivated based on the region and market demand. They differ in seed size, yield, oil content, nutritional composition and flavor (2). The oil composition and the level of protein are important determinants of the suitability of groundnut for the development of various products. Groundnut oil predominantly contains oleic acid (OA, 18:1), a monounsaturated fatty acid and linoleic acid (LA, 18:2), a polyunsaturated fatty acid, which together account for 75

- 85 % and it also contains 10 - 14 % of palmitic acid (16:0). The shelf life, stability and flavor of groundnut oil rely on the O/L ratio (3, 4). Due to the health benefits as well as preservation of oil shelf life, a high OA level and OA to LA (O/L) ratio are important quality requirements of groundnut oil. Most traditional cultivars of groundnut have an O/L ratio between 1.5 and 2 and several new varieties with a high O/L ratio (>9) have been developed (3 - 5). Groundnuts with high oil content such as Spanish and Runner are primarily cultivated for their high-quality cooking oil and several varieties with low oil contents (Virginia and Valencia) are cultivated for consumption and used as an ingredient for food and confectionery products (2).

India is one of the leading producers and exporters of groundnuts worldwide and most groundnut cultivators in India are small-scale farmers. Several new groundnut varieties have been produced and released over the years in India to enhance yield and productivity, by improving disease resistance and their adaptability to local ecological conditions (6). However, about 65 % of groundnut produce in India is stored for several months, by traders, oil extraction units, the food industry and farmers before transportation and final utilization (7). The most frequent and important problem encountered in the post-harvest storage of groundnut varieties is the decline in organoleptic quality, largely due to elevated levels of free fatty acid (FFA), lipid peroxidation (LPO) and oxidative rancidification of oil content during storage (8). The high fat/oil content and large proportion of unsaturated fatty acids (mainly LA) make them easily susceptible to oxidative deterioration during storage (9). This affects both their nutritional and market values. Several studies recommend the storage of groundnuts in hermetic bags/containers. This can limit/reduce moisture absorption, fungal contamination, insect infestation and oxidative rancidity. However, it leads to fermentation-associated seed deterioration through anaerobic respiration when stored for a longer time in airtight bags. This is the key reason most farmers and traders prefer to store them in open containers and cloth/jute in bulk (10, 11). In this background, we investigated the impact of the fatty acid profile and changes in their contents on seed viability and nutritional contents (protein and oil) of some popular (TMV 13, CO 6, VRI 8) and new (TMV 14 and BSR 2) groundnut varieties under normal storage conditions to ensure the maximum storage period for avoiding seed deterioration and preservation of nutritional quality.

Materials and Methods

All the chemicals and reagents used for various biochemical analyses were of analytical grade and obtained from Merck (India) and Sigma-Aldrich (India).

Seed collection and preparation

The groundnut varieties, namely TMV 13, TMV 14, CO 6, VRI 8 and BSR 2, were used in this study. They were cultivated using standard agronomic practices in the fields of Research Station under the Department of Seed Science, Tamil Nadu Agricultural University, Tamil Nadu, India. Freshly harvested mature groundnut varieties (with pods) were obtained from the Department of Seed Science, Tamil Nadu Agricultural University, India. The unshelled groundnuts were cleaned and properly dried to seed moisture content 7-8 % (below 8 % as per International Seed Testing Association, ISTA). They were separately packed in a cloth bag (3 kg of each) and stored in normal ambient conditions. Then the samples were drawn at monthly intervals for up to 7 months (P0 - P7) and the following parameters were evaluated. We carefully monitored the samples throughout the study to prevent fungal and insect infestation.

Analysis of physiological parameters

The groundnut sample (5 g) was weighed and spread on a pre-weighed dry dish for seed moisture analysis. Then it was dried in the air oven (at 105 °C) until a constant weight was obtained and the loss in mass was determined (n = 2) (12). The germination test was carried out based on the method described by ISTA (13), using two replicates (50 seeds per replicate) for each variety (n =2). The first count started on the 4th day and the final count on day 10. Then, the number of normal seedlings was counted and expressed as a germination percentage.

For the seed vigor (SV) index, the seedling length was measured (in cm) from tip of the shoot to tip of the root in twenty randomly selected normal seedlings from each variety on the 10th day of count (final count) (n =20). The SV index was calculated and expressed as a whole number (14) using the following formula. SV index = Germination (%) × [Root length (cm) + Shoot length (cm)]. The EC of the seed leachate was measured as described earlier (15), using a multirange benchtop conductivity meter (Hanna EC 215) and expressed as decisiemens per meter (dSm^{-1}). Two replicates of 25 seeds each were soaked in deionised water (50 ml, EC less than 5 dSm^{-1}) for 12 hours in a tightly closed glass jar and after that the EC of seed leachate was determined (n = 2).

Determination of biochemical parameters

Protein, oil and FFA contents: The total protein content was estimated colorimetrically using a modified biuret method (16) and expressed as a percentage. The oil content from groundnut seeds was extracted in a Soxhlet extractor using petroleum ether as a solvent (40-60 °C). The oil content was expressed in percentage using the following formula: Oil content % = (weight of oil/weight of groundnut seed) × 100 (17). The FFA content was estimated according to a standard method and expressed as a percentage (18).

LPO: The level of LPO was colorimetrically determined (in terms of malondialdehyde (MDA)) using thiobarbituric acid (TBA) method (19). Briefly, 500 mg of groundnut (without seed coat) was homogenized in 2 ml of 0.1 % trichloroacetic acid (TCA) and centrifuged for 10 min at 15000 × g. From this, 1 ml of supernatant was taken and 4 ml of 0.5 % TBA (prepared in 20 % TCA) was added. Then, the mixture was boiled at 95 °C for 20 min, cooled in an ice bath and centrifuged at 10000 × g for 15 min. The supernatant was collected and absorbance was measured at 532 nm. From this value, the nonspecific absorbance at 600 nm was subtracted and the concentration of MDA was calculated using an extinction coefficient (155 $mM^{-1}cm^{-1}$). The level of LPO was expressed in nanomoles of MDA per gram (nmol/g) groundnut weight.

Phytic acid: Groundnut (1g) was ground well and extracted with 0.5 M HNO_3 (20 ml) by continuous shaking for 3-4 hrs and filtered. Then, the filtrate (1.4 ml) was mixed with 1 ml of ferric ammonium sulfate solution (21.6 mg in 100 ml distilled water)

and boiled in a water bath (20 min). After cooling the content, 5 ml of isoamyl alcohol and 0.1 ml of ammonium thiocyanate solution were added and shaken well. Then centrifuged at 3000 rpm for 10 min. A series of standard solutions of sodium phytate (0.05-0.5 mg/ml) was prepared, made up to 1.4 ml with distilled water and analysed alongside the samples using the same procedure described above. The amyl alcohol layer was separated and read at 465 nm exactly 15 min after addition of ammonium thiocyanate. Isoamyl alcohol was used as a blank (20). A standard curve was plotted and the phytic acid content was calculated based on the following formula and expressed as mg/100 g dry weight basis. Phytic acid (mg/100 g groundnut) = [(C × V × 100)/W × V1 × 1000)], where C = concentration of sample for graph, V = total volume of the sample extract, W = weight of the sample, V1 = volume of aliquot taken.

Fatty acid composition: The fatty acid compositions of groundnut varieties were assessed through Gas Chromatography-Mass Spectroscopy (GC-MS) by converting FFAs into their methyl esters, as previously described (21). GC analysis was carried out on these methyl esters in a gas chromatograph (Agilent 5975C) equipped with a Flame Ionization Detector (FID) and a mass spectrometer (Agilent 5975C) with a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm). The percentage of each component was determined from the GC-FID peak areas and they were identified using WILEY, NIST and FLAVOR libraries for MS analysis.

Statistical analysis

All biochemical analyses were performed in triplicate (n = 3). The results are presented as mean values of triplicates. The data were analysed using a completely randomized design. Statistical difference among groups were analysed by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS software (Version 18.0). The Critical Difference (CD) was computed at 5 % probability level (22). The level of significance was set at $P < 0.05$.

Results

Changes in moisture content and seed weight during storage

The groundnut pods/kernels are widely stored with moisture content between 6 % and 8 % for maintaining seed quality (23, 24). The freshly harvested groundnut pods (TMV 13, TMV 14, CO 6, VRI 8 and BSR 2) were properly dried to below 8 % moisture content and maintained (ranged from 7.35 to 7.8 %) throughout the study. There was no notable change in the moisture content of all other varieties during the storage period, except for TMV 14, where moisture content increased from 6.95 % (initial) to 7.8 % (after 7 months). In addition, BSR 2 recorded a high moisture content (7.65 %) on day 0 of storage but was not altered throughout the storage period. Irrespective of varieties, the overall mean moisture content did not considerably increase (0.35 % increase from initial) after 7 months. Notably, a negligible fluctuation in moisture content was detected during the storage period which could be due to seasonal variations in atmospheric humidity and temperature.

The weight of the hundred seeds (HSW) varied between varieties after initial drying. VRI 8 (48.96g) had the highest HSW while TMV 14 (35.49g) and TMV 13 (36.77g) recorded the lowest HSW among the varieties used. However, there were no significant ($P < 0.05$) changes/decreases in HSW after 7 months of storage in all varieties. The reduction of mean HSW (considering all varieties together) from initial HSW was only 2.1 % and ranged between 0.08 % and 4.95 %. Among the varieties, BSR 2 showed a reduction of up to 5 % in HSW, but VRI 8 did not show any changes at the end of the storage period (Table 1). These results indicate that the seed weight was not significantly altered during the storage period.

Table 1. Changes in moisture content and seed weight during storage

Moisture content (%)						
Period	**TMV 13**	**TMV 14**	**CO 6**	**VRI 8**	**BSR 2**	**Mean**
P_0	7.20^a	6.95^a	7.15^a	7.15^a	7.65^a	**7.22**
P_1	7.05^a	6.95^a	7.15^a	7.20^a	7.45^a	**7.16**
P_2	7.25^a	7.30^a	7.40^a	7.40^a	7.45^a	**7.36**
P_3	7.40^a	7.40^a	7.25^a	7.55^a	7.45^a	**7.41**
P_4	7.45^a	7.45^a	7.50^a	7.60^a	7.50^a	**7.50**
P_5	7.40^a	7.40^a	7.50^a	7.50^a	7.65^a	**7.49**
P_6	7.40^a	7.40^a	7.55^a	7.60^a	7.65^a	**7.52**
P_7	7.35^a	7.80^a	7.55^a	7.50^a	7.65^a	**7.57**
Mean	**7.31**	**7.33**	**7.38**	**7.44**	**7.56**	
Difference*	**0.15**	**0.85**	**0.4**	**0.35**	**0**	**0.35**
	V	**P**	**Vx P**			
SEd	0.040	0.051	0.113			
CD (0.05)	0.079	0.101	0.225			
Hundred seed weight (g)						
Period	**TMV 13**	**TMV 14**	**CO 6**	**VRI 8**	**BSR 2**	**Mean**
P_0	36.77^a	35.49^a	46.05^a	48.96^a	40.84^a	**41.62**
P_1	36.38^a	34.93^a	44.96^a	48.94^a	40.98^a	**41.23**
P_2	36.13^a	34.93^a	44.61^a	48.98^a	40.86^a	**41.10**
P_3	36.06^a	35.92^a	45.86^a	48.77^a	40.76^a	**41.47**
P_4	35.12^a	36.38^a	45.91^a	47.31^a	40.77^a	**41.10**
P_5	35.96^a	36.28^a	47.07^a	48.22^a	40.48^a	**41.60**
P_6	36.26^a	34.78^a	46.09^a	47.92^a	39.84^a	**40.98**
P_7	35.35^a	34.77^a	44.80^a	48.92^a	38.82^a	**40.73**
Mean	**36.00**	**35.43**	**45.67**	**48.63**	**40.42**	
**Difference ** **	**3.89**	**2.03**	**2.71**	**0.08**	**4.95**	**2.14**
	V	**P**	**Vx P**			
SEd	0.328	0.415	0.929			
CD (0.05)	0.666	0.842	1.884			

*Difference in moisture content (P7-P0), ** % reduction in seed weight (P7 vs P0). P0 to P7 = Period in months, V = Variety, P = Period.

Each value is a mean from two replicates (n = 2). Means values within a row having same superscript letter (a) are not significant at $P < 0.05$.

Germination and vigor index reduces with an increase in age

The EC of seed leachate is one of the rapid tests for seed quality that negatively correlates with germination potential. We measured the EC value of groundnut seed leachate at monthly intervals (1 to 7 months). Irrespective of varieties, the initial (P0) mean EC value was 88.9 dSm^{-1} (mean), ranged from 85.4 to 92.4 dSm^{-1} and progressively increased after 3 months of storage. In all varieties, the EC value significantly ($P < 0.05$) increased more than >40 % (ranging between 44.7 % and 88.7 %) at the end of 7 months. The lowest EC values were recorded in BSR 2 (85.4 dSm^{-1}) and CO 6 (85.6 dSm^{-1}) on the initial day and increased up to 88 % (159.6 dSm^{-1} in BSR 2 and 161.5 dSm^{-1} in CO 6) after 7 months of storage. Among all varieties, TMV 13 (133.7 dSm^{-1}) and TMV 14 (131.9 dSm^{-1}) showed less upsurge of EC values (~45 % increase) after 7 months of storage (Table 2).

Next, we assessed the germination percentage and SV index at monthly intervals to monitor the viability and germinability of groundnut seeds during the storage period. Irrespective of varieties, the overall mean germination was 46 % on the initial day and reduced to 35 % at the end of 7 months. The germination and SV index were swiftly increased

Table 2. Changes in electrical conductivity (dSm^{-1}) during storage

Period	TMV 13	TMV 14	CO 6	VRI 8	BSR 2	Mean
P_0	92.4^{a}	90.6^{a}	85.6^{a}	90.4^{a}	85.4^{a}	**88.9**
P_1	92.6^{a}	89.4^{a}	86.6^{a}	91.7^{a}	85.5^{a}	**89.1**
P_2	90.9^{a}	93.2^{a}	88.2^{a}	91.0^{a}	88.7^{a}	**90.4**
P_3	93.3^{a}	94.5^{a}	90.4^{ab}	92.2^{a}	89.9^{a}	**92.0**
P_4	99.3^{ab}	101.5^{b}	99.2^{b}	103.5^{b}	103.9^{b}	**101.4**
P_5	110.6^{bc}	111.6^{b}	112.4^{c}	124.0^{c}	129.1^{c}	**117.5**
P_6	119.1^{c}	126.5^{c}	135.6^{d}	135.9^{d}	146.1^{d}	**132.6**
P_7	133.7^{d}	131.9^{c}	161.5^{e}	133.0^{d}	159.6^{e}	**143.9**
Mean	**104**	**104.9**	**107.4**	**107.7**	**111**	
Difference in Percentage *						
P3 vs P0	0.97	4.30	5.61	1.99	5.27	3.49
P4 vs P0	7.47	12.03	15.89	14.49	21.66	14.06
P5 vs P0	19.70	23.18	31.31	37.17	51.17	32.17
P7 vs P0	44.70	45.58	88.67	47.12	86.89	61.87
	V	**P**	**Vx P**			
SEd	0.487	0.616	1.378			
CD (0.05)	0.966	1.222	2.372			

*Percentage increase from the initial (vs P0)

P0 to P7 = Period in Months, V = Variety, P = Period

Each value is a mean from 2 replicates (n = 2). Mean values within a column having different superscripts (a-e) are significant (P < 0.05)

(P < 0.05) during the first month (P1), remained high up to 3 months of storage period (P3) and considerably decreased after 4 months (P4) of storage in all varieties. The reduction of germination was relatively high (P < 0.05) after 5 months (P6) of storage. Notably, the initial (P0) germination and SV index were relatively low in all varieties except VRI 8, possibly due to seed dormancy following drying. On day 0 of storage (P0), CO 6, a dormant variety, exhibited very low germination (18 %) and SV index (478) while VRI 8, a non-dormant variety, had remarkably high germination (88 %) and SV index (2772) among all varieties. In contrast, CO 6 showed a significant increase (P < 0.05) in germination and SV index, increasing up to 4.8-fold and 7.2-fold, respectively, at 3 months of storage, however, they rapidly declined during further storage periods. In addition, the mean germination and SV index of TMV 13 (72 % and 2352) and VRI 8 (71 % and 257) were relatively high among all varieties, irrespective of the storage period. Collectively, these results indicate that groundnut seeds with pods can be stored with the standard minimum germination percentage (70 %) for 4-5 months irrespective of the variety. The varieties such as TMV 13 and VRI 8 can maintain germination percentage (>70 % according to ISTA) and SV index up to 5 months of storage but CO 6 significantly loses their germinability and SV after 3 months (Table 3).

Biochemical changes in seeds of groundnut varieties with increase of storage period and age

The oil and protein contents are important determinants of nutritional quality and commercial values of groundnut kernels. First, we determined the fatty acid composition of freshly harvested groundnut varieties using GC-MS analysis. As shown in Table 4 and Fig. 1, OA (monounsaturated) and LA (polyunsaturated) acids constitute nearly 80 % of total FFAs in all varieties of groundnut oil. Among the varieties analysed, the OA level was high in CO 6 (51.04) and VRI 8 (50.68 %) and low in BSR 2 (44.51 %) while the level of LA was high in BSR 2 (35.13 %) and low in VRI 8 (27.90 %). Based on the O/L ratio, the groundnut varieties ranked in the following order: VRI 8 >CO 6>TMV 13>TMV 14>BSR2. VRI 8 and BSR 2 had the highest (2:1) and lowest (1.27:1) O/L ratio, respectively. In addition, palmitic, stearic and arachidonic acid levels ranged 13.56-14.44 %, 3.50-5.69 % and 1.09-3.0 %, respectively. Notably, trace amounts of squalene content were detected in VRI 8 (0.053 %) and BSR 2 (0.095 %).

Table 4. Fatty acid profile of groundnut varieties

Compound	TMV 13	TMV 14	CO 6	VRI 8	BSR 2
Oleic acid (O)	49.28	46.59	51.04	50.68	44.51
Linoleic acid (L)	30.28	32.41	29.72	27.90	35.13
Palmitic acid	13.75	14.44	13.89	14.22	13.56
Stearic acid	05.24	04.65	3.50	04.16	05.69
Arachidonic acid	01.42	01.88	1.93	03.00	01.09
Squalene (Antioxidant)	-	-		0.053	0.095
O/L ratio	**1.6:1** 50:30	**1.5:1** 45:30	**1.71:1** 51:30	**2:1** 50:25	**1.27:1** 45:35

Values are expressed as a percentage (%).

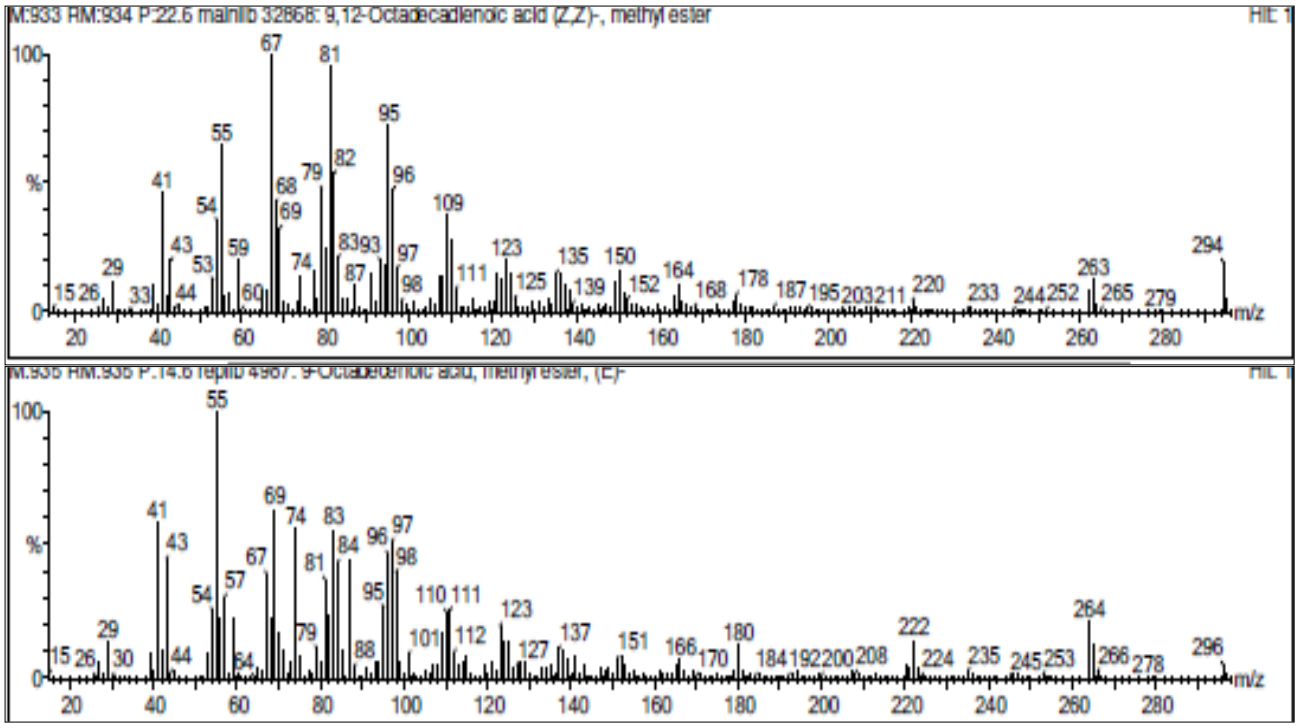
Table 3. Changes in the germination and vigor index during storage

Germination %							Vigor index						
Period	**TMV 13**	**TMV 14**	**CO 6**	**VRI 8**	**BSR 2**	**Mean**	**Period**	**TMV 13**	**TMV 14**	**CO 6**	**VRI 8**	**BSR 2**	**Mean**
P_0	54^{a}	38^{a}	18^{a}	88^{a}	30^{a}	**46**	**P_0**	1473^{a}	825^{a}	478^{a}	2772^{a}	912^{a}	**1292**
P_1	87^{b}	70^{b}	70^{b}	84^{ab}	72^{b}	**77**	**P_1**	2746^{b}	2373^{b}	2116^{b}	3139^{b}	2222^{b}	**2519**
P_2	84^{b}	75^{b}	88^{c}	86^{ab}	88^{c}	**84**	**P_2**	3417^{c}	2643^{bc}	3545^{c}	3798^{c}	3320^{c}	**3344**
P_3	82^{b}	74^{b}	86^{c}	88^{a}	84^{c}	**83**	**P_3**	3130^{c}	2846^{bc}	3442^{c}	3930^{c}	2885^{d}	**3247**
P_4	80^{bc}	75^{b}	65^{b}	79^{bc}	81^{c}	**76**	**P_4**	2859^{b}	2585^{bc}	2274^{b}	3263^{b}	2149^{b}	**2626**
P_5	75^{c}	68^{b}	51^{d}	72^{c}	56^{d}	**61**	**P_5**	2598^{b}	1207^{d}	1345	2783^{a}	1537^{e}	**1694**
P_6	63^{d}	53^{c}	31^{e}	57^{d}	45^{e}	**48**	**P_6**	1532^{a}	846^{a}	508^{a}	1110^{d}	686^{a}	**936**
P_7	53^{a}	42^{a}	18^{a}	40^{e}	24^{a}	**35**	**P_7**	1062^{d}	575^{a}	294^{a}	820^{d}	345^{e}	**619**
Mean	**72**	**62**	**53**	**71**	**60**		**Mean**	**2352**	**1737**	**1750**	**2577**	**1757**	
Fold change							**Fold change**						
P1 vs P0	1.61	1.84	3.89	0.95	2.40	**1.67**	**P1 vs P0**	1.86	2.88	4.43	1.13	2.44	**1.95**
P2 vs P0	1.56	1.97	4.89	0.98	2.93	**1.83**	**P2 vs P0**	2.32	3.20	7.42	1.37	3.64	**2.59**
P3 vs P0	1.52	1.95	4.78	1.00	2.80	**1.80**	**P3 vs P0**	2.12	3.45	7.20	1.42	3.16	**2.51**
P4 vs P0	1.48	1.97	3.61	0.90	2.70	**1.65**	**P4 vs P0**	1.94	3.13	4.76	1.18	2.36	**2.03**
P5 vs P0	1.39	1.79	2.83	0.82	1.87	**1.33**	**P5 vs P0**	1.76	1.46	2.81	1.00	1.69	**1.31**
P7 vs P0	0.98 (-1.9)	1.11 (10.5)	1.00 (0)	0.45 (-54.6)	0.80 (-20.0)	**0.76 (-23.9)**	**P7 vs P0**	0.72 (-27.9)	0.70 (-30.3)	0.62 (-38.5)	0.30 (-70.4)	0.38 (-62.2)	**0.48 (-52.1)**
	V	**P**	**Vx P**					**V**	**P**	**Vx P**			
SEd	0.616	0.78	1.744				**SEd**	82.17	103.93	232.41			
CD (0.05)	1.25	1.582	3.537				**CD (0.05)**	166.68	205.99	471.44			

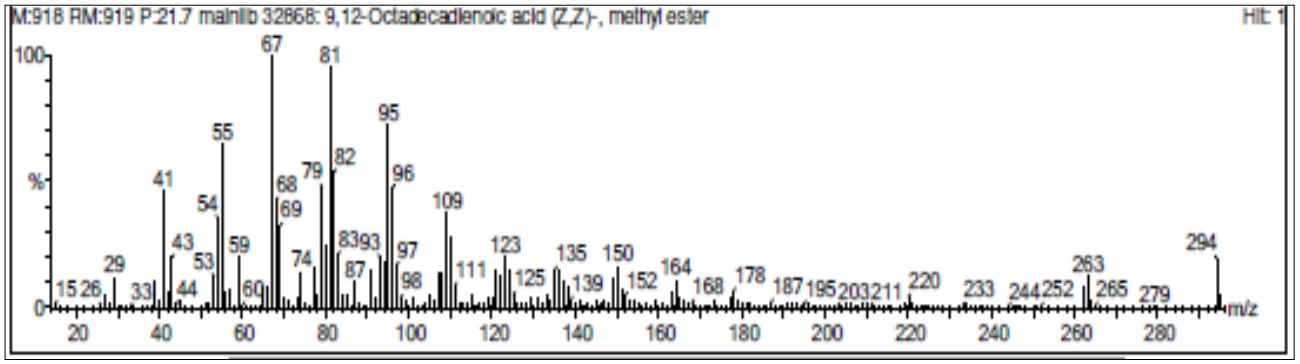
Values in the brackets indicate the % change from P0. P0 to P7 = Period in months, V = Variety, P = Period

Each value is a mean from 2 replicates (n = 2) for germination test and 20 samples for vigor index (n=20)

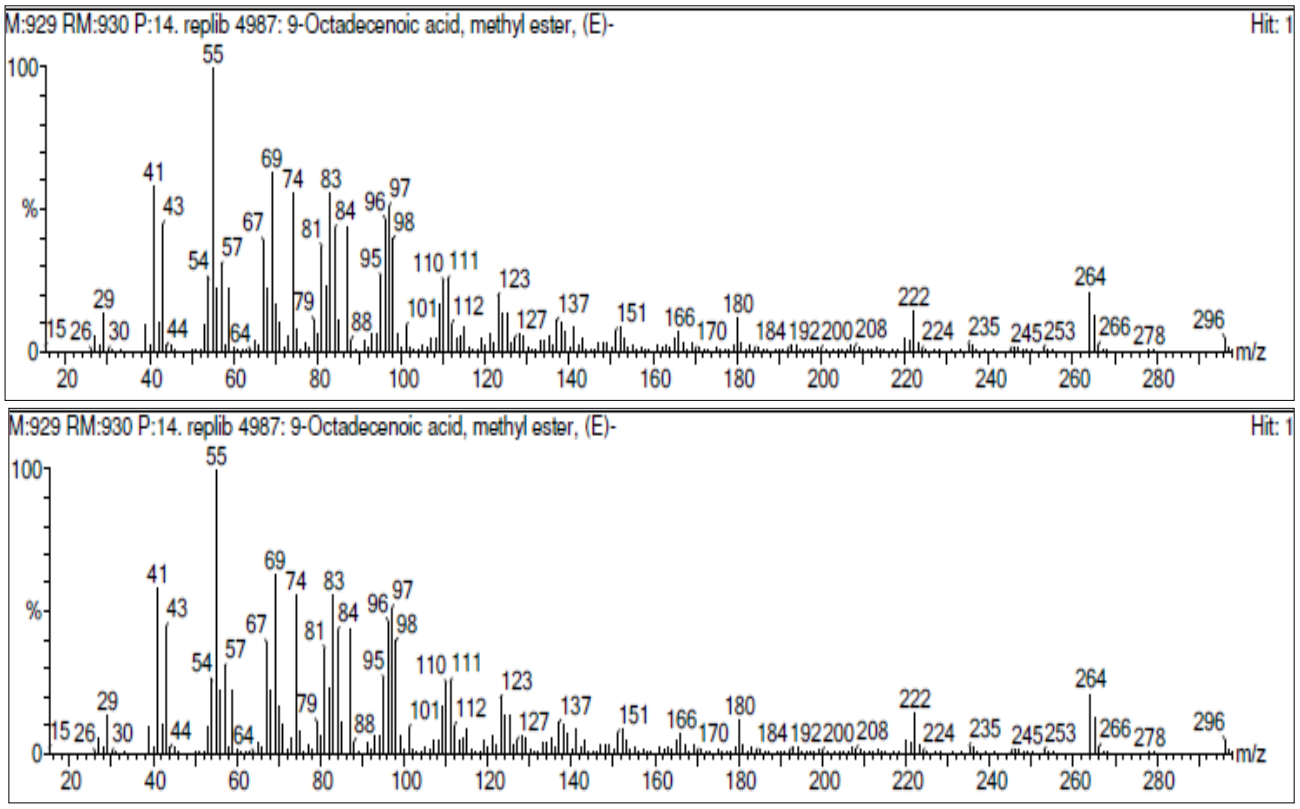
Means values within a column having different superscript (a-e) are significant (P < 0.05)



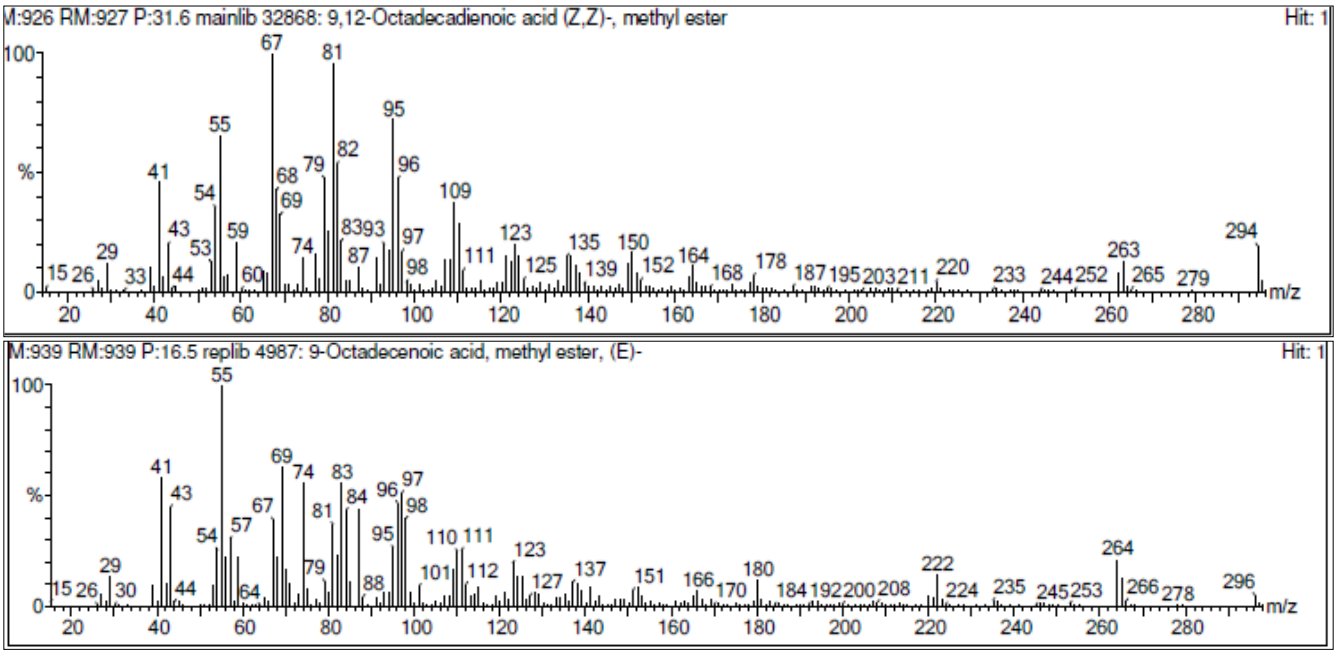
A. Chromatogram 1. TMV 13 GC- MS spectra for linoleic acid and oleic acid.



B. Chromatogram 2. TMV 14 GC- MS spectra for linoleic and oleic acid.



C. Chromatogram 4. VRI 8 GC- MS spectra for linoleic and oleic acid.



D. Chromatogram 5. BSR 2 GC- MS spectra for linoleic acid and oleic acid.

Fig. 1. Chromatogram of fatty acids of studied varieties.

As we observed that EC, seed germination and SV index were considerably altered after 4 months of storage, we estimated the oil and protein contents from 3rd month of storage. The percentage of oil content varied among the varieties (ranging between 45.70 % and 50.25 %) but was not significantly reduced ($P < 0.05$) throughout storage period. On day 0 of storage, BSR 2 recorded the lowest (45.70 %) and VRI 8 recorded the highest (50.25 %) oil content. Irrespective of varieties, the mean oil content was reduced by only 1.87 % (from 48.57 % at P0 to 46.7 % at P7) by the end of storage, which was about a 3.85 % reduction over the P0 of storage. In all varieties, a negligible reduction in oil content, less than 1 % decrease (<2 % decrease over P0) and not more than 1.75 % reduction (<4 % decrease over P0) were observed after 3 months and 5 months respectively. Among all varieties, TMV 13 had a reduction up to 3.95 % (8.04 % decrease over the P0) of oil content, but TMV 14 exhibited only a 0.2 % reduction from the initial (P0) oil content after 7 months of storage.

The average protein content, irrespective of varieties, was initially 22.94 % (ranging from 21.86 % to 25.51 %) and reduced to 20.91 % after 7 months of storage. Overall, there were no significant ($P < 0.05$) changes, only more than 1 % and up to 3.02 % (in TMV 14), in reduction of protein content was observed at the end of the storage period. Notably, VRI 8 recorded the highest protein content, 25.51 % at P0 and 23.89 % at P7, among the varieties used in this study. Notably, the lowest reduction (1.13 %) was observed in CO 6, followed by VRI 8 (1.62 %) after 7 months of storage period (P7) (Table 5). Together, these results reveal that only a slight/negligible variation in the oil and protein contents irrespective of the storage period despite there being a difference in the levels of oil and protein contents between varieties.

Table 5. Changes in seed oil and protein content during storage

	Seed oil content (%)						Protein content (%)					
Period	**TMV 13**	**TMV 14**	**CO 6**	**VRI 8**	**BSR 2**	**Mean**	**TMV 13**	**TMV 14**	**CO 6**	**VRI 8**	**BSR 2**	**Mean**
P_0	49.15^a	48.55^a	49.20^a	50.25^a	45.70^a	**48.57**	22.16^a	23.27^a	21.86^a	25.51^a	21.91^a	**22.94**
P_3	48.20^a	48.60^a	48.65^a	50.45^a	45.10^a	**48.20**	22.25^a	22.44^a	21.84^a	24.98^a	21.81^a	**22.66**
P_4	48.65^a	48.60^a	48.35^a	50.30^a	44.95^a	**48.17**	21.81^a	22.03^a	22.08^a	25.08^a	21.63^a	**22.53**
P_5	48.05^a	48.65^a	48.10^a	49.80^a	43.95^a	**47.71**	21.53^a	21.75^a	22.06^a	24.44^a	21.06^a	**22.17**
P_6	45.70^a	48.40^a	47.80^a	49.40^a	43.85^a	**47.03**	20.14^a	20.23^a	21.01^a	24.05^a	20.50^a	**21.18**
P_7	45.20^a	48.35^a	47.45^a	48.80^a	43.70^a	**46.70**	20.13^a	20.25^a	20.73^a	23.89^a	19.56^a	**20.91**
Mean	**47.49**	**48.53**	**48.26**	**49.83**	**44.54**		**21.33**	**21.66**	**21.59**	**24.66**	**21.08**	
	% reduction over P0						**Difference (from P0)**					
P3 vs P0	1.93	0.10	1.12	0.40	1.31	**0.76**	0.09	0.83	0.02	0.53	0.10	**0.28**
P5 vs P0	2.24	0.21	2.24	0.90	3.83	**1.77**	0.63	1.52	0.20	1.07	0.85	**0.77**
P7 vs P0	8.04	0.41	3.56	2.89	4.38	**3.85**	2.03	3.02	1.13	1.62	2.35	**2.03**
	V	**P**	**Vx P**				**V**	**P**	**Vx P**			
SEd	0.286	NS	0.699				0.188	NS	0.46			
CD (0.05)	0.586		1.435				0.386		0.945			

P0 to P7 = Period in months, V = Variety, P = Period. Each value is a mean from three replicates (n = 3). Means values within a column having same superscript letter (a) are not significant at $P < 0.05$

FFA contents and LPO level increases with increase of storage period

The levels of FFA contents and LPO products are closely associated with oil and nutrient quality, shelf life and seed deterioration in oil seeds including groundnut. The levels of FFAs and LPO ranged between 0.580 % - 0.710 % and 0.126 - 0.228 nmol/g respectively, on the initial day of storage (Table 6). FFA and LPO levels were comparatively higher in VRI 8 (0.710 % and 0.228 respectively) followed by TMV 14 (0.695 % and 0.190 respectively) on day 0 (P0) of storage. The overall mean FFA contents drastically increased ~4 fold (299 % increase over P0) and LPO increased ~2.66 fold (166 % increase over P0) after 7 months of storage. Moreover, FFA contents were comparatively high in VRI 8 (4.79-fold vs P0) and BSR 2 (4.73-fold vs P0), while the LPO level was high in CO 6 (3.23-fold vs P0) after 7 months. Next we determined the level of phytate content, which is an important factor that protects seeds against oxidative stress (25, 26). TMV 13 (280.65 mg/100g) and TMV 14 (280 mg/100g) had high phytic acid content in all 5 varieties. Irrespective of varieties, the phytic acid content reduced by more than 20 %, from 259.16 mg/100g (initial) to 202.31 mg/100g (P5), after 5 months of storage. In addition, a significant reduction ($P < 0.05$) up to 35.55 % (ranged between 25.02 % and 35.55 %), from 259.16 mg/100g (initial) to 184.47 mg/100g (P7), was observed by the end of the storage period. Notably, CO6 exhibited a comparatively low phytic acid content (84.4 mg/100g reduction from initial) after 7 months of storage. Together, our findings reveal that a reduction of phytic acid contents in groundnut varieties could be responsible for the increase of FFAs and LPO during the storage of groundnut seeds.

Discussion

The loss of quality and seed viability during storage is a crucial problem in most groundnut-cultivating countries, including India (27, 28). Thus, it is important to know the nutritional quality and shelf life of the groundnut varieties under normal storage conditions to ensure an optimum storage period to avoid the reduction of oil/nutritional quality and seed deterioration during storage. In this study, we found that popular varieties with high oil contents and OA, such as TMV 13 and VRI 8, can be stored under normal storage conditions (in cloth bags with moisture content <8 %) for more than 5 months and up to 6 months without significant loss of seed oil and protein contents as well as seed germination and SV index according to Indian Minimum Seed Certification Standards (IMSCS). Similarly, TMV 14, a variety with an O/L ratio of 1.5:1, can be stored for up to 5 months (with 70 % germination) without changes in the levels of oil and protein contents. In contrast, CO 6 variety has a comparatively low shelf-life of up to 3 months in terms of germinability and SV index, despite it having high OA content and no considerable changes in oil and protein contents even after 7 months of storage.

In oil seeds such as groundnuts, the chemical composition, primarily lipid contents, has a great influence on the shelf-life, germination and seed viability (29). The storage of groundnuts in ambient conditions with normal cloth bags and exposure to air intensifies the oxidative process of lipids in seeds and consequently increases the level of FFAs. This leads to the acceleration of oxidative rancidity, which affects the nutritional value and quality of groundnuts during storage (8, 9). Since lipid contents are key reservoirs for energy during the growth of an embryo in oil seeds, the alterations in seed lipid composition during the storage can affect the germination and vigor of groundnut seeds by influencing seed membrane lipid composition and permeability (29). Under normal storage conditions, FFAs progressively accumulate in groundnut seeds with the increase of age that are mobilized from triglycerides of oil due to enzymatic activity and auto-oxidation. In this context, we found that the LPO level is remarkably increased and accompanied by a several-fold increase of FFA contents in all five varieties after storage of 7 months.

Interestingly, the germination percentage and SV index are comparatively higher in varieties such as VRI 8, TMV 13 and TMV 14, up to 5 months, than in CO 6 and BSR 2, despite the levels of FFAs and LPO increased in aged seeds of all varieties. This disparity in germination and seed viability between these varieties might be associated with the variation in cell membrane integrity of seeds as indicated by a less upsurge of EC in VRI 8, TMV 13 and TMV 14 and high in CO 6 and BSR 2 varieties. In addition, the level of phytic acid, a vital contributor to seed germination and lifespan (25, 26), is comparatively high in VRI 8, TMV 13 and TMV 14. It is well-documented that phytic

Table 6. Changes in total free fatty acid content, lipid peroxidation products and phytic acid content during storage

	Total free fatty acid (%)					Lipid peroxidation product (nmol/g)						Phytic acid content (mg/100 g)					
Months	**P_0**	**P_7**	**Mean**	**Fold change***	**% Increase #**	**P_0**	**P_5**	**P_7**	**Mean**	**Fold change***	**% Increase #**	**P_0**	**P_5**	**P_7**	**Mean**	**Reduction level**	
Varieties																**P_5-P_0**	**P_7-P_0**
TMV 13	0.580^a	2.100^b	**1.340**	3.62	262.07	0.160^a	0.211^b	0.426^c	**0.266**	2.66	166.25	280.65^a	225.00^b	196.30^b	**233.98**	55.65	84.35
TMV 14	0.695^a	2.275^b	**1.485**	3.27	227.34	0.190^a	0.295^b	0.433^c	**0.306**	2.28	127.89	280.00^a	218.95^b	209.95^b	**233.30**	61.05	70.05
CO 6	0.640^a	2.235^b	**1.438**	3.49	249.22	0.126^a	0.207^b	0.407^c	**0.247**	3.23	223.02	237.40^a	177.75^b	153.00^b	**189.38**	59.65	84.40
VRI 8	0.710^a	3.400^b	**2.055**	4.79	378.87	0.228^a	0.334^b	0.632^c	**0.398**	2.77	177.19	255.30^a	198.55^b	188.05^b	**213.97**	56.75	67.25
BSR 2	0.615^a	2.910^b	**1.763**	4.73	373.17	0.182^a	0.273^b	0.460^c	**0.305**	2.53	152.75	242.45^a	191.30^b	175.05^b	**202.93**	51.15	67.40
Mean	**0.648**	**2.584**		**3.99**	**298.77**	**0.177**	**0.264**	**0.471**		**2.66**	**166.10**	**259.16**	**202.31**	**184.47**		**56.85**	**74.69**

	V	P	Vx P			V	P	Vx P			V	P	Vx P
SEd	0.039	0.025	0.055		**SEd**	0.004	0.003	0.007		**SEd**	1.072	0.831	1.857
CD (0.05)	0.088	0.056	0.124		**CD (0.05)**	0.009	0.007	0.015		**CD (0.05)**	2.306	1.786	3.994

Percentage increase from the initial (vs P0). P0 to P7 = Period in months, V = Variety, P = Period. Each value is a mean from three replicates (n =3). Means values within a row having different superscripts (a-c) are significant ($P < 0.05$)

acid, as a reservoir of phosphorus and mineral cations, supports metabolic processes involved in the early stage of seedling development and growth (30). It also reduces the degree of oxidative stress by preventing/scavenging reactive oxygen species, thereby preserving the viability of the embryo of the seed (26, 31). Despite the levels of LPO and FFAs being relatively high in VRI 8, the SV and germinability (as per IMSCS) are remarkably maintained with less damage to the cell membrane, as reflected by comparatively little increase of EC. This could be linked to high OA content and O/L ratio (2:1) in VRI 8 in addition to traces of squalene content, which is a peroxyl radical scavenger that protects PUFAs, including LA and arachidonic acid, from the oxidation/auto-oxidation (32). Furthermore, OA has 10-fold higher oxidative stability than LA (33) and it has a great impact on the shelf-life and flavor of groundnut (34).

Conclusion

From this study, we found that groundnut varieties such as VRI 8 and TMV 13, with a high OA content (about 50 %) and an O/L ratio of more than 1.5:1 can be stored for 5-6 months in a cloth bag with pod moisture content below 8 %. This method is suitable for both seed purposes (>70 % germination as per IMSCS) as well as for food processing purposes, as the protein and oil contents levels in these varieties are not altered during this period. However, CO 6 variety can only be stored for up to 3 months to ensure better germination and growth despite it having a high OA content (51.04 %) and O/L ratio. TMV 14 and BSR 2 can hold the minimum germinability requirement (>70 %) and they do not lose oil and protein contents for up to 4 months. This study strengthens the notion that the storage period varies for the groundnut varieties that largely depend on the levels of different chemical compositions, including fatty acids (primarily OA and LA) and antioxidants (phytic acid), in addition to cell membrane integrity of the seed. Overall, this study provides valuable information about the maximum storage period of groundnut varieties (VRI 8, TMV 13, TMV 14, BSR 2 and CO 6) with a negligible level of seed deterioration and minimal loss of germination potential, since the quality of groundnut seeds is essential for profitable production and high -quality yield as well as for high-quality food products.

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Authors' contributions

KC and RR involved in conceptualization, methodology, validation and drafted the original manuscript. SL and MU involved in prof reading of the original manuscript. KC and RR supervised the final process and fine-tuned the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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